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Host-driven subspeciation in the hedgehog fungus, *Trichophyton erinacei*, an emerging cause of human dermatophytosis

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Key words

epizootic fungal infections
microsatellite typing
multigene phylogeny
population genetics
skin infections
Trichophyton benhamiae complex
zoophilic dermatophytes

Abstract *Trichophyton erinacei* is a main cause of dermatophytosis in hedgehogs and is increasingly reported from human infections worldwide. This pathogen was originally described in the European hedgehog (*Erinaceus europaeus*) but is also frequently found in the African four-toed hedgehog (*Atelerix albiventris*), a popular pet animal worldwide. Little is known about the taxonomy and population genetics of this pathogen despite its increasing importance in clinical practice. Notably, whether there are different populations or even cryptic species associated with different hosts or geographic regions is not known. To answer these questions, we collected 161 isolates, performed phylogenetic and population-genetic analyses, determined mating-type, and characterised morphology and physiology. Multigene phylogeny and microsatellite analysis supported *T. erinacei* as a monophyletic species, in contrast to highly incongruent single-gene phylogenies. Two main subpopulations, one specific mainly to *Atelerix* and second to *Erinaceus* hosts, were identified inside *T. erinacei*, and slight differences in the size of microconidia and antifungal susceptibilities were observed among them. Although the process of speciation into two lineages is ongoing in *T. erinacei*, there is still gene flow between these populations. Thus, we present *T. erinacei* as a single species, with notable intraspecies variability in genotype and phenotype. The data from wild hedgehogs indicated that sexual reproduction in *T. erinacei* and de novo infection of hedgehogs from soil are probably rare events and that clonal horizontal spread strongly dominates. The molecular typing approach used in this study represents a suitable tool for further epidemiological surveillance of this emerging pathogen in both animals and humans. The results of this study also highlighted the need to use a multigene phylogeny ideally in combination with other independent molecular markers to understand the species boundaries of dermatophytes.

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INTRODUCTION

Trichophyton erinacei and other species from the *Trichophyton benhamiae* complex are mainly zoophilic fungal pathogens that are frequently transmitted to humans. These zoonotic infections, usually manifesting as highly inflammatory tinea corporis or tinea capitis, are common in pet owners and contribute significantly to numerous occupational infections in farmers, workers in livestock production, laboratory workers, pet shop workers, and other professions that require contact with animals (Halsby et al. 2014, Hubka et al. 2018b).

In the last decade, pathogens from the *T. benhamiae* complex have attracted considerable attention mainly due to the epizootic / epidemic spread of dermatophytosis caused by *T. benhamiae* var. *luteum* in guinea pigs and humans in Europe (Berlin et al. 2020, Čmoková et al. 2020). Similarly, the hedgehog pathogen *T. erinacei* is increasingly reported as a cause of dermatophytosis in hedgehog breeders or owners, but with a lower incidence. Unlike *T. benhamiae* var. *luteum*, the growing incidence of *T. erinacei* is not due to the emergence of a new virulent pathogen but rather to changes in preferences breeders have who increasingly buy hedgehogs as pets (Fig. 1). A worldwide

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distribution of *T. erinacei* is documented by numerous tinea cases from Europe (English et al. 1962, Romano et al. 2001, Schauder et al. 2007, Weishaupt et al. 2014, Perrier & Monod 2015, Abarca et al. 2017, Kargl et al. 2018, Lysková et al. 2018, Rivaya et al. 2020), Asia (Mochizuki et al. 2005, Lim et al. 2009, Rhee et al. 2009, Hsieh et al. 2010, Hui et al. 2017, Choi et al. 2018, Kim et al. 2018, Al Masaoodi et al. 2020, Ogawa et al. 2020, Watabe et al. 2021), America (Rosen 2000, Concha et al. 2012, Eid et al. 2014, Alejandra et al. 2018, Frantz et al. 2020, Rodríguez-Silva et al. 2021, Walsh et al. 2021) and Africa (Drira et al. 2015). Most cases described in literature were transmitted from pet African pygmy hedgehogs (syn. four-toed hedgehog; *Atelerix albiventris*). However, *T. erinacei* is also reported in wild European hedgehogs, i.e., West European hedgehogs (*Erinaceus europaeus*) and Northern white-breasted hedgehogs (*Erinaceus roumanicus*), which may represent risks for people, especially in wildlife rehabilitation centres or for dogs that may occasionally encounter hedgehogs in places of their hibernation (Le Barzic et al. 2021). Infections due to *T. erinacei* most often manifest as tinea manuum or tinea corporis localized on fingers, hands, wrists, or forearms (Fig. 2). Tinea faciei, kerion type of tinea capitis, tinea barbae and onychomycosis have also been documented in the abovementioned case reports.

Similar to *T. benhamiae* var. *luteum* in guinea pigs (Berlin et al. 2020), available epidemiological surveys showed high infection rates of *T. erinacei* in hedgehogs. The prevalence in *E. europaeus* ranged between 20–30 % in wild hedgehogs in France, Poland and Great Britain (English & Morris 1969, Gnat et al. 2021, Le Barzic et al. 2021) and up to 45 % in New Zealand (Smith & Marples 1964). The prevalence in *A. albiventris* ranged between 22–38 % in wild animals in Africa (Gregory & English 1975, Gregory et al. 1978) and 39–50 % in pet hedgehogs in Japan and Spain (Takahashi et al. 2003, Abarca et al. 2017). Hedgehogs are also the source of other potentially zoonotic bacterial, viral and fungal pathogens. Among these, *Staphylococcus aureus*, and especially methicillin-resistant *S. aureus* (MRSA) strains, belong to the most feared pathogens which have a high prevalence in hedgehogs (Bengtsson et al. 2017, Rasmussen et al. 2019). Interestingly, it was demonstrated that dermatophyte-infected hedgehogs provide a natural selective environment for MRSA because *T. erinacei* produces two antibiotics from the penicillin class (Dube et al. 2021, Larsen et al. 2022). Specific lineages of MRSA derived from hedgehogs have existed even before the era of antibiotics and their spread between livestock and humans has been confirmed (Larsen et al. 2022).

The geographic distribution of *T. erinacei* follows the distribution of hedgehogs (*Erinaceidae*) in Afro-Eurasia, with the highest diversity in Asia, where four of five genera occur, while the fifth genus *Atelerix* is found exclusively in Africa. Only two species of the genus *Erinaceus* are widespread in Europe, i.e., *E. europaeus* occurring in Western Europe and *E. roumanicus* in Eastern Europe (He et al. 2012), with the contact zone in Central Europe (Curto et al. 2019). Imperceptible morphological differences between these two species, especially in the contact zone, make exact identification difficult for non-experts (Černá Bolfíková et al. 2020). In addition to natural habitats, hedgehogs have been introduced in many non-native areas.

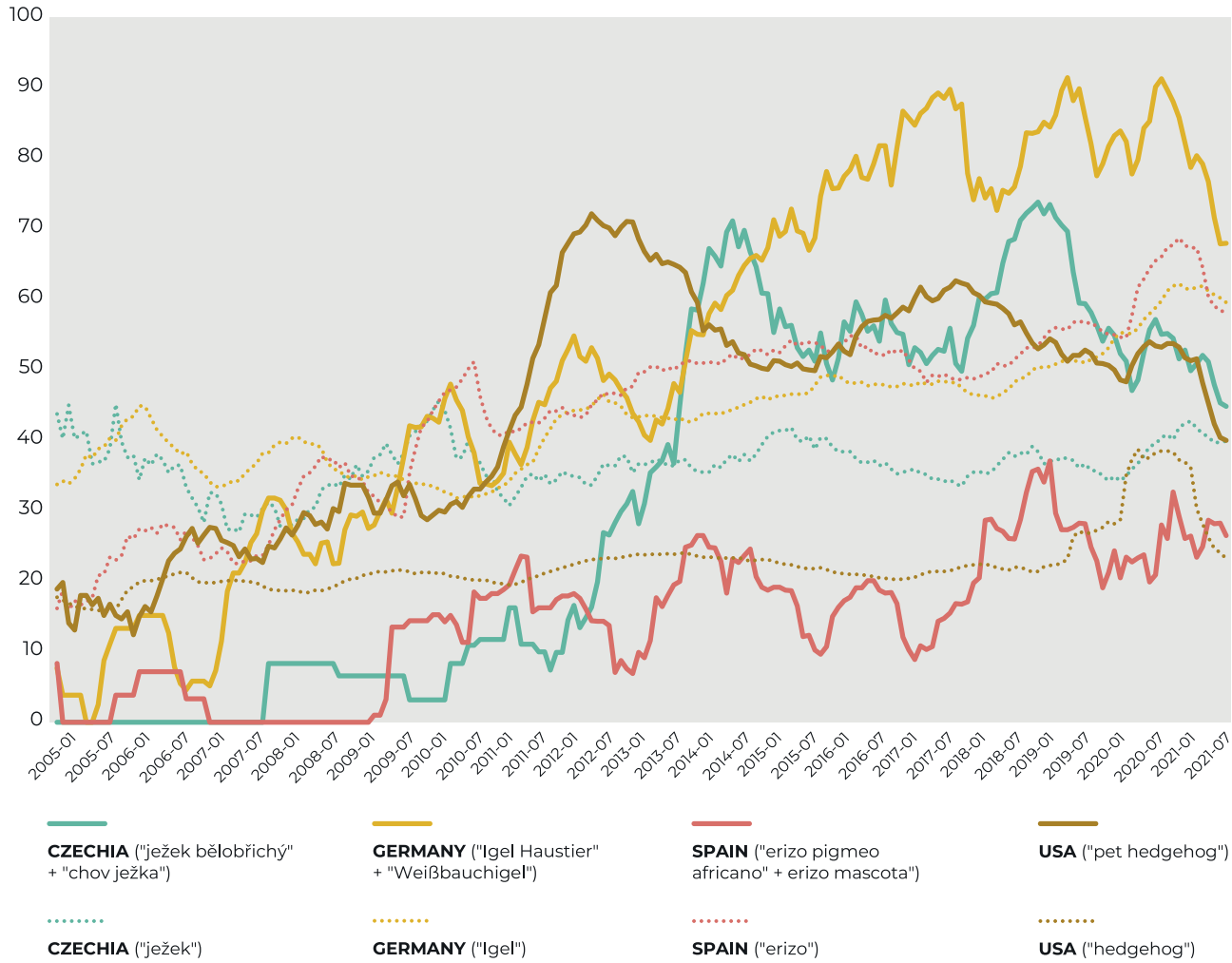

Fig. 1 Interest in hedgehogs as pet animals (solid line) compared to interest in hedgehogs in general (dotted line). Data were obtained from the Google Trends and show relative interest scaled on a range of 0 to 100 during 2005–2021 (measured as number of searches during half-year). Data are shown for four countries, and key words are listed in parentheses.

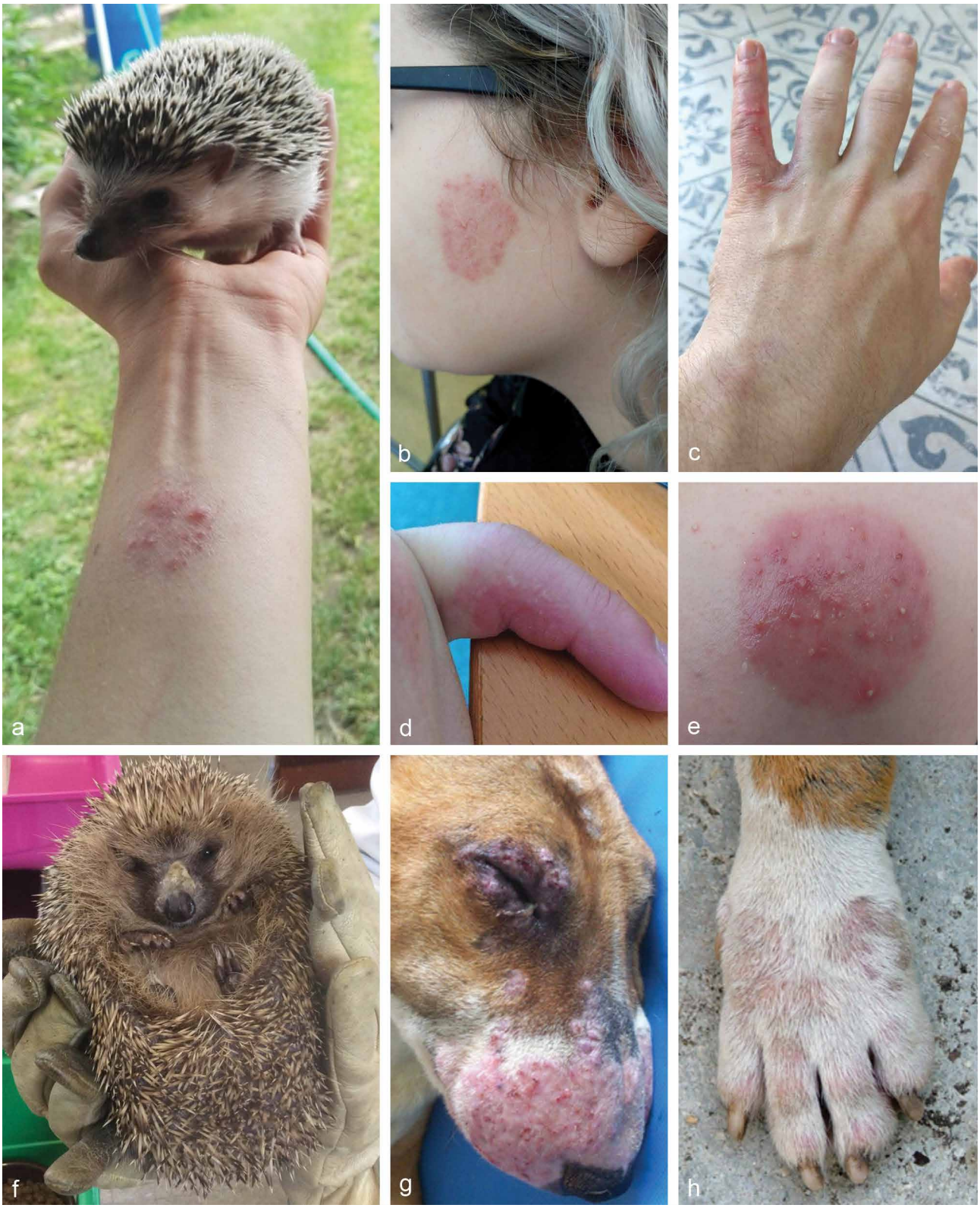

Fig. 2 Clinical manifestation of human (a–e) and animal (f–h) infections caused by *Trichophyton erinacei*. a. Tinea corporis on the left forearm (26-yr-old woman), erythaematous lesion covered with pustules, contact with a pet hedgehog (*Atelerix albiventris*) shown on the same picture, isolate IDE 740/19; b. tinea faciei (20-yr-old woman), erythaematous, scaly, sharply demarcated lesion on the left cheek, contact with a pet hedgehog (*A. albiventris*), isolate CCF 5775; c–d. erythaematous, scaly lesion on the little finger (20-yr-old man), contact with a pet hedgehog (*A. albiventris*), isolate SK 1826/19; e. tinea corporis (24-yr-old woman) on the outer side of the right thigh, lesion approximately 5 cm diam, pustules in the centre, contact with pet hedgehog (*A. albiventris*), isolate CCF 5209; f. hedgehog (*Erinaceus europaeus*) with erythematous dermatitis and scaly lesions above the muzzle; g–h. alopecia, erythema and pustules affecting head and limbs of a 4-yr-old Bull terrier, isolate CBS 124411.

The genus *Erinaceus* has been introduced to New Zealand, while *A. albiventris* and *Hemiechinus auratus* are considered invasive species in Spain. Worldwide, *A. albiventris*, *A. algirus* and their hybrids are kept as pets (Reeve 1994, He et al. 2012) or as research animals in laboratories (Lawn et al. 1995).

Erinaceus and *Atelerix* hedgehogs have been reported to be carriers of *T. erinacei* isolates with different genotypes and mating type gene idiomorphs, respectively (Takashio 1979, Takahashi et al. 2003, Schauder et al. 2007, Heidemann et al. 2010), suggesting that partially isolated populations co-evolved

with different hosts. However, whether the level of differentiation of these populations could meet the criteria for delimitation of separate species is not known. To answer this question, we collected *T. erinacei* isolates from different hedgehog genera and cases of human infections. The population structure and characteristics (e.g., genotype diversity and random mating) were examined using multilocus microsatellite typing (MLMT) and multilocus sequence typing (MLST) approaches. The association between population structure, host, geographic origin, antifungal susceptibility, mating-type gene distribution and phenotype was investigated to reveal patterns suggestive of biological divergence or (sub)speciation.

MATERIALS AND METHODS

Source of isolates

The isolates (n = 161) were collected from humans and hedgehogs and obtained in collaboration with clinical laboratories, hospitals, universities and culture collections (Table S1): National Veterinary School of Alfort (France); Autonomous University of Barcelona (Spain), Laboratory for Medical Microbiology (Germany); College of Veterinary Medicine, College of Bioresource Sciences, Nihon University (Japan); University of Turin (Italy); BCCM/IHEM Fungi Collection: Human and Animal Health (Belgium); CBS culture collection housed at the Westerdijk Institute (The Netherlands); and various institutions in the Czech Republic, i.e., Institute of Public Health in Ostrava and Ústí nad Labem, Hospital České Budějovice, General University Hospital in Prague, University Hospital in Pilsen, and the University Hospital Olomouc.

Selected isolates were deposited into the Culture Collection of Fungi (CCF), Department of Botany, Charles University, Prague, Czech Republic; and German Collection of Microorganisms and Cell Cultures (DSMZ), Leibniz Institute, Braunschweig, Germany.

Molecular studies

DNA was extracted from 7-d-old colonies using the Quick-DNA™ Fungal/Bacterial Miniprep kit (Zymo Research, USA). Amplification of the ITS rDNA region (ITS1-5.8S-ITS2 cluster) was performed using the primer set ITS1F and ITS4 (White et al. 1990, Gardes & Bruns 1993), the partial *gapdh* gene encoding glyceraldehyde-3-phosphate dehydrogenase using primers GPDF and GPDR (Kawasaki et al. 2011), the partial *tubb* gene encoding β-tubulin using primers Bt2a and Bt2b (Glass & Donaldson 1995), and the partial *tef1-α* gene encoding translation elongation factor 1-α using primers EF-DermF and EF-DermR (Mirhendi et al. 2015). Polymerase chain reaction (PCR) conditions were described by Sklenář et al. (2021). PCR products were purified using a Sap-Exo Kit (Jena Bioscience, Germany) and subsequent automated sequencing was performed at BIOCEV (Vestec, Czech Republic) using both terminal primers. The DNA sequences obtained in this study (unique genotypes only) were deposited into the GenBank database (www.ncbi.nlm.nih.gov) under the accession numbers listed in Table 1.

A fragment of the *MAT1-1-1* gene encoding a protein with an alpha-domain motif was amplified with the primer pairs MF3 and MF6 or MF3 and MF4, and a fragment of the *MAT1-2-1* gene encoding the high mobility group (HMG) domain was amplified with the primers TmHMG3S and TmHMG3R (Kano et al. 2012, Symoens et al. 2013). Several PCR products of each MAT idiomorph were subjected to DNA sequencing for verification of specificity, and the remaining samples were visualized on an electrophoretogram (1 % agarose gel with 0.5 µg/mL ethidium bromide) and identified according to the specific lengths of PCR products.

Table 1 Sequence accession numbers to unique multilocus genotypes of *Trichophyton erinacei*.

MLST genotype	Representative strain	Accession numbers				Corresponding MLMT genotype(s)
		ITS (genotype)	*tef1-α* (genotype)	*gapdh* (genotype)	*tubb* (genotype)	
G1 (35 strains)	CCF 5209	MZ314454 (a)	MZ320337 (a)	MZ320332 (a)	MZ320327 (a)	M1-M3
G2 (1 strain)	CBS 124411	MZ314453 (b)	MZ320336 (a)	MZ320331 (a)	MZ320326 (a)	M4
G3 (4 strains)	DSM 104923	MZ314456 (c)	MZ320339 (a)	MZ320334 (a)	MZ320329 (a)	M5-M6
G4 (8 strains)	CBS 511.73^{T}	LR794136 (a)	LR794267 (b)	LR794242 (a)	LR794292 (a)	M7-M9
G5 (23 strains)	CCF 6427	MZ314455 (a)	MZ320338 (a)	MZ320333 (b)	MZ320328 (a)	M10

MLST, multilocus sequence typing; MLMT, multilocus microsatellite typing

Table 2 Characteristics of alignments, partition-merging results and best substitution model for each partition according to Bayesian information criterion.

Alignment	Length (bp)	Variable position	Parsimony informative sites	Phylogenetic method	Partitioning scheme (substitution model)
ITS, *gapdh*, *tubb* & *tef1-α* (tree: Fig. 3)	2278	243	154	Maximum likelihood (ML)	Six partitions: ITS1 & ITS2 (TrN+G); 5.8S (K80); 1st codon positions of *gapdh* & *tef1-α* (F81+I); 1st codon positions of *tubb* & 2nd codon positions of *gapdh*, *tubb* & *tef1-α* (JC); 3rd codon positions of *gapdh*, *tubb* & *tef1-α* (HKY+I); introns of *gapdh*, *tubb* & *tef1-α* (K80+I+G)
ITS, *gapdh*, *tubb* & *tef1-α* (tree: Fig. 3)	2278	243	154	Bayesian inference	Five partitions: ITS1 & ITS2 & 3rd codon positions of *gapdh*, *tubb* & *tef1-α* (HKY+G); 5.8S (K80); 1st codon positions of *gapdh* & *tef1-α* (F81+I); 1st codon positions of *tubb* & 2nd codon positions of *gapdh*, *tubb* & *tef1-α* (JC); introns of *gapdh*, *tubb* & *tef1-α* (K80+I+G)
ITS (tree: Fig. 8)	592	88	53	ML	Two partitions: ITS1 & ITS2 (TrN+G); 5.8S (K80)
gapdh (tree: Fig. 8)	563	54	31	ML	Three partitions: 1st codon positions (F81+I); 2nd codon positions (JC); 3rd codon positions & introns (HKY+G)
tubb (tree: Fig. 8)	463	41	24	ML	Three partitions: 1st & 2nd codon positions (JC); 3rd codon positions (HKY+I); introns (K80)
tef1-α (tree: Fig. 8)	660	60	46	ML	Three partitions: 1st & 2nd codon positions (JC); 3rd codon positions (HKY); introns (K80+G)

Subtyping of *T. erinacei* using microsatellite markers was performed using the previously developed typing scheme for species from the *T. benhamiae* clade (Čmoková et al. 2020). The reaction volume of 5 µL for PCR contained 50 ng of DNA, 0.5 µL of the mixture of primers and 2.5 µL of Multiplex PCR Master Mix (Qiagen, Germany). The PCR conditions were chosen according to the manufacturer's recommendations. The PCR products (diluted in water 1 : 25) were mixed with 10 µL of deionized formamide and 0.2 µL of the GeneScan™ 600 LIZ size standard, denatured for 5 min at 95 °C, and subsequently analysed using an ABI 3100 Avant Genetic Analyzer.

Phylogenetic analysis

Alignments of the ITS, *gapdh*, *tubb* and *tef1-α* regions were performed using the FFT-NS-i option implemented in the MAFFT online service (Katoh et al. 2017). The alignments were trimmed, concatenated and then analysed using maximum likelihood (ML) and Bayesian inference (BI) methods. Suitable partitioning schemes and substitution models (Bayesian information criterion) for the analyses were selected using a greedy strategy implemented in PartitionFinder v. 2 (Lanfear et al. 2017) with settings allowing introns, exons, codon positions and segments of the ITS region to be independent datasets. The optimal partitioning schemes for each analysed dataset along with basic alignment characteristics are listed in Table 2.

ML trees were constructed with IQ-TREE v. 1.4.4 (Nguyen et al. 2015) with nodal support determined by ultrafast bootstrapping (BS) with 100 000 replicates. Trees were rooted with *Trichophyton rubrum*. Bayesian posterior probabilities (PP) were calculated for the concatenated dataset using MrBayes v. 3.2.6 (Ronquist et al. 2012). The analysis ran for 10^7 generations, two parallel runs with four chains each were used, every 1000th tree was retained, and the first 25 % of trees were discarded as burn-in. The convergence of the runs and effective sample sizes were checked in Tracer v. 1.6 (http://tree.bio.ed.ac.uk/software/tracer).

Statistical analysis of microsatellite data

Allele and binary data matrices were created using GeneMarker v. 1.51 (SoftGenetics, LLC, State College, PA, USA). The allele data matrix was reduced to ≤ 5 samples from each sampling site to minimize the effect of uneven sampling and analysed using a Bayesian model-based clustering algorithm with a clustering number (K) ranging from 1 to 10 in the software STRUCTURE (Pritchard et al. 2000). The admixture model and burn-in of 100 000 iterations followed by 1 M additional Markov chain Monte Carlo iterations were used to calculate 10 simulation runs. The optimal clustering number (K) was estimated by calculating ΔK (Evanno et al. 2005) using the script structure-sum (Ehrich 2006) in R v. 3.3.4 (R Core Team 2016).

To estimate the similarities between individuals, Jaccard's similarity coefficient was calculated on the previously generated binary matrix using the program FAMD (Schlueter & Harris 2006). A neighbour-joining tree based on Jaccard's similarity coefficient matrix was constructed and genetic distances were calculated using the same software. Genetic distances were used for the construction of the NeighborNet network in SplitsTree v. 4 (Huson & Bryant 2006).

The degree of gene flow among clusters was estimated for seven loci on the clone-corrected dataset using a pairwise fixation index (F_{ST}) calculated in Arlequin (Schneider et al. 2000). The degree of clonality or recombination within populations was estimated by calculating the index of association (I_A) in MultiLocus v. 1.3 (Agapow & Burt 2001) using a binary matrix (uncorrected and clone corrected dataset) and a matrix based on both microsatellite and sequence data. The index of association I_A is estimated to be nearly zero or zero if no linkage is noticed between the alleles of different loci (randomly distributed alleles); in that case random mating (panmixis) is suggested. The values observed in the examined populations were compared to those artificially generated by the 10 000 randomisations. The null hypothesis of recombination and linkage equilibrium is supported when observed and simulated values are close ($p > 0.05$), while significant differences between values support linkage disequilibrium and clonality.

Genetic diversity within population(s) was calculated from the frequencies of genetically distinct individuals using Nei's genotype diversity (Dg), and from the frequencies of alleles at individual loci using Nei's gene diversity (D) (Nei 1987, Kosman 2003). To obtain diversity values comparable between the clusters, the effective number of genotypes (G_{eff}) (Parker Jr 1979) was calculated based on the number of equally abundant genotypes necessary to reflect the value of a diversity measure. All three population indices (Dg, D, G_{eff}) were calculated from the binary data matrix using script AFLPdat (Ehrich 2006) in R v. 3.0.2.

Phenotypic studies

At least two randomly selected isolates from each MLST genotype (if available) were subjected to a more detailed analysis of macromorphology on Sabouraud glucose agar (SGA, Oxoid, Basingstoke, UK), malt extract agar (MEA, Oxoid, Basingstoke, UK) and potato dextrose agar (PDA, Himedia, Mumbai, India) at 25 °C. In addition, the growth on SGA at 30 and 37 °C was measured. The micromorphological features were measured in the same isolates and were recorded from cultures growing on MEA after 14–21 d of cultivation. The dimensions of conidia were recorded at least 25 times for each isolate.

The macromorphology of the colonies was documented using an Olympus SZ61, and colony colours were determined using the hexadecimal colour codes assigned according to the website https://coolors.co. Micromorphology was documented using an Olympus BX-51 microscope. Statistical differences in the size of spores were tested with one-way analysis of variance (ANOVA) followed by Tukey's honestly significant difference (HSD) test in R v. 3.3.4 (R Core Team 2016).

Statistical analysis of the antifungal susceptibility data

The raw data published by Shamsizadeh et al. (2021) were used for statistical analysis. The isolates were divided into two groups according to their assignment to the two clusters based on STRUCTURE analysis. Statistical differences in antifungal susceptibilities to eight antifungals (amorolfine, ciclopirox, efinaconazole, itraconazole, luliconazole, griseofulvin, terbinafine and tavaborole) were tested with one-way analysis of variance (ANOVA) followed by Tukey's honestly significant difference test (Tukey's HSD) in R v. 3.3.4 (R Core Team 2016).

RESULTS

Genotyping

In total, 161 isolates of *T. erinacei* were subjected to multilocus microsatellite typing (MLMT). Seven out of 10 markers, developed by Čmoková et al. (2020), were successfully amplified in *T. erinacei* (Table S1). A total of 71 samples were selected for multilocus sequence typing (MLST) using four genes (ITS, *gapdh*, *tubb* and *tef1-α*). The reduction in number was at the expense of samples from France, where we observed a high degree clonality among samples using MLMT.

Using the MLST approach, we observed a single genotype in *tubb*, two in *gapdh* and *tef1-a*, and three in ITS, which resulted in five multilocus genotypes designated G1–G5 (Fig. 3). Microsatellite markers showed higher levels of polymorphism

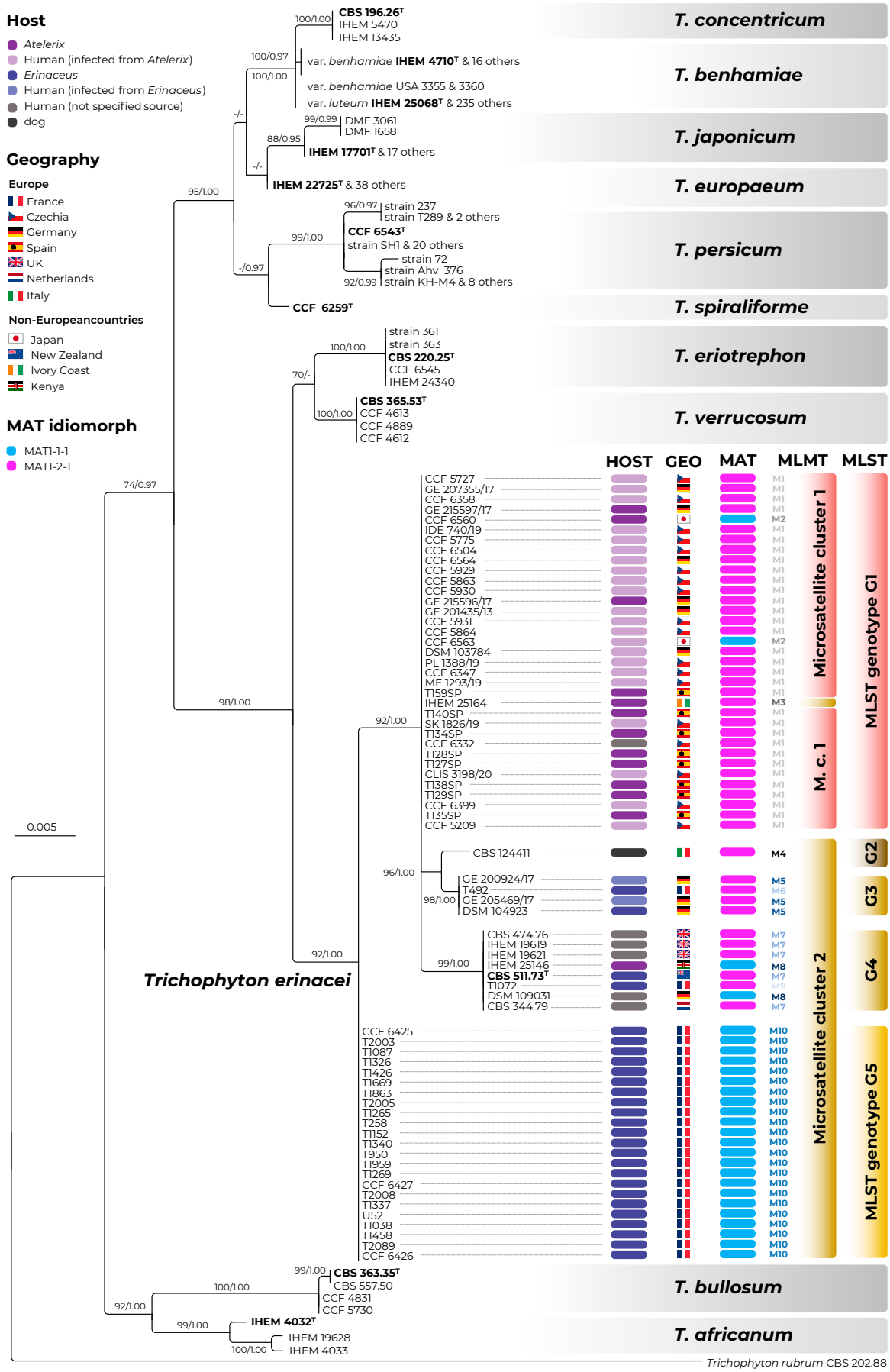

Fig. 3 Multilocus phylogeny of *Trichophyton benhamiae* complex inferred with the maximum likelihood method based on the ITS rDNA, *tef1-α*, *gapdh* and *tubb* loci (alignment characteristics, partitioning scheme and substitution models are listed in Table 2). A total of 71 *T. erinacei* isolates were characterized, included in the phylogeny, and the following information was plotted on the tree and designated by different colours: host from which isolate was obtained (column HOST), geographic origin (GEO), mating type gene idiomorph (MAT), multilocus genotype based on seven microsatellite markers (MLMT; M1-M10) and assignment of isolates into cluster based on analysis in STRUCTURE software (cluster 1: red, cluster 2: yellow) and multilocus genotype based on 4 loci sequences (MLST; G1-G5). Maximum likelihood bootstrap values and Bayesian posterior probabilities are appended to the nodes; only support values higher than 70 % and 0.95, respectively, are shown. The ex-type strains are designated with a superscripted T and **bold** print. *Trichophyton rubrum* was used as the outgroup.

(10 genotypes, M1-M10) in comparison to MLST. The most potent microsatellite locus was CT21b (5 genotypes), followed by TAG16 (4 genotypes), TCA16, TC17A, TC20 (3 genotypes each) and CT21 (1 genotype, monomorphic). The plot in Fig. 4 shows the dependence of genotype diversity as a function of an increasing number of microsatellite markers. From the course of the curve, enough loci (n = 7) were used to uncover the genetic diversity present in the examined set of *T. erinacei* strains.

Bayesian approach-based analysis supported the distribution of *T. erinacei* isolates (n = 161) into two clusters, designated here as cluster 1 and cluster 2, according to the ΔK peak (Fig. 5). Cluster 1 consists of two MLMT genotypes (M1 and M2) that correspond to MLST genotype G1 (except for IHEM 25164, which belongs to cluster 2). This cluster contains isolates exclusively (n = 33) from African hedgehogs and their owners (Fig. 3). M1 genotype isolates exhibited only the *MAT1-2-1* idiomorph and originated from Europe, while two M2 genotype isolates exhibited the *MAT1-1-1* idiomorph and originated from Japan. The induction of a sexual state by crossing opposite mating-type strains of M1 and M2 genotypes was not successful on agar medium supplemented with *Guizotia*

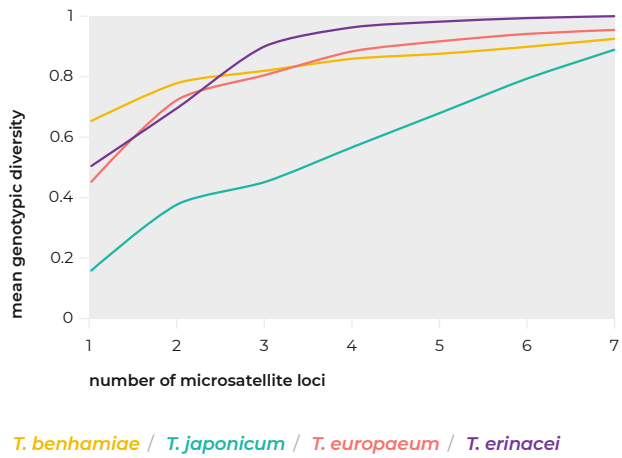

Fig. 4 Plot of mean genotypic diversity as a function of the number of microsatellite loci.

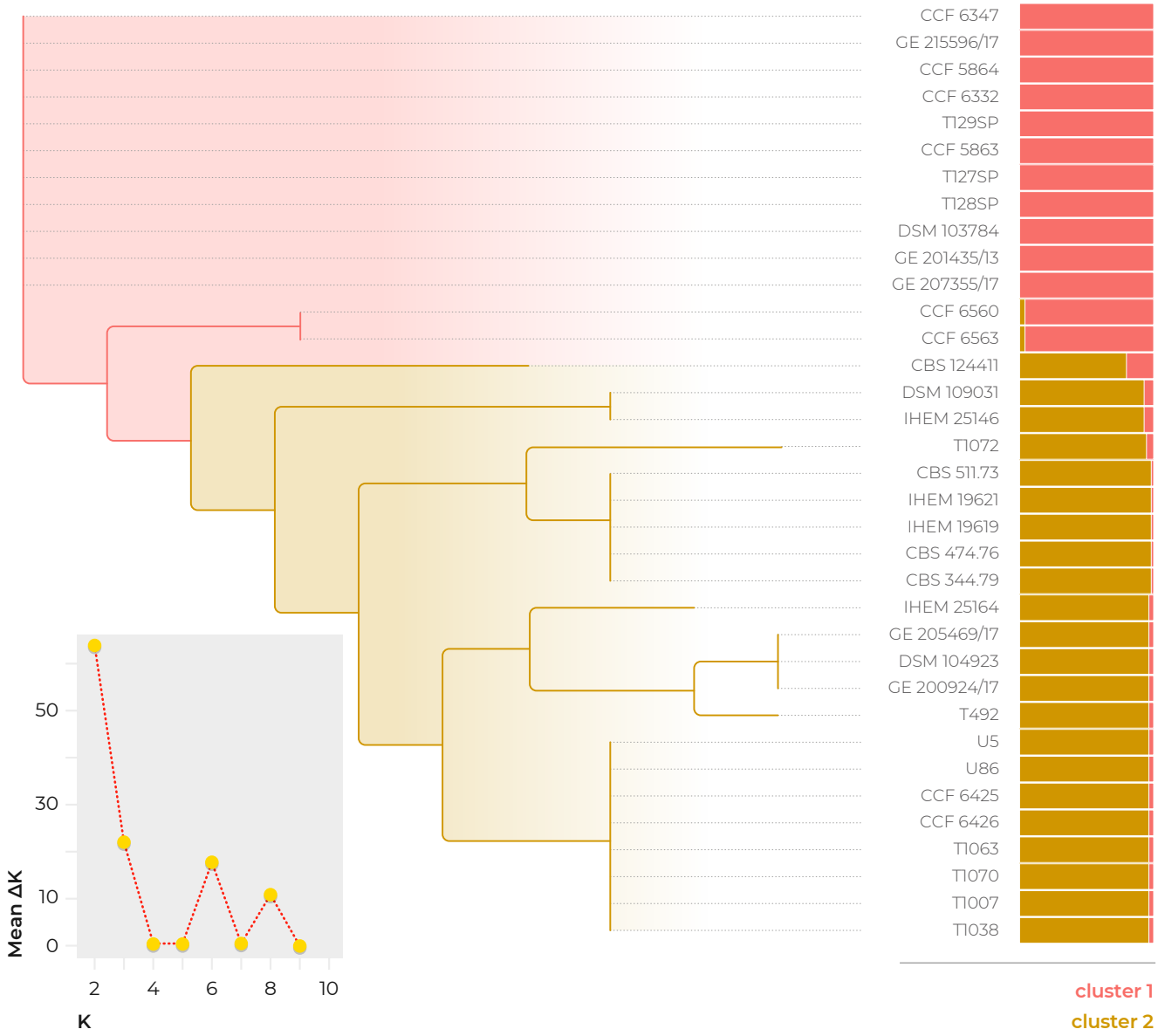

Fig. 5 Population structure plot for the distribution of *Trichophyton erinacei* isolates into two clusters (K = 2 according to the peak of ΔK; graph is shown in the lower left corner of the figure) based on seven microsatellite loci. The plot created with STRUCTURE software using Bayesian clustering is displayed vertically on the right side of the figure. Individual isolates (haplotypes) are represented by horizontal bars. The dataset was reduced to ≤ 5 samples from each locality to minimize the effect of uneven sampling. A neighbour-joining tree calculated from the multilocus microsatellite profiles using the Jaccard distance matrix measure in FAMD software is used for the comprehensive presentation of the results.

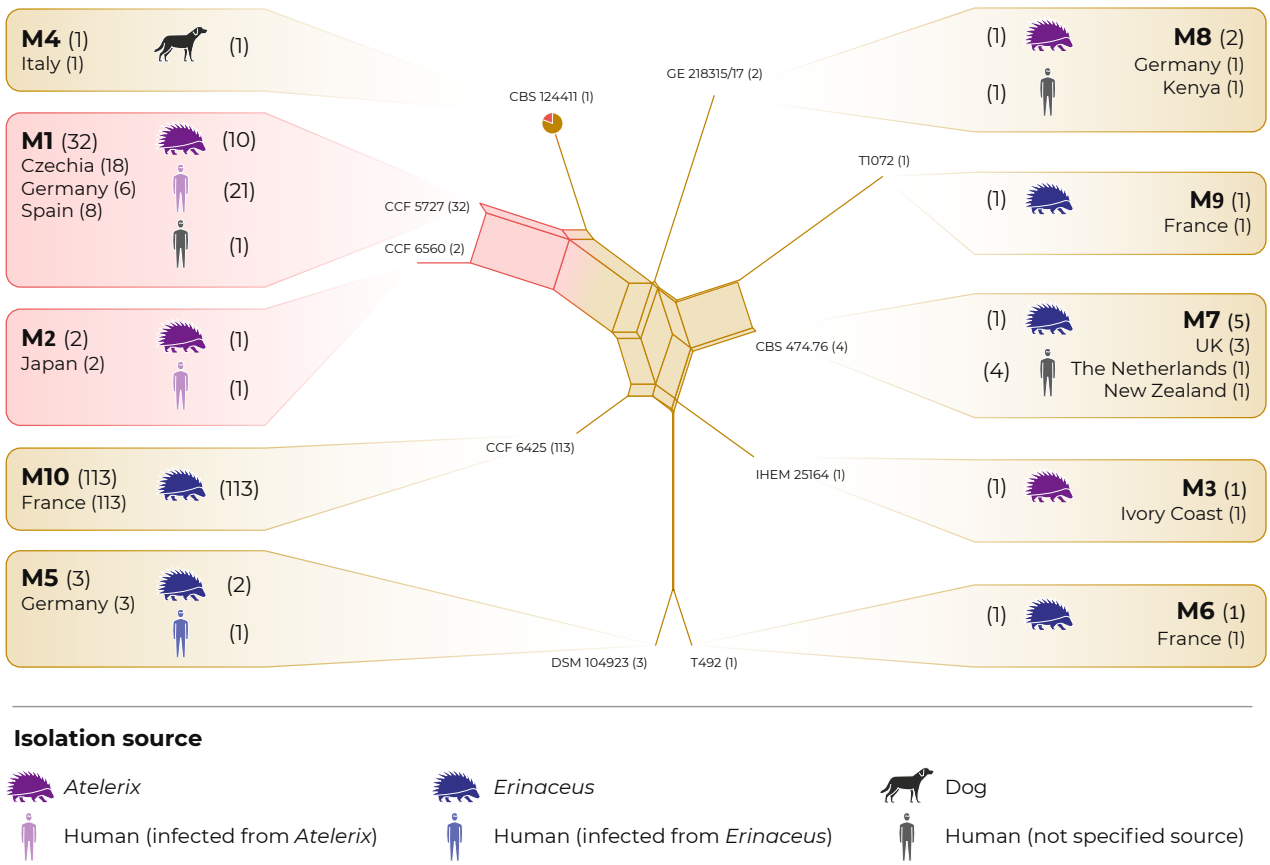

Fig. 6 Population structure of *Trichophyton erinacei* revealed by the analysis of seven microsatellite loci in 161 strains. The NeighborNet network was built with FAMD software and visualized in SplitsTree using the Jaccard index-based distance matrix. Geographic origin and host(s) are detailed separately for each multilocus genotype M1-M10. Subdivision of isolates into clusters based on STRUCTURE analysis (Fig. 5) is indicated by a colour gradient: cluster 1: red, cluster 2: yellow. The pie chart under the name of strain CBS 124411 indicates a genetic admixture between clusters. Detailed data associated with every isolate are listed in Table S1.

abyssinica seeds at 20 °C (Symoens et al. 2013, Čmoková 2015). Strain CBS 124411 showed a high level of admixture between clusters 1 and 2 (Fig. 5) but was assigned to cluster 2 by STRUCTURE. This strain is the only representative MLMT genotype M4 (MLST genotype G2) and was isolated from a dog dermatophytosis (Fig. 2) in Italy, and transmission from wild hedgehog of the genus *Erinaceus* is presumed.

Similar grouping of microsatellite genotypes was observed when the NeighborNet network was constructed with FAMD software and visualized in SplitsTree 4 using the Jaccard index-based distance matrix (Fig. 6). Genotypes M1 and M2 clustered together, and the next closest genotype was M4, a transitional genotype between cluster 1 and 2.

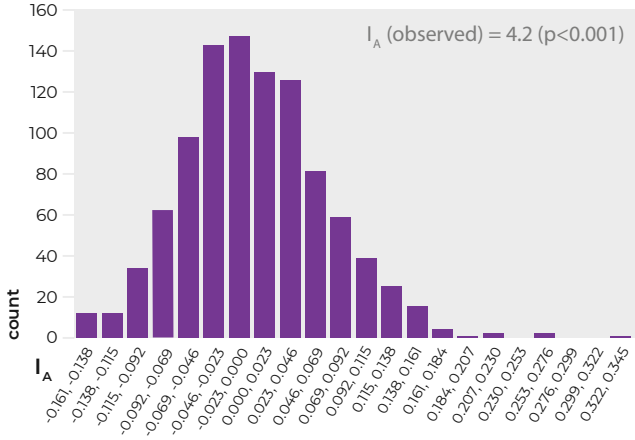

Fig. 7 Histogram of the simulated index of association (I_A) for cluster 2 isolates calculated from 10 000 permutations of randomization tests under a null model of allelic recombination; the observed value of I_A is 4.2, which is out of the 95 % confidence interval of the simulated values.

More diversified cluster 2 consisted of eight MLMT genotypes M3–M10 corresponding to strains from the remaining MLST genotypes G2–G5 (Fig. 3, 6). The cluster comprised mostly strains originating from hedgehogs of the genus *Erinaceus* in Europe, except for two isolates from wild hedgehogs of the genus *Atelerix* in Kenya (IHEM 25146) and Ivory Coast (IHEM 25164). Three German and one French isolate belonged to the M5 and M6 genotypes, respectively, and MLST genotype G3, all isolated from *Erinaceus* hedgehogs or human infection contracted from them.

The old strains isolated before 2000, including the ex-type strain of *T. erinacei* CBS 511.73 and the ex-type strain of *T. proliferans* CBS 474.76, mostly belonged to MLST genotype G4 and microsatellite genotypes M7–M9 (Fig. 3, 6). These strains were isolated from hedgehogs of the genera *Erinaceus* and *Atelerix* (strain from Kenya, IHEM 25146) and included strains of both mating types (mating experiments were not successful). The majority of strains from cluster 2 were segregated to the M10 genotype corresponding to MLST genotype G5. These strains originated exclusively from *Erinaceus* hedgehogs from France, and all showed *MAT1-1-1* gene idiomorph.

Population structure

Both clusters were genetically uniform as evidenced by low values of Nei's gene diversity (D), especially in cluster 1 (D = 0.07) compared to cluster 2 (D = 0.22). The low Nei's genotype diversity index in cluster 1 (Dg = 0.01) and cluster 2 (Dg = 0.22) reflects that they consisted of several abundant

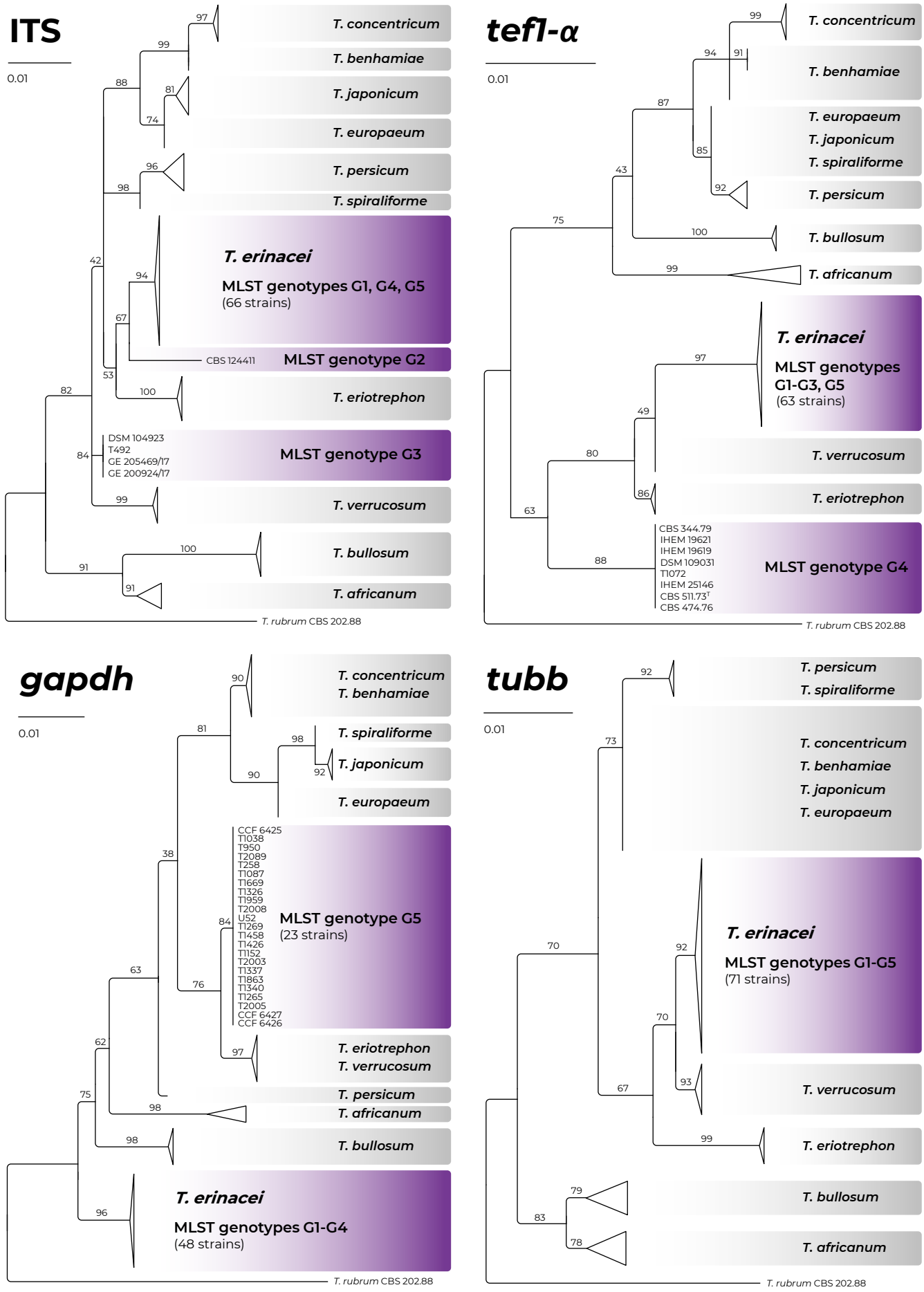

Fig. 8 Best scoring maximum likelihood tree based on single genes: ITS rDNA, *tef1-α*, *gapdh* and *tubb* (alignment characteristics, partitioning scheme and substitution models are listed in Table 2). *Trichophyton erinacei* strains are highlighted with violet colour; corresponding multilocus genotypes (MLST) from the multiple gene phylogeny (Fig. 3) are listed. Maximum likelihood bootstrap values are appended to the nodes; *Trichophyton rubrum* CBS 202.88 was used as the outgroup.

clones that were overrepresented due to uneven sampling. Clonal reproduction prevails in both clusters, which is supported by a low effective number of genotypes (Geff = 1.1 and 1.3, respectively) in comparison with the observed number of genotypes (G = 2 and 8, respectively).

The first cluster of *T. erinacei* consists of only two microsatellite genotypes, which is not enough to determine linkage among markers and make conclusions about the presence of sexual recombination in the dataset. The hypothesis about random mating was rejected in cluster 2 by calculating the index of association I_A on clone uncorrected data ($I_A = 4.4$, $p < 0.01$). Similarly, sexual recombination was also rejected for the complete dataset of all *T. erinacei* samples based on microsatellite data ($I_A = 4.2$, $p < 0.01$; Fig. 7) and for the dataset where MLMT data were combined with MLST data (data not shown). The hypothesis about random mating was not rejected on clone-corrected data in either case (cluster 2 and the whole dataset) but not at the significance level. We observed a relatively high proportion of fixed microsatellite alleles in both clusters, as evidenced by the relatively high fixation index ($F_{ST} = 0.85$, $p < 0.000001$), which means that there is only limited genetic information exchange between the clusters.

Phylogeny and position of T. erinacei in the T. benhamiae complex

For the multigene phylogeny based on ITS, *gapdh*, *tubb* and *tef1-α* loci, we used previously published alignments (Čmoková et al. 2021) that were enriched by 71 *T. erinacei* isolates for which we generated sequences in this study. The final alignment included 443 strains representing the entire species diversity of the *T. benhamiae* complex. Detailed alignment characteristics for all phylogenies together with the partitioning schemes and substitution models are listed in Table 2. The isolation source and accession numbers for DNA sequences are available in Table 1 and S1. The alignments were deposited in the Dryad Digital Repository (https://doi.org/10.5061/dryad.pzgmsbcnv).

Single-gene phylogenies were highly incongruent, and genes with more genotypes did not resolve *T. erinacei* strains as a monophyletic group (Fig. 8). The isolates were divided into three lineages in the ITS phylogeny. Four strains from *Erinaceus* hedgehogs, corresponding to MLST genotype G3, did not cluster with the majority of *T. erinacei* strains and were more closely related to *T. verrucosum* (Fig. 8). These strains had three unique substitutions in the ITS region and another three substitutions that were only shared with strain CBS 124411 (MLST genotype G2). The strain CBS 124411 was resolved on a long branch sister to the vast majority of *T. erinacei* strains (n = 66), and its ITS sequence contained five unique substitutions that were not shared with any other *T. erinacei* isolate (eight substitutions compared to the ex-type strain).

In the phylogeny based on the *tef1-α* gene, *T. erinacei* strains were divided into two groups. The majority of strains (n = 63) clustered with *T. verrucosum* and *T. eriotrephon*. Eight strains, corresponding to MLST genotype G4, also clustered with the species mentioned but were in a basal position to them. The *tef1-α* sequences of these strains associated with both *Erinaceus* and *Atelerix* hedgehogs contained 11 substitutions compared to the remaining *T. erinacei* strains.

The phylogeny based on the *gapdh* gene also divided isolates into two considerably remote groups. The French isolates from hedgehogs of the genus *Erinaceus*, corresponding to MLST genotype G5, clustered with *T. verrucosum* and *T. eriotrephon*, while the remaining strains were resolved in the basal position to other *T. benhamiae* complex species. In total, these two groups differed by 11 substitutions in the *gapdh* gene.

In contrast to single-gene phylogenies, the multigene phylogeny resolved *T. erinacei* as a monophyletic species (Fig. 3). Within the *T. benhamiae* complex, species were resolved into three major monophyletic clades, corresponding to *T. benhamiae*, *T. erinacei* and *T. bullosum* clades sensu Čmoková et al. (2020). *Trichophyton erinacei* formed an independent lineage within the *T. erinacei* clade, which also encompassed *T. verrucosum* and *T. eriotrephon*.

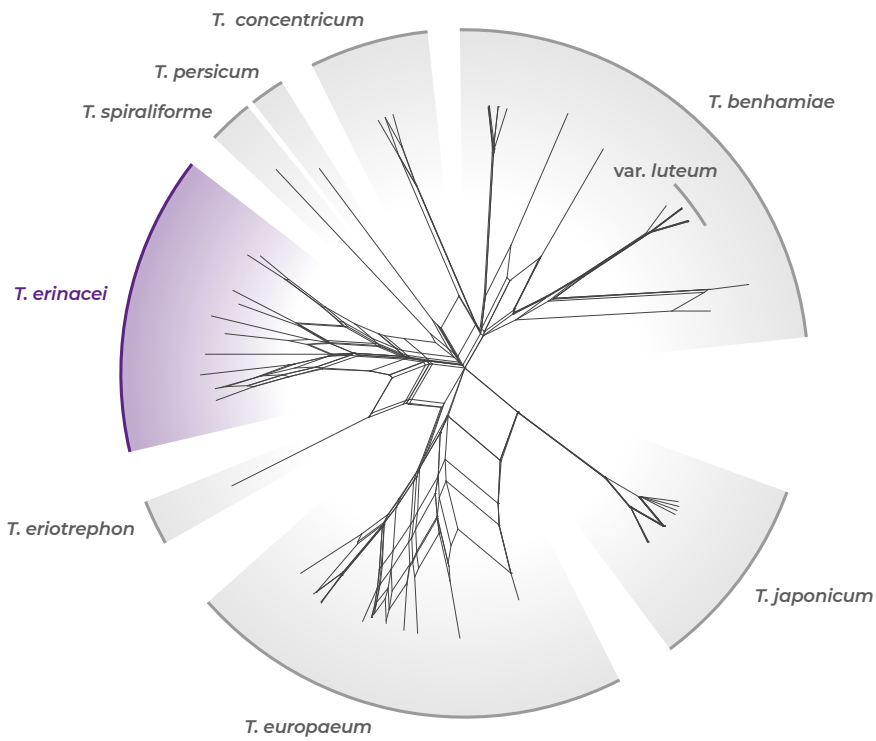

Fig. 9 Population structure of the *Trichophyton benhamiae* complex revealed by the analysis of seven microsatellite loci in 483 strains (Table S1). *Trichophyton verrucosum*, *T. bullosum* and *T. africanum* were not included because the markers could not be amplified in them. The NeighborNet network was built with FAMD software and visualized in SplitsTree using the Jaccard index-based distance matrix. *Trichophyton erinacei* strains are indicated by violet colour.

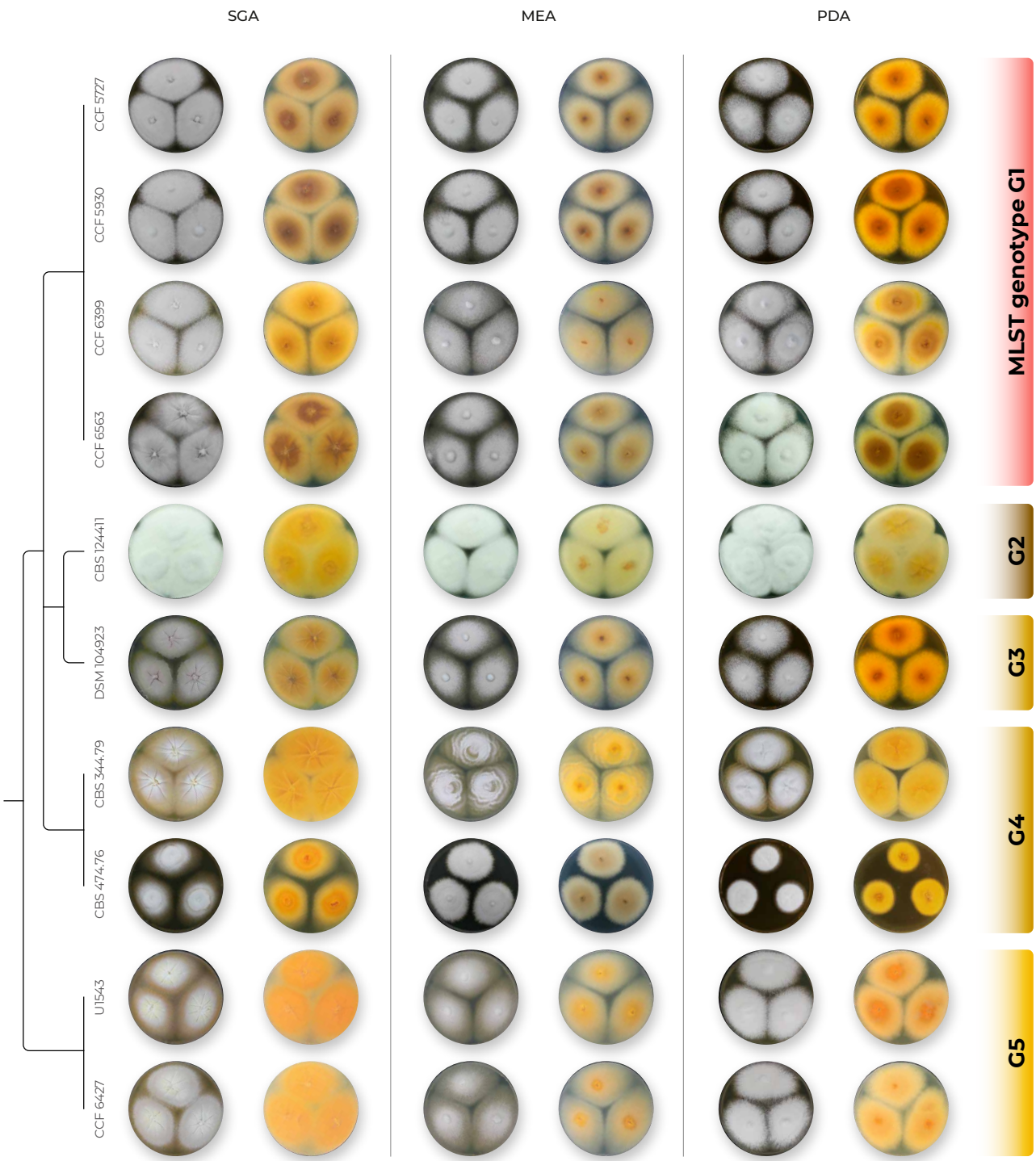

Fig. 10 Overview of the macromorphology of the *Trichophyton erinacei* isolates representing different multilocus genotypes. The isolates were cultivated on Sabouraud glucose agar (SGA), malt extract agar (MEA) and potato dextrose agar (PDA) for 14 d at 25 °C; obverse and reverse of colonies are shown.

In addition to DNA sequence markers, we also constructed a phylogenetic network based on seven microsatellite markers across the members of *T. benhamiae* and *T. erinacei* clades (except for *T. verrucosum*). In this network shown in Fig. 9, all *T. erinacei* strains clustered together in agreement with the results of the multigene phylogeny. The relationships between species resulting from this analysis were also similar to phylogeny (Fig. 3). *Trichophyton eriotrephon* was the most closely related species to *T. erinacei*, followed by a group of species from the *T. benhamiae* clade.

Phenotypic analysis

All *T. erinacei* stains were initially examined on MEA at 25 °C. For more detailed morphological examination, at least two strains (if available) of each MLST genotype were selected and cultivated on SGA, MEA and PDA media at 25 °C for 7 d with a subsequent measurement of micromorphological characteristics after 2 wk of cultivation. In total, 26 strains were examined.

There were no statistically significant differences in the growth rates or visible differences in the macromorphology of colonies (Fig. 10), which could be linked to MLST genotypes or microsatellite clusters. Colonies of strains from cluster 1 reached 22–26 mm diam (∅ = 24 mm) on MEA, 23–35 mm diam (∅ = 26 mm) on SGA and 17–32 mm diam (∅ = 21 mm) on PDA in 7 d at 25 °C. Colonies of strains from cluster 2 reached 19–45 mm diam (∅ = 28 mm) on MEA, 21–43 mm diam (∅ = 29 mm) on SGA, and 11–35 mm diam (∅ = 24 mm on PDA in 7 d at 25 °C. In particular, the strains isolated before 2000 showed

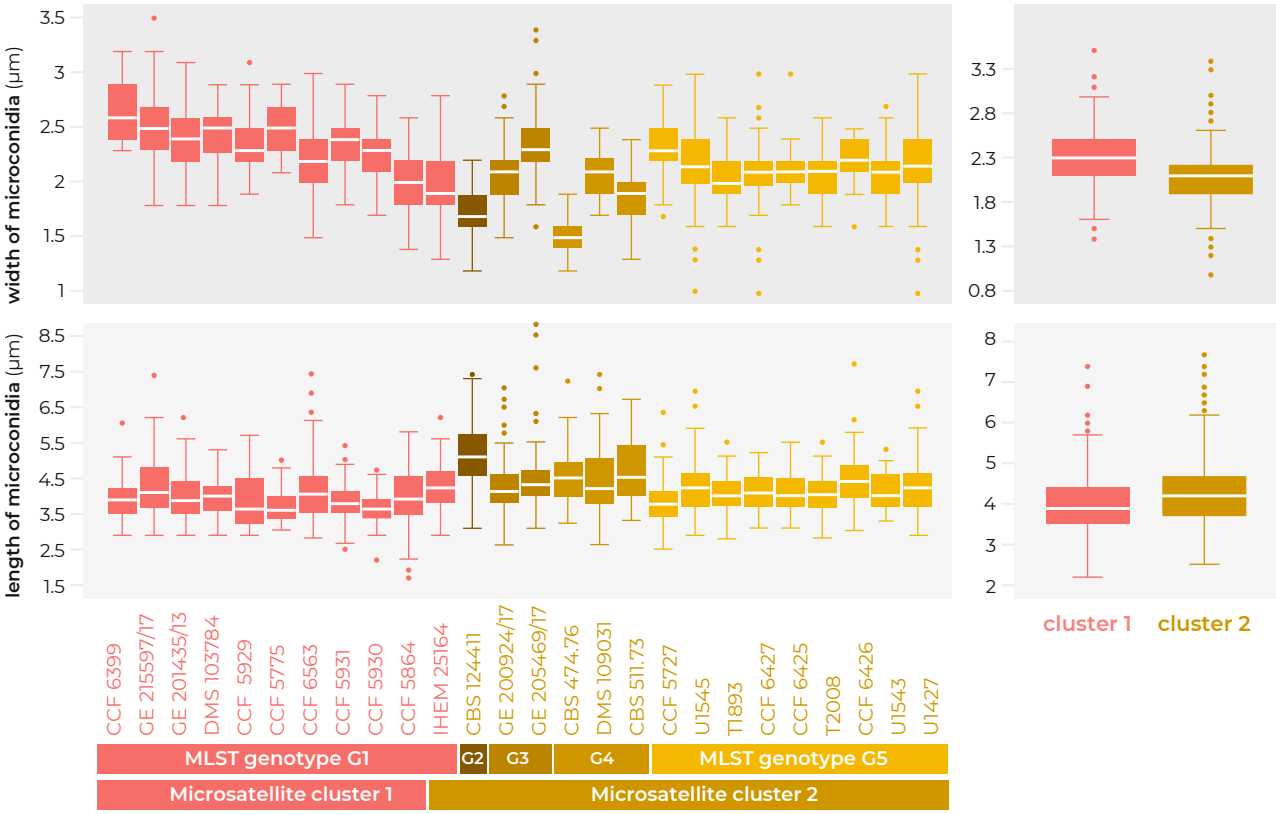

Fig. 11 Length and width of microconidia in *Trichophyton erinacei* isolates representing different microsatellite clusters/multilocus genotypes (MLST). The horizontal lines in boxplots indicate the mean value and interquartile range, whiskers span the 5 % and 95 % percentiles, and dots indicate extreme outliers.

cottony colonies and poor sporulation and thus could not be properly examined. Typical strains were characterized by finely to coarsely granular or powdery colonies that were flat, centrally raised or umbonate. Colony colour varied in individuals from white (#F2F3F4) to yellowish white (#F0EAD6). The reverse of colonies was pale-yellow (#F3E5AB) or brilliant yellow (#FADA5E) on all examined media after 14 d of cultivation. In some strains, the reverse colour in the centre of colonies turned brown (Fig. 10).

The dimensions and shape of microconidia and degree of sporulation differed slightly between isolates from cluster 1 and cluster 2. Cluster 1 isolates (n = 11) exhibited extensive production of microconidia on all media compared to cluster 2 isolates (n = 15), which did not sporulate so intensively, possibly a consequence of the slightly different arrangement of conidiophores. Conidiophores of cluster 1 strains showed mostly a pyramidal (grape-like) arrangement and therefore tended to produce larger quantities of spores, while conidiophores of cluster 2 were less extensively branched with conidia mostly sessile or borne along the conidiogenous hyphae on short protrusions or short lateral branches. The size and shape of microconidia also differed between clusters. Microconidia of cluster 1 strains were significantly wider and shorter (Fig. 11) than microconidia of cluster 2 ($p < 0.0001$) and were rather pyriform, 2.2–7.4 (3.9 ± 0.7) × 1.4–3.5 (2.3 ± 0.3) µm, while conidia of cluster 2 were predominantly clavate, 2.5–8.7 (4.2 ± 0.8) × 1–3.4 (2.1 ± 0.3) µm. Although sizes were significantly different, there was a large overlap in the dimensions, and significant interindividual differences between strains from the same cluster were detected. Therefore, the observed differences could not be used in the differentiation of clusters in practice.

Macroconidia were absent in most *T. erinacei* isolates. When present, they were rather sparse to rare, cigar-shaped or clavate, with a tapering rounded apex and truncate base, and

Table 3 Comparison of clinical and epidemiological data associated with strains representing different populations of *Trichophyton erinacei* (cluster 1 and cluster 2).

Source	Clinical data	Population[1]	
		cluster 1	cluster 2
Human	Number of samples	23	7
	Sex	women 86 %	women 75 %
	Age range (median)	15–46 (26)	2–28 (23)
	Site of infection	upper extremities (59 %), face (13 %), trunk (13 %)	upper extremities (100 %)
	Contracted from: *Erinaceus* / *Atelerix* / unknown	0 / 22 / 1	2 / 0 / 5
Animal	number of samples	11	120
	Erinaceus / *Atelerix* / dog	0 / 11 / 0	117 / 2 / 1
Total number of samples		34	127

[1] Delimited by STRUCTURE software.

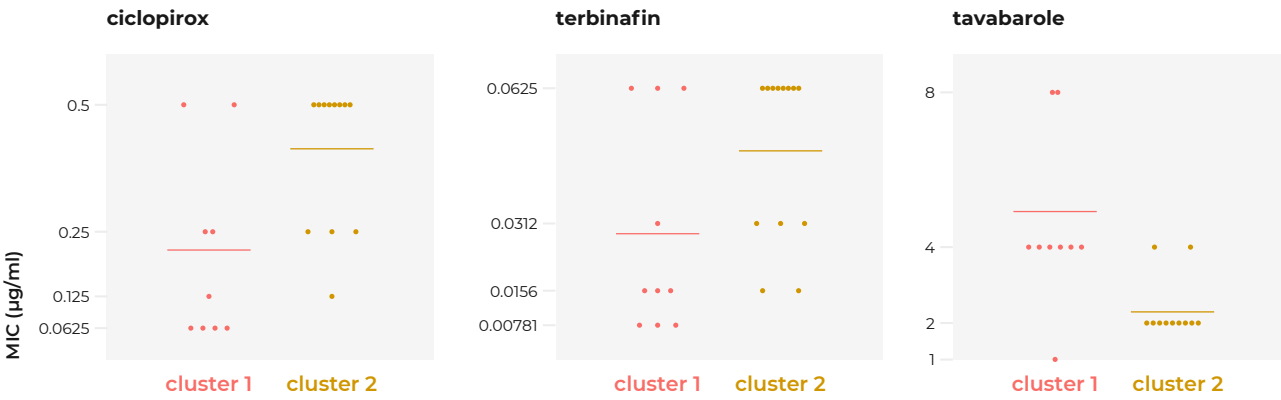

Fig. 12 Differences in antifungal susceptibilities in *Trichophyton erinacei* isolates from cluster 1 and cluster 2. Significant differences in susceptibility to ciclopirox, terbinafine and tavaborole were found (ANOVA, $p < 0.05$) while no statistically significant differences were found in susceptibility to the remaining five antifungals (see Results). The dots in the graph represent susceptibilities of individual isolates and geometric mean values are indicated by horizontal lines.

usually consisted of 2–8 cells (median 4), 25.5–33.5 (26.8 ± 3.5) × 4.3–6 (5 ± 0.6) µm. Chlamydospores were rarely present. No spiral hyphae were found even after 21 d of incubation.

Strain CBS 474.46, ex-type of *T. proliferans*, was characteristic by more intensive production of macroconidia and poor production of microconidia. The shape of its macroconidia was also different from the shape of macroconidia of the other examined strains, which were thin and long or flask-shaped, with pointed ends, and consisted of 2–6 cells (median 4), 10–72.5 (39 ± 19) × 2.5–5.5 (3.5 ± 1) µm. These macroconidia are often produced intercalarily in vegetative hyphae.

Clinical picture and epidemiology

Clinical and epidemiological data associated with strains examined in this study are summarised in Table 3. The spectrum of patients and clinical manifestations of human and animal infections due to *T. erinacei* did not differ between the two clusters. The human patients were within the range of 2–46 yr (median 23 and 26 yr, respectively) and were predominantly women (75 and 86 %, respectively). Infections usually manifested as tinea corporis (Fig. 2) localised on the upper extremities (predominantly hands), face and trunk. Infections in animals (mostly young individuals) were frequently asymptomatic or manifested as scaly skin or spine loss (Table S1).

Most human patients infected with isolates from cluster 1 (~ 96 %, 22 out of 23) reported contact with pet hedgehogs of the genus *Atelerix*. The animal strains from cluster 1 were all isolated from *Atelerix* hedgehogs (n = 11). Most isolates (~ 80 %, n = 101 of 127) belonging to cluster 2 were obtained from European hedgehogs (*Erinaceus* spp.) or were isolated directly from *Erinaceus* hedgehogs (n = 2). Cluster 2, however, also contained two samples from wild African hedgehogs of the genus *Atelerix* from Africa.

Antifungal susceptibility

The antifungal susceptibility to tavaborole, ciclopirox and terbinafine differed significantly (Fig. 12) between isolates from cluster 1 (n = 10) and cluster 2 (n = 14), while the results from the remaining antifungal drugs (amorolfin, griseofulvin, luliconazole, itraconazole and efinaconazole) showed no significant differences. Cluster 1 strains were significantly less susceptible to tavaborole, with MICs ranging between 1–8 µg/mL (median 4) than cluster 2 strains (2–4 µg/mL; median 2) (ANOVA, $p < 0.001$). In contrast, cluster 1 strains were more susceptible to ciclopirox with lower MICs (0.0625–0.5 µg/mL; median 0.1875) than the MICs from cluster 2 strains (0.125–0.5 µg/mL; median 0.5) (ANOVA, $p < 0.005$). Similarly, cluster 1 strains were more susceptible to terbinafine (MICs 0.00781–0.0625 µg/mL; median 0.0156) than cluster 2 strains (0.0156–0.0625 µg/mL; median 0.0625) (ANOVA, $p < 0.05$).

DISCUSSION

Hedgehogs have become fashionable pets in many countries worldwide. These animals are a potential source of many pathogens with zoonotic potential, including bacteria, viruses and fungi (Riley & Chomel 2005, Ruszkowski et al. 2021). Despite these facts, hedgehog-borne infections are generally of low public awareness. Together with the high prevalence of *T. erinacei* in hedgehogs, this low public awareness contributes to a significant increase in reported cases in recent years. Despite the growing importance of *T. erinacei* as a cause of zoonotic dermatophytoses, little is yet known about its taxonomy and diversification of populations depending on the different hedgehog hosts and the geographical origin of isolates. In this study, we collected the largest set of isolates from different hosts to date and examined population genetics using 11 genetic markers to address the questions mentioned.

Based on microsatellite data, the isolates of *T. erinacei* are distributed into two main subpopulations (clusters 1 and 2) with limited gene flow between them. The random mating hypothesis was rejected (I_A) for the whole species *T. erinacei* as well as for cluster 2 which is more diversified than cluster 1. However, this does not mean that there are no occasional recombination events between strains within and between subpopulations, rather reflecting that isolates examined in the present study spread clonally between hosts from which they were isolated. Most notably, isolates belonging to cluster 1 (MLMT genotypes 1 and 2 / MLST genotype G1) spread clonally between pet African hedgehogs, while cluster 2 strains spread predominantly between *Erinaceus* wild hedgehogs. The isolates of cluster 2 were more diverse in terms of genetic variability and ecology. Most of the isolates were obtained from European hedgehogs (*Erinaceus*) admitted to wildlife rehabilitation centres in France (Le Barzic et al. 2021), and except for two strains (T1072 and T492), all belonged to a single clone (MLMT genotype 10 / MLST genotype G5). The remaining minority of strains of cluster 2 were obtained from both *Erinaceus* and *Aleterix* hedgehogs from different countries and belonged to diverse MLST and MLMT genotypes (Fig. 3, 6).

From a genetic point of view, we can observe several signs of sexual reproduction/recombination in the population of *T. erinacei*. First, both mating type genes were present among isolates representing both clusters. Second, we observed incongruences between DNA sequence data and microsatellite data, and some isolates had significant admixture levels between

microsatellite clusters (CBS 124411; Fig. 5). The intraspecific genetic variability in *T. erinacei* is higher than the intraspecific genetic variability in other species of the *T. benhamiae* complex (Čmoková et al. 2020, 2021). Although the number of MLMT genotypes was comparable to the number of MLMT genotypes of *T. europaeum*, this species showed no variability in the DNA sequence data. A relatively high number of MLST genotypes detected among *T. erinacei* strains have also been observed in another sexual dermatophyte species, *T. mentagrophytes*, which also includes isolates of both mating types (Suh et al. 2018, Tang et al. 2021). Specific MLST genotypes in *T. erinacei* are substantiated by a relatively large number of substitutions that are present in certain strains, contributing to the polyphyletic nature of *T. erinacei* in the single-gene phylogenies (Fig. 8) and highlighted the pitfalls of constructing phylogeny in species groups where both sexual (*T. erinacei*) and clonal (e.g., *T. verrucosum* and *T. eriotrephon*) species are present. In general, the observed incongruence between single gene genealogies could be explained by past recombination/hybridization events or sharing of an ancestral polymorphism caused by incomplete lineage sorting, typical for recently diverged species (Hubka et al. 2018a, Steenkamp et al. 2018, Matute & Sepúlveda 2019, Kandemir et al. 2020). However, both the multigene phylogeny and network constructed from multilocus microsatellite data resolved *T. erinacei* as a monophyletic species (Fig. 3, 9).

In summary, we detected some level of substructuring into two subpopulations in *T. erinacei* that are associated with different hedgehog genera. These populations are not completely separated and there is a certain gene flow between them, probably due to the occasional rare transmission of pathogen between different hedgehog hosts. We also found minor differences in the micromorphology and antifungal susceptibilities between these two subpopulations, which are not large enough to recognise them as separate taxonomic entities, also supported by a coherent ecology strongly linked to hedgehogs and by similar growth parameters of strains belonging to different populations.

The soil environment enriched by keratin sources is necessary for the sexual reproduction of dermatophyte species (Weitzman & Summerbell 1995, Tang et al. 2021). According to this theory, anthropophilic and some zoophilic dermatophytes lost their sexuality due to lack of contact with soil habitat. Only some zoophilic *Trichophyton* species are believed to retain their ability to reproduce sexually, namely, *T. africanum*, *T. benhamiae*, *T. erinacei* and *T. mentagrophytes* (Hubka et al. 2018b, Čmoková et al. 2020, Metin & Heitman 2020, Tang et al. 2021). As far as we know, *T. erinacei* has not been cultivated directly from soil. However, its ability to survive in soil for more than one year was described by English & Morris (1969). Therefore, contaminated soil or hedgehog nests seem likely to be a source of infection to humans, dogs and other animals (English et al. 1962, Quaife 1966, English & Morris 1969, Romano et al. 2001).

Wild hedgehogs are in close contact with soil where sexual reproduction should take place and where genetic variability is expected to be generated by recombination. Therefore, we expected to find considerable genetic variability among 115 isolates from European hedgehogs from France (in the proximity of the Paris region) (Le Barzic et al. 2021). Surprisingly, this set of strains consisted mostly of clonal isolates (MLMT genotype 10) with *MAT1-1-1* gene idiomorph and only two strains, T1072 and T492, represented different genotypes and had an opposite mating type gene idiomorph, indicating that the sexual reproduction and de novo infection of hedgehogs from soil is a rare event and that clonal spread secured by horizontal spread from individual to individual strongly dominates. This spread occurs through asexual arthrospores on hairs or in skin scales.

Future population genetic studies should focus on collecting more strains from localities that were underrepresented in our dataset such as isolates from wild African hedgehogs of the genus *Atelerix*, as they seem to represent a connecting link between isolates of clusters 1 and 2. Two African strains were included in this study, IHEM 25164 from the Ivory Coast (Gregory et al. 1978) and IHEM 25146 from Kenya (Gregory & English 1975). Strain IHEM 25164 showed a conflicting classification in terms of various molecular markers – MLST genotype G1 typical for cluster 1 but the strain was placed in cluster 2. Strain IHEM 25146 was the only verified isolate from the genus *Atelerix* among strains of cluster 2. Encompassing more samples from wild African hedgehogs would probably result in a better understanding of the population structure and gene flow between subpopulations of *T. erinacei*. Additionally, future collections of strains should be performed in collaboration with zoologists to clearly identify the host at the species level. For instance, European and African hedgehogs sampled in hybrid zones may be either *E. europaeus* or *E. roumanicus* and *A. albiventris* or *A. algirus*, or respective hybrids between mentioned pairs of species. Clear identification of hosts would allow us to study the extent to which the pathogen is transmitted between hedgehog species and how the ecological barrier of the host species may influence the population genetics of the pathogen.

The molecular markers used in this study represent powerful tools for surveillance of infections due to *T. erinacei* and provide for the first-time insight into the population structure and spreading strategy of this emerging pathogen. As the popularity of hedgehogs as pet animals increases, an increasing number of human infections can be expected in the future. The spread of these infections underscores the need for closer collaboration between veterinarians, dermatologists, epidemiologists, and public health personnel to set up appropriate preventive measures.

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Declaration on conflict of interest The authors declare that there is no conflict of interest.

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Supplementary material

Table S1 List of *Trichophyton* strains used in the phylogenetic analysis and population genetic analyses.