

1 **Whole genome analysis illustrates global clonal population structure of the**
2 **ubiquitous dermatophyte pathogen *Trichophyton rubrum***

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35

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37

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40

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47 **Abstract**

48 Dermatophytes include fungal species that infect humans, as well as those which also
49 infect other animals or only grow in the environment. The dermatophyte species
50 *Trichophyton rubrum* is a frequent cause of skin infection in immunocompetent
51 individuals. While members of the *T. rubrum* species complex have been further
52 categorized based on various morphologies, the population structure and ability to
53 undergo sexual reproduction are not well understood. In this study, we analyze a large
54 set of *T. rubrum* and *Trichophyton interdigitale* isolates to examine mating types,
55 evidence of mating, and genetic variation. We find that nearly all isolates of *T. rubrum*
56 are of a single mating type, and that incubation with *T. rubrum* morphotype *megrinii*
57 isolates of the other mating type failed to induce sexual development. While the region
58 around the mating type locus is characterized by a higher frequency of SNPs compared
59 to other genomic regions, we find that the population is remarkably clonal, with highly
60 conserved gene content, low levels of variation, and little evidence of recombination. These
61 results support a model of recent transition to asexual growth when this species
62 specialized to growth on human hosts.

63

64 **Introduction**

65 Dermatophyte species are the most common fungal species causing skin infections. Of
66 the more than 40 different species infecting humans, *Trichophyton rubrum*, the major
67 cause of athlete's foot, is the most frequently observed [1,2]. Other species are more
68 often found on other skin sites, such as those found on the head, including *Trichophyton*
69 *tonsurans* and *Microsporum canis*. Some dermatophyte species only cause human
70 infections, including *T. rubrum*, *T. tonsurans*, and *T. interdigitale*. Other species,
71 including *Trichophyton benhamiae*, *Trichophyton equinum*, *Trichophyton verrucosum*,
72 and *M. canis*, infect mainly animals and occasionally humans, while others such as
73 *Microsporum gypseum* (*Nannizzia gypsea* [3]) are commonly found in soil and rarely
74 infect animals. In addition to the genera *Trichophyton* and *Microsporum*,
75 *Epidermophyton* and *Nannizzia* are other genera of dermatophytes that commonly
76 cause infections in humans [4]. The species within these genera are closely related

77 phylogenetically and are within the Ascomycete order Onygenales, family
78 Arthrodermataceae [3,5].

79

80 The *Trichophyton rubrum* species complex includes several “morphotypes,” many of
81 which rarely cause disease, and *T. violaceum*, a species that causes scalp infections
82 [3,6]. Some morphotypes display phenotypic variation, though these differences can be
83 modest. For example, *T. rubrum* morphotype *raubitscheckii* differs from *T. rubrum* in
84 production of urease and in colony pigmentation and colony appearance under some
85 conditions [7]. *T. rubrum* morphotype *megninii*, which is commonly isolated in
86 Mediterranean countries, requires L-histidine for growth unlike other *T. rubrum* isolates
87 [6]. However, little variation has been observed between these and other morphotypes
88 in the sequence of individual loci, such as the ITS rDNA locus; additionally, some of the
89 morphotypes do not appear to be monophyletic [3,6,8], complicating any simple
90 designation of all types as separate species. Combining morphological and multilocus
91 sequence typing (MLST) data has helped clarify relationships of the major genera of
92 dermatophytes and resolved polyphyletic genera initially assigned by morphological or
93 phenotypic data.

94

95 Mating has been observed in some dermatophyte species, although not to date in strict
96 anthropophiles including *T. rubrum* [9]. Mating type in dermatophytes, as in other
97 Ascomycetes, is specified by the presence of one of two idiomorphs at a single mating
98 type (*MAT*) locus; each idiomorph includes either an alpha box domain or HMG domain
99 transcription factor gene [10]. In the geophilic species *M. gypseum*, isolates of opposite
100 mating type (*MAT1-1* and *MAT1-2*) undergo mating and produce recombinant progeny
101 [10]. In the zoophilic species *T. benhamiae*, both mating types are detected in the
102 population and mating assays produced fertile cleistothecia [11], structures that contain
103 meiotic ascospores. In a study examining 600 isolates of *T. rubrum*, only five appeared
104 to produce structures similar to cleistothecia [12], suggesting inefficient development of
105 the spores required for mating. Sexual reproduction experiments of *T. rubrum* with
106 tester strains of *Trichophyton simii*, a skin infecting species that is closely related to *T.*
107 *mentagrophytes*, have been reported and one recombinant isolate was characterized,

108 consistent with a low frequency of mating of *T. rubrum* [13]. Further, sexual reproduction
109 of *T. rubrum* may be rare in natural populations, as a single mating type (*MAT1-1*) has
110 been noted in Japanese isolates [14], matching that described in the *T. rubrum*
111 reference genome of CBS 118892 [10].

112
113 Here we describe genome-wide patterns of variation in *T. rubrum*, revealing a largely
114 clonal population. This builds on prior work to produce reference genomes for *T. rubrum*
115 [15] and other dermatophytes [15,16]. Genomic analysis of two divergent morphotypes
116 of *T. rubrum*, *megrinii* and *soudanense*, reveal hotspots of variation linked to the mating
117 type locus suggestive of recent recombination. While nearly all *T. rubrum* isolates are of
118 a single mating type (*MAT1-1*), the sequenced *megrinii* morphotype isolate contains a
119 *MAT1-2* locus, suggesting the capacity for infrequent mating in the population.
120 Additionally, we examine variation in gene content across dermatophyte genomes
121 including the first representatives of *T. interdigitale*.

122

123 **Materials and methods**

124 **Isolate selection, growth conditions, and DNA isolation**

125 Isolates analyzed are listed in **Table S1**, including the geographic origin, site of origin,
126 and mating type for each. Isolates selected for whole genome sequencing were chosen
127 to maximize diversity by covering the main known groups. For whole genome
128 sequencing, 10 *T. rubrum* isolates and 2 *T. interdigitale* isolates were selected,
129 including representatives of the major morphotypes of *T. rubrum* (**Table S2**). Growth
130 and DNA isolation for whole genome sequencing were performed as previously
131 described [15].

132

133 For MLST analysis, a total of 80 *T. rubrum* isolates and 11 *T. interdigitale* isolates were
134 selected for targeted sequencing. Isolates were first grown on PDA medium (Difco) for
135 10 days at 25°C. Genomic DNA was extracted using an Epicentre Masterpure Yeast
136 DNA purification kit (catalog number MPY08200). Fungal isolates were harvested from
137 solid medium using sterile cotton swabs, transferred to microcentrifuge tubes, and
138 washed with sterile PBS. Glass beads (2 mm) and 300 µL yeast cell lysis solution

139 (Epicentre) were added to the tube to break down fungal cells, and the protocol
140 provided by Epicentre was then followed. The contents of the tube were mixed by
141 vortexing and incubated at 65°C for 30 minutes, followed by addition of 150 µl Epicentre
142 MPC Protein Precipitation Solution. After vortexing, the mixture was centrifuged for 10
143 minutes, followed by isopropanol precipitation and washing with 70% ethanol. The DNA
144 pellet was dissolved in TE buffer.

145
146 For mating assays, we investigated 55 *T. rubrum* and 9 *T. interdigitale* isolates
147 recovered from Adana and Izmir, Turkey. *T. simii* isolates CBS 417.65 MT -, CBS
148 448.65 MT + and morphotype *megninii* isolates CBS 389.58, CBS 384.64, and CBS
149 417.52 were also used in mating assays. DNA extraction was performed according to
150 the protocol described by Turin et al. [17]. These isolates were typed by ITS sequence
151 analysis. rDNA sequences spanning the internal transcribed spacer (ITS) 1 region were
152 PCR-amplified using the universal fungal primers ITS1 (5'-
153 TCCGTAGGTGAACCTGCGG3') and ITS4 (5'-CCTCCGCTTATTGATATGC-3') and
154 sequenced on an ABI PRISM 3130XL genetic analyzer at Refgen Biotechnologies using
155 the same primers (Ankara, Turkey). CAP contig assembly software, included in the
156 BioEdit Sequence Alignment Editor 7.0.9.0 software package, was used to edit the
157 sequences [18]. Assembled DNA sequences were characterized using BLAST in
158 GenBank.

159

160 **Multilocus sequence typing (MLST)**

161 A total of 108 isolates were subjected to MLST analysis (**Table S3**). For each isolate,
162 three loci (the *TruMDR1* ABC transporter [19], an intergenic region (IR), and an alpha-
163 1,3-mannosyltransferase (CAP59 protein domain)), with high sequence diversity
164 between *T. rubrum* CBS 118892 (GenBank accession: NZ_ACPH000000000) and *T.*
165 *tonsurans* CBS 112818 (GenBank accession: ACPI000000000), were selected as
166 molecular markers in MLST. The following conditions were used in the PCR
167 amplification of the three loci: an initial 2 min of denaturation at 98°C, followed by 35
168 cycles of denaturation for 10 sec at 98°C, an annealing time of 15 sec at 54°C, and an
169 extension cycle for 1 min at 72°C. The amplification was completed with an extension

170 period of 5 min at 72°C. PCR amplicons were sequenced using the same PCR primers
171 on an ABI PRISM 3130XL genetic analyzer by Genewiz, Inc. (**Table S4**).
172 Electropherograms of Sanger sequencing were examined and assembled using
173 Sequencher 4.8 (Gene Codes). Alternatively, sequences were obtained from genome
174 assemblies (**Table S5**).

175
176 To confirm the species typing for four isolates (MR857, MR827, MR816, and MR897),
177 the ITS1, 5.8S, and ITS2 region was amplified using the ITS5 (5'-
178 GAAGTAAAAGTCGTAACAAGG-3') and Mas266 (5'-
179 GCATTCCCAAACAACACTCGACTC-3') primers with initial denaturation at 94°C for 4
180 minutes, 35 cycles of denaturation at 94°C for 30 seconds, annealing at 60°C for 30
181 seconds, extension at 72°C for 1 minute, and final extension at 72°C for 10 minutes.
182 The reactions were carried out using a BioRad C1000 Touch thermocycler. ABI
183 sequencing reads were compared to the dermatophyte database of the Westerdijk
184 Fungal Biodiversity Institute. The sequences of MR857 and MR827 isolates were 99.6%
185 identical to that of the isolate RV 30000 of the African race of *T. benhamiae* (GenBank
186 AF170456).

187 188 **Mating type determination**

189 To identify the mating type of each isolate, primers were designed to amplify either the
190 alpha or HMG domain of *T. rubrum* (**Table S6**). For most isolates, PCR amplification
191 was performed using an Eppendorf epGradient Mastercycler, and reactions were
192 carried out using the following conditions for amplification: initial denaturation at 94°C
193 for 4 minutes, 35 cycles of denaturation at 94°C for 30 seconds, annealing at 55°C for
194 30 seconds, extension at 72°C for 1 minute, with a final extension at 72°C for 7 minutes.
195 For isolates from Turkey, PCR amplifications were performed with the same primers
196 using a Biorad C1000 Touch™ Thermal Cycler, and slightly modified conditions were
197 used for amplification: initial denaturation at 94°C for 5 minutes, 35 cycles of
198 denaturation at 95°C for 45 seconds, annealing at 55°C for 1.5 minutes, and extension
199 at 72°C for 1 minute, and final extension at 72°C for 10 minutes. The presence of the
200 alpha box gene, which is indicative of the *MAT1-1* mating type, or the HMG domain,

201 which is indicative of the *MAT1-2* mating type, was identified using primers
202 JOHE21771/WL and JOHE21772/WL, creating a 500-bp product, and JOHE21773/WL
203 and JOHE21774/WL, creating a 673-bp product, respectively. *Trichophyton rubrum* MR
204 851 was used as a positive control for *MAT1-1*, and morphotype *megninii* CBS 389.58,
205 CBS 384.64, CBS 417.52, and *T. interdigitale* MR 8801 were used as positive controls
206 for *MAT1-2*. The mating type was assigned based on the presence or absence of PCR
207 products on 1.5% agarose gels. For the whole genome sequenced isolates, mating type
208 was determined by analysis of assembled and annotated genes.

209

210 **Mating assays**

211 Mating assays were performed using both Medium E (12 g/L oatmeal agar (Difco), 1 g/L
212 MgSO₄·7H₂O, 1 g/L NaOH₃, 1g/L KH₂PO₄, and 16 g/L agar [20]) and Takashio medium
213 (1/10 Sabouraud containing 0.1% neopeptone, 0.2% dextrose, 0.1% MgSO₄·7H₂O, and
214 0.1%. KH₂PO₄). *MAT1-1* and *MAT1-2* isolates grown on Sabouraud Dextrose Agar
215 (SDA) for one week were used to inoculate both Medium E and Takashio medium
216 plates pairwise 1 cm apart from each other. The plates were incubated at room
217 temperature without Parafilm in the dark for 4 weeks. The petri dishes were examined
218 under light microscopy for sexual structures.

219

220 **Genome sequencing, assembly, and annotation**

221 For genome sequencing, we constructed a 180-base fragment library from each
222 sample, by shearing 100 ng of genomic DNA to a median size of ~250 bp using a
223 Covaris LE instrument and preparing the resulting fragments for sequencing as
224 previously described [21]. Each library was sequenced each on the Illumina HiSeq 2000
225 platform. Roughly 100X of 101 base-paired Illumina reads were assembled using
226 ALLPATHS-LG [22] run with assisting mode utilizing *T. rubrum* CBS118892 as a
227 reference. For most genomes, assisting mode 2 was used (ASSISTED_PATCHING=2)
228 with version R42874; for *T. interdigitale* H6 and *T. rubrum* MR1463 version R44224 was
229 used. For *T. rubrum* morphotype *megninii* CBS 735.88 and *T. rubrum* morphotype
230 *raubitschekii* CBS 202.88 mode 2.1 was used (ASSISTED_PATCHING=2.1) with
231 version R47300. Assemblies were evaluated using GAEMR

232 (<http://software.broadinstitute.org/software/gaemr/>); contigs corresponding to the
233 mitochondrial genome or contaminating sequence from other species were removed
234 from assemblies.

235
236 The *Trichophyton* assemblies were annotated using a combination of expression data,
237 conservation information, and *ab-initio* gene finding methods as previously described
238 [23]. Expression data included Illumina reads (SRX123796) from one RNA-Seq study
239 [24] and all EST data available in GenBank as of 2012. RNA-Seq reads were
240 assembled into transcripts using Trinity [25]. PASA [26] was used to align the
241 assembled transcripts and ESTs to the genome and identify open reading frames
242 (ORFs); gene structures were also updated in the previously annotated *T. rubrum*
243 CBS118892 assembly [15]. Conserved loci were identified by comparing the genome
244 with the UniRef90 database [27] (updated in 2012) using BLAST [28]. The BLAST
245 alignments were used to generate gene models using Genewise [29]. The *T. rubrum*
246 CBS 118892 genome was aligned with the new genomes using NUCmer [30]. These
247 alignments were used to map gene models from *T. rubrum* to conserved loci in the new
248 genomes.

249
250 To predict gene structures, GeneMark, which is self-training, was applied first;
251 GeneMark models matching GeneWise ORF predictions were used to train the other
252 *ab-initio* programs. *Ab-initio* gene-finding methods included GeneMark [31], Augustus
253 [32], SNAP [33] and Glimmer [34]. Next, EVM [35] was used to select the optimal gene
254 model at each locus. The input for EVM included aligned transcripts from Trinity and
255 ESTs, gene models created by PASA and GeneWise, mapped gene models, and *ab-*
256 *initio* predictions. Rarely, EVM failed to produce a gene model at a locus likely to
257 encode a gene. If alternative gene models existed at such loci, they were added to the
258 gene set if they encoded proteins longer than 100 amino acids, or if the gene model
259 was validated by the presence of a PFAM domain or expression evidence. Finally,
260 PASA was run again to improve gene-model structure, predict splice variants, and add
261 UTR.

262

263 Gene model predictions in repetitive elements were identified and removed from gene
264 sets if they overlapped TPSI predictions (<http://transposonpsi.sourceforge.net>),
265 contained PFAM domains known to occur in repetitive elements, or had BLAST hits
266 against the Repbase database [36]. Additional repeats were identified using a BLAT
267 [37] self-alignment of the gene set to the genomic sequence (requiring at least 90%
268 nucleotide identity over 100 bases aligned); genes that hit the genome more than eight
269 times using these criteria were removed. Genes with PFAM domains not found in
270 repetitive elements were retained in the gene set, even if they met the above criteria for
271 removing likely repetitive elements from the gene set.

272
273 Lastly, the gene set was inspected to address systematic errors. Gene models were
274 corrected if they contained in-frame stop codons, had coding sequence overlaps with
275 coding regions of other gene models or predicted transfer or ribosomal RNAs, contained
276 exons spanning sequence gaps, had incomplete codons, or with UTRs overlapping the
277 coding sequences of other genes. Transfer RNAs were predicted using tRNAscan [38],
278 and ribosomal RNAs were predicted with RNAmmer [39].

279
280 All annotated assemblies and raw sequence reads are available in NCBI (**Table S5**).

281

282 **SNP identification and classification**

283 To identify SNPs within the *T. rubrum* group, Illumina reads for each *T. rubrum* isolate
284 were aligned to the *T. rubrum* CBS 118829 reference assembly using BWA-MEM [40];
285 reads from the H6 *T. interdigitale* were also aligned to the *T. interdigitale* MR 816
286 assembly. The Picard tools (<http://picard.sourceforge.net>) AddOrReplaceReadGroups,
287 MarkDuplicates, CreateSequenceDictionary, and ReorderSam were used to preprocess
288 read alignments. To minimize false positive SNP calls near insertion/deletion (indel)
289 events, poorly aligned regions were identified and realigned using GATK
290 RealignerTargetCreator and IndelRealigner (GATK version 2.7-4 [41]). SNPs were
291 identified using the GATK UnifiedGenotyper (with the haploid genotype likelihood
292 model) run with the SNP genotype likelihood models (GLM). We also ran
293 BaseRecalibrator and PrintReads for base quality score recalibration on sites called

294 using GLM SNP and re-called variants with UnifiedGenotyper emitting all sites.
295 VCFtools [42] was used to count SNP frequency in windows across the genome (--
296 SNPdensity 5000) and to measure nucleotide diversity (--site-pi), which was normalized
297 for the assembly size. For comparison, the nucleotide diversity was calculated for the
298 SNPs identified in a set of 159 isolates of *C. neoformans* var. *grubii*, a fungal pathogen
299 that undergoes frequent recombination [43].

300
301 SNPs were mapped to genes using VCFannotator (<http://vcfannotator.sourceforge.net/>),
302 which annotates whether a SNP results in synonymous or non-synonymous change in
303 coding region. The total number of synonymous and non-synonymous sites across the
304 *T. rubrum* CBS 118829 and *T. interdigitale* MR 816 gene sets were calculated across all
305 coding regions using codeml in PAML (version 4.8) [44]; these totals were used to
306 normalize the ratios of non-synonymous to synonymous SNPs.

307
308 **Copy number variation**
309 To identify regions of *T. rubrum* that exhibit copy number variation between the isolates,
310 we identified windows showing significant variation in normalized read depth using
311 CNVnator [45]. The realigned read files used for SNP calling were input to CNVnator
312 version 0.2.5, specifying a window size of 1kb. Regions reported as deletions or
313 duplications were filtered requiring $p\text{-val}1 < 0.01$.

314
315 **Phylogenetic and comparative genomic analysis**
316 To infer the phylogenetic relationship of the sequenced isolates, we identified single
317 copy genes present in all genomes using OrthoMCL [46]. Individual orthologs were
318 aligned with MUSCLE [47] and then the alignments were concatenated and input to
319 RAxML [48], version 7.3.3 with 1,000 bootstrap replicates and model GTRCAT. RAxML
320 version 7.7.8 was used for phylogenetic analysis of SNP variant in seven *T. rubrum*
321 isolates, with the same GTRCAT model.

322
323 For each gene set, HMMER3 [49] was used to identify PFAM domains using release 27
324 [50]; significant differences in gene counts for each domain were identified using

325 Fisher's exact test, with p-values corrected for multiple testing [51]. Proteins with LysM
326 domains were identified using a revised HMM as previously described [15]; this HMM
327 includes conserved features of fungal LysM domains including conserved cysteine
328 residues not represented in the PFAM HMM model and identified additional genes with
329 this domain.

330

331 **Construction of paired allele compatibility matrix**

332 To construct SNP profiles, SNPs shared by at least two members of the *T. rubrum*
333 dataset were selected. Private SNPs are not informative for a paired allele compatibility
334 test because they can never produce a positive result. These profiles were then counted
335 across the genome to construct SNP profiles via a custom Perl script. We required
336 profiles to be present at least twice, to minimize the signal from homoplasic mutations.
337 Pairwise tests were then conducted between each of the profiles to look for all four
338 possible allele combinations, which would only occur via either mating or homoplasic
339 mutations.

340

341 **Linkage disequilibrium calculation**

342 Linkage disequilibrium was calculated for *T. rubrum* SNPs in 1kb windows of all
343 scaffolds with VCFtools version 1.14 [42], using the --hap-r2 option with a minimum
344 minor allele frequency of 0.2.

345

346 **Data Availability**

347 All genomic data is available in NCBI and can be accessed via the accession numbers
348 in Table S2. The NCBI GenBank accession numbers of the three MLST loci are listed in
349 Table S3.

350

351

352 **Results**

353 **Relationship of global *Trichophyton* isolates using MLST**

354 To examine the relationship of global isolates of *T. rubrum*, we sequenced three loci in
355 each of 104 *Trichophyton* isolates and carried out phylogenetic analysis. The typed

356 isolates included 91 *T. rubrum* isolates, 11 *T. interdigitale* isolates, and 2 *T. benhamiae*
357 isolates (**Table S1**). In addition, data from the genome assemblies of additional
358 dermatophyte species (*T. verrucosum*, *T. tonsurans*, *T. equinum*, and *M. gypseum*)
359 were also included. Three loci — the *TruMDR1* ABC transporter [19], an intergenic
360 region (IR), and an alpha-1,3-mannosyltransferase (CAP59 protein domain) — were
361 sequenced in each isolate. Phylogenetic analysis of the concatenated loci can resolve
362 species boundaries between the seven species (**Figure 1**). A large branch separates a
363 *T. benhamiae* isolate (MR857) from the previously described genome sequenced
364 isolate (CBS 112371) (**Figure 1**), and the sequences of two loci of a second *T.*
365 *benhamiae* isolate (MR827) were identical to those of MR857 (**Table S3**). Sequencing
366 of the ITS region of the MR857 and MR827 isolates revealed high sequence similarity to
367 isolates from the *T. benhamiae* African race (**Methods**), which is more closely related to
368 *T. bullosum* than isolates of *T. benhamiae* Americano-European race including
369 CBS112371 [52]. Otherwise, the species relationships and groups are consistent
370 between studies.

371
372 MLST analysis demonstrated that the *T. rubrum* isolates were nearly identical at the
373 three sequenced loci. Remarkably, of the 84 *T. rubrum* isolates sequenced at all three
374 loci, 83 were identical at all positions of the three loci sequenced (genotype 2, **Table**
375 **S3**). Only one isolate, 1279, displayed a single difference at one site in the *TruMDR1*
376 gene (genotype 3, **Table S3**). For the remaining six isolates, sequence at a subset of
377 the loci was generated and matched that of the predominant genotype. Thus, MLST
378 was not sufficient to discern the phylogenetic substructure in the *T. rubrum* population
379 that included six isolates representing different morphotypes (**Table S3**). Similarly, the
380 11 *T. interdigitale* isolates were highly identical at these three loci; two groups were
381 separated by a single nucleotide difference in the IR and the third group contained a 6-
382 base deletion overlapping the same base of the IR (genotypes 1, 5 and 6, **Table S3**).
383 Although most species can be more easily discriminated based on the MLST sequence,
384 *T. equinum* and *T. tonsurans* isolates differed only by a single transition mutation in the
385 IR, which illustrates the remarkable clonality of these species.

386

387 **Genome sequencing and refinement of phylogenetic relationships**

388 As MLST analysis was insufficient to resolve the population substructure of the *T.*
389 *rubrum* species complex, we sequenced the complete genomes of *T. rubrum* isolates
390 representing worldwide geographical origins and five morphotypes: *fischeri*, *kanei*,
391 *megninii*, *raubitschekii*, and *soudanense*. We generated whole genome Illumina
392 sequences for ten *T. rubrum* and two *T. interdigitale* isolates (**Table S2**). The sequence
393 of each isolate was assembled and utilized to predict gene sets. The *T. rubrum*
394 assembly size was very similar across isolates, ranging from 22.5 to 23.2 Mb (**Table**
395 **S5**). The total predicted gene numbers were also similar across the isolates, with
396 between 8,616 and 9,064 predicted genes in the ten *T. rubrum* isolates, and 7,993 and
397 8,116 predicted genes in the two *T. interdigitale* isolates (**Table S5**).

398
399 To infer the phylogenetic relationship of these isolates and other previously sequenced
400 *Trichophyton* isolates, we identified 5,236 single-copy orthologs present in all species
401 and estimated a phylogeny with RAxML [48] (**Figure 2A**). This phylogeny more
402 precisely delineates the species groups than that derived from the MLST loci and also
403 illustrates the relationship between the *T. rubrum* isolates (**Figure 2B**). The results of
404 this analysis suggest that the *fischeri* morphotype is not monophyletic, as one *fischeri*
405 isolate (CBS100081) is more closely related to the *raubitschekii* isolate than to the other
406 *fischeri* isolate (CBS 288.86). While a subset of seven *T. rubrum* isolates appear
407 closely related, others show much higher divergence, including the *soudanense* isolate,
408 the *megninii* isolate, the MR1459 isolate, and the CBS 118829 isolate representing the
409 reference genome. The *soudanense* isolate (CBS 452.61) was placed as an outgroup
410 relative to the other *T. rubrum* isolates; this is consistent with this isolate being part of a
411 clade more closely related to *T. violaceum* than to *T. rubrum* [6] and with the re-
412 establishment of *soudanense* isolates as a separate species [3].

413
414 To further classify the two *T. interdigitale* isolates, we assembled the ITS region of the
415 ribosomal DNA locus and compared the sequences to previously classified ITS
416 sequences, as *T. interdigitale* isolates differ from *T. mentagrophytes* at the ITS locus
417 [3,53]. For the two genomes of these species that we sequenced, MR816 was identical

418 to *T. interdigitale* at the ITS1 locus, whereas the H6 isolate appears intermediate
419 between *T. interdigitale* and *T. mentagrophytes*, containing polymorphisms specific to
420 each group (**Figure S1**). Genomic analysis of allele sharing across a wider set of *T.*
421 *interdigitale* and *T. mentagrophytes* isolates could be used to evaluate the extent of
422 hybrid genotypes and genetic exchange between these two species.

423

424 ***MAT1-1* prevalence and clonality in *T. rubrum***

425 To address if the *T. rubrum* population is capable of sexual reproduction, we surveyed
426 the *MAT* locus of all isolates. Using either gene content in assembled isolates or a PCR
427 assay to assign mating type, we found that 79 of the 80 *T. rubrum* isolates contained
428 the alpha domain gene at the *MAT* locus (*MAT1-1*). In addition, a set of 55 isolates from
429 Turkey were found to harbor the *MAT1-1* allele based on a PCR assay (**Figure S2**).
430 However, the *T. rubrum* morphotype *megrinii* isolate contained an HMG gene at the
431 *MAT* locus (*MAT1-2*) (**Figure 3, Table S1**). The presence of both mating types suggests
432 that this species could be capable of mating under some conditions. However the high
433 frequency of a single mating type strongly suggests that *T. rubrum* largely undergoes
434 clonal growth, although other interpretations are also possible (see **Discussion**). In
435 further support of this, a study of 206 *T. rubrum* clinical isolates from Japan noted that
436 all were of the *MAT1-1* mating type [14].

437

438 A closer comparison of the genome sequences of *T. rubrum* isolates also supports a
439 clonal relationship of this population. Phylogenetic analysis of the seven most closely
440 related *T. rubrum* isolates using SNPs between these isolates (see below) suggests that
441 the isolates have a similar level of divergence from each other (**Figure S3**). This
442 supports that these *MAT1-1* *T. rubrum* isolates have likely undergone clonal expansion.

443

444 To test for recombination that could reflect sexual reproduction within the *T. rubrum*
445 population sampled here, we conducted a genome-wide paired allele compatibility test
446 to look for the presence of all four products of meiosis (**Figure 4**). This test is a
447 comparison between two paired polymorphic sites in the population. While the presence
448 of three of the four possible allele combinations at two sites in a population is possible

449 through a single mutation and identity by descent, the presence of all four combinations
450 requires either recombination, or less parsimoniously, a second homoplastic mutation.
451 Four positive tests resulted from this analysis (out of 21 possible), including allele
452 combinations that occurred a minimum of 13 times. This may suggest that
453 recombination is a rare event arising through infrequent sexual recombination occurring
454 in this population although the same mutations and combinations arising via homoplasmy
455 (or selection) are difficult to exclude. Based on the number of triallelic sites in the
456 dataset (19), we would predict 9.5 homoplastic sites to have occurred by random
457 chance, which is similar to the number of sites responsible for the positive signals in the
458 compatibility test. In addition, linkage disequilibrium does not decay over increasing
459 distance between SNPs in *T. rubrum* (**Figure S4**), which further supports a low level of
460 recombination in this species; sequencing additional diverse isolates would help to
461 address if some isolates or lineages were more prone to recombination.

462

463 We also characterized the *MAT* locus of the newly sequenced *T. interdigitale* isolates
464 (H6 and MR816) and found that both contain an HMG domain gene. These *T.*
465 *interdigitale* isolates were more closely related to *T. equinum* (*MAT1-2*) and *T.*
466 *tonsurans* (*MAT1-1*) than *T. rubrum* (**Figure 2A**). To survey the mating type across a
467 larger set of *T. interdigitale* isolates, a set of 11 additional isolates from Turkey were
468 typed. Based on PCR analysis, all *T. interdigitale* isolates harbor the *MAT1-2* allele
469 (**Figure S2**).

470

471 The mating abilities of the isolates were tested by conducting mating assays with
472 potentially compatible isolates of *T. rubrum*, including the *magninii* morphotype, *T.*
473 *interdigitale*, and *T. simii* (**Table S7**). These experiments were conducted using both
474 Takashio and E medium at room temperature (approximately 21 to 22°C) without
475 Parafilm in the dark. Although the assay plates were incubated for longer than five
476 months, ascomata or ascomatal initials were not observed (**Figure S5**). While it is
477 possible that mating may occur under cryptic conditions [54], this data suggests that the
478 conditions tested are not sufficient for the initiation of mating structures in *T. rubrum*.

479

480 **Genome-wide variation patterns in *T. rubrum***

481 SNP variants were identified between *T. rubrum* isolates to examine the level of
482 divergence within this species complex (**Table S8**). On average, *T. rubrum* isolates
483 contain 8,092 SNPs compared to the reference genome of the CBS118892 isolate; this
484 reflects a bimodal divergence pattern where most isolates including three morphotypes
485 (*fischeri*, *kanei*, and *raubitscheckii*) have an average of 3,930 SNPs and two more
486 divergent isolates (morphotypes *megninii* and *soudanense*) have an average of 24,740
487 SNPs. The average nucleotide diversity (π) for all 10 *T. rubrum* isolates is 0.00054;
488 excluding the two divergent morphotypes, the average nucleotide diversity is 0.00031.
489 By comparison, the average nucleotide diversity of the fungal pathogen *Cryptococcus*
490 *neoformans* var. *grubii*, which is actively recombining as evidenced by low linkage
491 disequilibrium [43,55], is 0.0074, a level approximately 24-fold higher than that in *T.*
492 *rubrum* (**Methods**, [43]). Even higher levels of nucleotide diversity have been reported
493 in global populations of other fungi (see **Discussion**). A similar magnitude of SNPs
494 separate the two *T. interdigitale* isolates; 22,568 SNPs were identified based on the
495 alignment of H6 reads to the MR 816 assembly. Across all isolates, SNPs were
496 predominantly found in intergenic regions for both species, representing 76% and 81%
497 of total variants respectively (**Table 1**, **Table S8**). Within genes, the higher ratio of
498 nonsynonymous relative to synonymous changes among the closely related *T. rubrum*
499 isolates (**Table 1**) is consistent with lower purifying selection over recent evolutionary
500 time [56].

501
502 Examining the frequency of SNPs across the *T. rubrum* genome revealed high diversity
503 regions that flank the mating type locus in the two divergent isolates. Across all isolates,
504 some regions of the genome are over-represented for SNPs, including the smallest
505 scaffolds of the reference genome (**Figure 5**); these regions contain a high fraction of
506 repetitive elements [15]. The largest high diversity window unique to the *T. rubrum*
507 morphotype *megninii* was found in an ~810-kb region encompassing the mating type
508 locus on scaffold 2; a smaller high diversity region spanning the mating type locus was
509 found in the diverged *soudanense* isolate (**Figure 5**). The higher diversity found in this
510 location could reflect introgressed regions from recent outcrossing or could be

511 associated with lower recombination proximal to the mating type locus, resulting in
512 stratification of linked genes.

513

514 **Gene content variation in *T. rubrum* and *T. interdigitale***

515 To examine variation in gene content in the *Trichophyton rubrum* species complex, we
516 first measured copy number variation across the genome. Duplicated and deleted
517 regions of the genome were identified based on significant variation in normalized read
518 depth (Methods). We observed increased copy number only for two adjacent 26 kb
519 regions of scaffold 4 in two isolates (MR850 and MR1448) (**Figure S6**). Both of these
520 regions had nearly triploid levels of coverage (**Table S9**). While ploidy variation is a
521 mechanism of drug resistance in fungal pathogens, none of the 25 total genes in these
522 regions (**Table S10**) are known drug targets or efflux pumps. These regions include two
523 genes classified as fungal zinc cluster transcription factors; this family of transcription
524 factors was previously noted to vary in number between dermatophyte species [15]. A
525 total of 12 deleted regions (CNVnator p-val <0.01) ranging in size from 4 to 37 kb were
526 also identified in a subset of genomes (**Table S11**). Two of these regions include genes
527 previously noted to have higher copy number in dermatophyte genomes, a
528 nonribosomal peptide synthase (NRPS) gene (TERG_02711) and a LysM gene
529 (TERG_02813) (**Table S12**). Overall this analysis suggests recent gain or loss in
530 dermatophytes for a small set of genes including transcription factors, NRPS, and LysM
531 domain proteins.

532 We next examined candidate loss of function mutations in the *T. rubrum* species
533 complex. For the 8 closely related *T. rubrum* isolates, an average of 8.1 SNPs are
534 predicted to result in new stop codons, disrupting protein coding regions; in the
535 *soudanense* and *megrinii* isolates, an average of 58.5 SNPs result in new stop codons.
536 These predicted loss of function mutations do not account for previously noted
537 phenotypic differences between the morphotypes; no stop codons were found in the
538 seven genes involved in histidine biosynthesis (*HIS1-HIS7*) in the histidine auxotroph *T.*
539 *rubrum* morphotype *megrinii* or in urease genes in *T. rubrum* morphotype
540 *raubitscheckii*.

541

542 Comparison of the first representative genomes for *T. interdigitale* (isolates MR816 and
543 H6) to those of dermatophyte species highlighted the close relationship of *T.*
544 *interdigitale* to *T. tonsurans* and *T. equinum*. These three species are closely related
545 (**Figure 2**), sharing 7,618 ortholog groups, yet there are also substantial differences in
546 gene content. A total of 1,253 orthologs groups were present only in *T. equinum* and *T.*
547 *tonsurans* and 512 ortholog groups were present only in both *T. interdigitale* isolates.
548 However, there were no significant differences in functional groups between these
549 species based on PFAM domain analysis, suggesting no substantial gain or loss of
550 specific protein families. Two PFAM domains were unique to the *T. interdigitale* isolates
551 and present in more than one copy: PF00208, found in ELFV dehydrogenase family
552 members and PF00187, a chitin recognition protein domain. This chitin binding domain
553 is completely absent from the *T. equinum* and *T. tonsurans* genomes while in *T.*
554 *interdigitale* this domain is associated with the glycosyl hydrolase family 18 (GH18)
555 domain [57]. GH18 proteins are chitinases and some other members of this family also
556 contain LysM domains. We also examined genes in the ergosterol pathway for variation,
557 as this could relate to drug resistance; while this pathway is highly conserved in
558 dermatophytes [15], *T. interdigitale* isolates had an extra copy of a gene containing the
559 ERG4/ERG24 domain found in sterol reductase enzymes in the ergosterol biosynthesis
560 pathway. The *ERG4* gene encodes an enzyme that catalyzes the final step in ergosterol
561 biosynthesis, and it is possible that an additional copy of this gene results in higher
562 protein levels to help ensure that this step is not rate limiting.

563
564 These comparisons also highlighted the recent dynamics of the LysM family, which
565 binds bacterial peptidoglycan and fungal chitin [58]. Dermatophytes contain high
566 numbers of LysM domain proteins ranging from the 10 genes found in *T. verrucosum* to
567 31 copies found in *M. canis* (**Table S13, [15]**). Both the class of LysM proteins with
568 additional catalytic domains and the larger class consists of proteins with only LysM
569 domains, many of which contain secretion signals and may represent candidate
570 effectors [15], vary in number across the dermatophytes. Isolates from the *T. rubrum*
571 species complex have 16 to 18 copies of LysM proteins compared to the 15 found in the
572 previously reported genome of the CBS 118892 isolate (**Table S13**). One of the

573 additional LysM genes present in all of the newly sequenced isolates encodes a
574 polysaccharide deacetylase domain involved in chitin catabolism. There is also an
575 additional copy of a gene with only a LysM domain in 9 of the 10 new *T. rubrum* isolates
576 (**Table S13**). The genomes of the *T. interdigitale* isolates have only 14 genes containing
577 a LysM binding domain, and are missing a LysM gene encoding GH18 and Hce2
578 domains (**Figure S7**). Notably, this locus is closely linked to genes encoding additional
579 LysM domain proteins in some species (**Figure S7**). The variation observed in the LysM
580 gene family suggests that recognition of chitin appears to be highly dynamic based on
581 these differences in gene content and domain composition.

582

583 **Discussion**

584

585 In this study, we selected diverse *T. rubrum* isolates for genome sequencing, assembly,
586 and analysis and surveyed a wider population sample using MLST analysis. These
587 isolates include multiple morphotypes, which show noted phenotypic variation yet are
588 assigned to the same species based on phylogenetic analyses [3,53]. The *T. rubrum*
589 morphotype *soudanense* and *T. rubrum* morphotype *megninii* show higher divergence
590 from a closely related subgroup that includes the *kanej*, *raubitschekii*, and *fischerii*
591 morphotypes, as well as most other *T. rubrum* isolates.

592

593 Our MLST and whole genome analyses provide strong support that *T. rubrum* is highly
594 clonal and may be primarily asexual or at least infrequently sexually reproducing.
595 Across 135 isolates examined, 134 were from a single mating type (*MAT1-1*). Only the
596 *T. rubrum* type *megninii* isolate, Consistent with prior reports [3,53], only the *T. rubrum*
597 morphotype *megninii* isolates contains the opposite mating type (*MAT1-2*) while all
598 other *T. rubrum* isolates that are of *MAT1-1* type. Direct tests of mating between these
599 and other species did not find evidence for mating and sexual development. While
600 mating was not detected, studies in other fungi have required specialized conditions and
601 long periods of time to detect sexual reproduction [59]. As genes involved in mating and
602 meiosis are conserved in *T. rubrum* [15], gene loss does not provide a simple
603 explanation for the inability to mate. Sexual reproduction might occur rarely under

604 specific conditions such as specific temperatures as found for *Trichophyton onychocola*
605 [60], may be geographically restricted, as the opposite mating type *megninii* morphotype
606 is generally found in the Mediterranean [61], or could be unisexual as in some other
607 fungi such as *Cryptococcus neoformans* [62].

608
609 As MLST data provided no resolution of the substructure of the *T. rubrum* population,
610 we examined whole genome sequences for 8 diverse isolates. Analysis of the sequence
611 read depth revealed that while some small regions of the genome show amplification or
612 loss, there is no evidence for aneuploidy of entire chromosomes. Most of these *T.*
613 *rubrum* isolates contain an average of only 3,930 SNPs (0.01% of the genome) and
614 phylogenetic analysis revealed little genetic substructure. Two isolates were more
615 divergent with an average of 24,740 SNPs (0.06% of the genome); one of these was of
616 the recently proposed separated species *T. soudanense* [3], and the other was the *T.*
617 *rubrum* morphotype *megninii* isolate. While the similar level of divergence raises the
618 question of whether morphotype *megninii* isolates could also be a separate species, this
619 has not yet been proposed when considering additional phenotypic data in addition to
620 molecular data, however further study would help clarify species assignments. The low
621 level of variation is remarkable in comparison to other fungal pathogens; for example,
622 while *T. rubrum* isolates are identical at 99.97% of positions on average, isolates of
623 *Cryptococcus neoformans* var. *grubii* isolates are 99.36% identical on average [43,55].
624 Global populations of *Saccharomyces* have even higher reported diversity [63]. The low
625 diversity and the dependency on the human host for growth suggests that *T. rubrum*
626 may have a low effective population size impacted by the reduction of intra-species
627 variation by genetic drift. In addition, direct tests for recombination found a low level of
628 candidate reassortments that was not in excess of the estimated number of
629 homoplasmic mutations; further, as there was no apparent decay of linkage
630 disequilibrium over genetic distance, our analyses support the overall clonal nature of
631 this species. The high clonality observed in *T. rubrum* is also supported by MLST
632 analysis of eight microsatellite markers in approximately 230 *T. rubrum* isolates,
633 including morphotypes from diverse geographic origins [8]. With additional genome
634 sequencing geographic substructure may become more apparent; the fungal pathogen

635 *Talaromyces marneffeii* also displays high clonality yet isolates from the same country or
636 region are more closely related [64]. While low levels of diversity seems surprising in a
637 common pathogen, this is similar to findings in some bacterial pathogens including
638 *Mycobacterium tuberculosis* and *Mycobacterium leprae* [65,66], which also display high
639 clonality despite phenotypic variation.

640
641 LysM domain proteins are involved in dampening host recognition of fungal chitin [67]
642 and can also regulate fungal growth and development [68], yet their specific function in
643 dermatophytes and closely related fungi is not well understood. We also observed
644 variation in genes containing the LysM domain across the sequenced isolates, both in
645 the gene number and domain organization. LysM genes are present in higher copy
646 number in dermatophytes than related fungi in the Ascomycete order Onygenales [15].
647 Recent sequencing of additional non-pathogenic species in this order related to
648 *Coccidioides* revealed that most LysM copies found in dermatophytes have a homolog
649 [69]. Although this analysis excluded *M. canis* — the dermatophyte species with the
650 highest LysM count— this suggests that dermatophytes have retained rather than
651 recently duplicated many of their LysM genes. However, changes in the domain
652 composition of both genes with catalytic domains and those with only LysM domains,
653 many of which represent candidate effectors, highlights the dynamic evolution of the
654 LysM family in the dermatophytes. Studies of LysM genes in dermatophytes are needed
655 to determine whether these genes serve similar or different roles in these species.

656
657 *T. rubrum* is only found as a pathogen of humans, though this adaptation is more recent
658 relative to the related species that infect other animals or grow in the environment.
659 Unlike the obligate human fungal pathogen *Pneumocystis jirovecii* [70,71], *T. rubrum*
660 does not display widespread gene loss [15] indicative of host dependency for growth;
661 further, its genome size is also comparable to related dermatophyte species, supporting
662 no overall reduction [15]. The presence of a single mating type in the vast majority of
663 isolates and limited evidence of recombination suggests that sexual reproduction of *T.*
664 *rubrum* may have been recently lost or may be rarely occurring in specific conditions or

665 geographic regions. This may be linked to the specialization as a human pathogen, as
666 mating may be optimized during environmental growth in the soil [53].

667

668

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680

681 **Author contributions**

682 C.A.C., D.A.M., T.C.W. and J.H. conceived and designed the project. A.D., B.M, S.H.,
683 M. I., R.B., B.O., Y.G, N.M.M, and T.W. provided the isolates. W.L. and A.D. performed
684 the laboratory experiments. G.F.P, D.A.M, W.L, A.D., R.B.B, A.A, J.M., G,T.S., S.Y,
685 Q.Z, and C.A.C analyzed the data. C.A.C. and J.H. wrote the paper with input from all
686 authors. C.A.C. and J.H. supervised and coordinated the project.

687

688

689

690 **Table**

Table 1. Variation in *T. rubrum* SNP rate and class

Isolate	Total Number of SNPs	SNPs in CDS*	SYN*	NSY*	pN/pS*
<i>T. rubrum</i> MR1448	4,283	374	83	287	1.15
<i>T. rubrum</i> MR1459	2,188	436	103	317	1.02
<i>T. rubrum</i> MR850	4,203	387	88	289	1.09
<i>T. rubrum</i> D6	4,121	484	112	363	1.08
<i>T. rubrum</i> (morphotype <i>fischeri</i>) CBS 100081	4,199	409	94	307	1.09
<i>T. rubrum</i> (morphotype <i>fischeri</i>) CBS 288.86	4,147	375	84	283	1.12
<i>T. rubrum</i> (morphotype <i>kanei</i>) CBS 289.86	4,491	474	116	350	1.00
<i>T. rubrum</i> (morphotype <i>raubitschekii</i>) CBS 202.88	3,808	375	83	285	1.14
<i>T. rubrum</i> (morphotype <i>megninii</i>) CBS 735.88	26,406	7,328	3,069	4,185	0.45
<i>T. rubrum</i> (morphotype <i>soudanense</i>) CBS 452.61	23,073	6,253	2,377	3,808	0.53
<i>T. interdigitale</i> MR816	1,223,298	591,173	395,250	194,498	0.16
<i>T. interdigitale</i> H6	1,183,411	585,288	393,079	190,826	0.16

691 *CDS, coding sequence; SYN, synonymous SNP sites; NSY, non-synonymous SNP sites; pN/pS, (NSY/total NSY
 692 sites)/(SYN/total SYN sites).

693

694

695 **Figure Legends**

696 **Figure 1.** Phylogeny inferred from concatenated MLST sequences. Three MLST loci
697 (ABC transporter, outer membrane protein, and CAP59 protein) were amplified and
698 sequenced from 79 isolates and sequences were identified in an additional 19
699 assemblies. The concatenated sequence for each isolate was used to build a maximum
700 likelihood tree using MEGA 5.2. Isolate MR1168 is representative of 73 *T. rubrum*
701 isolates that have identical MLST sequences.

702

703 **Figure 2.** Phylogenetic relationship of *Trichophyton* isolates. A total of 5,236 single copy
704 genes were each aligned with MUSCLE; the concatenated alignment was used to infer
705 a species phylogeny with RAxML (GTRCAT model) with 1,000 bootstrap replicates
706 using either A. all species including the outgroup *M. gypseum* or B. only *Trichophyton*
707 *rubrum* isolates.

708

709 **Figure 3.** Alignment of the mating type locus of selected isolates. Mating type genes of
710 *T. rubrum* morphotype *megrinii* (CBS 735.88) and *T. rubrum* (CBS 188992) are shown
711 along the x- and y- axes, respectively, with regions aligning by NUCmer show in the
712 dotplot. The alignment extends into two hypothetical proteins (HP) immediately flanking
713 the alpha or HMG domain gene that specifies mating type. Most *T. rubrum* (*MAT1-1*)
714 isolates contain an alpha domain protein (blue) at the *MAT* locus. In contrast, the *T.*
715 *rubrum* morphotype *megrinii* isolate contains an HMG domain protein (green)
716 representing the opposite mating type (*MAT1-2*). All sequenced *T. interdigitale* isolates
717 are also of *MAT1-2* mating type including MR816. Gene locus identifiers are shown for
718 the genes flanking each locus (prefix TERG, H106, and H109).

719

720 **Figure 4.** Paired allele compatibility test suggests limited evidence for sexual
721 reproduction. A. A single example of a positive paired allele compatibility test from the
722 *T. rubrum* population. In this test, two loci are examined and typed across the
723 population. To perform a meaningful test, at least two individuals in the population must
724 share a variant allele at each site. Here alternative SNPs are depicted in red and
725 reference in white. Evidence for recombination is provided by any pairwise comparison

726 of two loci in which isolates are present where red-red, white-white, red-white, and
727 white-red combinations are all found (AB, Ab, aB, and ab) satisfying the allele
728 compatibility test and providing evidence for recombination. B. Paired allele
729 compatibility tests were performed for all isolates in the *T. rubrum* population across the
730 entire genome. SNP profiles were grouped into unique and informative allele patterns
731 and collapsed, with the number of occurrences of each profile across the genome listed.
732 Thus, the larger the number, the more common that SNP distribution is in the
733 population. Pairwise tests were then conducted for each combination of SNP profiles.
734 Reference nucleotides are indicated by white and variant by red. The pairwise matrix
735 displays the results of all of these tests; a green square in the pairwise matrix is
736 indicative of a positive test for the pairwise comparison and thus provides potential
737 evidence of recombination.

738
739 **Figure 5.** Genome-wide SNP frequency highlights hotspots. For each panel, the
740 frequency of SNPs in 5-kb windows is shown across the genome. The genome
741 assembly of isolate CBS 11892 was used for all comparisons, and scaffolds are ordered
742 along the x-axis with grey lines representing scaffold boundaries. Red dots indicate the
743 position of the mating type locus.

744

745 **Supplemental Figure and Table Legends**

746

747 **Figure S1. ITS sequence variation in *T. interdigitale*.** Aligned ITS sequence is shown
748 for four isolates, including the two for which whole genomes were sequenced (H6 and
749 MR816) and two previously characterized isolates (AF168124 and AY062119). Isolate
750 AY062119 has been re-classified as *T. mentagrophytes*. Variant sites are highlighted.

751

752 **Figure S2. Detection of *MAT1-1* in *T. rubrum* and *MAT1-2* in *T. interdigitale* by**
753 **PCR.** A. PCR-based determination of the *MAT1-1* alpha domain of *T. rubrum* isolates:
754 *T. rubrum* MR 851, *T. megninii* CBS 389.58, *T. megninii* CBS 384.64, and *T. megninii*
755 CBS 417.52. The alpha domain *MAT1-1* gene was identified from samples = 1-12 and
756 MR 851 *T. rubrum*; the alpha domain *MAT1-1* gene was not identified from *T. megninii*

757 CBS 389.58, *T. megninii* CBS 384.64, or *T. megninii* CBS 417.52. M: DNA ladder. B.
758 PCR-based determination of *MAT 1-2* HMG domain of *T. rubrum*: *T. rubrum* MR851, *T.*
759 *megninii* CBS 389.58, *T. megninii* CBS 384.64, and *T. megninii* CBS 417.52. The HMG
760 domain *MAT1-2* gene was identified from *T. megninii* CBS 389.58, *T. megninii* CBS
761 384.64, and *T. megninii* CBS 417.52, and was not identified from *T. rubrum* isolate = 1-
762 12 and *T. rubrum* MR 851, M: DNA ladder. C. PCR-based determination of *MAT1-2*
763 HMG domain of *T. interdigitale*. *T. interdigitale* isolates = 1-9, *T. interdigitale* MR8801
764 (positive control), and *T. rubrum* MR851 (negative control), M; DNA ladder. D. PCR
765 based determination of *MAT 1-1* alpha domain of *T. interdigitale* isolates. *T. interdigitale*
766 isolates = 1-9, *T. interdigitale* MR8801 (negative control), and *T. rubrum* MR851
767 (positive control). M: DNA ladder.

768

769 **Figure S3. Phylogenetic relationship and sharing of variant sites of sequenced *T.***
770 ***rubrum* isolates.** A. Phylogenetic relationship of *T. rubrum* isolates inferred using
771 RAxML (Methods). B. Classification of SNP sites based on conservation across the
772 sequenced isolates; unique: only in one isolate; shared: in two to seven isolates; fixed:
773 in all eight isolates.

774 **Figure S4. Lack of decay of linkage disequilibrium (LD) in *T. rubrum*.** LD (r^2) was
775 calculated for all pairs of SNPs separated by 0 to 300 kb and then averaged for every
776 1kb. LD values for each window were then calculated by averaging over all pairwise
777 calculations in the window.

778

779 **Figure S5. Mating assays.** A. Mating assay plate; *T. rubrum* and *T. interdigitale* on E
780 medium. B. Mating assay plate; *T. rubrum* and *A. simii* (a) E medium, (b) Takashio
781 medium eight weeks. C. *T. rubrum* and *T. megninii* on E medium for eight weeks.

782

783 **Figure S6. Read depth of sequenced isolates.** Reads from each isolate were aligned
784 to the *T. rubrum* reference genome and normalized read depth was computed for 5kb
785 windows. Read depth is even across the reference genome for most isolates, with small
786 regions of higher depth detected in some isolates.

787

788 **Figure S7. Variation in LysM-Hce gene cluster across sequenced dermatophytes.**

789 In *T. rubrum*, the LysM-Hce gene is closely linked to two other LysM genes; this
790 organization is most similar to that found in *M. canis*, although these genes are located
791 on two different scaffolds.

792

793 **Table S1. Properties of sequenced isolates.**

794 **Table S2. Accessions for sequenced genomes.**

795 **Table S3. MLST sequence, genotypes, and GenBank accession numbers.**

796 **Table S4. Primers for MLST gene amplification.**

797 **Table S5. *Trichophyton* genome assembly statistics.**

798 **Table S6. Primer pairs used for mating type determination of *T. rubrum***

799 **Table S7. Mating assays and results.**

800 **Table S8. Frequency of SNPs in *T. rubrum* and *T. interdigitale* isolates by mutation**
801 **type.**

802 **Table S9. Duplicated regions in sequence isolates.**

803 **Table S10. List of genes found in duplicated regions.**

804 **Table S11. Deleted regions in sequenced isolates.**

805 **Table S12. List of genes in deleted regions in sequenced isolate**

806 **Table S13. Genes containing the LysM binding domain in dermatophytes**

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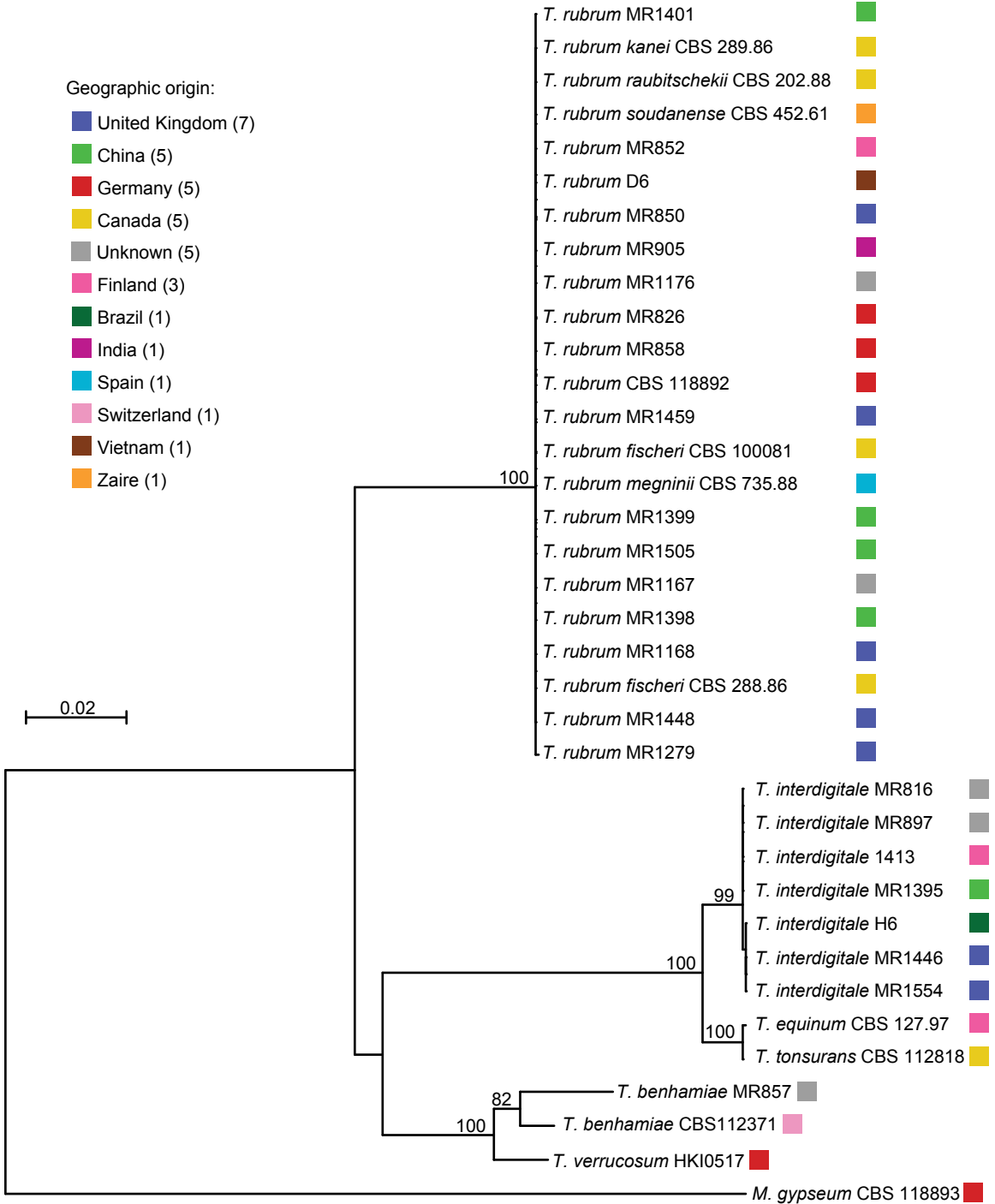
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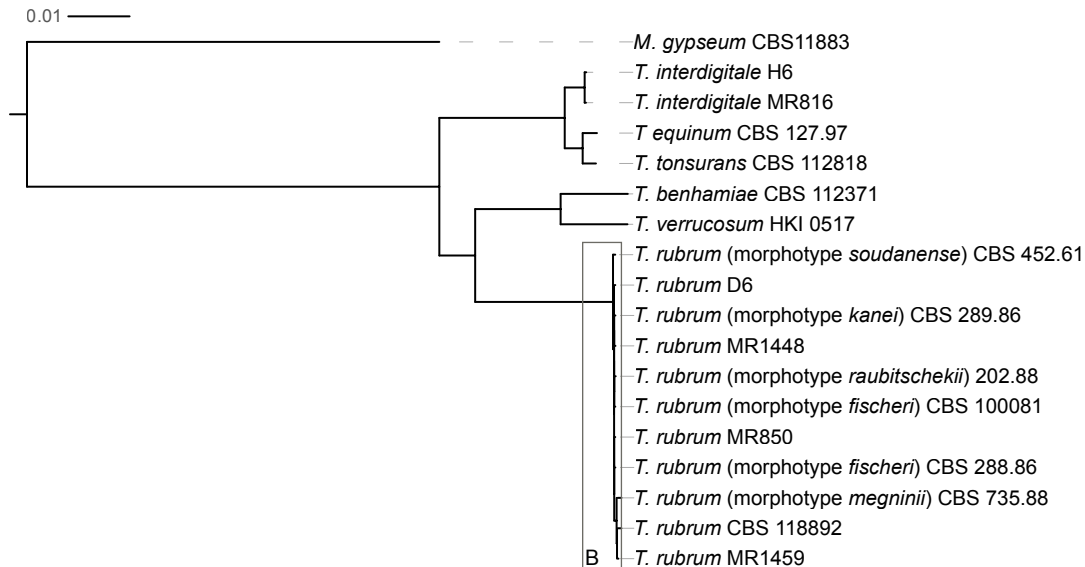
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1007

Geographic origin:

- United Kingdom (7)
- China (5)
- Germany (5)
- Canada (5)
- Unknown (5)
- Finland (3)
- Brazil (1)
- India (1)
- Spain (1)
- Switzerland (1)
- Vietnam (1)
- Zaire (1)



A.



B.

