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Highly Discriminatory Variable-Number Tandem-Repeat Markers for Genotyping of *Trichophyton interdigitale* Strains

Ines Drira,[a] Ines Hadrich,[a] Sourour Neji,[a] Nedia Mahfouth,[b] Houaida Trabelsi,[a] Hayet Sellami,[a] Fattouma Makni,[a] Ali Ayadi[a]

Laboratory of Fungal and Parasitic Molecular Biology, Faculty of Medicine of Sfax, University of Sfax, Sfax, Tunisia[a]; Laboratory Service, UHC-Hedi-Chaker, Sfax, Tunisia[b]

Trichophyton interdigitale **is the second most frequent cause of superficial fungal infections of various parts of the human body. Studying the population structure and genotype differentiation of *T. interdigitale* strains may lead to significant improvements in clinical practice. The present study aimed to develop and select suitable variable-number tandem-repeat (VNTR) markers for 92 clinical strains of *T. interdigitale*. On the basis of an analysis of four VNTR markers, four to eight distinct alleles were detected for each marker. The marker with the highest discriminatory power had eight alleles and a *D* value of 0.802. The combination of all four markers yielded a *D* value of 0.969 with 29 distinct multilocus genotypes. VNTR typing revealed the genetic diversity of the strains, identifying three populations according to their colonization sites. A correlation between phenotypic characteristics and multilocus genotypes was observed. Seven patients harbored *T. interdigitale* strains with different genotypes. Typing of clinical *T. interdigitale* samples by VNTR markers displayed excellent discriminatory power and 100% reproducibility.**

Trichophyton interdigitale is a member of the *Trichophyton mentagrophytes* species complex, which has often been reported as the most common causative agent of mycoses worldwide (1, 2), including Tunisia (3, 4). Identification and delineation of dermatophytes of this species still remain difficult, particularly because of phenotypic variation within and between isolates.

T. interdigitale can show wide variability in its phenotypic features, including the presence or absence of ornamental bodies (spiral hyphae) and the number and size of macroconidia and microconidia (5). These characteristics can change greatly during subculture and therefore are inappropriate for use as phenotypic strain markers. *T. interdigitale* has also been shown to possess genetic polymorphism. The nontranscribed spacer region of the ribosomal DNA was determined, and three individual subrepeat loci were identified (6).

In the last decade, genotyping approaches have proven useful for overcoming the challenge of dermatophyte taxonomy and enhancing the reliability and speed of dermatomycosis diagnosis (7–10). By PCR melting profile analysis, Leibner-Ciszak et al. (11), for instance, distinguished three genotypes (A, C, and D) among 29 clinical isolates of *T. interdigitale* from Lodz, Poland, and three genotypes (E, F, and G) among 10 isolates from Copenhagen, Denmark. Furthermore, Fréalle et al. (12) confirmed low genetic heterogeneity of the gene encoding the manganese-containing superoxide dismutase by internal transcribed spacer (ITS) locus sequence analyses. ITS data revealed four genotypes in a set of 86 *T. interdigitale* isolates from France, Germany, and China (2).

Recently, microsatellite markers have proven their utility for the detection of variability among dermatophytes (13). In other human-pathogenic dermatophytes, multilocus microsatellite typing has proved to be a promising tool for uncovering intraspecific diversity due to the high mutation rate of those markers (14). To our knowledge, no microsatellite typing methods for the assessment of *T. interdigitale* strain relatedness are currently available. Accordingly, the present study was undertaken to analyze four new variable-number tandem-repeat (VNTR) markers (two microsatellites and two minisatellites) in a global set of *T. interdigitale* isolates from patients with diverse clinical presentations. This study also aimed to determine whether a correlation between phenotypical characteristics and clinical manifestations exists and to explore the genetic structure of *T. interdigitale* strains.

MATERIALS AND METHODS

Fungal strains. A total of 92 clinical isolates (44 causing tinea pedis, 30 causing onychomycosis, 17 causing tinea corporis, and 1 causing tinea capitis) of *T. interdigitale* from 80 patients diagnosed at the Parasitology Mycology Laboratory of the Habib Bourguiba University Hospital (Sfax, Tunisia) were investigated. Among these clinical isolates, five quality control (QC) strains of *T. interdigitale* from temporally and geographically different sources were used to prove the robustness of the method. The characteristics of 87 isolates collected from 75 patients from Tunisia and 5 QC isolates collected from 4 patients from Libya and 1 patient from France are summarized in Table 1. The patient population had a sex ratio of 0.82 and an average age of 39.88 years, with extremes of 19 and 70 years. The isolates were preliminarily identified on the basis of macroscopic and microscopic characteristics; these findings were confirmed by analyses of the internal transcribed spacer 1 (ITS1), 5.8S, and ITS2 region rRNA sequences (15). Seven of these isolates were used as reference strains that were cultured in our diagnostic laboratory from specimens obtained from patients, identified by ITS sequence analysis, and deposited in GenBank (isolates TTIA 2224, TTIA 1317, TTIA 1003, TTIA 1001, TTIZ 0300, TTIZ 0509, and TTIZ 2402). One reference strain (LMA 95170.1) was obtained from Marseille, France. All strains were identified morphologically as *T. interdigitale*.

Six dermatophyte strains (*T. rubrum* TRN 2711; *T. violaceum* TVIO 262; *T. mentagrophytes* TM 2119, TM 3051, and TM 3026; and *T. erinacei* TERN 578) and five nondermatophyte strains (*Candida albicans* ATCC 90020 and CBS 2708, *Candida glabrata* ATCC 3153, and *Aspergillus flavus* CBS 12685.7 and JX 852615) were used in the specificity tests.

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Address correspondence to Ali Ayadi, ali.ayadi@rns.tn.

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TABLE 1 Origins of *T. interdigitale* isolates collected from 80 patients

Country and patient no.	Strain	Patient gender	Patient age (yr)	Mycosis
Tunisia				
1	1005.1	Female	35	Onychomycosis
	1005.2	Female	35	Tinea pedis
	1005.3	Female	35	Tinea pedis
2	2385	Male	32	Onychomycosis
3	2312	Male	43	Onychomycosis
4	0902	Male	54	Onychomycosis
5	2215	Female	46	Onychomycosis
6	0538.1	Male	54	Onychomycosis
	0538.2	Male	54	Tinea pedis
	0538.3	Male	54	Tinea pedis
7	2278.1	Male	52	Onychomycosis
	2278.2	Male	52	Tinea corporis
8	1252	Female	24	Onychomycosis
9	2608	Female	56	Onychomycosis
10	2395.1	Male	19	Tinea corporis
	2395.2	Male	19	Tinea corporis
	2395.3	Male	19	Onychomycosis
11	0295	Female	37	Onychomycosis
12	0207	Male	56	Onychomycosis
13	0809	Male	66	Onychomycosis
14	1105.1	Female	29	Tinea corporis
	1105.2	Female	29	Tinea corporis
	1105.3	Female	29	Onychomycosis
15	0338	Female	40	Onychomycosis
16	2749	Female	40	Onychomycosis
17	2616	Male	46	Onychomycosis
18	2672	Male	65	Onychomycosis
19	1425	Male	23	Onychomycosis
20	TTIA1003	Male	46	Onychomycosis
21	0904	Female	58	Onychomycosis
22	1094	Female	54	Onychomycosis
23	1034	Female	28	Onychomycosis
24	1071	Female	46	Onychomycosis
25	0770	Male	25	Onychomycosis
26	TTIA1317	Male	46	Onychomycosis
27	1635	Female	35	Onychomycosis
28	TTIA2224	Female	20	Tinea pedis
29	0612	Female	40	Tinea pedis
30	2938	Male	36	Tinea pedis
31	3058	Male	34	Tinea pedis
32	1522	Female	23	Tinea pedis
33	3083	Male	65	Tinea pedis
34	1139	Male	54	Tinea pedis
35	2254	Female	43	Tinea pedis
36	1781	Female	22	Tinea pedis
37	2326	Female	47	Tinea pedis
38	1068	Female	36	Tinea pedis
39	0346	Female	27	Tinea pedis
40	1986	Male	70	Tinea pedis
41	1736	Female	32	Tinea pedis
42	0896	Male	49	Tinea pedis
43	0985	Female	65	Tinea pedis
44	2297	Female	43	Tinea pedis
45	2850	Male	22	Tinea pedis
46	1039	Female	36	Tinea pedis
47	0972	Male	40	Tinea pedis
48	1550	Female	29	Tinea pedis
49	1009	Male	34	Tinea pedis
50	2397.1	Female	23	Tinea pedis
	2397	Female	23	Tinea pedis
51	0262	Female	50	Tinea pedis
52	0333	Male	28	Tinea pedis
53	1683.1	Male	26	Tinea pedis
	1683.2	Male	26	Tinea corporis
54	2313	Female	41	Tinea pedis
55	0774	Female	34	Tinea pedis
56	2223	Male	40	Tinea pedis
57	2580	Female	32	Tinea pedis
58	0722	Female	56	Tinea pedis
59	0564	Female	19	Tinea pedis
60	1733	Female	50	Tinea pedis
61	0875	Female	43	Tinea pedis
62	0975	Female	33	Tinea pedis
63	0435.1	Male	25	Tinea pedis
	0435.2	Male	25	Tinea corporis
64	3039	Female	61	Tinea pedis
65	0546	Male	56	Tinea corporis
66	TTIZ0509	Female	27	Tinea corporis
67	1693	Female	51	Tinea corporis
68	1463	Male	62	Tinea corporis
69	TTIA1001	Male	43	Tinea pedis
70	0456	Female	32	Tinea corporis
71	TTIZ2402	Male	23	Tinea corporis
72	0672	Female	45	Tinea corporis
73	2830	Male	65	Tinea corporis
74	TTIZ0300	Male	24	Tinea corporis
75	0745	Female	45	Tinea capitis
Libya				
1	Lib 017	Female	33	Onychomycosis
2	Lib 028	Female	45	Tinea corporis
3	Lib 298	Male	56	Onychomycosis
4	Lib144	Female	28	Onychomycosis
France, 1	LMA 95170.1	Unknown	Unknown	Tinea pedis

DNA extraction. DNA was extracted with a QIAamp DNA minikit (Qiagen) in accordance with the manufacturer's instruction and eluted with 50 μl of sterile water.

VNTR design. Genomic sequences of *T. interdigitale* were collected from published sequences available in the NCBI (http://ncbi.nlm.nih.gov), EMBL (http://www.embl.fr/), CBS (http://www.cbs.knaw.nl/collections/), and DDBJ (www.ddbj.nig.ac.jp/) databases. Sequences were analyzed for the presence of short tandem repeats with the Tandem Repeats Finder software (http://tandem.bu.edu/trf/trf.html). Two microsatellite and two minisatellite loci showing perfect repeat sequences (having 100% identity between repeat units) and highly repetitive sequences were selected. Imperfect repeats containing point mutations and/or insertions or deletions or having mismatches in the repeats were excluded. The analysis allowed for the generation of a flanking sequence for the microsatellites selected. Primers were then designed with the Primer (version 3) software (http://frodo.wi.mit.edu) and verified for specificity to *T. interdigitale* by BLASTn searches (http://blast.ncbi.nlm.nih.gov/Blast.cgi).

PCR amplification of VNTR markers with specific primers. PCRs were performed in a final volume of 25 μl containing 1 ng of genomic DNA, 0.5 μM each primer, 0.2 mM each deoxynucleoside triphosphate, 3 mM $MgCl_2$, and 2 U of GOTaq DNA polymerase (Promega) in 1× reaction buffer. PCR amplification was carried out in a thermocycler (Bio-

TABLE 2 Features of the four polymorphic VNTR PCR primers used to type 92 *T. interdigitale* isolates

Marker	Primer sequence (5′–3′)	Repeat unit sequence	Fragment size (bp)	No. of alleles	*D* value
VNTR15	GGAGCAGAAGAACGAAACCA CTGAGCCCAGAAGTGAGACC	GGCCTGCCATGTCTT	120–206	7	0.666
VNTR12	CTCCTCATGTTCCTCCGGTA GTTCAAAGGAGCAGGGTGAG	GCCATGGTCATG	197–249	4	0.406
VNTR8	CTTCCGTGCCTCTTTCTCTG TTCATTCCGGCTTATTACGG	CGTTCTGG	108–180	8	0.802
VNTR6	GCCAGAGTATGGCTGTTGGT AAGAGATGGACTGGCTCACC	GAACCA	124–232	6	0.787

Rad) and consisted of an initial denaturation at 94°C, followed by 30 cycles of 30 s of denaturation at 94°C, 30 s of annealing at 56°C, and 30 s of extension at 72°C and a final extension step of 30 min at 72°C. In each PCR, different fluorescent labels (6-carboxyfluorescein, 4,7,2′-trichloro-7′-phenyl-6-carboxyfluorescein, and nitrobenzoxadiazolyl [Applied Biosystems]) were used for the different markers to distinguish between the PCR products. The latter were diluted 10-fold with formamide. One microliter of the diluted PCR products was combined with 15 μl of formamide and 0.5 μl of LIZ[500] marker (Applied Biosystems Inc.). Following denaturation, the PCR products were resolved by capillary electrophoresis with polymer POP-7 in an ABI 310 genetic analyzer (Applied Biosystems Inc.). The injection and running parameters used were in accordance with the manufacturer's recommendations (Applied Biosystems Inc.). Analyses were performed with GeneScan software (Applied Biosystems Inc.).

Data analysis. The repeatability of VNTR typing was evaluated by using five different DNA preparations of the same isolate and 10 repeated analyses of the same DNA preparation under standardized conditions.

The reproducibility of VNTR typing was evaluated by using five different DNA preparations of the same isolate (which was passaged nine times) and repeated analysis of the same DNA preparation under different amplification conditions.

The Simpson index of diversity, *D* (16), was computed for each marker and each possible marker combination so as to determine the most parsimonious combination yielding a *D* value of >0.95, a sufficiently high discriminatory power recommended for typing experiments. The degree of similarity was calculated by applying the Dice coefficient test with the NTSYSpc 2.1 software numerical taxonomy and multivariate analysis system (17). The fixation index (F_{ST}) of all loci was estimated from 1,000 bootstrap repetitions with the ARLEQUIN software package (18). The unweighted-pair group method using average linkages (UPGMA) (19) was used to analyze and compare the individual genotype with the NTSYSpc software. The confidence of the clusters was tested by applying a bootstrap analysis with 1,000 replicates with Free-Tree 0.9.1.50 (20) software. Isolates possessing alleles with the same number of repeat units in all loci were defined as a clonal cluster. To analyze the genetic diversity of the sample and to test for clonality versus recombination in *T. interdigitale*, the overall and in-population indexes of association (I_A) were calculated with Multilocus 1.3b software (21). The data obtained were compared with the null hypothesis of random mating (random association of alleles from different DNA loci). When the null hypothesis was rejected, a clonal population structure was suggested. The genotype frequencies of each marker were calculated with the GENEPOP software version 1.2 (22).

The SPSS software version 12.0 (SPSS) was used to calculate the Pearson correlation coefficient (*r*) and to measure the strength of a linear association between two variables (phenotypic characteristics and multilocus genotypes). All of the cultures examined were the same age (3 weeks.). The value $r = 1$ means a perfect positive correlation, $r = 0$ means no correlation, and $r = -1$ means a perfect negative correlation.

Similarity coefficient. The Dice index was used to determine the genetic distance between *T. interdigitale* strains, and the Dice similarity measure was used to follow the genetic variability of strains over time.

RESULTS

A total of 150 VNTRs were found, with 80% consisting of repeat units of >10 nucleotides (minisatellites) and 20% containing repeat units ranging from 3 to 8 nucleotides in length. Eight markers that met the selection criteria were retained for further selection studies with the BLASTN program. Four markers were found to match the selection criteria (>12 repeats and a 100% match) and were therefore maintained for further assays: one hexanucleotide, one octanucleotide, one dodecanucleotide, and one pentadecanucleotide.

The reproducibility of each marker, i.e., the ability to assign an identical type to the same isolate, was 100%. These markers appear to be specific to *T. interdigitale*. The primers used to amplify the microsatellite flanking regions (Table 2) were selected on the basis of *in silico* specificity to *T. interdigitale*.

Specificity of VNTR markers. Amplification of the four VNTR markers by using genomic DNA of six dermatophyte strains (*T. rubrum* TRN 2711; *T. violaceum* TVIO 262; *T. mentagrophytes* TM 2119, TM 3051, and TM 3026; and *T. erinacei* TERN 578) and five nondermatophyte strains (*Candida albicans* ATCC 90020 and CBS 2708, *Candida glabrata* ATCC 3153, and *Aspergillus flavus* CBS 12685.7 and JX 852615) suggested that the four new loci were specific for *T. interdigitale*. *In vitro* PCR amplification was not observed with the *T. rubrum*, *T. violaceum*, *T. mentagrophytes*, and *T. erinacei* strains or the nondermatophyte strains.

Genetic diversity. On the basis of an analysis of 92 isolates, four to eight distinct alleles were detected for each VNTR marker (Fig. 1). The highest discriminatory power for a single locus was obtained with the VNTR8 marker, which had eight distinct alleles and a *D* value of 0.802 (Table 2). A four-marker combination (VNTR6, VNTR8, VNTR12, and VNTR15) yielded 29 multilocus genotypes with a *D* value of 0.969 (Table 3).

Cluster analysis. The dendrogram resolved three major clusters with a high level of bootstrap support (values of >50). Cluster A contained 27 strains from Tunisia and 3 QC strains from Libya, cluster B consisted of 42 strains from Tunisia and 1 QC strains from France, and cluster C comprised 18 strains from Tunisia and 1 QC strain from Libya. Group A was a homogeneous group; it

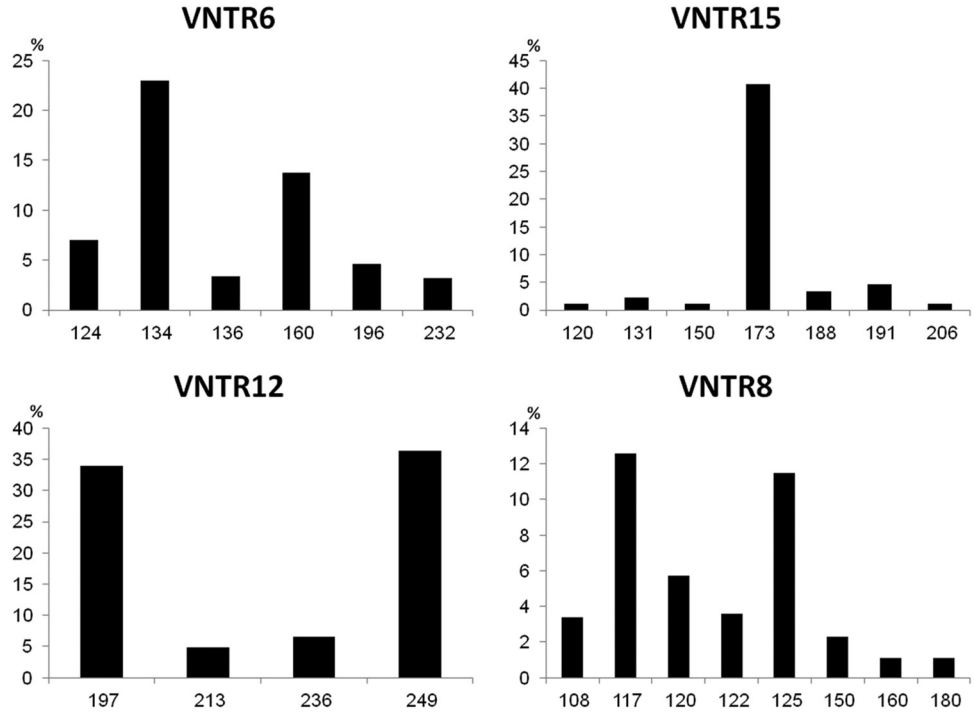

FIG 1 Allele size distribution from the analysis of *T. interdigitale* isolates. *x* axes, allele size; *y* axes, isolate frequency.

contained 27 strains from Tunisia and 3 from Libya, which were all isolated from onychomycosis patients. Cluster B was dominated by strains isolated from tinea pedis (42/43), including one QC strain from France. Cluster C consisted of 15 strains from Tunisia and 1 QC strain from Libya, which were all isolated from tinea corporis patients (16/19). While reference strains of *T. interdigitale* types I and II were grouped in clusters A and B, the strains of *T. interdigitale* types III* and III were grouped in cluster C (Fig. 2).

Phenotypically, the morphological features of colonies isolated from onychomycosis (cluster A) and tinea pedis (cluster B) patients typically had powdery and velvety growth forms, respectively, with a white color on the front and a brownish color on the back. However, colonies isolated from tinea corporis patients (cluster C) mostly had a granular appearance with a beige color on the front and a yellowish-to-brownish back. Microscopic examination showed microconidia in all of the cultures that were predominantly subspherical. Macroconidia were rarely observed, and spiral hyphae were markedly present in the three clusters but with different frequencies, i.e., 22.3% in group A, 16.6% in group B, and 28% in group C.

Pearson's coefficient ($r = 0.4$, P value $= 0.0001$) showed that there was a correlation between phenotypic characteristics and multilocus genotypes. The powdery appearance and white color were associated, respectively, with tinea pedis and onychomycosis populations, whereas the granular appearance and beige color were associated with the tinea corporis population.

Similarity coefficient. The pairwise Dice coefficients of similarity between strains ranged from 0.27 to 1 (Table 4). Cluster A was distantly related to cluster B with a Dice value of 0.64, and cluster C included genetically divergent strains with a Dice value of <0.6 (Fig. 2).

In the eight cases where two strains were recovered from a single patient, identical multilocus genotypes for both isolates were obtained in one case (no. 50) that was isolated from a tinea pedis patient (Table 4). In seven cases, patients were infected in different body areas by multiple genotypes of *T. interdigitale*. Genotypic diversity was detected in the same patients (patients 6, 7, 10, 14, 53, and 63) with a low similarity index (0.27), indicating exposure to a highly heterogeneous genetic population of *T. interdigitale*. Patient 1 had onychomychosis (one strain) and tinea pedis (on the foot [one strain] and between the toes [one strain]), with the three strains showing different genotypes whose Dice similarity index was 0.64.

Interpopulation distance. The findings revealed that cluster A contained strains from the onychomycosis population, cluster B included strains from the tinea pedis population, and cluster C consisted of strains from the tinea corporis population. The genetic diversity per locus (F_{ST} value) was calculated for the three populations and was noted to range between 0.067 and 0.357, with

TABLE 3 Discriminatory indices (*D* values) of different VNTR markers

Locus combination	No. of profiles	*D* value
VNTR15-VNTR6	10	0.703
VNTR12-VNTR15	11	0.753
VNTR12-VNTR6	13	0.768
VNTR15-VNTR8	14	0.842
VNTR12-VNTR8	16	0.869
VNTR6-VNTR8	17	0.871
VNTR12-VNTR6-VNTR8	21	0.892
VNTR12-VNTR15-VNTR6	23	0.832
VNTR12-VNTR15-VNTR8	24	0.901
VNTR6-VNTR8-VNTR15	25	0.934
VNTR6-VNTR8-VNTR12-VNTR15	29	0.969

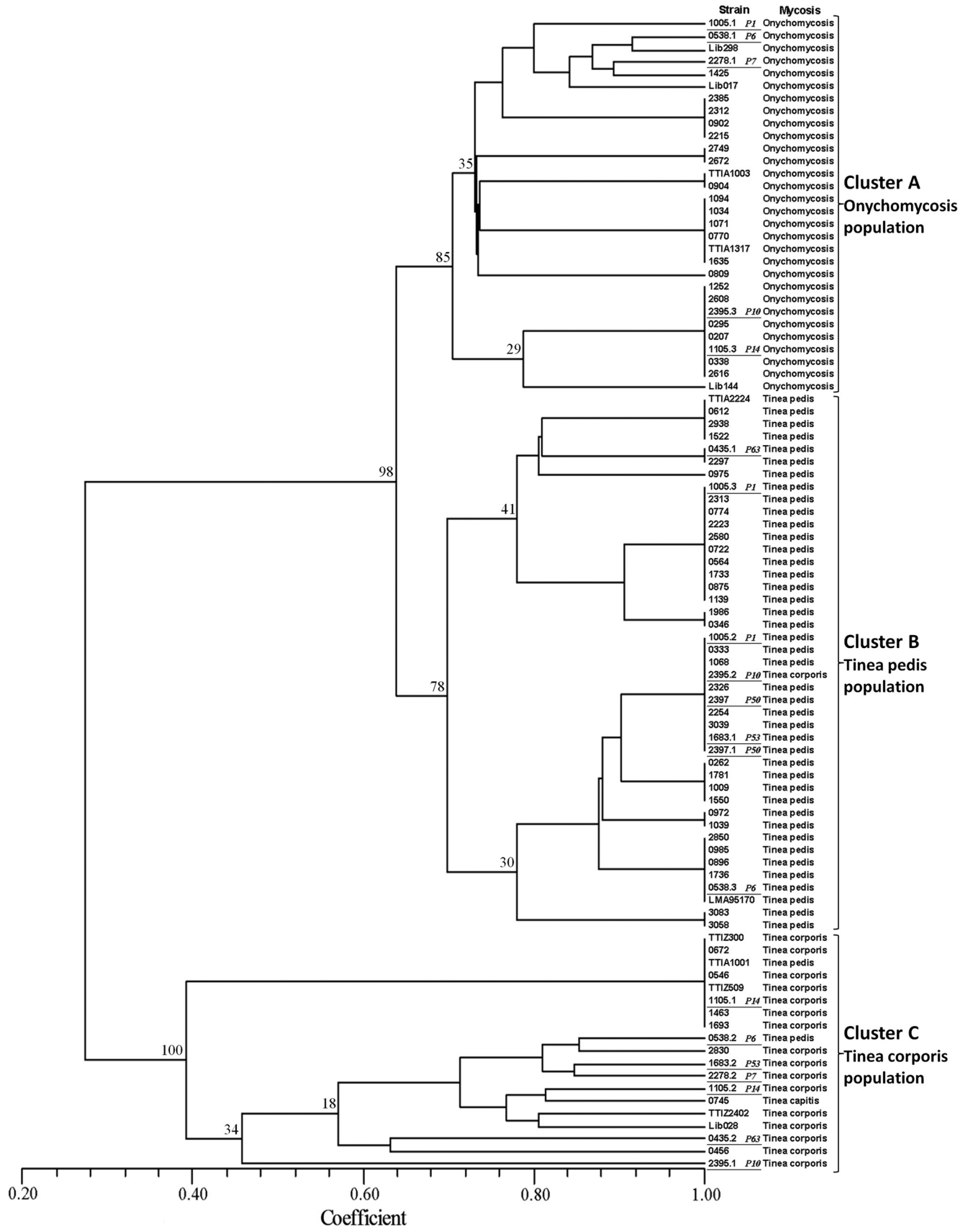

FIG 2 UPGMA dendrogram based on the Dice similarity coefficient from the analysis of four VNTR markers in 92 *T. interdigitale* isolates. The isolates were identified by the culture collection number. Eight isolates were used as reference strains, i.e., 4TTIA, Tunisian *T. interdigitale* anthropophilic ITS types I and II, 3TTIZ, Tunisian *T. interdigitale* zoophilic ITS types III and III* and LMA95170, and an anthropophilic *T. interdigitale* isolate from Marseille, France. In eight cases, multiple strains of *T. interdigitale* were isolated from different body sites (underlined). The robustness of the groups was tested by applying a bootstrap analysis with 1,000 replicates.

TABLE 4 Genotypes and similarity indexes of the four VNTR markers from the eight cases where multiples strains were isolated from different body sites

Patient no. and strain	No. of isolates with: VNTR15	VNTR8	VNTR12	VNTR6	Mycosis	Dice index
1						0.64
1005.1	1	2	6	1	Onychomycosis	
1005.2	1	1	1	1	Tinea pedis	
1005.3	1	1	2	1	Tinea pedis (intertrigo)	
6						
538.1	1	2	1	5	Onychomycosis	0,27
538.2	1	2	2	5	Tinea pedis (intertrigo)	
538.3	1	1	1	1	Tinea pedis	
7						
2278.1	1	2	1	3	Onychomycosis	0.27
2278.2	2	2	7	1	Tinea corporis	
10						
2395.1	2	5	5	4	Tinea corporis	0.27
2395.2	1	1	4	1	Tinea corporis	
2395.3	1	3	1	1	Onychomycosis	
14						
1105.1	1	1	1	1	Tinea corporis	0.27
1105.2	1	2	1	1	Tinea corporis	
1105.3	1	3	1	1	Onychomycosis	
50						
2397.1	1	1	1	1	Tinea pedis	1
2397	1	1	1	1	Tinea pedis (intertrigo)	
53						
1683.1	1	1	1	1	Tinea pedis	0.27
1683.2	2	2	6	1	Tinea corporis	
63						
435.1	1	1	4	1	Tinea pedis	0.27
435.2	2	1	3	1	Tinea corporis	

an average value of 0.21 ($P < 0.001$), indicating a genetic differentiation among the three populations. This fixation index showed that 21% of the genetic variability of *T. interdigitale* strains occurred when there was transmission from one colonized site to another. The Maynard I_A value used to detect the association between alleles at different loci provided evidence that there was a clonal population structure for the *T. interdigitale* isolates ($I_A = 0.47$ and $P < 0.01$).

DISCUSSION

The VNTR markers used in the present study yielded promising results in terms of the identification of intraspecies polymorphisms in *T. interdigitale* strains. In fact, a few studies in the literature have demonstrated the utility of microsatellite markers for the detection of dermatophytes, including *T. rubrum* (23) and *T. violaceum* (24). Gräser et al. (23) identified 55 multilocus genotypes, allowing subdivision of the species *T. rubrum* into two populations.

The four novel VNTR markers presented in this work showed high levels of discrimination and specificity for interstrain differentiation of *T. interdigitale*. In addition to a high degree of discrimination and reproducibility, VNTR analysis has several other advantages over other DNA-based typing assays. Because the assay is PCR based, VNTR analysis requires relatively small amounts of template DNA. The high degree of discrimination ($D = 0.957$) achieved for *T. interdigitale* is in good agreement with previous reports on the typing of other fungi by polymorphic microsatellite marker analysis. For the pathogenic yeast *C. albicans*, the discriminatory power based on polymorphic microsatellite marker analysis was reported to range from 0.87, with only three markers for 60 strains (25), to 0.97, for the typing of 114 strains (26). Likewise, a high level of discrimination ($D = 0.989$) was observed when four polymorphic microsatellite markers were used to type *A. fumigatus* isolates (27). In addition to yielding efficient and rapid results, this method is relatively cost-effective, with the possibility of a further reduced cost by applying multiplex assays with primers labeled with different fluorescent dyes. This tool will not only enhance the therapeutic management of cutaneous infections but also improve current epidemiological knowledge of atypical strains and, more particularly, the sites for which they have a predilection. The VNTR analysis represents a user-friendly and affordable solution for epidemiological studies of superficial mycoses by clinical labs and reference centers.

The PCR melting profile technique was not able to distinguish several genotypes among *T. interdigitale* isolates, which could presumably be attributed to the low frequency of DNA changes among strains originating from the same region (28).

The findings from the VNTR system proposed here showed that the *T. interdigitale* strains analyzed could be divided into three populations. The structuring of *T. interdigitale* populations was in conjunction with their predilection for the human host. Independently of their geographical origins, the *T. interdigitale* species were grouped according to infection sites. This result can be explained by similar climatic conditions at the three sites from which the samples originated. PCR melting profile analysis, however, showed that the zoophilic *T. interdigitale* strains originating from Poland were different from the zoophilic strains originating from Denmark (28). To study the evolution of populations of *T. interdigitale* strains and determine the geographic distribution of these populations, further studies involving larger samples from other countries are required.

The F_{ST} index showed that 21% of the genetic variability of *T. interdigitale* strains occurred when there was transmission from one colonized site into another. The association between alleles at different loci ($I_A = 0.47$) provided evidence that there was a clonal population structure for the *T. interdigitale* isolates. An entirely clonal mode of reproduction was previously considered quite unusual for fungi other than dermatophytes (29). The requirement for sexuality seems to apply primarily to environmental fungi and less to fungi with life cycles that are completed on mammals and with host-to-host transmission (10). In this respect, the present study reported on the presence of genetic diversity among *T. interdigitale* species isolated from different sampling sites on the same patient. Gräser et al. (23) identified distinctive genotypes of *T. rubrum* isolated from the same patient but at different sampling sites. In the present study, 7.2% of the patients (seven patients) were infected with multiple *T. interdigitale* strains that were genet-

ically divergent ($D = 0.27$), indicating the pathogenic power of each strain.

The analysis established a morphological correlation between phenotypic characteristics and genetic variability within the *T. interdigitale* populations. This result is in accordance with the findings of Heidemann et al. (2), who reported that *T. interdigitale* types I and II were normally the etiological agents of tinea pedis and onychomycosis, respectively, with type II being more frequent in both infection types. They also reported that type III was associated predominantly with animal and human strains causing tinea corporis.

The typing scheme, which was applied for epidemiology studies in the present work, also has the potential to address other significant issues. It may be used, for example, to detect markers of virulence and drug resistance in specific genotypes.

In conclusion, the findings of this study reveal that VNTR analysis has high discriminatory power and can be used efficiently in future epidemiological studies of *T. interdigitale*. The identification of the genotypes and population structure of *T. interdigitale* could be a significant marker for further investigations of virulence factors.

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REFERENCES

1. **Cafarchia C, Weigl S, Figueredo L, Otranto D.** 2012. Molecular identification and phylogenesis of dermatophytes isolated from rabbit farms and rabbit farm workers. Vet. Microbiol. **154:**395–402. http://dx.doi.org/10.1016/j.vetmic.2011.07.021.
2. **Heidemann S, Monod M, Gräser Y.** 2010. Signature polymorphisms in the internal transcribed spacer region relevant for the differentiation of zoophilic and anthropophilic strains of *Trichophyton interdigitale* and other species of *T. mentagrophytes* sensu lato. Br. J. Dermatol. **162:**282–295. http://dx.doi.org/10.1111/j.1365-2133.2009.09494.x.
3. **Neji S, Chakroun M, Dammak Y, Trabelsi H, Makni F, Cheikhrouhou F, Sellami H, Marrekchi S, Meziou J, Ayadi A.** 2012. Les mycoses superficielles: profil épidémiologique et mycologique des différents champignons isolés au CHU de Sfax (Tunisie). J. Mycol. Med. **22:**103–104. http://dx.doi.org/10.1016/j.mycmed.2011.12.019.
4. **Neji S, Makni F, Cheikhrouhou F, Sellami A, Sellami H, Marreckchi S, Turki H, Ayadi A.** 2009. Epidemiology of dermatophytoses in Sfax, Tunisia. Mycoses **52:**534–538. http://dx.doi.org/10.1111/j.1439-0507.2008.01651.x.
5. **Beraldo RM, Gasparoto AK, de Siqueira AM, Tranches Dias AL.** 2011. Dermatophytes in household cats and dogs. Rev. Bras. Cienc. Vet. **18:**85–91. http://www.uff.br/rbcv/ojs/index.php/rbcv/article/view/229.
6. **Mochizuki T, Tanabe H, Kawasaki M, Ishizaki H, Jackson CJ.** 2003. Rapid identification of *Trichophyton tonsurans* by PCR-RFLP analysis of ribosomal DNA regions. J. Dermatol. Sci. **32:**25–32. http://dx.doi.org/10.1016/S0923-1811(03)00030-6.
7. **De Hoog GS, Guarro J, Gene J, Figueras MJ.** 2000. Atlas of clinical fungi, 2nd ed. American Society for Microbiology, Washington, DC.
8. **Faggi E, Pini G, Campisi E, Bertellini C, Difonzo E, Mancianti F.** 2001. Application of PCR to distinguish common species of dermatophytes. J. Clin. Microbiol. **39:**3382–3385. http://dx.doi.org/10.1128/JCM.39.9.3382-3385.2001.
9. **Faggi E, Pini G, Campisi E.** 2002. PCR fingerprinting for identification of common species of dermatophytes. J. Clin. Microbiol. **40:**4804–4805. http://dx.doi.org/10.1128/JCM.40.12.4804-4805.2002.
10. **Gräser Y, De Hoog S, Summerbell RC.** 2006. Dermatophytes: recognizing species of clonal fungi. Med. Mycol. **44:**199–209. http://dx.doi.org/10.1080/13693780600606810.
11. **Leibner-Ciszak J, Dobrowolska A, Staczek P.** 2010. Evaluation of a PCR melting profiles of *Trichophyton rubrum* and *Trichophyton interdigitale*. J. Med. Microbiol. **59:**185–192. http://dx.doi.org/10.1099/jmm.0.013458-0.
12. **Fréalle E, Rodrigue M, Gantois N, Aliouat CM, Delaporte E, Camus D, Dei-Cas E, Kauffmann-Lacroix C, Guillot J, Delhaes L.** 2007. Phylogenetic analysis of *Trichophyton mentagrophytes* human and animal isolates based on MnSOD and ITS sequence comparison. Microbiology **153:**3466–3477. http://dx.doi.org/10.1099/mic.0.2006/004929-0.
13. **Hryncewicz-Gwóźdź A, Jagielski T, Kalinowska K, Baczynska D, Plomer-Niezgoda E, Bielecki J.** 2012. Stability of tandemly repetitive subelement PCR patterns in *Trichophyton rubrum* over serial passaging and with respect to drug pressure. Mycopathologia **174:**383–388. http://dx.doi.org/10.1007/s11046-012-9565-4.
14. **Fisher MC, Aanensen D, de Hoog GS, Vanittanakom N.** 2004. Multilocus microsatellite typing system for *Penicillium marneffei* reveals spatially structured populations. J. Clin. Microbiol. **42:**5065–5069. http://dx.doi.org/10.1128/JCM.42.11.5065-5069.2004.
15. **Li HC, Bouchara JP, Hsu MM, Barton R, Su S, Chang TC.** 2008. Identification of dermatophytes by sequence analysis of the rRNA gene internal transcribed spacer regions. J. Med. Microbiol. **57:**592–600. http://dx.doi.org/10.1099/jmm.0.47607-0.
16. **Hunter PR, Gaston MA.** 1988. Numerical index of the discriminatory ability of typing systems: an application of Simpson's index of diversity. J. Clin. Microbiol. **26:**2465–2466.
17. **Rohlf FJ.** 2000. NTSYSpc: numerical taxonomy and multivariate analysis system, version 2.11. Exeter Software, Setauket, NY.
18. **Excoffier L, Laval G, Schneider S.** 2005. Arlequin (version 3.0): an integrated software package for population genetics data analysis. Evol. Bioinform. Online **1:**47–50. http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2658868/.
19. **Sneath PHA, Sokal RR.** 1973. Numerical taxonomy: the principles and practice of numerical classification. Freeman & Co., San Francisco, CA.
20. **Pavlicek A, Hrdá S, Flegr J.** 1999. Free-Tree—freeware program for construction of phylogenetic trees on the basis of distance data and bootstrap/jackknife analysis of the tree robustness. Application in the RAPD analysis of genus *Frenkelia*. Folia Biol. (Praha) **45:**97–99.
21. **Agapow PM, Brut A.** 2001. Indices of multilocus linkage disequilibrium. Mol. Ecol. Notes **1:**101–102. http://dx.doi.org/10.1046/j.1471-8278.2000.00014.x.
22. **Roze D, Rousset F.** 2008. Multilocus models in the infinite island model of population structure. Theor. Popul. Biol. **73:**529–542. http://dx.doi.org/10.1016/j.tpb.2008.03.002.
23. **Gräser Y, Frohlich J, Presber W, de Hoog S.** 2007. Microsatellite markers reveal geographic population differentiation in *Trichophyton rubrum*. J. Med. Microbiol. **56:**1058–1065. http://dx.doi.org/10.1099/jmm.0.47138-0.
24. **Ohst T, de Hoog GS, Presber W, Stavrakieva V, Gräser Y.** 2004. Origins of microsatellite diversity in the *Trichophyton rubrum-T. violaceum* clade (Dermatophytes). J. Clin. Microbiol. **42:**4444–4448. http://dx.doi.org/10.1128/JCM.42.10.4444-4448.2004.
25. **Dalle F, Franco N, Lopez J, Vagner O, Caillot D, Chavanet P, Cuisenier B, Aho S, Lizard S, Bonnin A.** 2000. Comparative genotyping of *Candida albicans* bloodstream and nonbloodstream isolates at a polymorphic microsatellite locus. J. Clin. Microbiol. **38:**4554–4559.
26. **Sampaio P, Gusmao L, Alves C, Pina-Vaz C, Amorim A, Pais C.** 2003. Highly polymorphic microsatellite for identification of *Candida albicans* strains. J. Clin. Microbiol. **41:**552–557. http://dx.doi.org/10.1128/JCM.41.2.552-557.2003.
27. **Bart-Delabesse E, Humbert JF, Delabesse E, Bretagne S.** 1998. Microsatellite markers for typing *Aspergillus fumigatus* isolates. J. Clin. Microbiol. **36:**2413–2418.
28. **Leibner-Ciszak J, Dobrowolska A, Krawczyk B, Kaszuba A, Staczek P.** 2010. Evaluation of a PCR melting profile method for intraspecies differentiation of *Trichophyton rubrum* and *Trichophyton interdigitale*. J. Med. Microbiol. **59:**185–192. http://dx.doi.org/10.1099/jmm.0.013458-0.
29. **Taylor J, Jacobson D, Fisher M.** 1999. The evolution of asexual fungi: reproduction, speciation and classification. Annu. Rev. Phytopathol. **37:**197–246. http://dx.doi.org/10.1146/annurev.phyto.37.1.197.