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Molecular Characterisation and Phylogenetic Analysis of Dermatophytic Fungi Isolated from Tinea Capitis in Northwest Nigeria Using Sequence of the *28S rRNA*

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Abstract: The detection and identification of fungal DNA from clinical samples is one of the fundamental approaches in biomedicine. The incidence, distribution, and control of dermatophytes has progress significantly and the use of phylogenetic species concepts based on rRNA regions have enhanced the taxonomy of dermatophyte species; however, the use of *28S rDNA* genes has certain limitations. This gene has been used in dermatophyte taxonomy with limited enumeration; we appraised the sequence disparity within and among groups of the species, the gene ranking in identification, phylogenetic analysis, and taxonomy of 32 strains of eight dermatophyte species. In this study, a set of primers was adopted to amplify the target followed by a partial sequencing of the *rDNA*. The utilization of a pairwise nucleotide differentiation, an affinity was observed among eight dermatophyte species, with disparity among species ranging from 0 to 197 base pair (bp). Intra-species bp differences were found within strains of *Trichophyton eriotrephon*, *Trichophyton bullosum*, *Trichophyton simii* (Trichophyton genus), *Microsporum audouinii,* and *Trichophyton tonsurans* (Microsporum and Trichophyton genus, respectively); however, only some strains of *Trichophyton eriotrephon* were found to be invariant having three genotypes. *Trichophyton tonsurans* exhibited most intra-species variability. The characterization and construction of a phylogenetic tree of *28S rDNA* gene on dermatophyte species provide a bedrock of an additional finding of connections between species. However, *28S rRNA* capture provides a novel method of effective and sensitive detection of dermatophytes lodged in human skin scale. We report for the first time the emergence of *T. eriotrephon*, *T. bullosum*, *T. simii*, *T. benhamiae,* and *Ctenomyces serratus* dermatophytes from *Tinea capitis* in Nigeria.

Keywords: *Tinea capitis*; *28S rDNA* gene; phylogenetics; Sokoto; Trichophyton; *Ctenomyces*

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Lay Abstract

The incidence, distribution, and control of dermatophytes has progress significantly and a superior knowledge of the phylogenetic understanding of dermatophytes may provide a master plan in preventing *Tinea capitis* transmission and infection, thus treatment. Few studies have reported on the molecular characterization of dermatophytes in Nigeria and not much attempt has been set down into the phylogenetic analysis, statistics, and subsequent submission of these sequence into gene repositories (https://www.ncbi.nlm.nih.gov/nuccore?term=MT893932+%3A+MT893963%5Baccn%5D&cmd=DetailsSearch&log$=activity, accessed on 27 August 2020). To the best of our understanding, this study is the first of its kind in Sokoto, successfully depositing nucleotide sequences of dermatophyte

strains into the GenBank database. This was done to further populate the Genebank database, particularly using *28S rRNA*, whose information is limited.

1. Introduction

Dermatophytes are a group of fungi with a genera consisting of *Trichophyton*, *Microsporum*, and *Epidermophyton* that causes dermatophytosis by influencing keratinised tissues (skin, scalp, hair, and nails) of human and animal hosts [1]. The species of dermatophytes, which tend to cause scalp ringworm, may differ from country-to-country and from region-to-region [2].

Tinea capitis is a dermatophytosis of the hair and scalp skin, correlated with clinical symptoms and signs of inflammation and hair loss, which includes thickened, scaly, and boggy swellings, or as raised red rings (ringworm). Others are severe itching of the scalp, dandruff, and bald patches, where the fungus has rooted itself in the skin [3]. Sources of ringworm include anthropophilic, zoophilic, and geophilic, and is highly contagious amongst children, especially school pupils, with rare reports in adults [4]. The epidemiology and prevalence of this infection varies among regions, populations, lifestyles, migration, drug therapies, and socioeconomic conditions [5]. *Microsporum audouinii* and *Microsporum canis* have been reported to be the main causative agents in western and Mediterranean Europe while *Trichophyton* species (*T. schoenleinii*) is the most prevalent agent in Eastern Europe and Africa [6].

Characterisation, identification, and classification of dermatophytes were conducted conventionally by the use of clinical and gross examination of colonies from culture, microscopically (macro- and micro-conidia). The use of biochemical testing is used as confirmation. However, this method of characterisation is time-consuming and needs experts to interpret results of the morphology [7]. The use of recent molecular methods of characterisation of the polymerase chain reaction (PCR) technique has provided a faster, accurate, and reliable means of identification, especially in infections directly from nail fragments, since it is possibly the most complex structure of the skin [8]. DNA fragments have been noticed as the main dermatophyte genetic markers (ribosomal DNA) regions [9]. The *28S rDNA* regions are a good balance for detecting differences between conservancy and variability in organisms and are, hence, potentially useful markers to study the relationships of populations and closely related species in microorganisms. However, they are scarce in databases. The goal of the study is to (i) recognise the prevalence and phylogenetic affiliation among dermatophyte strains of *Tinea capitis* from primary school pupils in Sokoto State-owned primary schools, with a view to ascertain the genetic diversity, conservancy, and variability of the strains based on *28S rRNA* gene sequencing. (ii) Phylogenetic relationships of *28S rRNA* gene analysis in the segregation of anthropophilic from zoophilic dermatophytes.

2. Materials and Methods

2.1. Study Centre

The present research work was carried out on strains from Sokoto State, located in the extreme northwest of Nigeria, between longitudes 4°8′ and 6°54′ and latitude 12° N and 13°58′ N. The state has a population of nearly 5.4 million people, covers a terrestrial area of 32,000 km, and shares a border with the Republic of Niger. The major ethnic groups in the state are the Hausa and Fulani groups. Over 80 percent of the people in the state practice agriculture (husbandry and crops) as their major source of income [10].

2.2. Dermatophyte Clinical Strains

In this study, an ethical permission was obtained from the Sokoto State Ministry of Health Ethical Committee (SKHREC/088/017) and informed consent was obtained from all participants involved in the study. Clinical strains obtained from the heads of 125 participants (boys and girls between the ages of 4 and 10) presented with scaling and/or hair loss was suggestive of Tinea capitis and typically had two or more of pruritus, hair

loss, scaling, erythema, and posterior cervical adenopathy from the Sokoto state-owned primary school. Study participants were excluded if a kerion was present. Affected areas were cleansed with 70% *v*/*v* ethanol, allowed to dry, and light scrapings from the edge of the lesions were taken using a blunt sterile scalpel blade. The specimens were placed in clean white envelopes with each participant code labelled. Clinical strains were selected for sequencing after being previously characterised using standard microbiological procedures (10% KOH, cycloheximide and chloramphenicol, urea hydrolysis, 1% peptone agar, and the 40 strains of dermatophytes were cultured onto the Sabouraud dextrose agar (SDA) at 32 °C for up to 2 weeks), DNA extraction, and PCR amplification.

2.3. Dermatophyte DNA Extraction and PCR Amplification

Genomic DNA was harvested using Qiagen (Hilden, Germany) DNA extraction kit in adherence to the manufacturer's protocols; Aliquot volume containing culture micelles from colonies cultured from SDA were sampled (1–3 $\times$ 10^5) and transferred to a 1.5 mL e-tube, and centrifuged at 10,000 rpm for 7 min. The supernatant was poured off and the cells were re-suspended with Hank's balanced salt solution in a total volume of 200 µL. The samples were freeze on pellets in a −80 °C freezer for 60 min. Qiagen Proteinase K of 20 µL was pipetted into the bottom of a 1.5 mL microcentrifuge tube and 200 µL of both cell suspension and Buffer AL were added. This was vortex for 15 s and incubated in a 56 °C water bath for 10 min. The content were centrifuged to remove droplets formed at the top. Ethanol (100%) of 200 µL was added to the content of the mixture and mix by vortexing for 15 s and briefly centrifuged, which was later added the to a QIAamp spin column and again centrifuged at 13,200 rpm in the Eppendorf 5415R microcentrifuge for 1 min at room temperature. Buffer AW1 (500 µL) was added to the Eppendorf 5415R microcentrifuge containing residual contaminants and centrifuged at 10,000 rpm for 1 min at room temperature. The spin column was removed and place in another clean, labelled collection tube, of which 500 µL of Buffer AW2 was added and centrifuged at 13,200 rpm in the Eppendorf 5415R microcentrifuge, for 3 min at room temperature. The concentration of the extracted DNA was measured with the NanoDrop spectrophotometer ND-1000 and 50 ng was taken for use in PCR. For each strain, DNA fragment (about 298 bp) of the *28S rRNA* gene were amplified using 28S rRNA primer of forward 5′-ACAGGGATTGCCCCAGTA-3′,reverse 5′-CTTGTTCGCTATCGGTCTC-3′, according to methods previously described by Kim et al. [11].

A total of 25 µL volume contained 12.5 µL of Qiagen Top Taq master mix, 1 µL of each primer, 5.5 µL of nuclease free water, and 5 µL of DNA template. The reaction mix was centrifuged briefly and transferred to the thermocycler at 3 min of hot-start at 94 °C, 30 s of denaturation at 94 °C, 30 s of annealing at 50 °C, and 1 min of extension at 72 °C. The entire process was repeated for 35 cycles, with the final extension at 72 °C for 10 min.

2.4. Sequencing

The amplicons were re-amplified and purified using Qiagen DNA kits according to the manufacturer's recommendations. The purified PCR products were packaged and sent for Sanger sequencing at Inqaba Biotec South Africa.

2.5. Nucleotide Blast

The *28S rRNA* sequence data obtained were entered into the Basic Local Alignment Search Tool (BLASTN) of the National Centre for Biotechnology Information (NCBI) database and compared with information provided by Centraalbureau voor Schimmelcultures (CBS).

2.6. Sequence Analysis

The sequence (forward and reverse) chromatograms of each sample were amended to improve the alignment precision using MEGA 7 software; BioEdit software version 7.0.5 was used for pairwise contrast and multiple alignment to determine similarities and differ-

ences among the nucleotides. Pairwise affinity values were calculated and phylogenetic trees were constructed using the neighbour-joining (NJ) method with the Tamura–Nei parameter as a substitution model, as implemented in MEGA 7. The reliability of internal branches was assessed using the bootstrap method with 500 replicates. The consensus nucleotide sequence data determined in this study were deposited in the GenBank under the accession numbers MT893932–MT893963 (Table 1).

Table 1. Clinical strains and accession number of dermatophytes used in this study.

Accession Number	Organism	Isolate ID (Ugk)
MT893932–MT893941	*Trichophyton eriotrephon*	1, 7, 10, 14, 16, 19, 20, 22, 31, 40
MT8939342–MT893943	*Trichophyton bullosum*	2, 23
MT893944–MT893955	*Trichophyton simii*	3, 4, 5, 6, 8, 9, 15,2 4, 26, 29, 34, 37
MT893956	*Trichophyton benhamiae*	11
MT893957	*Trichophyton rubrum*	13
MT893958–MT893960	*Trichophyton tonsurans*	33, 35, 39
MT893961–MT893962	*Microsporum audouinii*	30, 32
MT893963	*Ctenomyces serratus*	38

3. Results

The use of *28S rRNA* gene sequences for differentiation and phylogenetic studies of the dermatophytes species was achieved using a part of the gene that was amplified for 32 strains, with sizes of the region ranging from 239 to 347 base pair (bp). Partial sequences of approximately 809–1350 bp, corresponding to the smaller sub-unit of gene *28S rRNA*, were obtained from the GenBank and aligned with the study sequences. The smallest size was found in *Trichophyton simii*, comprising of 239 bp, and the longest in *Trichophyton tonsurans* with 347 bp. Most of the *Trichophyton species* had identical sizes, between 247 and 248 bp.

Multiple sequence alignment showed a fundamental heterogeneity within species of dermatophytes. Figure 1 shows the multiple sequence alignment of *28S rRNA* gene in these dermatophytes with an evolutionarily conserved nucleotide region of 239–245, which could be useful in designing a primer used in dermatophyte characterisation, while genetic variance is seen to be limited to fragments of 1–111, 116–159, 166–184, 191–193, 199–204, 209–215, and 230–239. Conserved regions found within intra-species of *T. eriotrephon* (221–234 bp), *T. bullosum* (114–137 bp), *T. simii* (61–81, 121–155 and 194–212 bp), *T. tonsurans* (17–26 and 152–161 bp), and *M. audouinii* (60–80, 90–157, 172–190, and 193–249 bp) as seen in Table 2. Pairwise nucleotide alignment of *28S rRNA* gene sequences in tested dermatophytes showed a mean distance of 0.17 ± 0.03 between the species; this is shown in Table 3. Interspecies divergence ranged from 0 bp between some strains of *T. eriotrephon (Ugk 16)* and *T. bullosum (Ugk 2),* to 197 bp between *T. tonsurans* and *M. audouinii* (197 bp), which conform to the largest distance that was observed between *T. tonsurans* (Ugk 39) and *M. audouinii* (Ugk 32). The nucleotide sequences of *T. eriotrephon* and *T. bullosum* were identical (Ugk 2, 10, 16, and 22). Meanwhile, the intra-species differences were found within strains of *T. eriotrephon*, *T. bullosum*, *T. simii*, *M. audouinii,* and *T. tonsurans* by 0–81 (Ugk 10/22 at 0 bp, Ugk 1/14 at 81 bp), 41 bp (Ugk 2/23), 2–42 bp (Ugk 6/3, Ugk3/8, Ugk 4/6 at 2 bp and Ugk 34/29 at 42 bp), 30 bp (Ugk 30/32 at 30 bp), and 13–192 bp (Ugk 33/35 at 13 bp, Ugk 35/39 at 192 bp), respectively (Table 3); however, only strains of *T. eriotrephon* (Ugk 10, 16 and 22) were found to be invariant, having three *28S rRNA* genotypes. *T. tonsurans* exhibited most intra-species variability.

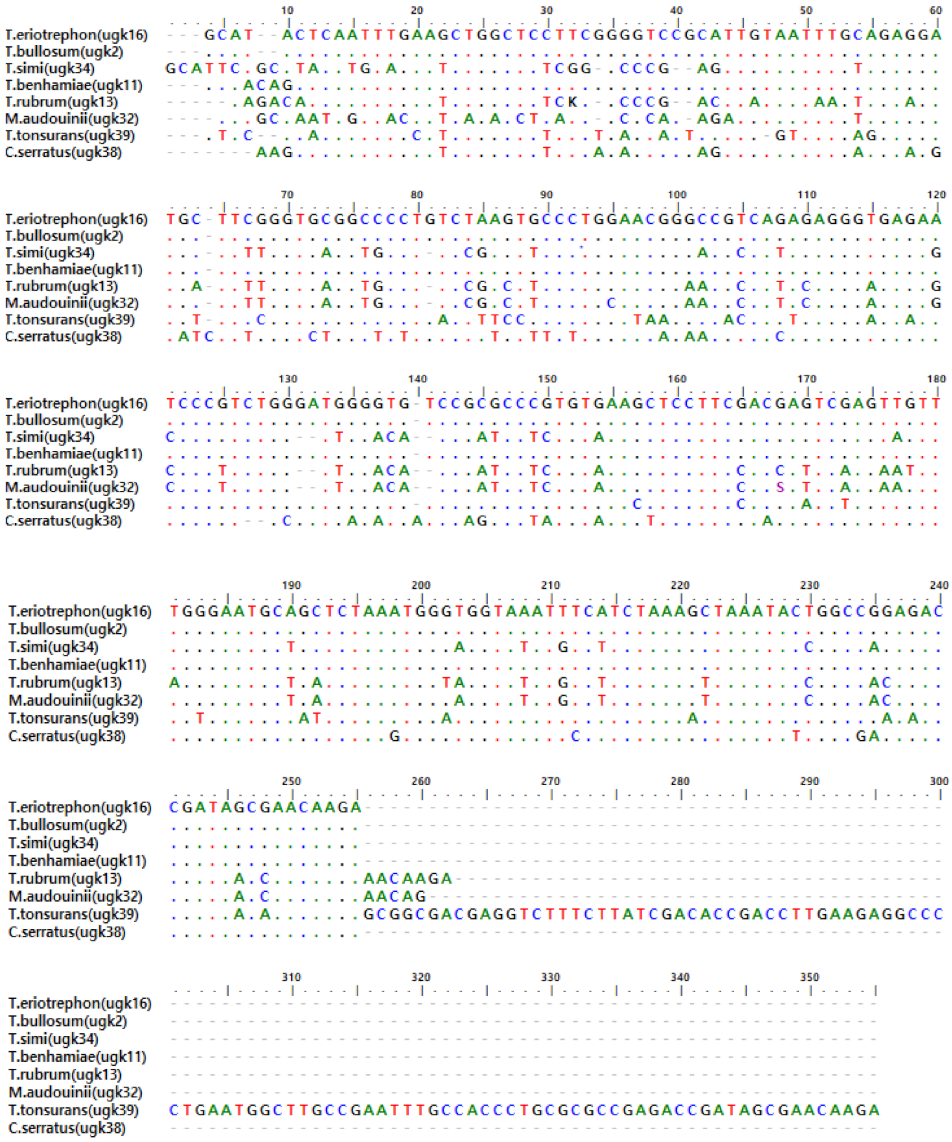

Figure 1. Multiple sequence alignment of eight (8) nucleotide sequences of the *28S rRNA* gene of dermatophytes isolates from Sokoto. The coloured bases indicate regions of nucleotides diversity among isolates. The identical residues are represented with dots. The sequence layout is set to wrap at every 60 residues.

Table 2. Clinical isolated species of dermatophytes used in this study for partial sequence analysis of the *28S rRNA* gene, fragment size, and the range of intra-species variations and conserved regions within the species, are shown.

Species (Tested Strain Number)	LS (bp)	Range of Intra-Species Variations	Intra-Species Conserved Region
T. eriotrephon (10)	247–253	0–81	221–234
T. bullosum (2)	248–249	41	114–137
T. simii (12)	239–251	2–42	61–81, 121–155, 194–212
T. benhamiae (1)	250	-	-
T. rubrum (1)	248	-	-
T. tonsurans (3)	247–347	13–192	17–26,152–161
M. audouinii (2)	248–251	30	60–80, 90–157,172–190,193–249
C. serratus (1)	246	-	-

LS: Sequence length or fragment size; -: Not applicable.

Table 3. Sequence differences based on pairwise sequence comparison of 28S rDNA gene between dermatophytes.

	Seq->	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32
1	ugk1	ID																															
2	ugk2	20	ID																														
3	ugk3	66	56	ID																													
4	ugk4	66	56	2	ID																												
5	ugk5	71	59	10	10	ID																											
6	ugk6	66	57	2	2	9	ID																										
7	ugk7	72	60	89	90	93	89	ID																									
8	ugk8	66	57	2	4	9	2	88	ID																								
9	ugk9	66	58	6	6	12	8	94	8	ID																							
10	ugk10	20	1	57	57	58	56	59	56	59	ID																						
11	ugk11	22	7	58	58	60	57	62	58	57	7	ID																					
12	ugk13	94	85	35	37	43	35	91	35	41	84	88	ID																				
13	ugk14	81	68	91	91	97	90	38	91	96	67	73	100	ID																			
14	ugk15	68	59	6	5	6	4	91	5	10	58	59	39	93	ID																		
15	ugk16	20	0	56	56	59	57	60	57	58	1	7	85	68	59	ID																	
16	ugk19	23	4	58	58	59	57	62	57	60	3	10	85	69	59	4	ID																
17	ugk20	21	6	58	58	60	58	60	58	60	6	7	88	71	60	6	9	ID															
18	ugk22	20	1	57	57	58	56	59	56	59	0	7	84	67	58	1	3	6	ID														
19	ugk23	56	41	75	74	76	75	30	76	78	42	42	91	44	76	41	45	42	42	ID													
20	ugk24	83	74	21	23	29	21	88	21	27	73	75	17	98	25	74	74	75	73	84	ID												
21	ugk26	81	71	17	17	26	19	92	19	21	72	74	25	98	22	71	73	73	72	82	15	ID											
22	ugk29	90	81	34	33	38	32	91	34	39	80	84	12	95	33	81	81	83	80	87	19	24	ID										
23	ugk30	94	86	38	40	44	39	99	39	36	86	83	15	109	42	86	87	87	86	93	20	28	21	ID									
24	ugk31	24	10	57	56	63	58	56	59	55	11	14	81	64	61	10	14	13	11	38	71	67	77	80	ID								
25	ugk32	88	80	42	42	44	41	102	42	44	79	84	34	107	43	80	80	83	79	91	33	37	29	35	79	ID							
26	ugk33	92	83	33	33	39	31	93	33	38	82	82	13	99	34	83	83	84	82	86	16	23	9	19	79	33	ID						
27	ugk34	66	62	12	11	14	12	99	13	7	62	61	46	99	13	62	63	64	62	83	32	27	42	39	61	44	42	ID					
28	ugk35	92	82	34	34	43	36	94	36	38	83	87	12	101	39	82	84	86	83	88	19	22	13	16	77	32	13	44	ID				
29	ugk37	95	86	40	42	46	41	99	41	38	86	84	19	108	44	86	87	88	86	92	24	28	23	10	80	37	21	42	16	ID			
30	ugk38	62	52	70	69	71	68	88	69	74	51	54	96	89	68	52	53	54	51	73	87	85	93	100	54	98	94	78	95	100	ID		
31	ugk39	164	152	187	186	188	185	145	186	191	151	153	190	158	186	152	154	153	151	136	183	186	189	196	149	197	187	195	192	196	176	ID	
32	ugk40	22	5	56	56	60	56	60	57	57	5	9	85	68	58	5	8	9	5	41	73	71	81	84	11	80	80	60	83	84	52	150	ID

The cladistics, which show the classifications of organisms based on evolutionary relatedness for 32 sequences, representing species, are presented in Figure 2. The analysis of these sequences gave species primary habitat, as shown in Figure 2. Closely related species in different groups formed have formed a well-supported clades in the *28S rRNA* gene tree, as shown in Figure 2. For example, *T. eriotrephon* (Ugk 22) and *T. bullosum* (Ugk 2) at 90% bootstrap value, Ugk 23 and Ugk 7 at 90% bootstrap value.

The phylogenetic tree of *28S rDNA* sequences revealed a cluster consisting of anthropophilic and zoophilic. *Trichophyton species* of *T. eriotrephon*, *Trichophyton benhamiae,* and *T. bullosum* were found in a cluster, which were all zoophilic, a cluster consisting of *T. simii*, *T. rubrum*, *M. audouinii*, and *T. tonsurans*, which were both anthropophilic and Zoophilic, thus indicating that all of the anthropophilic isolates in this cluster were of zoophilic origin.

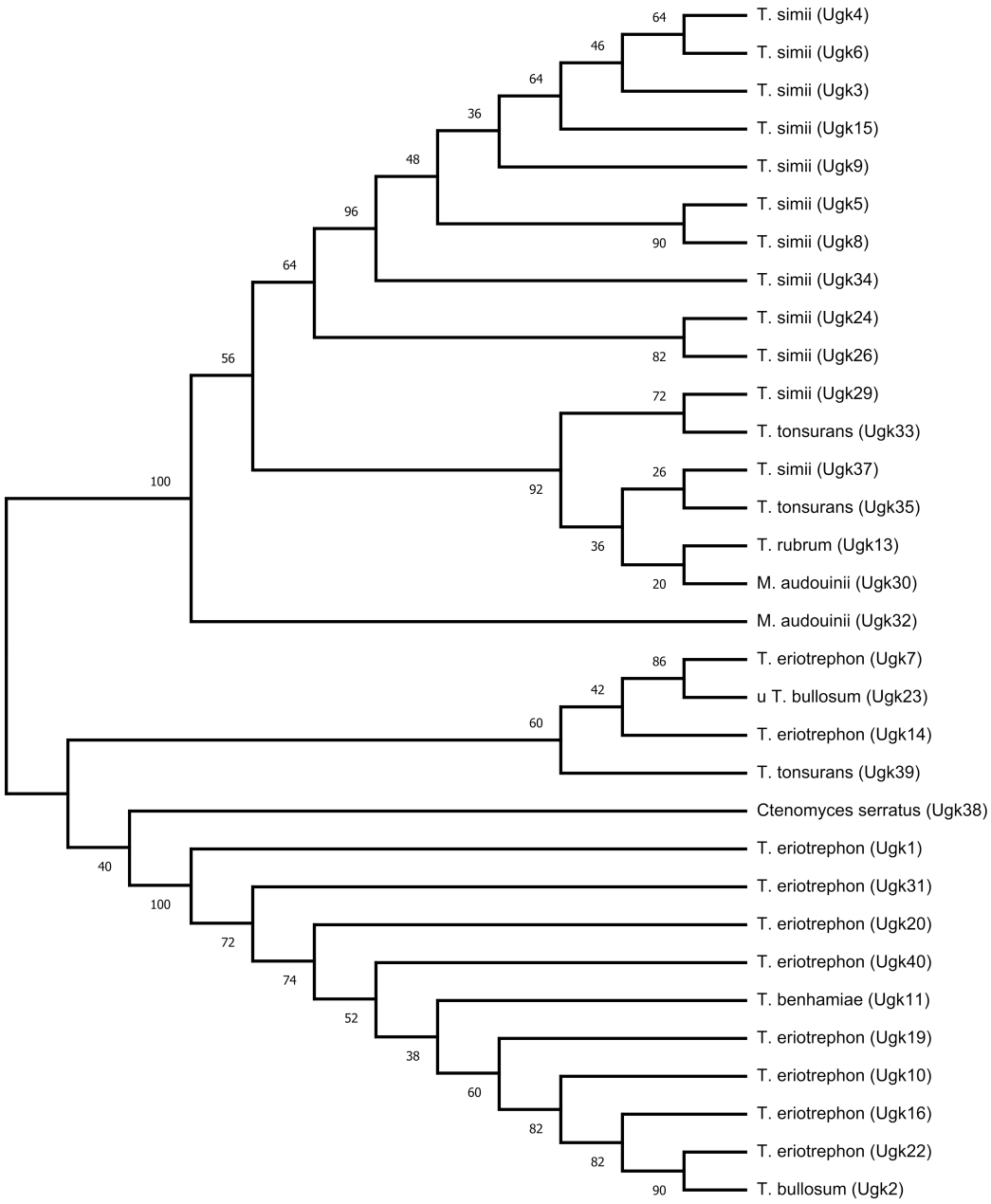

Figure 2. Phylogenetic tree of 32 representative dermatophyte species based on analysis of *28S rRNA* gene sequences. The evolutionary history was inferred using the neighbour-joining (NJ) method.

4. Discussion

The incidence of dermatophytes isolated from *Tinea capitis* in this study has found that dermatophyte infection remains a major problem in Africa, especially Nigeria. It is commonly found among families in certain localities, especially primary school pupils, where shelter and hygiene are unhealthy and, as well, northern Nigeria, where animal husbandry is prominent in most homes [12]. It affects children from less than four to ten years of age. The incidence in males, 22 (75%), is three times higher than the incidence in females, 10 (25%) as seen in Table 4. This might be attributed to the fact that boys' health practices include participating in animal rearing shares of caps, combs, and unsterile blades and clippers during barbing. These results are in agreement with studies by Dogo et al. [4]. Hay and Ashbee [13] also mentioned that erroneous health practices of boys, including the use of other combs and caps, and fewer hair washings than girls, have been associated with dermatophyte infections. Moreover, females have less exposure to sporting facilities

and institutions [1]. These erroneous health practices are why incidence rate in males was almost thrice the incidence rate in females.

Table 4. Age and sex distribution of forty patients with dermatophytes infection.

	Age (Years)			
Sex	**<5**	**>5 (6–10)**	**Total**	**Percentage (%)**
Males	8	22	30	75
Females	4	6	10	25
Total	12	28	40	100
Percentage (%)	30	70	100	

It is a fact that certain species of dermatophyte species are known to affect certain body areas. For example, *T. rubrum* is dominantly found in onychomycoses, whereas *M. canis* is prevalent in *Tinea capitis* and *Tinea corporis*. However, in contrast, some species of dermatophytes are never (or are rarely) isolated from a particular dermatophyte infection. This study reveals that *T. simii* and *T. eriotrephon* were most prevalent in *T. capitis* where both recorded 37.5% and 31.25% prevalence, respectively. Sen and Rasul [14] reported *T. simii* (10%) as one of the prevalent dermatophytes in *Tinea capitis*. The predominance of *Trichophyton* species as the causative agent of *Tinea capitis* (ringworm of the head) is not unexpected. Most studies found *T.* spp. as the most common etiological agent of *Tinea capitis* [5]. *Trichophyton* spp. accounted for 90% of *Tinea capitis* in this study. This is higher as compared to a study by Dogo et al. [4], who accounted for 37.8%, and in agreement with Ahmed et al. [15], who reported 90% of *T.* spp. Other *T.* spp. and *M.* spp. reported in this study were T. *bullosum*, *T. benhamiae*, *T. rubrum*, *T. tonsurans,* and *M. audouinii,* accounting for 6%, 3%, 3%, 9%, and 6%, respectively. Ansari et al. [16] reported 5.4% for *T. benhamiae* in *T. capitis.* A study in Belgium by Sacheli et al. [8] reported a prevalence of *T. benhamiae* (2.1%), which is less than the result obtained. It is worthy to note that *Ctenomyces serratus* was among the fungi isolated in *Tinea capitis* infection from this study and no studies have reported the isolation of these fungi from *Tinea capitis,* to the best of our knowledge. Characterisation of the dermatophyte species causing fungal infection is identified using traditional conventions in Nigeria laboratories. Rapid and accurate identification of dermatophytes, especially using PCR, will provide a platform in prescribing appropriate treatment to the infection. The sequences can be with rDNA sequences from the NCBI/European Molecular Biology Laboratory (EMBL) GenBank database.

5. Sequences Analysis

The use of *28S rRNA* region as a target for both phylogenetic analysis and molecular species identification of dermatophytes has provided a better understanding of the taxonomy and evolution of the species. However there are areas of conflict in regards to this genetic marker, because of low nucleotide differences amongst closely related species, such as *T. bullosum (Ugk 2)/T. eriotrephon (Ugk 10*, *16,* and *22)* can be difficult; the discrimination of some closely related species were also reported in a study by Ahamdi et al., [17]. This shows that the target of *28S rRNA* requires investigation of additional molecular markers for the further identification of these closely related dermatophytes. Phylogenetic relationships obtained from *28S rRNA* gene analysis resulted in the identification of *Trichophyton* and *Microsporum* species and their segregation from zoophilic (*Trichophyton eriotrephon*, *Trichophyton bullosum*, *Trichophyton benhamiae,* and *Trichophyton simii)* and anthropophilic (*Trichophyton rubrum*, *Trichophyton tonsurans,* and *Microsporum audouinii)* species. The length of *28S rRNA* sequences (239–347 bp) across these different dermatophyte strains had variations, such that these genes were usually conserved between them.

The differences in sequence length between the different dermatophytes are mainly due to length variation in the non-coding regions of an RNA transcript, or the DNA encoding it, which are eliminated by splicing before translation. The phylogenetic sequence analysis, as shown in Figure 2, shows a cluster consisting of both primary habitat (anthro-

pophilic and zoophilic) of *Trichophyton* species, *T. eriotrephon* (Ugk 7, 14)/*T. tonsurans* (Ugk 39)/*T. bullosum* (Ugk 2), supported by a bootstrap value of 99%, and a cluster of zoophilic *Trichophyton* species of *T. eriotrephon* (Ugk10, 16, 19, 20, 22, 31, and 40)/*T. benhamiae* (Ugk 11)/*T. bullosum* (Ugk 2). There is also a cluster of primary habitat consisting of anthropophilic and zoophilic of both *Trichophyton* and *Microsporum* species *M. audouinii* (Ugk 32 and 30)/*T. tonsurans* (Ugk 33 and 35)/*T. rubrum* (Ugk13)/*T. simii* (Ugk 3, 45, 8, 9, 15, 24, 26, 29, 34, and 37) supported by a bootstrap value of 99%. This observation has shown a potential probability that the taxon has an animal-associated ancestry as their primary habitat. The lengths of *28S rRNA* sequences of the different strains in a cluster consisting of Ugk 1, 31, 20, 11, 40, 19, 10, 16, 22, and 2 ranged from 247 to 250 bp (Table 3); thus, indicating that these species are very closely related. The biodiversity of differentiated species *T. eriotrephon (Ugk 16)* and *T. bullosum (Ugk 2)* showed 90% (0 bp difference) similarity. Our data suggest that *28S rRNA* is not useful for species differentiation of *T. eriotrephon (Ugk 22)* and *T. bullosum (Ugk 2*), which are on the same internode (bootstrap value 90%) and, thus, needed an additional marker for accurate identification.

The *28S rRNA* gene sequence analysis showed that most of the anthropophilic strains were from animal origins. As reported by some researchers, hedgehogs, chicken, horses, and guinea pigs are carriers of these dermatophyte strains. Northern Nigeria is into rearing of these animals and, as such, possible transmission of these strains to humans. The close relationship between the strains of all these species (Zoophilic and anthropophilic) in the phylogenetic tree is also supported by *28S rRNA* gene sequence data.

6. Conclusions

Most species causing fungal infections, especially *Tinea capitis* in Nigeria, are identified using traditional conventional methods, which are generally time consuming with wrongful identification of the causative species, thus necessitating for a rapid and accurate identification and characterisation in providing standard and appropriate prescription treatment. PCR targeting the *28S rDNA* region is considered as a gold standard in the identification and characterization of dermatophytes from human skin [18]. There are studies that describe the extraction of DNA directly from human samples (nails) for the identification of the infecting dermatophytes [19]. The use of amplified fragments of the *28S-rDNA* gene contain regions of differentiation amongst these species [20]. These facts have provided a potential use of this marker in a confirmatory technique for dermatophyte-specific PCR targeting the *28S rRNA* gene, in characterisation and accurate identification, especially in *Tinea capitis* infection [21]. Based on the above proven facts, we consider *28S rRNA* PCR as the gold standard for this study. To the best of our understanding, this study is the first of its kind in Sokoto to have successfully deposited nucleotide sequences of studied dermatophyte strains into the GenBank database.

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