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ORIGINAL ARTICLE

Genotyping of intraspecies polymorphisms of *Sporothrix globosa* using partial sequence of mitochondrial DNA

Hirokazu Mochizuki | **Kazushi Anzawa** | **Takashi Mochizuki**

Department of Dermatology, Kanazawa Medical University, Uchinada, Japan

Correspondence
Takashi Mochizuki, Department of Dermatology, Kanazawa Medical University, 1-1 Daigaku, Uchinada, Kahoku, Ishikawa, 920-0293, Japan.
Email: mocizuki@kanazawa-med.ac.jp

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Abstract

Restriction fragment length polymorphism (RFLP) of mitochondrial DNA (mtDNA) had been used for molecular identification of *Sporothrix* spp., which is the causative fungi of sporotrichosis and the most prevalent deep-seated dermatomycosis. Also, mtDNA-RFLP had been used to investigate the molecular epidemiology of sporotrichosis. While the current standard for molecular diagnosis is performed by sequence analysis of the calmodulin gene (*CAL*), correspondence between the results from *CAL* and mtDNA is of diagnostic and epidemiological interest. Here, we investigated the correspondence between *CAL* and mtDNA used for molecular identification of *Sporothrix globosa* and *S. schenckii*, which are two major species. We also investigated and propose molecular markers suitable to describe the epidemiology of *S. globosa*, which is considered as a species with few intraspecific polymorphisms. Eighty-seven strains morphologically identified as *S. schenckii sensu lato* were investigated. They were identified as group A (17 types, 17 strains) or B (14 types, 70 strains) by mtDNA-RFLP. Partial sequences of *CAL*, internal transcribed spacer, and spacer between atp9 and cox2 genes of mtDNA of these strains were determined. All group A strains corresponded to *S. schenckii*, and group B to *S. globosa*. The sequences of the amplicons targeted on the spacer region in mtDNA of *S. globosa* ranged 510–515 bp in length and exhibited 10 molecular variations, whereas *CAL* indicated seven molecular variations. In conclusion, most of the *S. schenckii sensu lato* strains isolated from Japanese sporotrichosis patients were confirmed as *S. globosa*, because group B, which comprised the majority of strains, matched perfectly with *S. globosa* by the *CAL* sequencing study. We proposed sequence variations in the spacer between atp9 and cox2 genes of mtDNA as a suitable molecular epidemiological marker for *S. globosa*.

KEYWORDS
calmodulin gene, genotyping, mitochondrial DNA, *Sporothrix globosa*, *Sporothrix schenckii*

1 | INTRODUCTION

Sporotrichosis is the most predominant and worldwide deep-seated dermatomycosis. The causative fungi, *Sporothrix* spp., which inhabits soil, causes lesions when inoculated into skin or subcutaneous tissue by tiny wounds. *Sporothrix schenckii* had long been regarded as the only species causing sporotrichosis until Marimon *et al.*[1,2] conducted molecular characterization of morphologically identified *S. schenckii* isolates using several genes including calmodulin (*CAL*), and proposed a new taxonomy comprising *S. schenckii* (*sensu stricto*) with

some new species, *S. brasiliensis*, *S. globosa*, and *S. mexicana*. Now, the taxonomy morphologically identified as *S. schenckii* is understood to be a species complex (*S. schenckii sensu lato*). Historically, Ishizaki *et al.*[3–5] investigated genetic polymorphisms between *S. schenckii sensu lato* strains by restriction enzyme fragment length polymorphisms (RFLP) of mitochondrial (mt)DNA from the late 1980s to early 2000s, and revealed two major groups, A and B, in the species. Later, groups A and B were divided into 17 genotypes and 14 genotypes, respectively, by studies using isolates from many countries in the four continents, Eurasia, the Americas, Africa, and Australia.[6] Although the method using DNA extracted from the mitochondrial fraction recovered from homogenized fungal cells may be considered obsolete, it is still considered the most sensitive method for investigating intraspecific polymorphisms.

Here, we investigated how genotypes defined by RFLP of mtDNA[3–6] correspond to the latest taxonomy composed of *S. schenckii* and *S. globosa*, the latter being the most important causative species of sporotrichosis in Asia including Japan.[7] We examined a partial sequence of mtDNA, where the existence of diversity was predicted in a previous sequence study,[8] to determine whether it may be used for genotyping *S. globosa* to study the epidemiology of sporotrichosis.

2 | METHODS

2.1 | Fungal strains

Thirty-one strains of *S. schenckii sensu lato* maintained in our department (Table 1) were selected. They were identified as *S. schenckii* based on their morphological characteristics when they were registered at our department, and their genotypes were determined by RFLP of mtDNA (Mt-RFLP types). The panel of 31 strains comprised a representative strain of each of 31 Mt-RFLP types; among them, 17 genotypes were classified as group A and 14 as group B.[3–6] These isolates originated from Japan, the USA, China, Australia, Argentina, Mexico, Venezuela, Costa Rica, South Africa, and India.

An additional 56 group B strains isolated from different regions of Japan were included in this study (Table 2). Overall, 17 strains in group A and 70 in group B were investigated.

2.2 | Preparation of template DNA

Fungal DNA was extracted from colonies grown on potato dextrose agar slants or plates, as previously described[9] with slight modification. Briefly, small amounts of mycelial mat rinsed with 70% ethanol were ground in 200 μl of lysis buffer (200 mmol/L Tris-HCl, pH 7.5, 0.5% sodium dodecylsulfate, 250 mmol/L NaCl, 25 mmol/L ethylenediaminetetraacetic acid). The homogenates were heated at 100°C for 5 min, followed by the addition of 100 μl of 3 mol/L sodium acetate (pH 7.0), centrifuged, and 300 μl of isopropanol was added to the supernatant. The precipitated DNA pellets were washed in 70% ethanol, dried, and dissolved in 100 μl of 10 mmol/L Tris-HCl (pH 8.0) solution.

2.3 | Species identification by *CAL* and internal transcribed spacer (ITS) of ribosome RNA genes

Partial sequence of *CAL* was determined with primers CL1 and CL2A,[1,2,7] and two supplemental primers f1 and r1 designed for 3′- and 5′-ends (Table 3). Sequences near the 3′-end were determined with primers CL2A and f1 and near the 5′-end with primers CL1 and r1, respectively. The polymerase chain reaction (PCR) conditions included an initial cycle of 5 min at 94°C, followed by 35 cycles of 50 s at 94°C, 50 s at 55°C, 1 min at 72°C, and a single extension of 7 min at 72°C.[1,2] The sequence of ITS of ribosomal RNA gene was determined with primers ITS1 and ITS4 (Table 3)[10] as described.[1,2,7] If the strains whose nucleotide sequence did not completely match with the National Center for Biotechnology Information (NCBI) database (www.ncbi.nlm.nih.gov/BLAST/), their conidial shape, assimilation pattern, and limitation of growth temperature were examined for species level identification.

2.4 | Genotyping using mtDNA

A primer pair 975-8038F and 975-9194R (Table 3) was used for amplification of intergenic spacer region between atp9 and cox2 genes of mtDNA (Figure 1)[8] with the PCR conditions as follows: degeneration at 94°C for 4 min, then 35 cycles of 1 min at 94°C, 2 min at 58°C, and 1.5 min at 72°C. The targeted region revealed the greatest difference between a group A strain (ATCC 10268) and a group B strain (KMU 2052).[8] Amplicons were sequenced and grouped into varieties, and subjected to RFLP with *Ase* I (New England Biolabs).[8,11]

3 | RESULTS

3.1 | Species identification of the fungal strains based on sequence of *CAL*

The *CAL* sequences of 17 strains in group A were 817–822 bp in length, among which 14 were identical to *S. schenckii* registered in the NCBI database (four strains, i.e., Mt-RFLP types 19, 25, 26, and 29), or clustered together with the type strain of *S. schenckii* CBS359.36 (Figure 2). Two of the remaining four strains in group A (Mt-RFLP types 14 and 17) demonstrated the cluster of *S. schenckii* in the ITS tree (Figure S1). The other two (Mt-RFLP types 18 and 22) were identified as *S. schenckii* by physiological and morphological characters showing positivity for assimilation tests of sucrose and raffinose, growth at 37°C, and sessile pigmented conidia, consistent with those of *S. schenckii*. Identification of these two strains was consistent with the ITS tree (Figure S1).

The *CAL* sequences of all 14 representative strains in group B were 821–823 bp in length, with the strains clustered in a single branch together with type strain *S. globosa* CBS292.55 (Figure 2). The additional 56 strains in group B were sequenced. Seven variations were found and named Cal-gl 1–7 in this study (Table S1).

TABLE 1 Representative strains of each mtDNA RFLP type used in this study

No.	KMU number	Origin	Mt-RFLP types[a]	Mt-RFLP groups[a]	GenBank/EMBL/DDBJ accession no.		
					CAL	ITS	Mt-seq[b]
1	975	USA	1	A	LC635382	LC636163	LC635763
2	2286	Central Japan	2	A	LC635383	LC636164	LC635764
3	2500	Central Japan	3	A	LC635384	LC636165	LC635765
4	2747	South Japan	4	B	LC635385	LC636166	LC635766
5	3311	Central Japan	5	B	LC635386	LC636167	LC635767
6	2750	South Japan	6	B	LC635387	LC636168	LC635768
7	3360	Central Japan	7	B	LC635388	LC636169	LC635769
8	2741	West Japan	8	B	LC635389	LC636170	LC635770
9	2760	South Japan	9	B	LC635390	LC636171	LC635771
10	2763	South Japan	10	B	LC635391	LC636172	LC635772
11	2687	South Africa	11	A	LC635392	LC636173	LC635773
12	3314	Central Japan	12	B	LC635393	LC636174	LC635774
13	2762	South Japan	13	B	LC635394	LC636175	LC635775
14	3580	Costa Rica	14	A	LC635395	LC636176	LC635776
15	3504	USA	15	A	LC635396	LC636177	LC635777
16	3652	Argentina	16	A	LC635397	LC636178	LC635778
17	3655	Argentina	17	A	LC635398	LC636179	LC635779
18	3617	Venezuela	18	A	LC635399	LC636180	LC635780
19	3627	Venezuela	19	A	LC635400	LC636181	LC635781
20	3621	Venezuela	20	B	LC635401	LC636182	LC635782
21	3912	Australia	21	B	LC635402	LC636183	LC635783
22	3492	USA	22	A	LC635403	LC636184	LC635784
23	3998	South Africa	23	A	LC635404	LC636185	LC635785
24	4303	China	24	B	LC635405	LC636186	LC635786
25	4383	Mexico	25	A	LC635406	LC636187	LC635787
26	4385	Mexico	26	A	LC635407	LC636188	LC635788
27	4386	Mexico	27	B	LC635408	LC636189	LC635789
28	4384	Mexico	28	A	LC635409	LC636190	LC635790
29	4390	Mexico	29	A	LC635410	LC636191	LC635791
30	4398	Mexico	31	A	LC635411	LC636192	LC635792
31	4432	India	32	B	LC635412	LC636193	LC635793

Note: KMU number: registration number in Kanazawa Medical University.

Abbreviations: *CAL*, calmodulin gene; DDBJ, DNA Data Bank of Japan; EMBL, European Molecular Biology Laboratory; ITS, internal transcribed spacer; mtDNA, mitochondrial DNA; RFLP, restriction fragment length polymorphism.

[a]Mt-RFLP: genotypes and groups determined by RFLP of mtDNA.[3–6]

[b]Mt-seq: partial sequence of mitochondrial DNA determined by primers 975-8038F and 975-9194R.

Consequently, all group A strains corresponded to *S. schenckii*, and group B to *S. globosa*. No other species such as *S. brasiliensis* and *S. mexicana* were included in the series.

3.2 | Genotyping based on sequence of mtDNA

Partial sequence of mtDNA of 17 strains belonging to group A, which corresponds to *S. schenckii*, and 70 strains of group B, which corresponds to *S. globosa*, were determined and phylogenetic trees were produced (Figure 3). The topology of each branch on the tree appeared more widely distributed than that on the *CAL* tree (Figure 2). In detail, the size of the amplicons of 17 strains of group A ranged 513–1116 bp, containing a spacer 343–946 bp in length, comprising 16 variations named Mt-sch 1–16 in this study. The size of the amplicons of group B strains ranged 510–515 bp, containing a spacer 340–345 bp in length, comprising 10 variations named Mt-gl 1–10 in this study (Table S2). The match of 70 strains was: Mt-gl 5, 30 strains; followed by Mt-gl 1, 18 strains; Mt-gl 2, eight strains; Mt-gl 3, five strains; Mt-gl, three strains;

TABLE 2 mtDNA RFLP group B (*Sporothrix globosa*) strains isolated in Japan used in this study

No.	KMU number	Geographic background of isolates[a]	Mt-RFLP types[b]	GenBank/EMBL/DDBJ accession no		Genotypes	
				CAL	Mt-seq[c]	Cal-gl[d]	Mt-gl[e]
1	2679	Central	4	LC635794	LC635952	1	4
2	2688	West	4	LC635795	LC635953	1	4
3	2747	South	4	LC635385	LC635766	1	4
4	3021	Central	4	LC635796	LC635954	1	4
5	3112	North	4	LC635797	LC635955	1	4
6	3191	Central	4	LC635798	LC635956	1	7
7	3392	South	4	LC635799	LC635957	1	4
8	3479	West	4	LC635800	LC635958	1	4
9	3877	West	4	LC635801	LC635959	1	4
10	4061	Central	4	LC635802	LC635960	1	7
11	4078	Central	4	LC635803	LC635961	1	4
12	4131	West	4	LC635804	LC635962	1	4
13	4193	Central	4	LC635805	LC635963	1	7
14	4230	South	4	LC635806	LC635964	1	4
15	4257	West	4	LC635807	LC635965	1	4
16	4526	Central	4	LC635808	LC635966	1	4
17	4670	South	4	LC635809	LC635967	1	4
18	6488	Central	4	LC635810	LC635968	1	7
19	6799	South	4	LC635811	LC635969	1	4
20	2746	South	5	LC635812	LC635970	4	1
21	2778	South	5	LC635813	LC635971	5	1
22	2824	North	5	LC635814	LC635972	4	1
23	3041	Central	5	LC635815	LC635973	5	1
24	3308	North	5	LC635816	LC635974	4	1
25	3311	Central	5	LC635386	LC635767	1	1
26	3341	Central	5	LC635817	LC635975	1	1
27	3874	Central	5	LC635818	LC635976	1	1
28	4073	Central	5	LC635819	LC635977	4	1
29	4244	West	5	LC635820	LC635978	4	1
30	4453	North	5	LC635821	LC635979	1	1
31	4669	South	5	LC635822	LC635980	1	1
32	4710	Central	5	LC635823	LC635981	1	1
33	6326	Central	5	LC635824	LC635982	5	1
34	6637	Central	5	LC635825	LC635983	4	1
35	6705	Central	5	LC635826	LC635984	4	1
36	6798	South	5	LC635827	LC635985	1	1
37	2750	South	6	LC635387	LC635768	1	4
38	3376	Central	6	LC635828	LC635986	1	4
39	3515	Central	6	LC635829	LC635987	1	2
40	3604	West	6	LC635830	LC635988	1	4
41	3693	West	6	LC635831	LC635989	1	4
42	3705	Central	6	LC635832	LC635990	1	4
43	4130	West	6	LC635833	LC635991	6	4
44	4238	South	6	LC635834	LC635992	1	7

(Continues)

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TABLE 2 (Continued)

No.	KMU number	Geographic background of isolates[a]	Mt-RFLP types[b]	GenBank/EMBL/DDBJ accession no		Genotypes	
				CAL	Mt-seq[c]	Cal-gl[d]	Mt-gl[e]
45	6084	North	6	LC635835	LC635993	1	4
46	6429	West	6	LC635836	LC635994	1	9
47	2647	Central	7	LC635837	LC635995	5	8
48	3360	Central	7	LC635388	LC635769	1	2
49	3507	Central	7	LC635838	LC635996	1	2
50	4115	North	7	LC635839	LC635997	1	2
51	4129	South	7	LC635840	LC635998	1	2
52	4256	West	7	LC635841	LC635999	1	7
53	4648	South	7	LC635842	LC636000	1	2
54	6085	West	7	LC635843	LC636001	1	2
55	6684	South	7	LC635844	LC636002	1	2
56	6796	South	7	LC635845	LC636003	7	8
57	2741	West	8	LC635389	LC635770	1	5
58	2736	West	9	LC635846	LC636004	1	10
59	2760	South	9	LC635390	LC635771	1	4
60	3398	West	9	LC635847	LC636005	1	4
61	4132	West	9	LC635848	LC636006	1	7
62	4219	Central	9	LC635849	LC636007	1	4
63	2763	South	10	LC635391	LC635772	1	4
64	3314	Central	12	LC635393	LC635774	1	1
65	2762	South	13	LC635394	LC635775	1	4

Note: KMU number: registration number in Kanazawa Medical University.

Abbreviations: CAL, calmodulin gene; DDBJ, DNA Data Bank of Japan; EMBL, European Molecular Biology Laboratory; mtDNA, mitochondrial DNA; RFLP, restriction fragment length polymorphism.

[a]Geographic background of isolates: the regions of Japan geographically divided into four parts: Central (central Japan; central to eastern Honshu), West (western Japan; Shikoku, western Honshu), South (southern Japan; Kyushu), and North (northern Japan; northern Honshu, Hokkaido).

[b]Mt-RFLP: genotypes and groups determined by RFLP of mitochondrial DNA.[3–6]

[c]Mt-seq: partial sequence of mitochondrial DNA determined by primers 975-8038F and 975-9194R.

[d]Cal-gl: genotypes based on variations of sequence of calmodulin gene.

[e]Mt-gl: genotypes based on variations of sequence of mitochondrial DNA determined by primers 975-8038F and 975-9194R.

TABLE 3 Primers used in this study

Target	Primers	Sequence
Calmodulin gene, partial[1]		
	CL1	GA(GA)T(AT)CAAGGAGGCCTTCTC
	CL2A	TTTTTGCATCATGAGTTGGAC
Near the 3′-end	f1	AACAACGGCACCATTGACTT
Near the 5′-end	r1	GTCGACCTCGTTGATCATGT
Internal transcribed spacer[10]		
	ITS1	TCCGTAGGTGAACCTGCGG
	ITS4	TCCTCCGCTTATTGATATGC
Mitochondrial DNA, partial[8]		
	975-8038F	GCTAGAAATCCTTCTTTAAGAGGAC
	975-9194R	CCTTCCATTTGAGGTGTAGC

Mt-gl 6, two strains; and of Mt-gl 7, Mt-gl 8, Mt-gl 9, and Mt-gl 10, one strain each. The Mt-gl typing and Mt-RFLP typing[3–6] revealed incompatibility. However, only Mt-gl 1 corresponded exactly to Mt-RFLP type 5.

These sequence variations were examined by RFLP analysis,[5] but only five polymorphisms were detected among *S. schenckii* strains and none among *S. globosa* strains (Figure S2). The variations of *S. globosa* strains could not be detected using commercially available restriction enzymes in silico (data not shown).

4 | DISCUSSION

The present study revealed that groups A and B of *S. schenckii sensu lato* classified by RFLP of mtDNA[3–6] correspond to *S. schenckii* and *S. globosa*, respectively. The molecular epidemiology of 257 strains isolated before 1990 in Japan had comprised 14 group A strains,

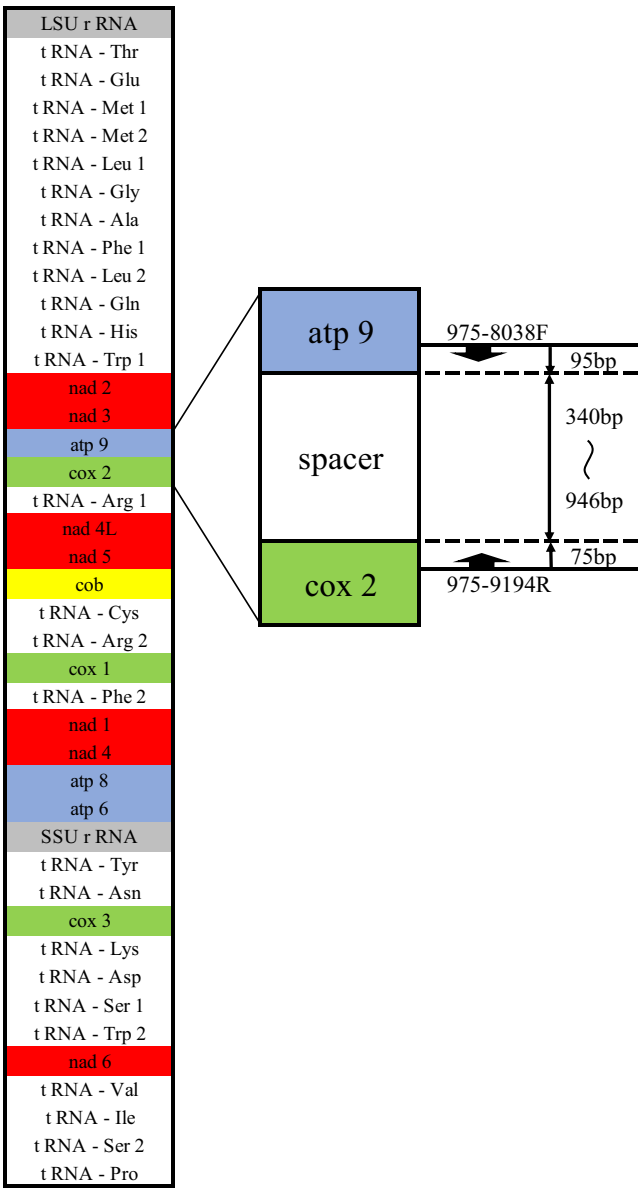

FIGURE 1 Structure of mitochondrial DNA of *Sporothrix schenckii sensu lato* and target of the primers used in the study

and 243 group B strains.[4] Therefore, it can be regarded that 14 of 257 strains (5.4%) were *S. schenckii*, and 243 of 257 (94.6%) *S. globosa*. A previous molecular epidemiological study using *CAL* and ITS found nine strains (3.0%) of *S. schenckii* and 291 (97.0%) of *S. globosa* among 300 Japanese isolates collected independently.[7] The present study indicated that the major causative species of Japanese sporotrichosis is *S. globosa*. No causative species other than *S. schenckii* and *S. globosa* has been found among Japanese strains so far.

Sporotrichosis has distinctive characteristics and is known as an endemic mycosis, which is widespread.[12] In Japan, sporotrichosis tends to be concentrated in specific regions such as large river basins, but such regions exist in geographically distant locations. In addition, human activities involving contact with wood, plants, moss, and so forth have sometimes been associated with outbreaks of sporotrichosis,[12,13] which may affect the epidemiological distribution of *Sporothrix* spp. Since a case of simultaneous infection in a human by genetically distinct strains was reported,[14] molecular markers that can detect polymorphisms within a species are useful to study epidemiology.

Several molecular markers have been applied to track and monitor sporotrichosis. In particular, *S. globosa* is known to have low diversity[15–17] and considered to require sensitive markers. Intraspecific polymorphisms of *CAL* or ITS have been detected in only a few varieties among *S. globosa* strains.[8] Amplified fragment length polymorphism (AFLP) analysis, which detects differences in the length of fragments sandwiched between restriction enzyme cleavage sites, divided 225 clinical isolates of *S. globosa* from China into eight distinct clusters.[15] Multilocus microsatellite analysis is another sensitive method[17,18] and microsatellite markers have been reported for genotyping of *S. globosa* which enabled amalgamation of 120 isolates from China into three distinct clusters.[17] However, peaks for microsatellite markers sometimes shift due to differences in electrophoresis conditions and primer modification processes, and special attention is needed in inter-laboratory comparison.[19] The most sensitive marker is RFLP analysis of mtDNA,[3–6] which albeit a non-PCR-based complicated and time-consuming method, found 14 polymorphisms among *S. globosa* strains. However, the RFLP analysis was sometimes difficult to compare banding profiles and could be confused by bands of similar size or conditions of electrophoresis. In recent days, nucleotide sequence analysis has become easier, and highly variable regions of genes are targeted as molecular markers. As one candidate for this purpose, Kawasaki *et al.*[8] proposed the intergenic region between atp9 and cox2 genes based on sequence comparison of completely determined mtDNA of KMU975 (group A) and KMU2052 (group B) (Figure 1). Using the primer pair 975-8038F and 975-9194R, 10 polymorphisms were detected among 70 strains, which is fewer variations than that of RFLP analysis of whole molecule of mtDNA, yet more sensitive than sequence analysis of *CAL* which revealed seven variations among these strains. In addition, it is easier to sequence the partial mtDNA gene compared to *CAL* due to their smaller size. This marker may contribute to understanding the route of transmission of *Sporothrix*, especially when the source was assumed to be in the environment such as plants and soil, pet animals, or in family onset cases.

We tried to find correspondence of the present Mt-gl types with the geographic origins of *S. globosa*. The 65 Japanese strains were isolated from four provinces of Japan: southern Japan (Kyushu), western Japan (Shikoku, western Honshu), central Japan (central to eastern Honshu), and northern Japan (northern Honshu, Hokkaido). However, the strains in each of the four provinces were found to be genetically polymorphic; namely 18 strains from southern Japan comprised five genotypes (Mt-gl 1, 2, 4, 7, 8), 16 strains from western Japan seven genotypes (Mt-gl 1–5, 9, 10), 25 from central Japan five genotypes (Mt-gl 1, 2, 4, 7, 8), and six strains from northern Japan three genotypes (Mt-gl 1, 2, 4).

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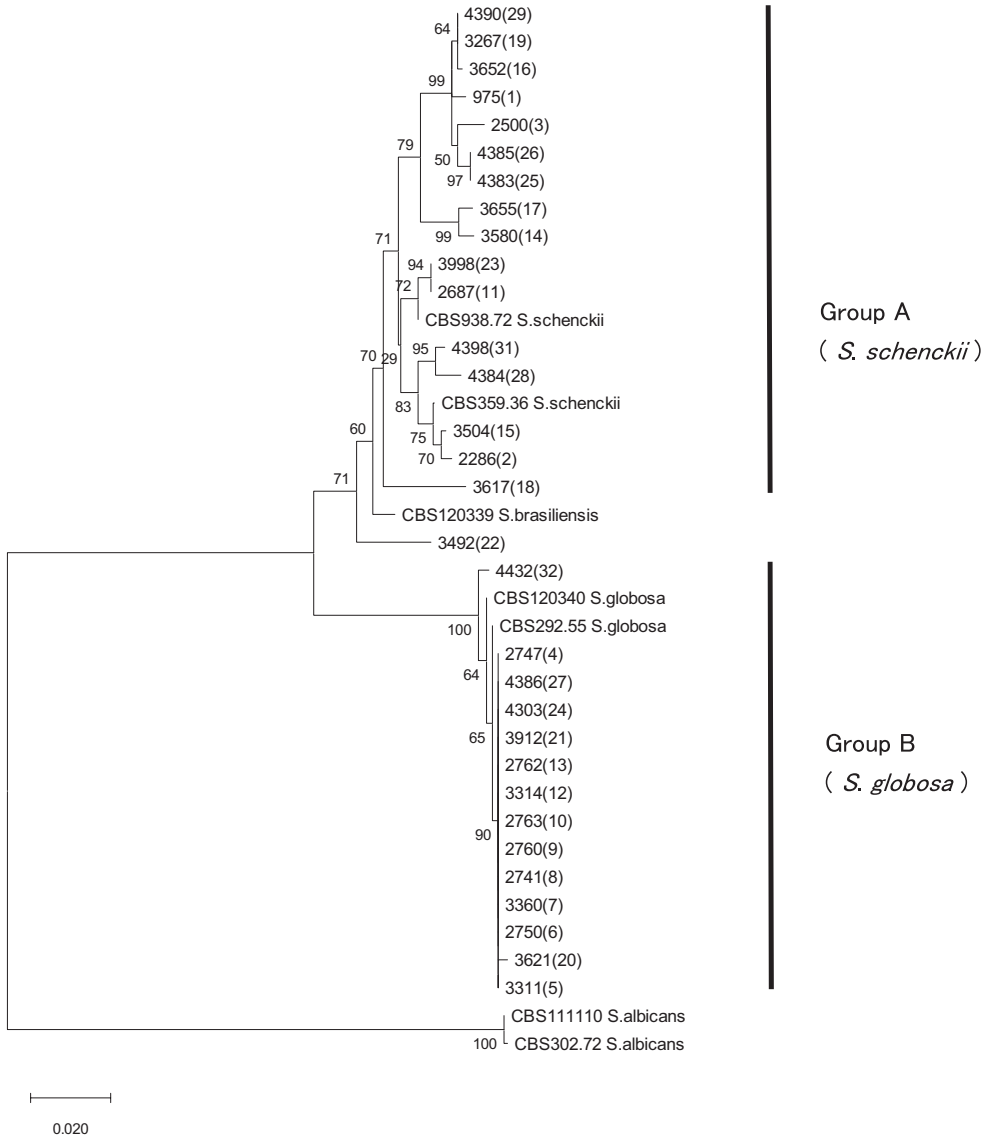

FIGURE 2 Phylogenetic tree of *Sporothrix schenckii sensu lato* based on partial sequence of calmodulin gene. All 17 strains from each mitochondrial (mt)DNA restriction fragment length polymorphism (RFLP) type in group A were clustered with type strain *S. schenckii* CBS 359.36, and all 14 in group B with ex-type strain *S. globosa*, CBS 120340, respectively. Twelve variations were found among group A strains and three among representative strains in group B. The mtDNA RFLP types are shown in parentheses. Neighbor-joining method

Genotype Mt-gl 4, the most common, was found in 27 among 65 strains, and isolated from all four provinces in Japan. Mt-gl 4 was also found among isolates from China, Mexico, and Australia, suggesting global distribution. Genotypes Mt-gl 1 (18 strains), and Mt-gl 2 (eight strains) were also found in all four Japanese provinces. The proportion of Mt-gl 1 among genotypes was low in western Japan but high in central-east Japan. The proportion of Mt-gl 4 among the isolates was higher in southern and western Japan, and lower in central and northern Japan. However, no particular genotype was responsible for the endemic in Japan. In China, AFLP genotyping was reported to reflect regional differences,[15] but in Japan, many people inhabit relatively small areas and farming was prevalent, so it is postulated that genotypes were affected by human activities. In addition, 18 strains of Mt-gl 1 isolated from Japan have three types of *CAL* variations, and combining these markers makes more detailed genotyping of *S. globosa* possible.

The relationship between genotypes and virulence is of clinical interest. In a few strains belonging to Mt-gl 1 and Mt-gl 4, we attempted to find differences in thermotolerance and minimum inhibitory concentration (MIC) for some antimycotics, which may influence their pathogenicity (Table S3), but comprehensive studies of a larger number of samples are needed to make any reliable conclusion. We would like to determine the genotype as an attribute of the maintained culture collection for further study.

In conclusion, the present study revealed that groups A and B of *S. schenckii sensu lato* classified by RFLP of mtDNA[1–4] corresponded to *S. schenckii* and *S. globosa*, respectively. *S. globosa* is the main pathogen of sporotrichosis in Asia, including Japan, but it is genetically less variable than *S. schenckii*. For molecular epidemiology, sequence information of the amplicons targeted on the spacer between apt9 and cox2 genes of mtDNA by the primer pair 975-8038F

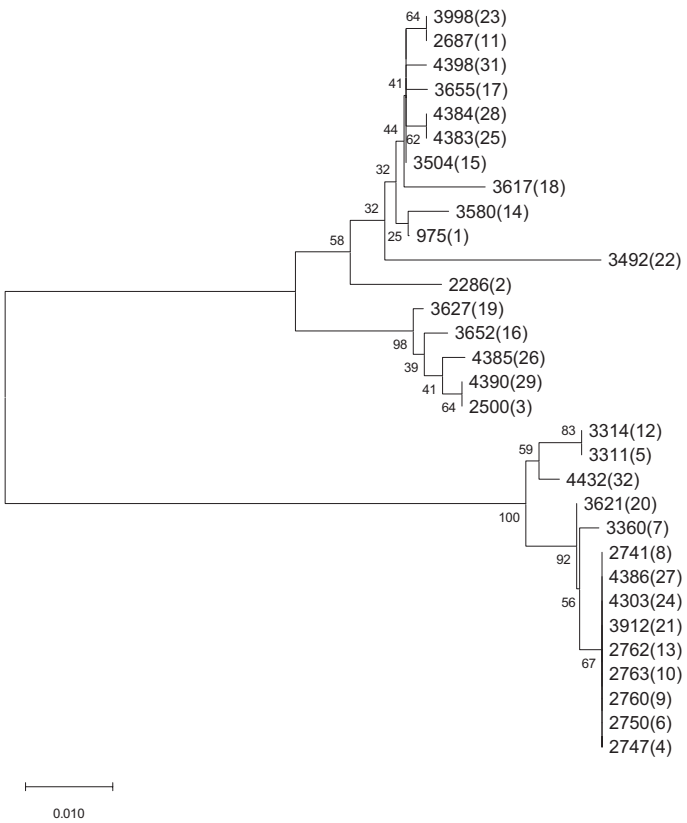

FIGURE 3 Phylogenetic tree of *Sporothrix schenckii sensu lato* based on partial sequence of mitochondrial (mt)DNA by primers 975-8038F and 975-9194R. All 17 strains from each mtDNA restriction fragment length polymorphism (RFLP) type in group A, namely *S. schenckii*, were clustered together, and all 14 in group B, namely *S. globosa*, were clustered together, respectively. Fourteen variations were found among group A strains, and five among representative strains in group B. The mtDNA RFLP types are shown in parentheses. Neighbor-joining method

and 975-9194R has indicated higher discriminatory power than that of *CAL*, and we propose to adopt this region for a useful marker for molecular epidemiology of *S. globosa*.

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CONFLICT OF INTEREST

None declared.

ORCID

Takashi Mochizuki https://orcid.org/0000-0002-3793-980X

REFERENCES

1. Marimon R, Gené J, Cano J, Trilles L, Dos Santos Lazéra M, Guarro J. Molecular phylogeny of *Sporothrix schenckii*. *J Clin Microbiol*. 2006;44:3251–6.
2. Marimon R, Cano J, Gené J, Sutton DA, Kawasaki M, Guarro J. *Sporothrix brasiliensis*, *S. globosa*, and *S. mexicana*, three new *Sporothrix* species of clinical interest. *J Clin Microbiol*. 2007;45:3198–206.
3. Suzuki K, Kawasaki M, Ishjzaki H. Analysis of restriction profiles of mitochondrial DNA from *Sporothrix schenckii* and related fungi. *Mycopathologia*. 1988;103:147–51.
4. Takeda Y, Kawasaki M, Ishjzaki H. Phylogeny and molecular epidemiology of *Sporothrix schenckii* in Japan. *Mycopathologia*. 1991;116:9–14.
5. Ishizaki H, Kawasaki M, Aoki M, Vismer H, Muir D. Mitochondrial DNA analysis of *Sporothrix schenckii* in South Africa and Australia. *Med Mycol*. 2000;38:433–6.
6. Ishizaki H, Kawasaki M, Anzawa K, Mochizuki T, Chakrabarti A, Ungpakorn R, *et al*. Mitochondrial DNA analysis of *Sporothrix schenckii* in India, Thailand, Brazil, Colombia, Guatemala and Mexico. *Nippon Ishinkin Gakkai Zasshi*. 2009;50:19–26.
7. Suzuki R, Yikelamu A, Tanaka R, Igawa K, Yokozeki H, Yaguchi T. Studies in phylogeny, development of rapid identification methods, antifungal susceptibility, and growth rates of clinical strains of *Sporothrix schenckii* complex in Japan. *Med Mycol J*. 2016;57:E47–57.
8. Kawasaki M, Anzawa K, Mochizuki T, Ishizaki H. New strain typing method with *Sporothrix schenckii* using mitochondrial DNA and polymerase chain reaction restriction fragment length polymorphism (PCR-RFLP) technique. *J Dermatol*. 2012;39:362–5.
9. Makimura K, Tamura Y, Mochizuki T, Hasegawa A, Tajiri Y, Hanazawa A, *et al*. Phylogenetic classification and species identification of dermatophyte strains based on DNA sequence of nuclear ribosomal internal transcribed spacer 1 regions. *J Clin Microbiol*. 1999;37:920–4.
10. White TJ, Bruns T, Lee S, Taylor J. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Sninsky JJ, White TJ, editors. *PCR Protocols -a Guide to Methods and Application*. New York: Academic Press Inc.; 1990. p. 315–22.
11. Watanabe S, Kawasaki M, Mochizuki T, Ishizaki H. RFLP analysis of the internal transcribed spacer regions of *Sporothrix schenckii*. *Nihon Ishinkin Gakkai Zasshi*. 2004;45:165–75.
12. Chakrabarti A, Bonifaz A, Gutierrez-Galhardo MC, Mochizuki T, Li S. Global epidemiology of sporotrichosis. *Med Mycol*. 2015;53:3–14.
13. Coles FB, Schuchat A, Hibbs JR, Kondracki FK, Salkin IF, Dixon DM, *et al*. A multistate outbreak of sporotrichosis associated with sphagnum moss. *Am J Epidemiol*. 1992;136:475–87.
14. Kobayashi H, Kawasaki M, Ishizaki H, Fukushiro R, Matsumoto R. A case of sporotrichosis caused by two genetically different *Sporothrix schenckii* strains. *Mycopathologia*. 1990;112:19–22.

15. Zhao L, Cui Y, Zhen Y, Yao L, Shi Y, Song Y, *et al*. Genetic variation of *Sporothrix globosa* isolates from diverse geographic and clinical origins in China. *Emerg Microbes Infect*. 2017;6:e88.
16. Moussa TAA, Kadasa NMS, Al Zahrani HS, Ahmed SA, Feng P, Gerrits van den Ende AHG, *et al*. Origin and distribution of *Sporothrix globosa* causing sapronoses in Asia. *J Med Microbiol*. 2017;66:560–9.
17. Gong J, Zhang M, Wang Y, Li R, He L, Wan Z, *et al*. Population structure and genetic diversity of *Sporothrix globosa* in China according to 10 novel microsatellite loci. *J Med Microbiol*. 2019;68:248–54.
18. Taylor JW, Fisher MC. Fungal multilocus sequence typing - it's not just for bacteria. *Curr Opin Microbiol*. 2003;6:351–6.
19. Yamada S, Anzawa K, Mochizuki T. Molecular epidemiology of *Microsporum canis* isolated from Japanese cats, dogs and pet owners by multilocus microsatellite typing fragment analysis. *Jpn J Infect Dis* (in press). https://doi.org/10.7883/yoken.JJID.2020.809

SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

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