Original Articles

Kerion celsi due to Arthroderma incurvatum infection in a Sri Lankan child: species identification and analysis of area-dependent genetic polymorphism

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A three-year-old Sri Lankan boy residing in Japan developed a nodule on his scalp after visiting Sri Lanka. Two months later, the lesion increased in size to 22 × 19 mm², and was identified as an erythematous nodule with alopecia. Direct examination of the infected hair shafts indicated fungal hyphae outside the shafts. The fungus was identified as Microsporum gypseum following mycological examination. The sequence of the internal transcribed spacer 1 region of ribosomal RNA gene (ITS1 rDNA) exhibited 95.7–100.0% homology with that of Arthroderma incurvatum. The patient was successfully treated with a 6-week itraconazole course. We also examined DNA samples from eight clinical isolates of A. incurvatum. Alignments of ITS1 sequences of these strains and our isolate, showed gaps in the 64-bp positions 140–142 and 141–143 of the 205–207-bp ITS1 alignment. We performed phylogenetic analysis using the neighboring (NJ) method based on the ITS1 sequences of the present isolate and twenty related strains. Fifteen A. incurvatum strains were divided into East Asia and non-East Asia clusters. The present isolate belonged to the non-East Asia cluster, suggesting that the patient was infected outside Japan. Moreover, the trees suggested area-dependent genetic polymorphism of A. incurvatum.

Keywords Arthroderma incurvatum, Microsporum gypseum, kerion celsi, itraconazole, internal transcribed spacer 1 region of ribosomal RNA gene (ITS1 rDNA)

Introduction

The teleomorphs of the Microsporum gypseum complex were classified as Arthroderma gypseum, Arthroderma fulvum, and Arthroderma incurvatum based on mating studies. A. incurvatum was initially identified as Nannizzia incurvata gen. nov. sp. nov. by Stockdale in 1963 [1]. She reported that N. incurvata, Nannizzia gypseai, and Nannizzia fulva are heterothallic and exhibit slight but constant differences in their colonial and microscopic appearances on agar media and in the form of the peridial hyphae and appendages of their cleistothecia [2]. Distinguishing between these three species is difficult. Cross-mating does not occur among them [2], but mating tests are biologically definitive. However, it is often difficult for tester strains to retain their mating ability; moreover, the tests are time-consuming and cannot be performed routinely. Molecular identification techniques are currently the practical methods for the identification of teleomorphs.

In Japan, a strain of N. incurvata was isolated from the soil in 1971 [3]. To our knowledge, only 27 stocked strains of A. incurvatum exist globally, and 18 of them have originated in Japan. This would suggest that the

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DOi: 10.3109/13693786.2012.671968
telemorph of \textit{A. incurvatum} was present earlier in Japan. Unfortunately, the rDNA sequences of \textit{A. incurvatum} have not been sufficiently analyzed, and the genotypic polymorphism associated with geographical distribution has never been reported. In the present study, we identified, through mycological examination and molecular identification, an isolate of \textit{A. incurvatum} from a Sri Lankan boy with kerion celsi. In order to elucidate the exact location where the infection occurred, we analyzed the ITS regions of the species using several strains from Japan and other countries.

**Case**

The patient was a three-year-old Sri Lankan boy residing in Japan. His parents were born in Sri Lanka and immigrated to Japan when he was 6 months old. From December 2009 to February 2010, the family resided in Sri Lanka, where the boy developed a 5-mm skin-colored nodule with yellow crust on his scalp at the beginning of February 2010. After his return to Japan, he visited a doctor and was treated with cryosurgery. After 10 days, the lesion was inflamed and exhibited a purulent discharge. The patient was unsuccessfully treated with antibiotics. He visited our hospital in April 2010. Clinical examination showed a 22 × 19 mm² erythematous, painful nodule associated with pustules and alopecia (Fig. 1).

Direct microscopy of the affected hair with potassium hydroxide (KOH) solution indicated the presence of fungal hyphae outside the hair shafts (Fig. 2).

The patient was otherwise healthy without any immunosuppressive disorders and had not experienced any trauma. His family history did not show any similar conditions. He did not have any pets, although he occasionally played with dogs, cats, and birds. He also enjoyed playing in the sand.

**Materials and methods**

**Fungal strains**

The 12 isolates of \textit{A. incurvatum}, six isolates of \textit{A. gypseum}, and five isolates of \textit{A. fulvum} used in this study are described in Table 1.

**Mycological examination**

Potato dextrose agar (PDA; Difco Laboratories, Detroit, MI, USA) slants supplemented with cycloheximide (500 mg/l) and chloramphenicol (50 mg/l) were used for primary culture. The present isolate (strain IFM 59508) and \textit{A. incurvatum} (IFM 5905, IFM 5906, IFM 47488, IFM 49903, IFM 49904, KMU 2981, KMU 2982, KMU 2986, and KMU 2987), \textit{A. gypseum} (IFM 5309, IFM 46928 and IFM53877), and \textit{A. fulvum} (IFM 5308, IFM 48151 and IFM 48152) isolates were cultured on PDA to generate giant colonies (25°C, 14 days) and for slide culture studies (25°C, 11 days). The sizes of a hundred macroconidia of each strain of \textit{A. incurvatum}, \textit{A. gypseum}, and \textit{A. fulvum} were measured after the isolates were grown on PDA slants at 28°C for 10 days.

**Mating tests**

The isolate IFM 59508 was crossed with each of the following tester strains of the indicated mating types (+) and (−): \textit{A. incurvatum} IFM 5306 (+), IFM 5305 (−), IFM 49903 (+), IFM 49904 (−), KMU 2981 (+), KMU 2982 (−), KMU 2986 (+), and KMU 2987 (−); \textit{A. gypseum} IFM 5309 (+) and IFM 5309 (−); and \textit{A. fulvum} IFM 47491 (+) and IFM 47492 (−). The isolate was inoculated on 1/10-diluted Sabouraud agar containing salts, 0.2% glucose (Wako Chemical Co., Ltd, Osaka, Japan), 0.1% neopeptone (Difco Laboratories), 0.1% MgSO$_4$.7H$_2$O, 0.1% KH$_2$PO$_4$, and 2% agar.

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region of rDNA was amplified through PCR by using the
illustra Hot Start Mix RTG (GE Healthcare, Piscataway,
NJ, USA) with the primer pair ITS5 (5’/H11032-GGAAGTAAAAG
TCGTAAGAAGG-3’/H11032) and ITS4 (5’/H11032-TCCTCCGCTTATTG
ATATGC-3’/H11032). PCR was performed using 35 cycles, each
consisting of denaturation at 95 °C for 30 s, primer annealing
at 55 °C for 30 s, and primer extension at 72 °C for 60 s. The
PCR products were purified using the QIAquick PCR Puri-
fication Kit (Qiagen, Valencia, CA, USA), and the DNA
sequences were determined using the Genetic Analyz-
er3130xl (PE Applied Biosystems, Carlsbad, CA, USA)
(Bacto-agar, Difco Laboratories) (also called Takashio
Medium), along with each of the above mentioned strains and
incubated at 25°C for 8 weeks. Each pair was also incubated
on plain agar with hair shafts obtained from an infant.

Molecular identification

Preparation of genomic DNA samples and 16S rDNA
sequencing

DNA samples were prepared using the GenTLE High
Recovery Kit (TAKARA Bio Inc., Shiga, Japan). The ITS
region of rDNA was amplified through PCR by using the
illustra Hot Start Mix RTG (GE Healthcare, Piscataway,
NJ, USA) with the primer pair ITS5 (5’-GGAAGTAAAAG
TCGTAAGAAGG-3’) and ITS4 (5’-TCCTCCGCTTATTG
ATATGC-3’). PCR was performed using 35 cycles, each
consisting of denaturation at 95°C for 30 s, primer annealing
at 55°C for 30 s, and primer extension at 72°C for 60 s. The
PCR products were purified using the QIAquick PCR Puri-
fication Kit (Qiagen, Valencia, CA, USA), and the DNA
sequences were determined using the Genetic Analyzer
3130xl (PE Applied Biosystems, Carlsbad, CA, USA)

Table 1  Isolates examined in this study.

<table>
<thead>
<tr>
<th>Species (mating type)</th>
<th>Strains</th>
<th>Origin</th>
<th>GenBank/EMBL/DDBJ accession numbers</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arthroderma incurvatum (−)</td>
<td>IFM 5905</td>
<td>Unknown</td>
<td></td>
</tr>
<tr>
<td>Arthroderma incurvatum (+)</td>
<td>IFM 5906</td>
<td>Unknown</td>
<td></td>
</tr>
<tr>
<td>Arthroderma incurvatum (−)</td>
<td>IFM 46456</td>
<td>N-22</td>
<td>AB613256**</td>
</tr>
<tr>
<td>Arthroderma incurvatum (+)</td>
<td>IFM 46457</td>
<td>N-23</td>
<td>AB613258**</td>
</tr>
<tr>
<td>Arthroderma incurvatum (−)</td>
<td>IFM 47488</td>
<td>IMI 104076</td>
<td></td>
</tr>
<tr>
<td>Arthroderma incurvatum (+)</td>
<td>IFM 49903</td>
<td>JCM 1905</td>
<td>AB592337**</td>
</tr>
<tr>
<td>Arthroderma incurvatum (−)</td>
<td>IFM 49904</td>
<td>IMI 86518 = JCM 1906</td>
<td>AB613255**</td>
</tr>
<tr>
<td>Arthroderma incurvatum (+)</td>
<td>IFM 2981</td>
<td>Human skin</td>
<td>AB613251**</td>
</tr>
<tr>
<td>Arthroderma incurvatum (−)</td>
<td>IFM 2982</td>
<td>Human skin</td>
<td>AB613252**</td>
</tr>
<tr>
<td>Arthroderma incurvatum (−)</td>
<td>IFM 2986</td>
<td>Human skin</td>
<td>AB613253**</td>
</tr>
<tr>
<td>Arthroderma incurvatum (−)</td>
<td>IFM 2987</td>
<td>Human skin</td>
<td>AB613254**</td>
</tr>
<tr>
<td>Arthroderma incurvatum (ND)*</td>
<td>IFM 59508</td>
<td>Human skin</td>
<td>AB613257**</td>
</tr>
<tr>
<td>Arthroderma gypseum (−)</td>
<td>IFM 5309</td>
<td>Human skin</td>
<td></td>
</tr>
<tr>
<td>Arthroderma gypseum (−)</td>
<td>IFM 5310</td>
<td>Human skin</td>
<td></td>
</tr>
<tr>
<td>Arthroderma gypseum (−)</td>
<td>IFM 46928</td>
<td>Human skin</td>
<td>AB193701</td>
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<tr>
<td>Arthroderma gypseum (ND)</td>
<td>IFM 47490</td>
<td>CBS 171.64</td>
<td></td>
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<tr>
<td>Arthroderma gypseum (+]</td>
<td>IFM 53877</td>
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<td>Arthroderma gypseum (ND)</td>
<td>IFM 53930</td>
<td>Human skin</td>
<td>AB461864</td>
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<td>Arthroderma gypseum (−)</td>
<td>IFM 5308</td>
<td>Unknown</td>
<td>AB193715</td>
</tr>
<tr>
<td>Arthroderma gypseum (+)</td>
<td>IFM 47491</td>
<td>IMI 86179</td>
<td>AB193718</td>
</tr>
<tr>
<td>Arthroderma gypseum (−)</td>
<td>IFM 47492</td>
<td>IMI 86180</td>
<td>AB193719</td>
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<td>Arthroderma gypseum (ND)</td>
<td>IFM 48151</td>
<td>Soil</td>
<td></td>
</tr>
<tr>
<td>Arthroderma gypseum (ND)</td>
<td>IFM 48152</td>
<td>Soil</td>
<td></td>
</tr>
</tbody>
</table>

*Present isolate; ND, not determined; **ITS1 rDNA analyzed in this research.

Fig. 3  A giant colony of strain IFM 59508 incubated on potato dextrose agar (PDA) at 25°C for 14 days. A yellowish-white and powdery thin colony appeared (a). Slide-culture examination indicated fusiform macroconidia and club-shaped microconidia on PDA at 25°C for 11 days; the bar indicates 50 μm (b).
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with a BigDye Terminator v1.1 Sequencing standard Kit (PE Applied Biosystems). The sequences were assembled using the Genetyx-Mac program (Genetyx Corp., Tokyo, Japan).

Certain ITS1 sequences were aligned with the new sequences determined in this study by using the Genetyx-Mac program (Genetyx Corp.). The ITS1 sequences included those of *Arthroderma fulvum* IFM 47491 (DDBJ/EEMBL/GenBank accession no. AB193718) [4], *A. gypseum* (DDBJ/EEMBL/GenBank accession no. AJ970144) [5], *A. fulvum* (DDBJ/EEMBL/GenBank accession no. AM000035) [6], *A. gypseum* IFM 41066 (DDBJ/EEMBL/GenBank accession no. AB193684) [4], *A. gypseum* CBS 170.64 (DDBJ/EEMBL/GenBank accession no. AF168128) [7], *A. gypseum* IFM 53930 (DDBJ/EEMBL/GenBank accession no. AB461864) [4], *A. incurvatum* CBS 174.64T (DDBJ/EEMBL/GenBank accession no. AJ970153), and *A. incurvatum* IFM 41290

Fig. 4  The giant colonies of *Arthroderma incurvatum*, *Arthroderma gypseum*, and *Arthroderma fulvum* incubated on PDA at 28°C for 14 days.
In vitro with isolate and that from 20 related strains. The tree was rooted on the ITS1 sequences on the rRNA gene from the present Ph. 

The neighbor-joining (NJ) [9] tree was constructed based on the ITS1 sequences from Arthroderma incurvatum (IFM 46793 (DDBJ/EEMBL/GenBank accession no. AB193689) from Nagasaki, Japan [8]; A. incurvatum IFM 46926 (DDBJ/EEMBL/GenBank accession no. AB193700) from Amami, Japan [8]; A. incurvatum IFM 41067 (DDBJ/EEMBL/GenBank accession no. AB193685) from Okinawa, Japan [8]; A. incurvatum IFM 41133 (DDBJ/EEMBL/GenBank accession no. AB193700) from Peking, China [8]; and A. incurvatum IFM 41133 (DDBJ/EEMBL/GenBank accession no. AB193687) from Brazil [8].

Phylogenetic analysis

The neighbor-joining (NJ) [9] tree was constructed based on the ITS1 sequences on the rRNA gene from the present isolate and that from 20 related strains. The tree was rooted with *Epidermophyton floccosum* as an out-group.

In vitro antifungal susceptibility testing

The antifungal sensitivities of the isolate were assessed using a microbroth dilution kit for Yeast-like Fungi DP Eiken trays (Eiken Chemical Co. Ltd, Tokyo, Japan) that were developed on the basis of the Clinical and Laboratory Standards Institute (CLSI) document M27 by the manufacturer. The 100% inhibitory concentration (IC$_{100}$) (amphotericin B) and the 50% inhibitory concentration (IC$_{50}$) (flucytosine, fluconazole, itraconazole, miconazole, micafungin, and voriconazole) values were determined according to a standard protocol that included the guidelines of CLSI M38-A2 test [10] after incubating at 30°C for 96 h. 

Table 2 Sizes of macroconidia in *Arthroderma gypseum*, *Arthroderma fulvum* and *Arthroderma incurvatum*.

<table>
<thead>
<tr>
<th>Species &amp; strain</th>
<th>Length (μm)</th>
<th>Breadth (μm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Range</td>
<td>Average</td>
</tr>
<tr>
<td><em>Arthroderma incurvatum</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IFM 47488</td>
<td>37.6–80.4</td>
<td>62.2 ± 8.3</td>
</tr>
<tr>
<td>KMU 2982</td>
<td>39.3–71.8</td>
<td>56.3 ± 6.9</td>
</tr>
<tr>
<td>IFM 59508</td>
<td>44.5–75.2</td>
<td>61.1 ± 6.0</td>
</tr>
<tr>
<td><em>Arthroderma gypseum</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IFM 5309</td>
<td>41.0–71.8</td>
<td>58.5 ± 6.1</td>
</tr>
<tr>
<td>IFM 46928</td>
<td>41.0–65.0</td>
<td>52.7 ± 5.4</td>
</tr>
<tr>
<td>IFM 47490</td>
<td>42.8–75.2</td>
<td>60.6 ± 7.0</td>
</tr>
<tr>
<td><em>Arthroderma fulvum</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IFM 5308</td>
<td>35.9–75.2</td>
<td>55.1 ± 8.0</td>
</tr>
<tr>
<td>IFM 48151</td>
<td>41.0–66.7</td>
<td>52.1 ± 4.7</td>
</tr>
</tbody>
</table>

The sizes of 100 macroconidia in each strain of *A. incurvatum*, *A. gypseum* and *A. gypseum* were measured after they had been grown on PDA slants at 28°C for 10 days.

Mating tests

No reaction was obtained when the isolate was crossed with six strains of *A. incurvatum* mating type (−). No gymnothecia were observed when the isolate was crossed with six strains of *A. incurvatum* mating type (+), although confronting lines were observed when the isolate was crossed with two strains of *A. incurvatum* mating type (+). 

Results

Mycological examination

Yellowish-white colonies with a powdery surface were observed on the primary culture on PDA slants supplemented with cycloheximide (500 mg/l) and chloramphenicol (50 mg/l) and incubated at room temperature (PDA; 25°C; 14 days) (Fig. 3a). Slide cultures (PDA; 25°C; 11 days) exhibited four to seven celled fusiform, thin-walled, macroconidia and globose, unicellular microconidia (Fig. 3b).

Giant colonies (PDA; 28°C; 14 days) of *A. incurvatum*, *A. gypseum*, and *A. fulvum* were yellowish-white and powdery (Fig. 4). The periphery of the colonies of *A. gypseum* appeared fastigiated and in a dendritic form. The colonies of *A. incurvatum* IFM 5305, IFM 5306, IFM 49903, IFM 49904, KMU 2981, KMU 2982, KMU 2986, and KMU 2987 were in villous degeneration, and no colony was similar to IFM 59508. 

The average sizes of macroconidia of *A. incurvatum* (IFM 47488, IFM 59508, and KMU 2982), *A. gypseum* (IFM 5309, IFM 49903, IFM 49904, KMU 2981, KMU 2982, KMU 2986, and KMU 2987) were measured after they had been grown on PDA slants at 28°C for 10 days.

The sizes of 100 macroconidia in each strain of *A. incurvatum*, *A. gypseum* and *A. gypseum* were measured after they had been grown on PDA slants at 28°C for 10 days.
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In order to examine their mating ability, mating tests were conducted between the tester strains (/H11001/) and (/H11002/) of *A. incurvatum*, but no gymnothecia were observed. No reaction was observed when the isolate was crossed with either mating type of *A. gypseum* or *A. fulvum*.

**Molecular identification**

A total of 205 bases of ITS1 rDNA were analyzed. A BLAST search showed that the sequence of the ITS1 region was 95.7–100.0% homologous to that of *A. incurvatum*. This sequence was 70.3% homologous to that of *A. gypseum* AB193684 and 71.4% homologous to that of *A. fulvum* AB193717. The present isolate IFM 59508 was the most related to *A. incurvatum* among the three teleomorphic species.

Alignments of ITS1 sequences of *A. incurvatum*, including those of the present isolate, exhibited certain gaps, particularly at 64-bp positions 140–142 and 141–143 of the 205–207-bp ITS1 alignment. Alignments of ITS1 sequences of *A. fulvum* exhibited 15-bp gaps at positions 143–145 and 144–146 (Fig. 6). The presence of the gaps strongly suggested that the present isolate was *A. incurvatum*.

**Phylogenetic analysis**

The NJ tree of ITS1 rDNA of the *M. gypseum* complex contained the *A. fulvum*-*A. gypseum* cluster (Fig. 7B) and the *A. incurvatum* cluster (Fig. 7A).

Fifteen strains of *A. incurvatum* were divided into two clusters: East Asia (Fig. 7a) and non-East Asia clusters (Fig. 7b).

**In vitro antifungal susceptibility testing**

In vitro susceptibility testing of strain IFM 59508 indicated its susceptibility to itraconazole (IC$_{50}$ value, <0.015 μg/ml), amphotericin B (IC$_{100}$ value, 0.125 μg/ml), fluconazole (IC$_{50}$ value, 2 μg/ml), miconazole (IC$_{50}$ value, 0.125 μg/ml), micafungin (IC$_{50}$ value, <0.03 μg/ml), and voriconazole (IC$_{50}$ value, <0.03 μg/ml) (Table 3).

**Treatment and prognosis**

According to the results of morphological and physiological studies and homology searches for the ITS1 sequence, the isolate was identified as *M. gypseum* with the teleomorphic genotype *A. incurvatum*. The child was diagnosed with kerion celsi due to *A. incurvatum*. Treatment with 70 mg/day (5 mg·kg$^{-1}$·day$^{-1}$) oral itraconazole was initiated. The erythematous alopecic lesions with follicular pustules improved after two weeks. Hair growth from the follicle was then observed, and culture examination for the dermatophyte provided negative results six weeks after the initiation of treatment, after which the treatment was discontinued. The hair almost completely grew back after three months (Fig. 8). Finally, he was cured with no clinical or serological adverse effects.
Fig. 6 Alignment of ITS1 sequences of the *Microsporum gypseum* complex. The nine sequences determined in this study and twelve previously reported sequences of ITS1 rDNA were aligned using the Genetyx-Mac program (Genetyx Corp). ‘-’ indicates gaps added to permit alignment.
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**Discussion**

In the present study, we report the first documented case of infection by *A. incurvatum* outside Japan. We previously reported a case of kerion celsi caused by *A. gypseum* along with a review of 96 cases in Japan [4], none of which included any immigrants or visitors from abroad. The latter is probably the result of the fact that kerion celsi generally occurs in children, and foreigners residing in Japan for work are mostly adults. The clinical symptoms and the form of hair invasion observed in this case were typical of kerion celsi, and there were no differences among the teleomorphs. The present patient was successfully treated with itraconazole for six weeks without any side-effects.

Table 3  *In vitro* antifungal susceptibility testing of strain IFM 59508 to various antifungal agents.

<table>
<thead>
<tr>
<th>Antifungal Agent</th>
<th>MIC (amphotericin B) (μg/ml)</th>
<th>50% IC (others) value (μg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amphotericin B</td>
<td>0.125</td>
<td></td>
</tr>
<tr>
<td>Flucytosine</td>
<td>&gt;64</td>
<td></td>
</tr>
<tr>
<td>Fluconazole</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Itraconazole</td>
<td>&lt;0.015</td>
<td></td>
</tr>
<tr>
<td>Miconazole</td>
<td>0.125</td>
<td></td>
</tr>
<tr>
<td>Micafungin</td>
<td>&lt;0.03</td>
<td></td>
</tr>
<tr>
<td>Voriconazole</td>
<td>&lt;0.03</td>
<td></td>
</tr>
</tbody>
</table>

The 100% inhibitory concentration (IC<sub>100</sub>) (amphotericin B) and 50% inhibitory concentration (IC<sub>50</sub>) (others) values of the antifungal agents against the present isolate were determined after incubation for 96 hours at 30°C using a microbroth dilution kit for Yeast-like Fungi DP Eiken trays (Eiken Chemical Co. Ltd).

Fig. 7 The neighbor-joining (NJ) tree based on ITS1 sequences in the rRNA gene from the present isolate and twelve related strains. *Epidermophyton floccosum* was used as an out-group. The numbers above the branches indicate the percentage of bootstrap samplings. A, cluster of *Arthroderma incurvatum*; B, cluster of *Arthroderma fulvum*-*Arthroderma gypseum*; a, East Asia cluster; b, non-East Asia cluster; K nuc, thousands of nucleotides.

Fig. 8 Three months after the initiation of treatment, the lesion on the scalp disappeared, and the patient’s hair grew back almost completely.
the length:breadth ratio (Table 2), but were less than those reported by Stockdale [2]. Discriminating *A. incurvatum*, *A. gypseum*, and *A. fulvum* from the other forms of macroconidia has certain difficulties. Mating behavior is a biologically definitive factor for identification of a teleomorph, but is difficult to assess when tester strains are deteriorative. Interestingly, five strains of *M. gypseum* from Japan (Nagasaki, Amami and Okinawa), Brazil, and China (Peking) conserved at the Medical Mycology Research Center at Chiba University were reclassified as *A. incurvatum* after ITS rRNA sequence analysis [8], but not with mating tests.

The ITS1 rDNA sequences of dermatophytes are well known [11]. We analyzed the entire ITS region of eight *A. incurvatum* strains, i.e., four strains from Japan, two from UK, and two from Costa Rica (Table 1, Fig. 6). We observed that alignments of ITS1 sequences were the most effective method for differentiating among the three teleomorphs as was reported by Makimura [11,12]. Thus, we concluded that rapid and accurate identification of *A. incurvatum* could be achieved through estimating the form of macroconidia and by analyzing the ITS1 region.

This is the first reported case of infections caused by *A. incurvatum*, although such infections might have occurred in Japan. In 1971, Fujiyama isolated a strain of *A. incurvatum* from soil collected at Sendai, Japan [3]. It should be emphasized that *A. incurvatum* has been present in Japan for a long period, although the frequency of isolation is less than that of *A. gypseum*.

We compared the nucleotide sequence of ITS1 rDNA obtained from the present isolate, IFM 59508, with those of fourteen strains of *A. incurvatum*. Phylogenetic analysis using an NJ tree indicated that strains of *A. incurvatum* were divided into two clusters, i.e., the East Asia cluster, including strains from Japan and China (Fig. 7a), and the non-East Asia cluster, including strains from countries other than Japan and China (Fig. 7b). This suggests area-dependent genetic polymorphism. Thus, strains from Japan and the eastern part of China share a common region in the ITS1 rDNA sequence. The present patient’s infection was believed to have occurred in Sri Lanka which is supported by the fact that IFM 59508 belongs to the non-East Asia cluster, suggesting that the patient was infected outside Japan. No information is available regarding the non-East Asia cluster because few stock strains are present. If more strains from the western part of China, South and Southeast Asia, Oceania, the European Continent, North and South America, and Africa are analyzed, then additional information will be available regarding area-dependent genetic polymorphism.

Acknowledgements

We thank Prof Takashi Mochizuki and Dr Masako Kawasaki (Department of Dermatology, Kanazawa Medical University) for providing KMU 2981, 2982, 2986, and 2987. We also thank Dr Katsukiyo Yazawa and Ms Kiyomi Kagami (Medical Mycology Research Center, Chiba University) for technical advice and assistance.

Declaration of interest: The authors report no conflicts of interest. The authors alone are responsible for the content and the writing of the paper.

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This paper was first published online on Early Online on 26 March 2012.