Case Report

Nannizzia incurvata as a rare cause of favus and tinea corporis in Cambodia and Vietnam

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Abstract

Nannizzia (*N.*) *incurvata* (formerly *Microsporum incurvatum*) represents a geophilic dermatophyte which has been previously classified as belonging to the species complex of *N. gypsea* (formerly *Microsporum gypseum*). A 42-year-old Vietnamese female from Saxony, Germany, suffered from tinea corporis of the right buttock after she returned from a 2-week-visit to her homeland Vietnam. From skin scrapings of lesions, *N. incurvata* grew on Sabouraud's dextrose agar. Treatment by ciclopirox olamine cream twice daily for 4 weeks was successful. A 6-year-old Cambodian boy living near river Mekong with contact history to chicken, dogs and cattle suffered from tinea faciei and capitis. Symptoms of the favus-like tinea capitis and tinea faciei were erythema and scaly patches with areas of alopecia. *N. incurvata* grew on Sabouraud′ s dextrose agar. The boy was treated with oral terbinafine 125 mg daily, topical miconazole cream and ketoconazole shampoo. The symptoms healed within 4 weeks of treatment. Cultivation of the samples revealed growth of *N. incurvata*. For confirmation of species identification, the isolates were subject to sequencing of ITS (internal transcribed spacer) region of the rDNA, and addition of the "translation elongation factor 1 α" (TEF 1 α) gene. Sequencing of the ITS region showed 100% accordance with the sequence of *N. incurvata* deposited at the NCBI database under the accession number MF415405. *N. incurvata* is a rare, or might be underdiagnosed geophilic dermatophyte described in Sri Lanka and Vietnam until now. This is the first isolation of *N. incurvata* in Cambodia, and the first description of favus in a child due to this dermatophyte.

Key words: Dermatophyte, DNA-sequencing, terbinafine, tinea capitis, tinea faciei

Introduction

Nannizzia (*N.*) *incurvata* (formerly *Microsporum* [*M.*] *incurvatum*) represents a geophilic dermatophyte previously classified as belonging to the species complex of *N. gypsea* (formerly *M. gypsea*). According to the new taxonomy of dermatophytes, *N. incurvata* has to be considered as a separate species.[1] Due to its geophilic character and origin, this fungus can be transmitted from soil to human beings causing tinea corporis and tinea manuum. Until now, there are only few descriptions of infections in animals and humans.[2] Two patients with an infection caused by *N. incurvata* are presented in this study. Both *N. incurvata* strains were identified microscopically by culture and by Sanger sequencing of the internal transcribed spacer (ITS) region of the rDNA, and addition of the "translation elongation factor 1 α" (TEF 1 α) gene.

Case Reports

Patient 1

History and clinical picture

A 42-year old Vietnamese female from Saxony, Germany, suffered from tinea corporis of the right buttock after she

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returned from a 2-week visit to her homeland Vietnam. Annular scaly dry erythematous lesions appeared in the region of the right hip [Figure 1a]. Family members of the woman were not affected.

Mycological diagnostics

From skin scrapings of the centrifugal lesions, the cultures on Sabouraud's dextrose agar with and without cycloheximide (Actidion®), fast-growing fungal colonies developed. The thallus of the dermatophyte was flat, fast-growing, initially white, but quickly becoming beige to cinnamon brown stained [Figure 1b]. The surface appeared powdery with spreading peripheral hyphae bundles, while the reverse was yellowish-brown stained [Figure 1c]. Microscopically, a multitude of spindle-like (ellipsoid), relatively short (30–50 µm long), thick-walled, echinulate macroconidia with half-round tops were seen [Figure 1d]. They were present in bundles at the end of hyphae or also as single macroconidia at the end of a conidiophore. An abundance of macroconidia were located separately on all mycelia. Clavulate and sessile microconidia together with single chlamydospores extend alongside the hyaline septate hyphae [Figure 1e]. Based on these macro-morphological and microscopic features, the geophilic dermatophyte species *N. gypseum* was suspected. Other members of the so-called *N. gypsea*-complex, however, were considered as differential diagnoses, e. g., *N. fulva* and *N. incurvata*.

Figure 1a: Tinea corporis due to *Nannizzia incurvata* in a 42-year-old woman after a journey to Vietnam. Centrifugal erythematosquamous lesion in the region of the right hip

Figure 1b: Tinea corporis due to *Nannizzia incurvata* in a 42-year-old woman after a journey to Vietnam. *Nannizzia incurvata* isolated from the skin lesion of the aforementioned patient

Treatment

There was no improvement after treatment with clotrimazole + betamethasone dipropionate ointment (due to wrong diagnosis as a psoriasiform inflammatory dermatosis), ciclopirox olamine cream, and mometasone furoate. After changing to ciclopirox olamine cream monotherapy twice daily for 4 weeks, the tinea corporis lesions healed.

Patient 2

History and clinical picture

A 6-year old Cambodian boy living near river Mekong (water and forest region) at a village with history of contact with chicken, dogs and cattle suffered from tinea faciei and tinea capitis. Family members were not affected. The disease started at an age of four years. Initial lesion was on the scalp, later extended to the face [Figure 2a]. Symptoms of the favus-like tinea capitis and tinea faciei were erythema, scaly patches with areas of alopecia, but no pustules [Figure 2b]. Main complaints were pruritus and alopecia. The child was treated first by a pediatrician for 12 weeks with griseofulvin 250 mg once daily, but without improvement. Hepatitis B and C and HIV tests were negative. In *Microsporum* or *Nannizzia* species, a typical green-yellowish fluorescence should be expected. From skin scrapings, *N. incurvata* grew on Sabouraud′s dextrose agar. Later on, at the department of dermatology in Phnom Penh, Cambodia, the boy received oral terbinafine 125 mg daily, topical miconazole, ketoconazole

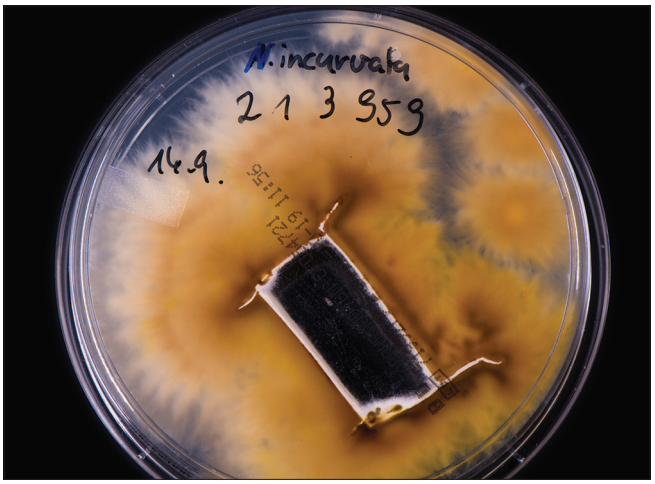

Figure 1c: Tinea corporis due to *Nannizzia incurvata* in a 42-year-old woman after a journey to Vietnam. Reverse side of the colonies

shampoo, oral cetirizine and multivitamin preparations. The symptoms improved after 2 weeks of treatment. Treatment was continued for 1 month.

Molecular identification of the dermatophyte species

Molecular biological diagnostics

For confirmation of the suspected dermatophyte species, sequencing of the ribosomal DNA (rDNA), mainly the regions ITS 1, 5.8S rRNA, ITS 2 and the translation elongation factor (TEF)-1 α gene were done for both strains as described[3-5] to identify dermatophytes at a species level via the extracted DNA from fungal cultures. This required PCR amplification of a ~ 900 bp DNA fragment using universal primers that bind to flanking pan-fungal sequence regions. The following gene sequences were used as probes for sequencing of the ITS region of the rDNA:

V9G 5'-TTACGTCCCTGCCCTTTGTA-3' and LSU266 5'-GCATTCCCAAACAACTCGACTC-3'.

The length of the analyzed region in the TEF-1 α gene varied from 709 to 769 nucleotides among the various dermatophyte species. Primers EF1a-F 5'CACATTAACTTGGTCGTTATCG 3' and EF1a-R 5'CATCCTTGGAGATACCAGC3' were used for sequencing.[3]

The sequence of each strain was compared to sequences of type strains from the databases. Based on the principle of similarity search (BLASTn search), individual strains were identified down to the species level by utilizing the validated Online Dermatophyte Database of the Westerdijk Fungal Biodiversity Institute (formerly Centraalbureau voor Schimmelcultures CBS), Utrecht, The Netherlands ("www.westerdijkinstitute.nl"). In addition, we compared sequences of our samples with those contained in the very comprehensive database of the National Centre for Biotechnology Information (NCBI) in Bethesda, Maryland, USA.

In the ITS region, 100% accordance was found with the reference sequence of *N. incurvata* deposited at the Database of the NCBI under the Accession number MF415405 and MF415404, respectively. For the TEF 1 α gene, the sequence KM678069 of *N. incurvata* is available. Due to several revisions of previously submitted sequences, this sequence, however, is deposited under the wrong species name *N. gypsea*. The phylogenetic analysis of the strains based on ITS region of the rDNA and of the TEF 1 α-gene in a dendrogram allows genetic differentiation between *N. incurvata* and the close related species *N. gypsea* and *N. fulvum* [Figure 3a and b].

Deposition of the isolates in strain collections and gene databases

Both ITS and TEF1 α gene sequences of the two strains are deposited at the NCBI database, the ISHAM-ITS-Database and at the "Fungal MLST Database." The strains were deposited at the German Collection of Microorganisms and Cell Cultures (DSMZ, Braunschweig, Germany).

NCBI deposition: ITS region *N. incurvata* strain 213959/2017 (patient 1)—NCBI accession number MF 415405.1. ITS region *N. incurvata* strain 211859/2017 (patient 2)—NCBI accession number MF415404.1.

Deposition at the culture collection DSMZ in Braunschweig, Germany: *N. incurvata* strain 213959/2017 (patient 1)—

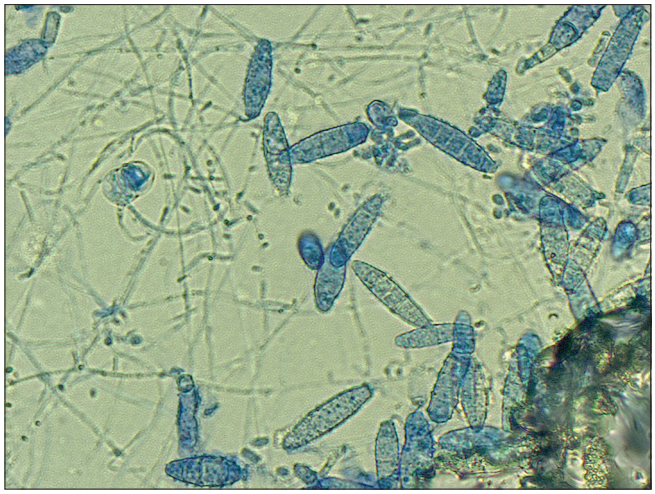

Figure 1d: Tinea corporis due to *Nannizzia incurvata* in a 42-year-old woman after a journey to Vietnam. Microscopically, a multitude of spindle formed (ellipsoid), relatively short (30–50 µm long), thick walled, echinulate macroconidia with half round tops were seen. Clavulate and sessile microconidia alongside the hyaline septate hyphae (Lactophenol cotton blue preparation)

Figure 1e: Macroconidia together with single big chlamydospore (Lactophenol cotton blue preparation)

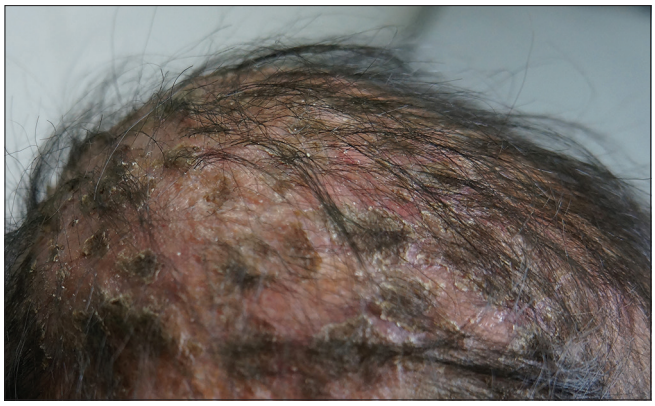

Figure 2a: Tinea capitis and tinea faciei in a 6-year-old Cambodian boy living in a rural region of the country.Favus-like tinea capitis with erythema, scaly patches within huge areas of alopecia

DSM106637. *N. incurvata* strain 211859/2017 (patient 2)—DSM106636.

Sequencing of the TEF1-α gene: For strain 213959/2017, no cluster to compare was available. Strain 211859/2017 clustered with KM678105.1 from the NCBI database. Note: Due to the new taxonomy and nomenclature change, KM678105.1 was first assigned as *N. gypsea*, but has to be considered now as *N. incurvata*.

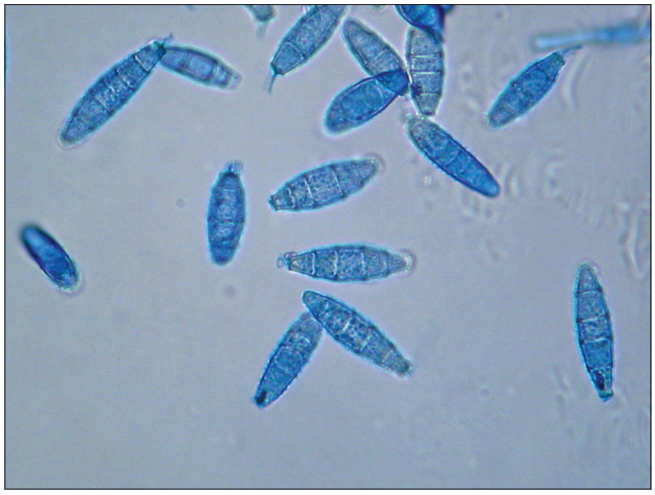

Figure 2c: Tinea capitis and tinea faciei in a 6-year-old Cambodian boy living in a rural region of the country. Spindle formed (ellipsoid) macroconidia with half-round tops. Lactophenol cotton blue preparation

Discussion

Taxonomy of *Nannizzia incurvata*

N. incurvata represents a rare geophilic dermatophyte, here isolated from two patients from Vietnam and Cambodia. *N. incurvata* was first described in 1961 by Stockdale, at that time under the species name *M. incurvatum*.[6] In 1963, *N. incurvata* was referred as belonging to the so called *N. gypsea* (formerly *M. gypseum*) complex, which included besides *N. gypsea* (formerly *M. gypseum*) the other morphologically closely related geophilic species *N. fulva* (formerly *M. fulvum*) and *N. incurvata*.[7,8]

Figure 2b: Tinea capitis and tinea faciei in a 6-year-old Cambodian boy living in a rural region of the country. Tinea faciei with hyperkeratotic, papular and dry squamous lesions in the central parts of the face

Figure 2d: Tinea capitis and tinea faciei in a 6-year-old Cambodian boy living in a rural region of the country. Clavulate microconidia at the hyphae. Lactophenol cotton blue preparation

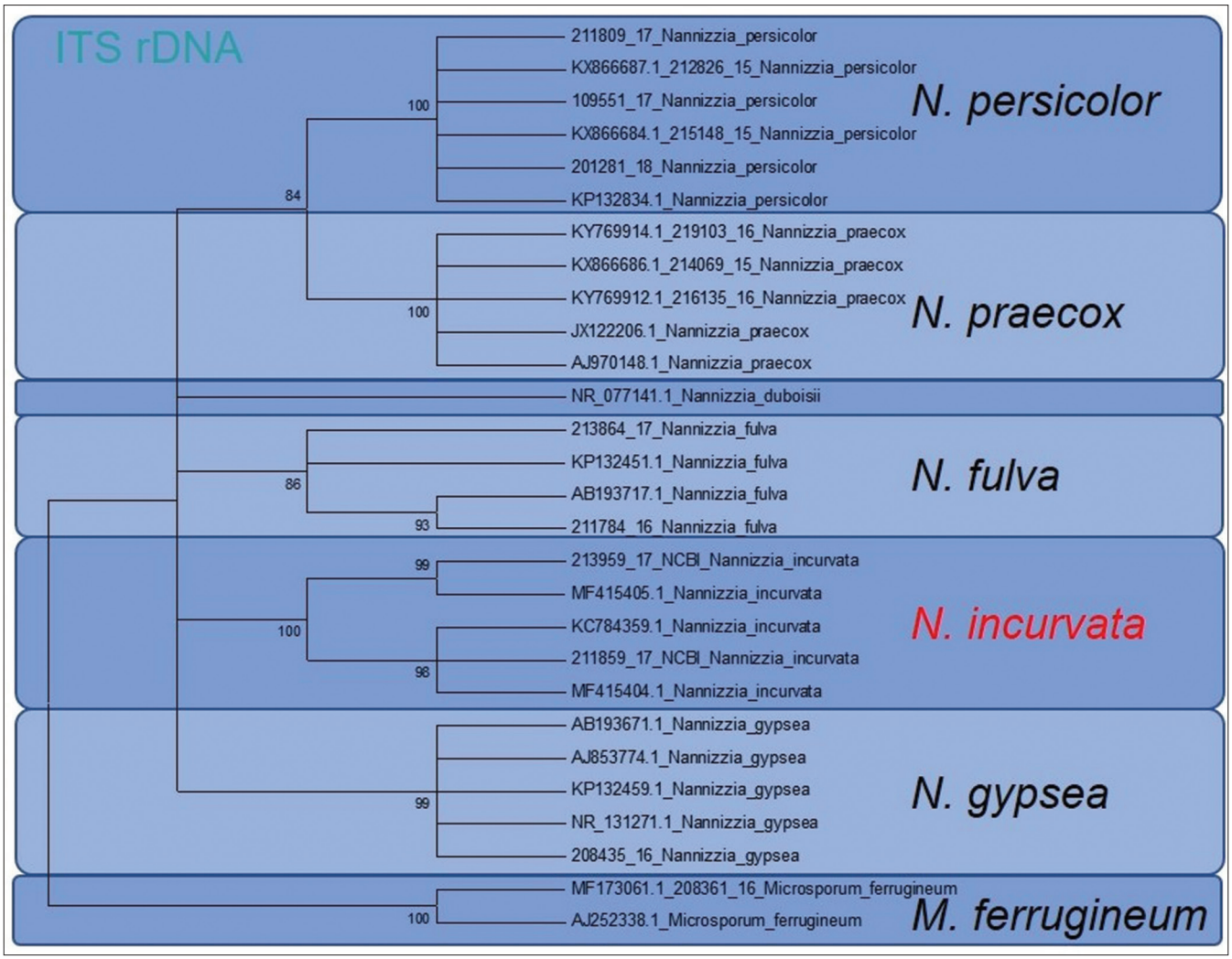

Figure 3a: Phylogenetic analysis of both reported patients isolated *Nannizzia incurvata* strains. Based on ITS region of the rDNA, the dendrogram showed clear genetic differentiation between *Nannizzia incurvata* and the closely related species *Nannizzia gypsea* and *Nannizzia fulva*

Now, according to the new taxonomy, the genus *Nannizzia* has 10 species: *N. aenygmaticum*, *N. corniculata*, *N. duboisii*, *N. fulva*, *N. gypsea*, *N. incurvata*, *N. nana*, *N. persicolor*, *N. praecox* and *N. perplicata*.[1,9]

According to the recently published molecular-based new taxonomy of dermatophytes, *N. incurvata* is considered as a separate geophilic species within the clade C: *Nannizzia*. Although rare, or underdiagnosed, this dermatophyte can also cause human infections.[1] This is the first isolation of *N. incurvata* in Cambodia, and the first description of favus in a child due to this dermatophyte.

Morphologic features of *Nannizzia incurvata*

Colonies of the dermatophytes belonging to the genus *Nannizzia* are described in the review of De Hoog *et al*. as mostly cottony to powdery, whitish to brown, with a cream-colored, brown or red pigmentation of the surface.[1] Hyphae are thin-walled and hyaline. Sexual states of *Nannizzia* were described, after mating, like with *Arthroderma* genus.[10,11] First impression of the fast-growing dermatophyte *N. incurvata* on Sabouraud′ s dextrose agar is that the bright brown to cinnamon brown stained granular to powdery appearing colonies resemble the species *N. gypsea*. Morphologically, it is difficult or even impossible to differentiate *N. gypsea* from *N. incurvata*. The reverse side of the colonies was yellowish bright brown, but also a reddish yellow to wine red staining is possible. The history of a journey to or living in Southeast Asia, in particular, the region of Vietnam and neighboring countries, should be a reason to look more deeper for this species, not the least to use molecular methods of sequencing for exact species identification. Microscopically, clavate, thin-walled sessile microconidia located alongside hyphae are seen [Figure 2 d].[2] Macroconidia are described as abundant, echinulate, thick-walled, ellipsoid, (3-) 4-6(-9)- and septate, 8–12 × 30–50 µm [Figure 2 c].

Geographic distribution of dermatophytoses due to *Nannizzia incurvata*

Infections due to the ubiquitous dermatophyte *N. gypsea* occur worldwide. Tinea capitis and tinea corporis due to *N. gypsea* are common and were reported from a

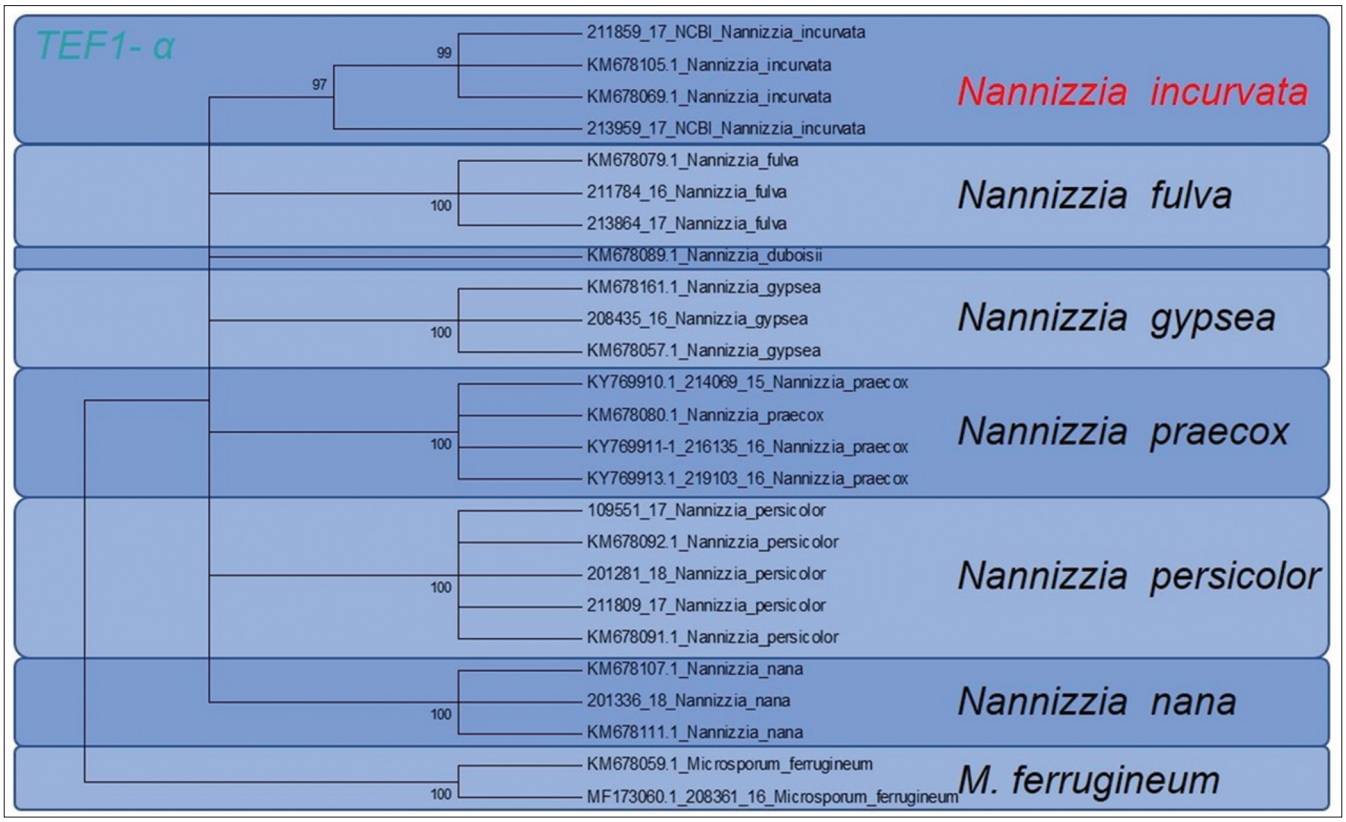

Figure 3b: Phylogenetic analysis of both reported patients isolated *Nannizzia incurvata* strains. Sequencing of the translation elongation factor 1 α-gene allowed clear phylogenetic distinction between *Nannizzia incurvata* strains and the closely related species *N. gypsea* and *Nannizzia fulva*

multitude of countries.[12,13] In Sri Lanka, *N. gypsea* was the commonest fungus isolated from children.[14] Contrary to that, dermatophytoses due to *N. incurvata* are rarely reported; they were described in a few distinct countries only. The latest report originated from Southeast Asia, Vietnam.[15] In Taiwan, *N. incurvata* was found to be cause of cat favus in four animals.[16] In Brazil, in 692 soil samples, the formerly two teleomorphic species of *M. gypseum* (*Arthroderma* [*A.*] *gypseum* and *A. incurvatum*) were isolated in approximately 19% samples.[17] The enzymatic activities (expression of keratinase and elastase) of these geophilic dermatophytes may play an important role in the pathogenicity and underlines the probable adaptation of this fungus to the animal parasitism. Using the phenotypical and molecular analysis, *Microsporum* identification and their teleomorphic states will provide a useful and reliable identification system.

In 2012, *N. incurvata* was isolated from a Sri Lankan child residing in Japan and suffering from favus.[18] Other countries, where *N. incurvata* was described in the past, were the US,[19] Japan[20-23] and France.[24] Rezaei-Matehkolaei *et al*.[25] reported a 4-year-old Iranian boy who developed erythematous, itchy and annular lesion on his face. The etiological agent was *M. gypseum* identified based on its morphologic features. However, ITS sequencing of the DNA revealed that the isolate showed 98% homology to *M. incurvatum* strain CBS 172.64 (re-classified as *N. incurvata*). Recently, tinea corporis presenting as disseminated verrucous plaques caused by *A. incurvatum* (re-classified as *N. incurvata*) in a young Indian boy was published.[26]

Molecular identification of *Nannizzia incurvata* by sequencing of the rDNA

Garcia *et al.* from Brazil found that several dermatophyte species have a full-length PRP8 intein with a homing endonuclease belonging to the family LAGLIDADG, which is a powerful additional tool for identifying and classifying dermatophytes.[27] Phylogenetically confirmed *Epidermophyton floccosum* was in the same clade as the *Arthroderma gypseum* complex, *M. audouinii* was close to *M. canis*, which allowed differentiating *A. gypseum* (*N. gypsea*) from *A. incurvatum*.

Here, sequencing of the ITS1-region of the rDNA enabled a clear distinction between *N. incurvata* and the other closely related *Nannizzia* species *N. gypsea* and *N. fulva*, but also *N. praecox* and *N. persicolor* [Figure 3a]. The same was found by sequencing the TEF 1 α gene [Figure 3b].

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Declaration of patient consent

The authors certify that they have obtained all appropriate patient consent forms. In the form, the patients have given their consent for their images and other clinical information to be reported in the journal. The patients understand that their names and initials will not be published and due efforts will be made to conceal their identity, but anonymity cannot be guaranteed.

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Conflicts of interest

There are no conflicts of interest.

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