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. gypseum). 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 female from Saxony, Germany, suffered from tinea corporis of the right buttock after she 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 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 septated 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. gypseum-complex, however, were considered as differential diagnoses, e.g., N. fulva and N. incurvata.

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 change 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 to 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].

Figure 1a: Tinea corporis due to Nannizzia incurvata in a 42-year-old woman after a journey to Vietnam. Centrifugal erythematousquamous 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 afore mentioned patient.

Figure 1c: Tinea corporis due to Nannizzia incurvata in a 42-year-old woman after a journey to Vietnam. Reverse side of the colonies.
Main complaints were pruritus and alopecia. The child was treated first by 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 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 described3-5 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’-TTACGTCCCTGGCCCTTTGTA-3’ and LSU266 5’-GCATTCGCAAACAACACTGACTC-3’.

The length of the analyzed region in the TEF-1 α gene varied from 709 to 769 nucleotides among the various dermatophyte species. PrimersEF1a-F 5’-CACATTAACTTTGCTGTATCG 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 itself 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

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**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 septated hyphae (Lactophenol cotton blue preparation).

**Figure 1e:** Macroconidia together with single big chlamydospores (Lactophenol cotton blue preparation).
Discrimination

Taxonomy of Nannizzia incurvata

N. incurvata represents a rare geophilic dermatophyte, here isolated from two patients from Vietnam and Cambodia.

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.

Deposition at the culture collection DSMZ in Braunschweig, Germany: N. incurvata strain 213959/2017 (patient 1)—DSM106637. N. incurvata strain 211859/2017 (patient 2)—DSM106636.

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

Discussion

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415405.1. ITS region N. incurvata strain 211859/2017 (patient 2)—NCBI accession number MF415404.1.
**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. Hyphae are thin-walled and hyaline. Sexual states of *Nannizzia* were described, after mating, like with the *Arthroderma* genus. 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

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*N. incurvata* was first described in 1961 by Stockdale, at that time under the species name *M. incurvatum*. 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*. 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*. 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. This is the first isolation of *N. incurvata* in Cambodia, and the first description of favus in a child due to this dermatophyte.
Nannizzia incurvata in Cambodia and Vietnam

Figure 3b: Phylogenetic analysis of both from the here 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 close related species N. gypseae and Nannizzia fulva.

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. gypseae occur worldwide. Tinea capitis and tinea corporis due to N. gypseae are common and were reported from a multitude of countries.12,13 In Sri Lanka, N. gypseae 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 Japan20-23 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. gypseae) 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. perlicata* [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.

**References**


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