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Fungal melanonychia caused by *Onychocola canadensis*: first records of nail infections due to *Onychocola* in the Czech Republic

DAVID STUCHLÍK[1], KAREL MENCL[2], VÍT HUBKA[3*], MAGDALENA SKOŘEPOVÁ[4]

[1]Regional Hospital Pardubice, Department of Dermatology, Czech Republic
[2]Regional Hospital Pardubice, Department of Clinical Microbiology, Czech Republic
[3]Charles University in Prague, Faculty of Science, Department of Botany, Benátska 2, 128 01 Praha 2, Czech Republic;
vit.hubka@seznam.cz
[4]Charles University in Prague, First Faculty of Medicine, Dermatological Clinic, Czech Republic
*corresponding author

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Onychocola canadensis is a non-dermatophyte filamentous fungus with an unusual ecology. Hitherto, *O. canadensis* has been isolated only from human nails and skin, although attempts to isolate it from the environment have been unsuccessful. We describe two new cases of onychomycosis caused by *O. canadensis* with dissimilar clinical appearance. The first infection manifested itself as distal and lateral onycholysis with conspicuous black pigmentation. As far as we know, this is the first description of *O. canadensis* onychomycosis in the Czech Republic. In connection with this case, the authors emphasise the importance of mycological laboratory examination of dark nail lesions. Based on photodocumentation, a second case of onychomycosis due to *O. canadensis* was identified retrospectively. This case manifested itself as distal and lateral subungual onychomycosis with yellow discoloration, which is more typical of *O. canadensis* onychomycosis. Morphological characteristics important for discrimination of *O. canadensis* from other medically important fungi are discussed.

Key words: onychomycosis, *Arachnomyces nodosetosus*, fungal infection, elderly people, soil fungi.

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Onychocola canadensis je nedermatofytická vláknitá houba s neobvyklou ekologií. Dosud byla *O. canadensis* izolována pouze z lidských nehtů a kůže. Pokusy o izolaci houby z jiných substrátů byly neúspěšné. V následujícím textu popisujeme dva nové případy onychomykózy způsobené *O. canadensis* s rozdílnými klinickými projevy. První případ se manifestoval jako distální a laterální onycholýza s nápadně černou diskolorací. V souvislosti s tímto případem autoři zdůrazňují důležitost mykologického vyšetření tmavě zbarvených nehtových lézí. Pokud víme, je tento případ vůbec prvním případem onychomykózy způsobené *O. canadensis* v České republice. Druhý případ onychomykózy způsobené *O. canadensis* byl zpětně identifikován na základě fotodokumentace. Postižený nehet se manifestoval jako distální a laterální subunguální onychomykóza se žlutou diskolorací. Tento fenotyp je více příznačný pro onychomykózu způsobenou *O. canadensis*. Dále jsou diskutovány morfologické znaky důležité pro odlišení *O. canadensis* od jiných lékařsky významných hub.

INTRODUCTION

Onychocola canadensis is a non-dermatophyte agent of onychomycosis. It is a relatively new species of fungus that occurs mainly in temperate climates and is probably associated with soil. This fungus was described based on three isolates from Canadian cases of onychomycosis (Sigler & Congly 1990). Since then, cases in New Zealand (Sigler et al. 1994), France (Contet-Audonneau et al. 1997, Koenig et al. 1997), Great Britain (Campbell et al. 1997, O´Donoghue et al. 2003), Italy (Fanti et al. 2003), Belgium (Esbroeck et al. 2003, Sijs et al. 2007) and Spain (Torres-Sangiao et al. 2006) have been reported. Recently, four cases were reported from Slovakia (Volleková & Lisalová 2009). It typically affects elderly gardeners and farmers. The mean age of patients in the previously described cases is almost 70. Most of the cases are described as distal and lateral subungual onychomycosis or white superficial onychomycosis similar to the appearance that might be expected with dermatophytes or other non-dermatophyte moulds such as *Acremonium* spp. Association with melanonychia has as yet been mentioned only in a case described by Gupta et al. (1998).

The description of *Arachnomyces nodosetosus* – teleomorphic stage of *O. canadensis* (Sigler et al. 1994) was followed by several taxonomical studies focused on the position of the genus *Arachnomyces* and its anamorph *Onychocola* (5 species described to date) within the Ascomycota (Gibas et al. 2002a, Gibas et al. 2002b, Gibas et al. 2004). It resulted in the creation of the new order *Arachnomycetales* within the *Eurotiomycetes* and description of several new species of the genus *Arachnomyces*. Except of *A. nodosetosus*, there are reports about several cases of isolation of three other *Arachnomyces* species and *O. sclerotica* (teleomorph not described) from nails or skin generally of unclear etiological significance (Sigler et al. 1994, Gibas et al. 2002a, Gibas et al. 2002b, Gibas et al. 2004).

MATERIAL AND METHODS

Patient 1. A 51-year-old woman diagnosed with onychodystrophy and black discoloration of the right great toenail of several years' duration (Fig. 1B). She reported no recent progression of the lesion. Due to the long time lapse she could not recall the circumstances that led to the development of the lesion. She recalled no major trauma of the nail but minor repeated traumatisation of the distal nail plate could not be excluded. There was no history of foreign travels except for a holiday in Spain in summer 1997. She used to work in her garden. The family history and personal history of the patient were insignificant.

The toenail showed partial onycholysis, the affected part was thickened and dystrophic. Conspicuous black discoloration was present (Fig. 1B). Because of melanonychia it was crucial to exclude subungual melanoma. Another differential diagnosis was subungual haematoma.

Patient 2. The second patient was an otherwise healthy 68-year-old woman. The patient described progressive discoloration of the left great toenail during the last year. She reported that she often took part in all-day hiking trips. She remembered a trauma of the nail due to tight shoes a year ago. A short time after the trauma, slight discoloration appeared. No history of foreign travels was reported.

The affected nail was raised by hyperkeratosis, friable at the distal border, and demonstrated yellow discoloration (Figs. 1G, H).

Laboratory investigations. Specimens for mycological examination (KOH mount and cultivation) were collected by scraping off the nail plate and observed microscopically in 20 % KOH. Nail fragments were inoculated on slants of Sabouraud glucose agar (SGA) with and without chloramphenicol (Bio-Rad) and incubated at 27 °C.

The macromorphology of the subcultures was observed on a set of media (the producer of the commercial media is referred to in parenthesis, all other media were prepared according to Atlas 1997): MEA (malt extract agar, Oxoid), SGA (Carl Roth), PDA (potato dextrose agar), OA (oatmeal agar), CY20S (Czapek yeast extract sucrose 20 % agar), CDA (Czapek dox agar), PCA (potato carrot agar) and SNA (Synthetic nutrient agar). Cultures grew at a temperature of 25 °C. Growth at a temperature of 37 °C was also tested.

Lactophenol cotton blue wet mount preparations from subcultures growing at 25 °C were prepared at weekly intervals for a period of 8 weeks. Photographs were taken on an Olympus BX-51 microscope using Nomarski contrast or phase contrast.

DNA analysis. Genomic DNA was isolated from 14-day-old cultures using the Microbial DNA Isolation Kit (MoBio Laboratories, Inc.). The region including ITS1, 5.8S r DNA and ITS2 was amplified using primers ITS5 (White et al. 1990) and ITS4S (Kretzer et al. 1996). The SSU rDNA was amplified using the NS1 (White et al. 1990) and NS24 (Gargas & Taylor 1992) primer set. A partial sequence of LSU rDNA was amplified with the NL1 (O'Donnell 1993) and LR6 (Vilgalys & Hester 1990) primer sets. PCR amplification was performed using the same cycling conditions for all three rDNA regions and followed procedures described in Kolařík et al. (2004). PCR product purification and sequencing was carried out at Macrogen Inc. (Seoul, South Korea). Using BLAST similarity search, the obtained sequences were compared with sequences originating from taxonomical monographs deposited on the GenBank server.

RESULTS

Laboratory investigations

Microscopic examination of the nail scrapings in KOH showed septate branched hyphae and masses of arthroconidia (Figs. 1A, C, F). In both mentioned cases slow growth of a pure culture of a non-dermatophyte hyphomycete was observed on SGA with chloramphenicol (Figs. 1D, E). After 3 weeks at 27 °C the colonies were restricted, initially greyish white but gradually darkening. The colony reverse was dark grey-brown to black. After 6 weeks the colonies in the prime culture were almost brown-black. This pigmentation was less accented in the subcultures. The same fungus was isolated and observed in direct microscopy during a subsequent visit.

Therapy

In case 1, an ointment containing 40 % urea without antifungals was applied for atraumatic nail avulsion. When the patient returned after 3 weeks for debridement of the softened nail plate, and a third specimen was collected from the subungual debris. The same fungus grew again in pure culture. The epidermis of the nail bed showed no melanin pigmentation so that subungual melanoma and haematoma could be excluded. Topical antifungal therapy with a 1 % ciclopirox solution was applied and the diseased nail grew out gradually. After nine months of therapy, clinical appearance of the nail was normal and mycological control was negative.

In the second case, a daily oral dose of terbinafine (250 mg) was administered for three months. After 10 months, the nail plates exhibited healthy growth. Mycological examination was negative.

Macro– and micromorphology of subcultures

Growth on all tested media was slow. Colony diameters reached about 10 mm or less after 3 weeks at 25 °C. Growth was even more restricted at 37 °C. On MEA (Fig. 2C), SAB (Fig. 1E) and PDA (Fig. 2B), the colonies were initially glabrous and became velvety or tomentose after 2 weeks due to aerial hyphae that grew on surface of the colonies. The margin of the colonies was irregularly lobate and the height of the colonies increased. They became conical and radially wrinkled. The colour was white, grey-white or yellow-white, old colonies acquired a brownish colour through brown hyphae that had appeared in culture after some weeks. The colour of the reverse was brownish and became dark-brown after some weeks. Submerged mycelium with radial arrangement was a dominant component on nutrient-weak media (PCA, SNA) and CDA. Colonies on OA showed yeast-like

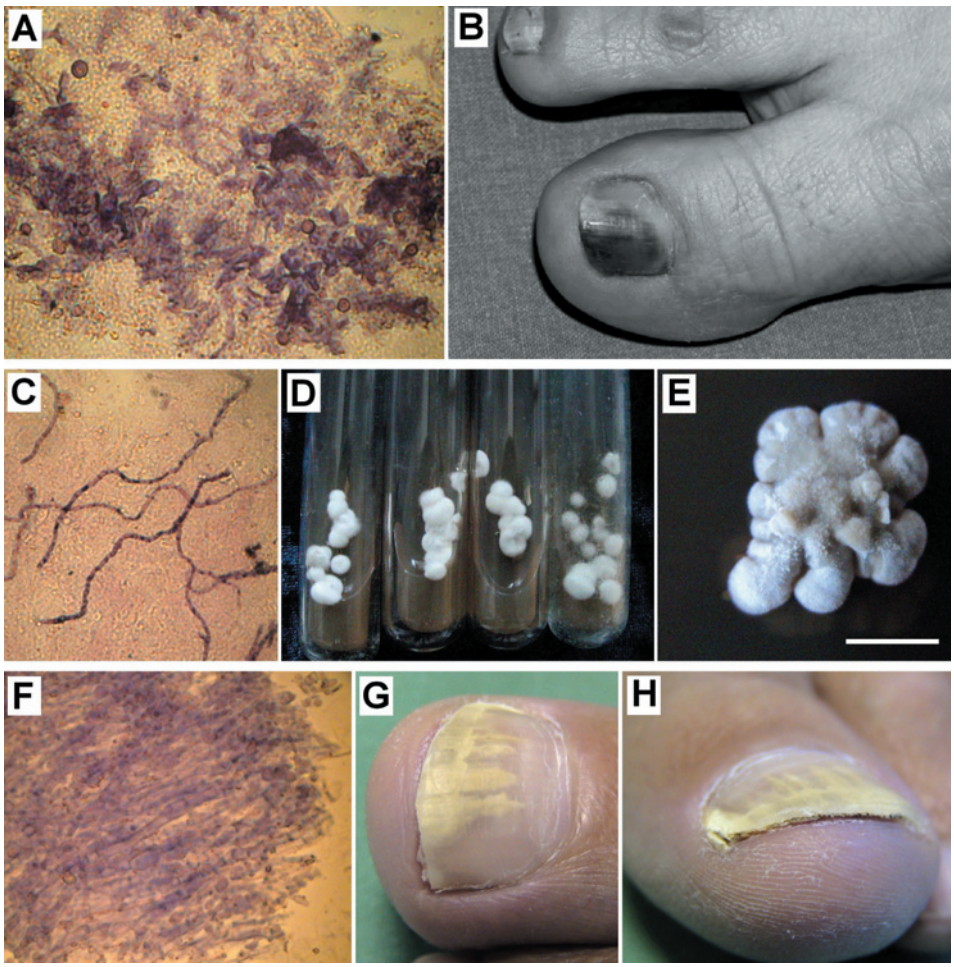

Fig. 1. Onychomycosis by *Onychocola canadensis*. Distal and lateral onycholysis with dark discoloration of the nail (B). Colonies on slants of SGA (D) and on an SGA plate after 21 days (E, scale bar 5 mm). Septate hyphae and arthroconidia in nail debris. KOH mount stained with Parker ink (A, C, F). Affected nail (case 2) raised by hyperkeratosis, friable at the distal border, with yellow discoloration (G, H).

growth and remained glabrous. The most abundant aerial mycelium was developed on CY20S (Fig. 2D).

Micromorphological features were described from MEA, SGA and PDA, on which essentially the same image was observed and all typical morphological characters were well developed. At first, the young vegetative hyphae were smooth, hyaline and up to 2.5 µm wide. Finally, they became roughly verrucose (Fig. 2E). Fertile hyphae were poorly differentiated from vegetative mycelium.

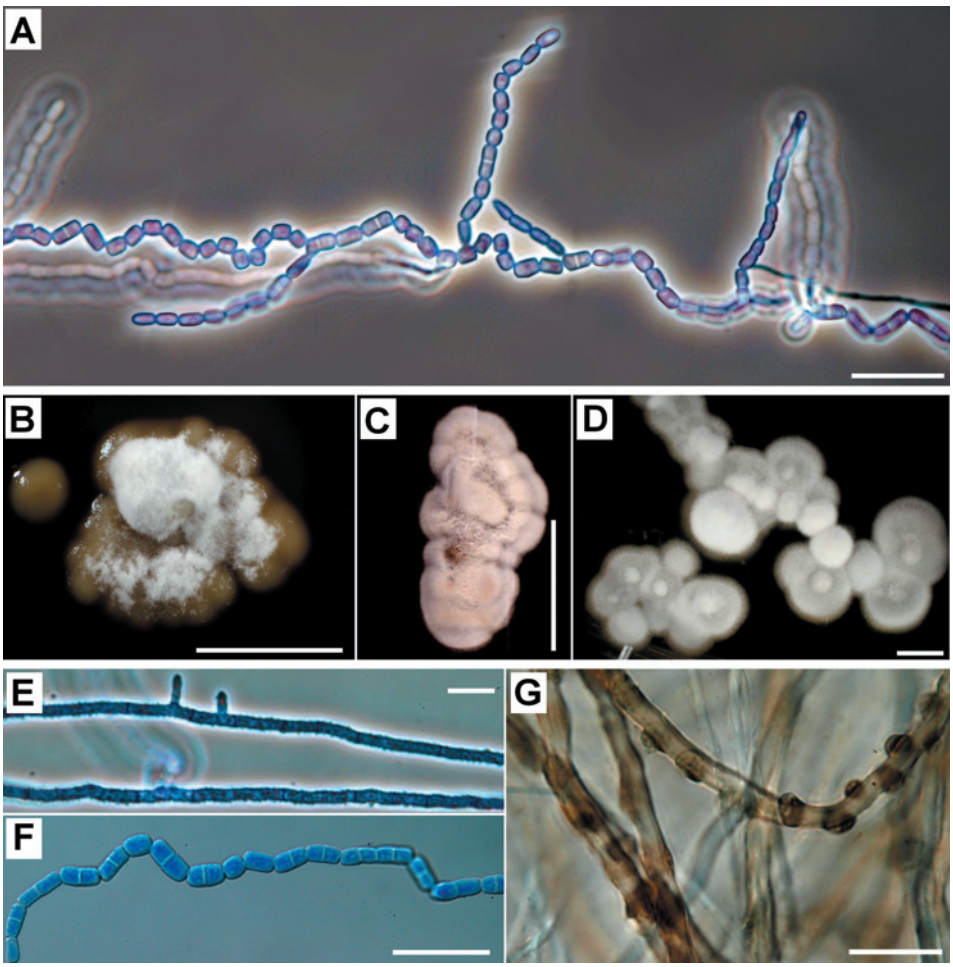

Fig. 2. *Onychocola canadensis*. Fertile hyphae consisting of elliptic arthroconidia seceded by fracture (A, F, scale bar 20 µm). Hyaline, rough-walled hyphae (E, scale bar 10 µm) and pigmented hyphae with dark brown nodosities (G, scale bar 10 µm). Colony detail on PDA (B), MEA (C) and CY20S (D) after five weeks at 25 °C; scale bar 5 mm.

Arthroconidia were profusely present in culture after 3–4 weeks of cultivation. They were hyaline, smooth and ellipsoidal with 1 septum or non-septate (Fig. 2A, F). Conidia were released intercalarily or terminally due to constriction of the septum or due to rhexolysis. They often persisted in chains. Brown, infrequently septate hyphae (up to 6 µm wide) were present mainly in old cultures (Fig. 2G). Dark brown nodosities occurred abundantly on their walls.

The case 1 isolate is maintained in the Culture Collection of Fungi (CCF), Department of Botany, Charles University in Prague, as CCF 3957. The case isolate from the second report was identified retrospectively. This identification was possible thanks to accurate photo-documentation of colony morphology and microscopic preparations of the isolate. However, we were not able to revive the isolate.

Molecular data analysis

The morphological identification was supported by rDNA sequence data. The sequence obtained from ITS region (602 bp) showed 100 % similarity to the type strain of *A. nodosetosus* UAMH 5344 (Gibas et al. 2004). The SSU rDNA sequence (814 bp) was 100 % similar to *A. nodosetosus* strain UAMH 6106 (Gibas et al. 2002a). The partial LSU (798 bp) sequence was 100 % similar to *A. nodosetosus* strain CBS 313.90 (Sugiyama and Mikawa 2001).

The sequences were deposited in the GenBank database under accession numbers HM205102–HM205104.

DISCUSSION

To our knowledge, isolation of *O. canadensis* has not yet been reported from the Czech Republic. Four cases have been recently reported from Slovakia (Volleková & Lisalová 2009).

Onychocola canadensis is a fungus with an unusual ecology. Based on published data, *O. canadensis* has been isolated only from clinical material and has been reported from countries located in the temperate climate zone. However, there are some omissions in this distribution. In particular, *O. canadensis* has not been recorded from the USA.

Onychomycosis due to *O. canadensis* manifests itself mostly as distal and lateral onychomycosis or white superficial onychomycosis. Onychomycosis associated with brown-black nail discoloration (melanonychia, patient 1) is unusual and has been reported only in one case by Gupta et al. (1998). It is necessary to exclude subungual melanoma and subungual haematoma in a differential diagnosis.

O. canadensis has been isolated more frequently in elderly females. The patients often worked as gardeners or farmers. This suggests probable infection from soil (Gupta et al. 1998). This is in agreement with two new reports presented here.

A number of morphological features such as growth parameters, colony morphology and micromorphology make the fungus practically unmistakable. On the other hand, the rare isolation of *O. canadensis* (only dozens of cases worldwide so far) makes its determination nontrivial. The most important features in identifying the fungus are growth parameters, presence of elliptic arthroconidia in

chains together with rough vegetative hyphae and also brown hyphae with dark superficial deformities. All important micro-morphological features (including arthroconidial chains and brown hyphae) develop well in 4–6 week-old cultures. Examination of cultures older than 4 weeks growing on solid media such as SAB, MEA and PDA at 25 °C is likely to lead to a correct determination.

The culture morphology and microscopic appearance before the third week of cultivation may imitate *Trichophyton rubrum* and this might cause misidentification. Conversely, atypical isolates of *T. rubrum* may resemble *O. canadensis* in some features. The occasionally cited report of onychomycosis caused by *O. canadensis* from Turkey (Erbagci et al. 2002) was revised by R.C. Summerbell and the strain was re-identified as atypical *T. rubrum* (Fanti et al. 2003). The possibility to mistake *O. canadensis* for *Scytalidium*, which can appear in our geographical latitude as an etiological agent of imported mycosis, was discussed by Sigler et al. (1990) and Volleková & Lisalová (2009).

In summary, we have reported these two cases because *O. canadensis* is infrequently detected as a human pathogen and had not yet been reported from Czech Republic. Moreover, both cases showed strikingly different clinical appearance. Onychomycosis due to *O. canadensis* is characterised by chronicity and slowly progressing infection, and patients usually appear several months or years after its beginning. It is possible that *Onychocola* spp. are relatively common in dermato-mycological specimens but are overlooked due to their slow growth.

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