

Toenail infection caused by *Onychocola canadensis* gen. et sp. nov.

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(Accepted 15 June 1990)

Three cases of great toenail infection are described in which a slow-growing arthroconidial hyphomycete was isolated repeatedly and in pure culture. Direct microscopy revealed hyaline, round to barrel-shaped arthroconidia, hyaline hyphae of varying width, and broad thick-walled brownish hyphae. Three additional isolates were obtained from clinical specimens, for which the results of direct microscopy were unknown or negative. The fungus was resistant to cycloheximide, sensitive to common antifungal drugs by susceptibility tests *in vitro* and sensitive to benomyl. It was urease positive, hydrolysed casein and tyrosine but not xanthine or hypoxanthine, showed no specific nutritional requirements but grew better on carbohydrate-free media, assimilated 12 carbohydrates and potassium nitrate, and failed to perforate hair. The fungus is described as *Onychocola canadensis* Sigler gen. et sp. nov., and it is compared to *Scytalidium lignicola*, *Scytalidium hyalinum* and the *Scytalidium* synanamorph of *Natrassia mangiferae* (*Hendersonula toruloidea*).

A variety of non-dermatophyte fungi have been implicated in nail infections, but it is often difficult to evaluate the significance of the isolation of a filamentous fungus from nail. English [5] suggested three criteria by which a mould could be evaluated as a potential pathogen: (i) any dermatophyte isolated should be considered to be the presumptive pathogen, (ii) direct microscopy should demonstrate mycelial filaments, and (iii) five of 20 inocula should yield the same organism. Among the most commonly reported species of non-dermatophyte isolated from nail are *Scopulariopsis brevicaulis*, *Aspergillus versicolor*, *Aspergillus terreus* and *Acremonium* species (as “*Cephalosporium*”) [5]. In recent years, *Hendersonula toruloidea*, now renamed *Natrassia mangiferae* [29], and *Scytalidium hyalinum* have been well documented as agents of recalcitrant toe- and fingernail infection, primarily in individuals of Indian, Caribbean and African origin [2, 7, 9, 14, 15, 17, 20]. Several studies have described their epidemiology and geographic distribution, and reports of infection in individuals from non-endemic areas suggest that these pathogens are more widespread than previously thought [6]. The factors involved in the transmission of these infections are

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still unknown [16]. A 3-year investigation of over 4000 cases of nail, sole and palm infection in Ontario, Canada [28], concluded that non-dermatophyte moulds accounted for 3.3% of confirmed etiologic agents from nail infection. *S. brevicaulis*, *N. mangiferae* (as *H. toruloidea*), and *Aspergillus sydowii* were the most common species, and *S. hyalinum* was reported for the first time from Canada. *N. mangiferae* and *S. hyalinum* are unusual among the known etiologic agents of onychomycosis in that they produce arthroconidia in culture.

This report describes three cases of great toenail infection, from which a slow-growing arthroconidial fungus was isolated repeatedly and in pure culture. No dermatophytes were isolated in any instance, and mycelial filaments and arthroconidia were demonstrated by direct microscopy. The same fungus has been isolated on three other occasions, from an arm, palm and great toenail. Direct microscopy of the scrapings from the arm was negative, and results are unknown in the two other cases. Morphological and physiological evidence suggest that the fungus differs from *Scytalidium* species known to cause onychomycosis, and it is described as *Onychocola canadensis* Sigler gen. et sp. nov.

CASE REPORTS

Case 1

A 52-year-old white woman was referred to a dermatologist in March 1982 because of an infection of her right great toenail. According to her family physician, the toenail had appeared abnormal for many years but remained asymptomatic. When the patient was 10-years-old the nail had been injured as a result of a cow stepping on her foot. At the time of presentation the patient was in good health and had no previous skin or nail problems; she had never been abroad. Examination disclosed a dull and rather greyish nail. The nail plate was distally separated from the nail bed, heaped up and thickened. There was marked accumulation of soft, subungual debris beneath the nail plate. The surrounding skin and other toenails appeared normal. A KOH preparation of the subungual keratin revealed hyaline arthroconidia, 2.3–4.5 μm wide, abundant septate, hyaline, sinuous, distorted hyphae of variable width, 1.8–3.4 μm , and a few short, septate, thick-walled, light-brownish hyphae, 2.3–6.8 μm wide (Fig. 1). Cultures of the subungual material on Sabouraud's glucose agar (SAB, Gibco Laboratories, Madison, WI), SAB containing chloramphenicol (50 $\mu\text{g ml}^{-1}$), gentamicin (20 $\mu\text{g ml}^{-1}$) and cycloheximide (500 $\mu\text{g ml}^{-1}$) (SAB-CCG), and Littman Oxgall agar (LITTMAN; BBL Microbiology Systems, Cockeysville, MD), supplemented with streptomycin at 66.6 $\mu\text{g ml}^{-1}$, yielded a pure growth of a slow-growing, glabrous, light-greyish mould (425M) after 2 weeks incubation at 28°C in the dark. Sporulation was not evident microscopically. After an additional 5–6 weeks of incubation, the fungus developed a fluffy, dull-whitish aerial growth. Microscopic examination showed abundant hyaline, 0–1 septate arthroconidia and the isolate was initially identified as a *Scytalidium*-like fungus. Repeat KOH examinations of nail material on two separate occasions were positive for hyphae and the same fungus was cultured. No dermatophytes or yeasts were isolated.

Before susceptibility data were available, the patient was treated by complete removal of the subungual debris followed by topical application of 4% thymol in chloroform twice a day. In June 1982, the toenail appeared healed and thymol treatment was discontinued. Direct microscopy of nail and subungual scales at this time showed a few distorted, light-brownish hyphae and hyaline arthroconidia. Cultures were negative for fungi and bacteria. In April 1983, examination showed normal regrowth of the nail and the nail plate had reattached to its bed. Direct microscopy of nail scrapings and subungual keratin revealed sparse hyphal fragments devoid of cytoplasmic contents. No micro-organisms were cultured.

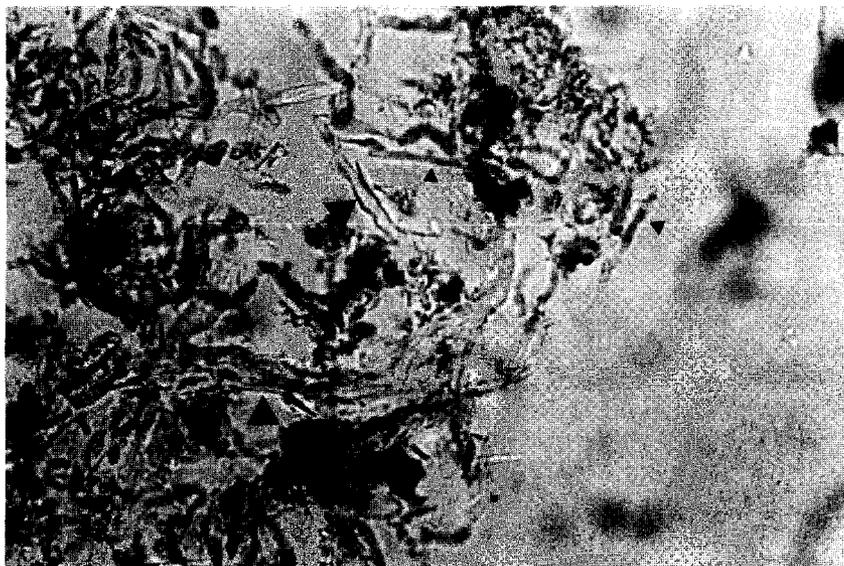


FIG. 1. Direct microscopy of nail specimen from case 1, showing numerous hyphae of varying width (small arrowheads) and broader, thick-walled hyphae (large arrowheads), $\times 600$.

Case 2

An otherwise healthy 67-year-old woman presented in January 1984 with an infection of her left great toenail of 2 years' duration. She travelled frequently to the United States, but denied visits to any tropical countries; she worked frequently in her garden. There was no pain associated with the infection. There had been no trauma to the involved nail. Examination revealed that one third of the lateral nail plate was affected. The surface was light-yellow, the nail plate was thickened and friable, and there was marked accumulation of light-brownish subungual debris. Other toe- and fingernails appeared normal. KOH preparations of the subungual keratin showed round to barrel-shaped arthroconidia measuring $2.3\text{--}4.5\ \mu\text{m}$ in width, septate, hyaline, tortuous hyphae of variable width ($1.8\text{--}4.5\ \mu\text{m}$ in diameter) and thick-walled, light-brownish hyphae. Culture on SAB-CCG and LITTMAN yielded pure growth of a light-greyish, non-sporulating mould (209M) after 10 days incubation. Subculture onto peptone agar and sheep blood agar enhanced the production of arthroconidia after 14 days incubation. Examination of repeat specimens of nail on two occasions were positive by direct microscopy and culture. No other fungi were isolated. The patient refused treatment and was lost to follow-up.

Case 3

A 46-year-old woman visited a physician in July 1987 because of a thickening of her left great toenail which was of several years' duration. The affected nail showed a dull-greyish discoloration, hyperkeratosis and onycholysis. The surrounding skin and other toe- and fingernails appeared normal. There had been no trauma to the great toe. The patient was in good physical condition and had not travelled outside Canada. A KOH preparation of the subungual keratin showed septate, hyaline, branching hyphae of variable width and hyaline, round to barrel-shaped arthroconidia (Fig. 2), measuring $3.5\text{--}6(8)\ \mu\text{m}$ long $\times 2.5\text{--}3.8\ \mu\text{m}$ wide, rounding up to $5\ \mu\text{m}$ in diameter. Cultures of the nail scrapings yielded multiple colonies of *O. canadensis*. Repeat specimens on two

separate occasions were positive by direct microscopy and culture. No other fungi were isolated. The specimen collected in July 1987 was retained at 4°C and it was possible to reisolate the same fungus 33 months after initial collection. In January 1988, the toenail was surgically excised; no antifungal therapy was given. The new toenail grew normally, but in September 1988 the patient noted the reappearance of the discoloration of the nail plate and the left sole appeared to be infected. She was treated with tolnaftate cream and gentian violet paint for 6 months with no apparent clinical improvement. Follow-up examination was not available.

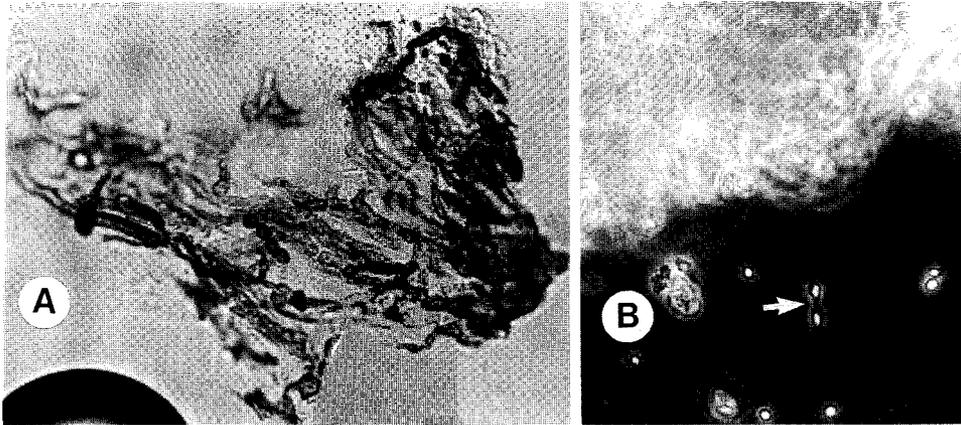


FIG. 2. Direct microscopy of nail specimen from case 3. (A) Arthroconidia and hyphae of varying widths stained with methylene blue, $\times 525$ brightfield. (B) Chain of two arthroconidia with collapsed intervening cell (arrow), $\times 395$, phase contrast.

METHODS

Cultural studies

Isolates from Cases 1–3 were deposited in the University of Alberta Microfungus Collection as UAMH 4596 (425M), 5344 (209M) and 5893 (1897M) respectively. A fourth isolate has been obtained recently in pure culture (UAMH 6637=439M) from an arm lesion on a 24-year-old male residing in Saskatchewan. Direct microscopic examination was negative for hyphae. Two additional isolates had been referred to the Ontario Ministry of Health, Toronto, but the results of direct microscopy were unavailable; UAMH 6106 (FR 385) came from the right palm of a male patient, and UAMH 6043 (FR 2252:87) came from the left great toenail bed of a male. The gross colony morphology of each isolate was examined on SAB (Difco), potato dextrose agar (PDA; Difco Laboratories, Detroit, MI), and Pablum cereal agar without antibiotics (CER) [19] at 25°C. Colony color codes are derived from the color standard of Kornerup & Wanscher [11]. Growth rates at 23, 28 and 37°C on different media were determined by transferring a 5mm disk from the periphery of a 3-week-old colony on blood agar (BA; Tryptic soy agar (Gibco)+5% sheep's blood) to the centre of 100mm Petri plates containing either BA, 2% phytone peptone agar (phytone peptone (BBL) 20 g, agar 20 g, distilled water 1l), SAB or PDA, and measuring radial diameters of the colonies, minus the width of the original inoculum, after 3 weeks incubation. Cultures at 28 and 37°C were incubated in the dark; all other cultures were exposed to

fluorescent light during normal working hours. CER was the medium used for slide culture preparations. Nail specimens were examined in 20% KOH or in methylene blue stain.

Susceptibility testing

Minimal inhibitory concentrations (MICs) to antifungal antibiotics were determined by the National Reference Centre, Edmonton, according to the method of Sekhon & Funk [22].

Physiological tests

All isolates were studied for urease activity in Christensen's urea broth [10] and results read at 14 days; decomposition of casein, tyrosine, xanthine and hypoxanthine after 18 days growth; assimilation of carbohydrates and nitrate [26] read at 21 days; requirements for vitamins thiamine, inositol and nicotinic acid on *Trichophyton* agars (Difco), examined after 12 days incubation at 28°C; hair penetration [1] after 3–4 weeks incubation; tolerance to benomyl by comparing growth rates on modified Melin-Norkrans medium [12] unamended and amended with 2 µg ml⁻¹ benomyl [27], and tolerance to cycloheximide at concentrations of 0.4 mg ml⁻¹ by recording growth rates on mycosel agar. All tests were performed at room temperature (23–25°C) except where noted above. *O. canadensis* was compared with isolates of *N. mangiferae* (UAMH 4755 and 6027, both Form 1 types; 6392, Form 2 type; and 6394, Form 3 type [16]), *S. hyalinum* (UAMH 4069 ex-type, 4070, 4760, 5954, 6031, 6032) and *Scytalidium lignicola* (UAMH 1502 ex-type, 4831) for urease activity, tolerance to benomyl and cycloheximide, and ability to grow at 37°C. The ability of *N. mangiferae* to assimilate carbohydrates and nitrate was tested for a single strain (UAMH 5396).

RESULTS

Description of the fungus

Onychocola Sigler gen. nov.

Fungi Imperfecti, Hyphomycetes.

Mycelium hyalinum. Conidiophora distincta absunt. Hyphae conidiogenae ex hyphis ramosis lateralibus transformantur. Arthroconidia hyalina, catenulata, prope septa constricta, fractione cellularum adiacentium leviter-tunicatorum (rhexolysi) vel aliquando schyzolysi cellularum adiacentium difficulter secedentia, sine disiunctoribus coniunctoribusve. Arthroconidia late ellipsoidea vel cylindrica. Reproductio sexualis ignota.

Typus: *O. canadensis* Sigler

Onychocola canadensis Sigler sp. nov.

Coloniae in agaro ad 25°C lente crescunt, planae vel glabrae, deinde elevatae, rotundae, post 42 dies 14–20 mm diam. attingentes, sericae vel gossypionae vel lanosae, luteo-albae vel luteo-griseae. Incrementum exigue tardius ad 37°C. Hyphae septatae, hyalinae, 1.5–3 µm latae, conidiophora absunt. Hyphae fertiles aegre distinctae, primo septatae sine constrictionibus prope septa, cellulae singillatim tumentes et prope septa constrictae; conidia temere in locis intercalaribus vel in ordine basipetale in ramis lateralibus formata. Conidia (arthroconidia) fractione rhexolysi regionis leviter-tunicatae hyphae adiacentis vel conidii imperfecti formati secedentia, deinde cristam materiae tunicae in tunicis extremis conidii disiuncti vel catenae conidicae ferentia. Arthroconidia interdum schyzolysi conidiorum adiacentium secedentia. Arthroconidia late ellipsoidea, cylindrica vel irregulariter formata, 0–1 septata, sed saepe

in catenis occurrentia, levia ad exigue verrucosa, hyalina ad pallide-lutea, $4-8 \times 2-5 \mu\text{m}$ in 0-septatis, $8-17 \times 2.5-5.5 \mu\text{m}$ in 1-septatis, aliquando ad $6.5 \mu\text{m}$ attingentia. Chlamydo sporae raras; teleomorphosis ignota.

Typus: Coloniae exsiccatae ex UAMH 5344, isolatae a ungui pedis laevi feminae 67 annorum, H. Congly, Regina, Saskatchewan.

Growth on all media was slow. Within 2 weeks at 25°C , colonies were flat and rather glabrous with sparse tufts of aerial mycelium, gradually increasing in height and becoming domed, reaching heights of 3–5 mm with abundant aerial hyphae, but remaining restricted to diameters of only 14–20 mm by 6 weeks on SAB, PDA and CER (Figs 3A, C–D). At 6 weeks, colonies were velvety, cottony or woolly, sometimes fasciculate, more glabrous on SAB than on other media, yellowish-white or yellowish-grey (3-4A2/4B2), reverse brownish-grey, olive-grey or dark grey-brown (3D2/4F2/6F2), with scant, greenish diffusing pigment below colonies on CER, margin glabrous, irregular or lobed. On firmer media such as PDA or SAB, the colonies depressed the agar below sometimes causing it to crack (Fig. 3B). Colonies were similar in diameter and texture on cycloheximide-containing medium (mycosel agar, BBL) after 2 weeks at 25°C (Fig. 3B). Growth rates on PDA and SAB were comparable at all temperatures with mean colony diameters of 5.4, 6.5, and 2.3 mm on PDA, and 7.5, 7.6 and 2.2 mm on SAB at 23, 28 and 37°C respectively, after 21 days. In contrast, growth was enhanced on BA and peptone agar, with mean colony diameters of 12.5, 14.2 and 5.6 mm, and 9.4, 10.9 and 5.0 mm respectively at the three temperatures. *O. canadensis* was not osmophilic.

Sporulation occurred slowly, being enhanced on BA, peptone agar and CER, but poor on SAB. Vegetative hyphae were hyaline, $1.5-3 \mu\text{m}$ wide, septate, sometimes swollen at the septum (racquet hyphae) (Fig. 3G), smooth or finely verrucose. Fertile hyphae were scarcely differentiated, initially septate without constrictions at the septa (Fig. 3E), with individual cells swelling and becoming constricted at the septa (Figs 3E–G); conidia formed randomly in the intercalary position (Fig. 3G), or in basipetal order on lateral branches (Figs 3F–G, 4A, C). Conidia (arthroconidia) seceded by fracture (rhexolysis of a thin-walled region of the adjacent hypha or of an incompletely formed conidium (Figs 4B, E–F), and then bore a frill of wall material on end walls of the detached conidium or conidial chain (Figs 4E–F). Arthroconidia occasionally seceded by schizolysis of adjacent conidia, and a remnant of outer wall was sometimes visible at high magnification (Fig. 4D). Arthroconidia were broadly ellipsoidal, cylindrical or irregularly shaped, 0–1 septate, but often persisted in chains, smooth to minutely-warty, hyaline to pale-yellow, $4-8 \times 2-5 \mu\text{m}$ if 0-septate, $8-17 \times 2.5-5.5 \mu\text{m}$ if 1-septate, sometimes rounding up to $6.5 \mu\text{m}$. Chlamydo spores were rare.

Cultures on BA, examined after 12 weeks incubation, were cottony or woolly, yellowish-white and spreading. Microscopically, broad, sparsely septate, brown hyphae, measuring up to $6.5 \mu\text{m}$ in width, and bearing irregular, raised, darker brown knobs (Fig. 5) were abundant. These hyphae may represent the broad, brownish hyphae observed in the KOH preparations of the subungual keratin. Two isolates (5344 and 6106) developed hyphae in which the apical regions were coiled or spiralled (Fig. 6), suggesting the possibility of an ascomycete teleomorph, but no ascumatal initials have been observed.

Susceptibility tests

Susceptibility testing of isolates from Cases 1–3 showed the following minimal inhibitory concentrations *in vitro*: griseofulvin, $0.19 \mu\text{g ml}^{-1}$, miconazole, range $0.19-0.39 \mu\text{g ml}^{-1}$, ketoconazole, range $0.39-0.78 \mu\text{g ml}^{-1}$, 5-fluorocytosine (5-FC; flucytosine), $0.78 \mu\text{g ml}^{-1}$, amphotericin B, $0.39 \mu\text{g ml}^{-1}$. For 5-FC, only the isolate from Case 1 was tested; for ketoconazole, only the isolates from Cases 1 and 3 were tested.

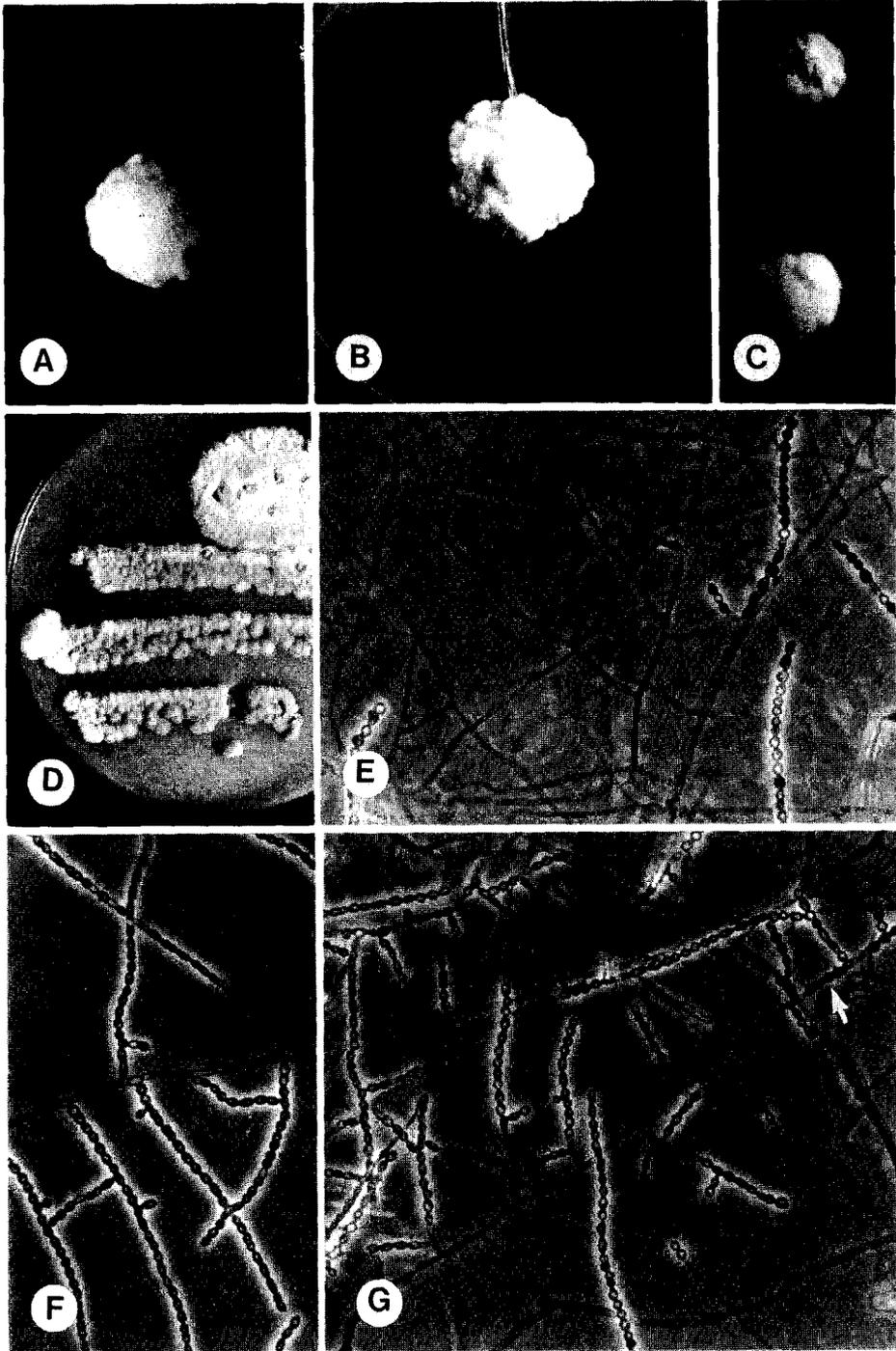


FIG. 3. *O. canadensis*. (A-D) Colonies at 25°C. (A) After 6 weeks on PDA, 5344, $\times 65$, (B) After 7 weeks on mycosel, 4596, $\times 0.9$, (C) After 6 weeks on SAB, top, 5433, bottom, 4596, $\times 0.86$, (D) After 20 days on CER, showing early stages of growth and tufts of aerial hyphae developing at periphery, arrow, 5344, $\times 0.7$. (E) Vegetative hyphae and fertile hyphae showing early stage of arthroconidium development, 6043, $\times 395$. (F-G) Conidia forming in intercalary position (arrow) or in basipetal order on lateral branches, 6043, $\times 395$.

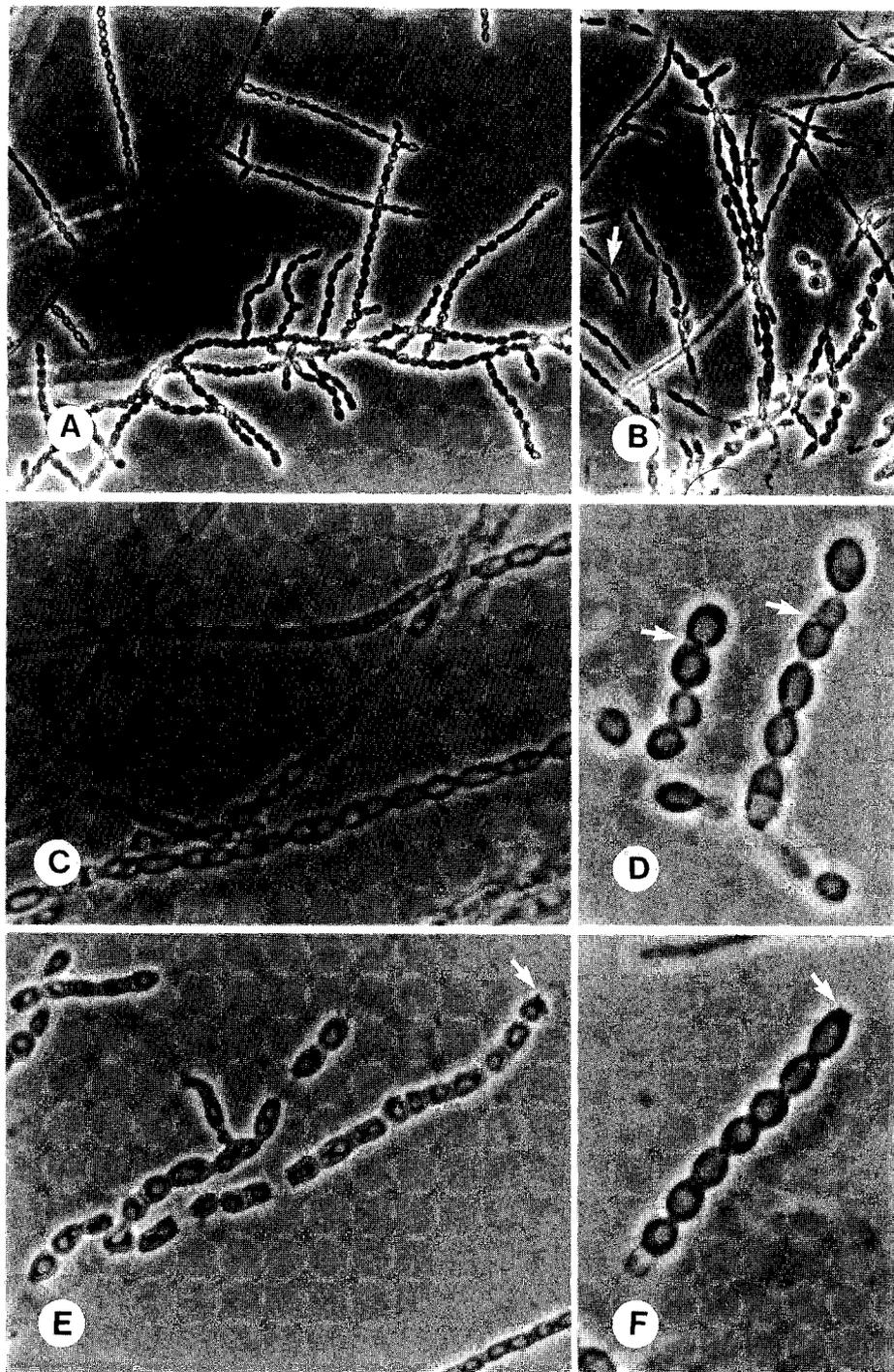


FIG. 4. *O. canadensis*. (A) Arthroconidia in persistent chains, showing irregular swelling, 6043, $\times 395$, (B) Chains of conidia with intermediate thin-walled cells which often collapse (arrow) and fracture, 6043, $\times 395$, (C) Development of arthroconidia in basipetal order, 5344, $\times 960$, (D) Mature conidia seceding by schizolysis. Remnant of outer wall may be seen, arrow, 6106, $\times 1290$, (E-F) Conidia seceding by fracture of thin-walled intervening cells leaving frill of wall material on end cell, arrows, (E) 6043, $\times 960$, (F) 5893, $\times 1290$.

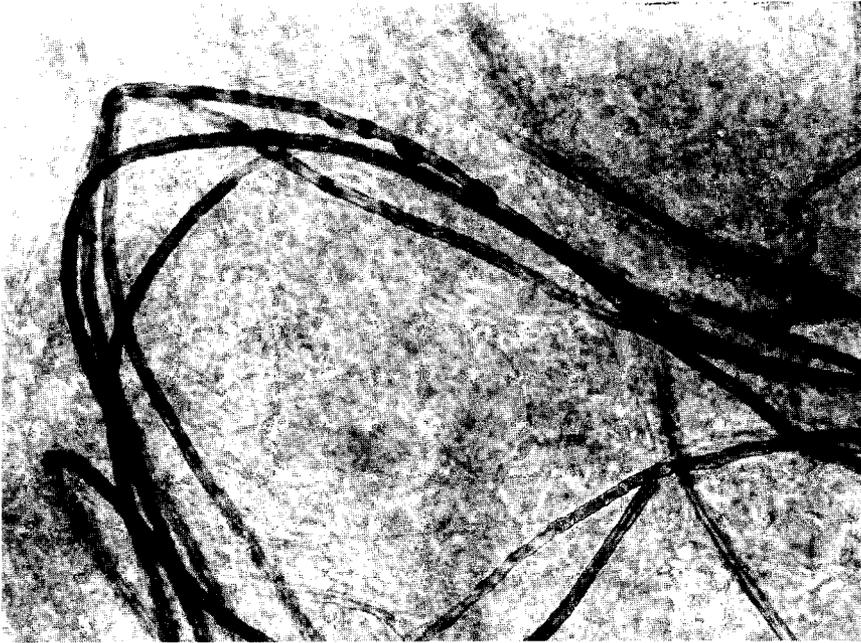


FIG. 5. Broad, sparsely septate, brown hyphae bearing irregular, raised, darker brown knobs, in old cultures on BA, 6106, $\times 460$.

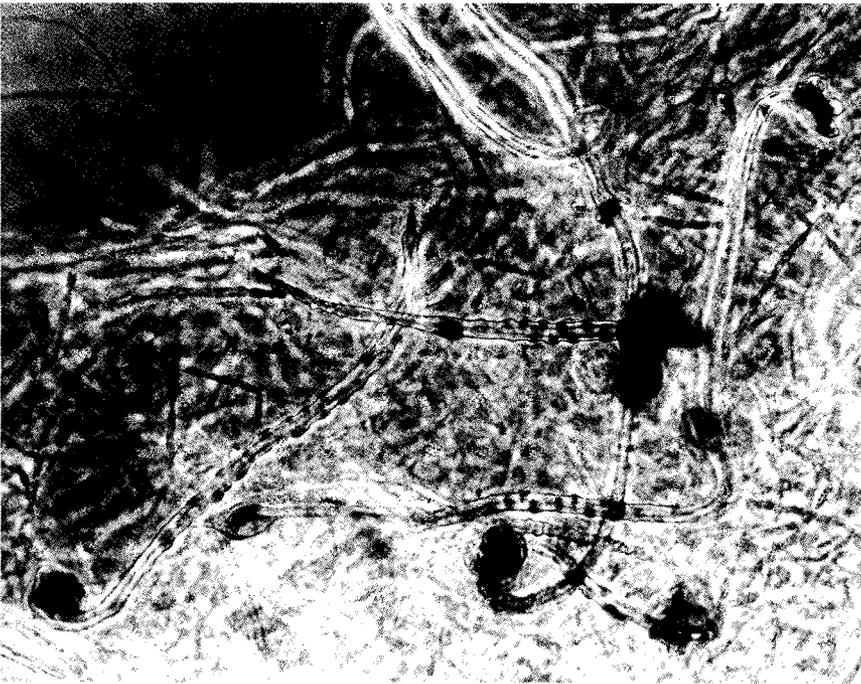


FIG. 6. Dark-brown, apically coiled or spiralled hyphae in old cultures on BA, 6106, $\times 460$.

Physiological tests

All isolates of *O. canadensis* hydrolysed casein and tyrosine, but not xanthine or hypoxanthine. They showed no specific requirements for vitamins thiamine, inositol or nicotinic acid, assimilated dextrose, maltose, sucrose, lactose, galactose, melibiose, cellobiose, inositol, xylose, raffinose, trehalose and dulcitol, potassium nitrate and peptone. Peptone supported better growth than KNO_3 . Hair perforation tests were negative. All isolates hydrolysed urea within 14 days. All isolates of *N. mangiferae* and *S. lignicola* were urease negative, whereas three of five isolates of *S. hyalinum* were weakly positive. Both *O. canadensis* and *S. lignicola* were completely inhibited by benomyl at $2 \mu\text{g ml}^{-1}$. Four of six isolates of *S. hyalinum* were partially inhibited and two completely inhibited by benomyl. The pycnidial forms of *N. mangiferae* showed greater tolerance to benomyl than non-pycnidial forms which were greatly restricted. *O. canadensis* was resistant to cycloheximide at 0.4 mg ml^{-1} , but all other taxa were sensitive. However, two of four strains of *N. mangiferae* and three of six strains of *S. hyalinum* overcame the sensitivity after 2 or 3 weeks incubation. *O. canadensis* grew more slowly at 37°C whereas all other taxa were thermotolerant, with growth rates approximately the same at 37°C as at 25°C , except for one strain of *S. lignicola* (4831) which was greatly inhibited at 37°C . The single strain of *N. mangiferae* tested demonstrated the same carbohydrate and nitrate assimilation characteristics as *O. canadensis*.

DISCUSSION

Clinically, the onychomycosis in our patients resembled tinea unguium. Trauma to the nail could have predisposed our first patient to nail infection, but nail invasion occurred in Case 3 without prior trauma. Onychomycosis due to non-dermatophyte moulds is common in elderly individuals [5], such as our second patient who appeared to have no underlying abnormality. Whether *O. canadensis* is a primary pathogen of nail, like *N. mangiferae* and *S. hyalinum* [9], requires verification from additional cases. That it is able to invade nail is supported by the demonstration of non-dermatophyte hyphae and arthroconidia by direct microscopy and by the repeated isolation of the fungus in pure culture without evidence of dermatophyte infection. The arthroconidia were more rounded than the cylindrical arthroconidia with truncate ends usually seen in dermatophyte infections, and collapsed intervening cells (Fig. 2B) resembled those seen in culture. The hyphae of varying width resembled those of *N. mangiferae* and *S. hyalinum* in nail. Although pigmented hyphae have been reported from some *N. mangiferae* infections, the thick-walled, light-brownish hyphae (Fig. 1) of *O. canadensis* are unusual. Since the broader, knobbed hyphae (Fig. 5) occurred in older cultures especially on BA, we speculate that the thick-walled, brownish hyphae seen in the specimens may occur in chronic infections.

Because of similarities in the clinical features, direct microscopy and formation of arthroconidia in culture, we initially questioned whether our isolates might represent aberrant forms of *S. hyalinum*, similar to the slow-growing, sparsely sporulating, non-pycnidial Form 3 isolates of *N. mangiferae* [16]. It has been suggested that the Form 3 isolates, in which the environmental habitat is not yet known, may represent an adaptation to an anthropophilic form of transmission [16]. Although the majority of infections due to these agents occur in natives of the West Indies or West Africa, a recent report had documented evidence of infection in native-born North Americans [6]. In contrast to cases from endemic areas in which the incidence of infection is greater in males than females (3:1), females appear to be more predisposed to infection in non-endemic areas [6]. The three infections due to *O. canadensis* occurred in native-born Canadian females.

O. canadensis differs from *S. hyalinum* in its susceptibility to antifungal antibiotics,

in physiology and in cultural characteristics. *O. canadensis* had a MIC of 0.19 $\mu\text{g ml}^{-1}$ to griseofulvin, compared to a reported MIC of $>100 \mu\text{g ml}^{-1}$ for *S. hyalinum* [20]. Susceptibility tests indicated that a number of antifungal agents, including ketoconazole at a MIC range of 0.39–0.78 $\mu\text{g ml}^{-1}$, were highly active against *O. canadensis*. Zaatari *et al.* [30] reported a MIC of 0.125 $\mu\text{g ml}^{-1}$ to ketoconazole for *S. hyalinum*, but observed no improvement in their patient's subcutaneous infection after 6 months of therapy; in contrast, Moore *et al.* [17] found the MIC of *S. hyalinum* to ketoconazole to be $>50 \mu\text{g ml}^{-1}$. Hay [8] commented on the resistance of the fungus *in vitro* to common antifungal drugs and the difficulty in management of *S. hyalinum* infections. Our first patient's infection was successfully treated by removal of the subungual debris followed by topical application of thymol, an alkyl derivative with antifungal activity. None of the antifungal agents used in the susceptibility tests were given to our patients, hence their therapeutic effectiveness could not be determined. Further clinical studies will be required to assess the value of antimycotic therapy in the management of nail lesions caused by *O. canadensis*.

O. canadensis differs from *S. hyalinum* in its resistance to cycloheximide at 0.4 mg ml^{-1} and sensitivity to benomyl at 2 $\mu\text{g ml}^{-1}$. Isolates of *S. hyalinum* varied in their sensitivity to benomyl; some isolates overcame their sensitivity to cycloheximide after 2–3 weeks. The two species may be further contrasted by their daily growth rates on SAB of 0.37 mm day^{-1} for *O. canadensis* compared with 8.7 mm day^{-1} for *S. hyalinum*.

Due to its formation of hyaline arthroconidia and isolation from human nail, we initially considered our fungus to be classifiable in the genus *Scytalidium* since this genus already accommodates two etiologic agents of onychomycosis. However, when the process of arthroconidium development in the new fungus was compared with that of the type species, *S. lignicola*, this placement was not satisfactory. In recent years, *Scytalidium* has become a repository for a number of species which differ significantly from the type. The main reason that the taxonomy of *Scytalidium* has been troublesome is that Pesante [21] erected the form-genus based on the co-occurrence of two anamorphs: (i) bacilliform hyaline conidia, which he called 'conidia vera', and (ii) dematiaceous intercalary conidia, which he called 'chlamydoconidia'. Based on a limited sample of representative strains and the description and illustration of Ellis [4] for *S. lignicola*, Sigler & Carmichael [23, 24] interpreted the latter structures as dematiaceous arthroconidia and described several species which differed from the type in forming only dematiaceous arthroconidia. Examination of additional isolates of *S. lignicola* has shown that the dematiaceous structures are more appropriately considered chlamydospores [25] and that the hyaline arthroconidial synanamorph is the most representative morph. The newly described, *Scytalidium circinatum* [25], which appears to be related to *S. lignicola* in both habitat and conidium development, provides grounds for a possible revision of the genus.

A recent proposal [29] to synonymize *S. lignicola* with the arthroconidial synanamorph of *N. mangiferae*, as *S. dimidiatum*, appears to have been made without examination of type material or living cultures of *S. lignicola*. This is unhelpful, since on at least one occasion [3], *S. lignicola* has been incorrectly identified as the cause of subcutaneous phaeoerythromycosis in man. The senior author examined the culture from this case (obtained from M. R. McGinnis as NCMH 1255=UAMH 4755) and demonstrated pycnidia characteristic of *N. mangiferae* (Sigler, unpublished data). Although the differences between *S. lignicola* and the *S. mangiferae* anamorph of *N. mangiferae* have been described previously [24, 25], it seems appropriate to reiterate them here since they provide the basis for our decision to erect a new taxon.

S. lignicola is distinguished by the uniformly narrow, fertile hyphae and hyaline cylindrical arthroconidia (2.5 μm or less in width). Intercalary and terminal chlamydospores develop by swelling and subsequent melanization of portions of the vegeta-

tive hyphae [24, 25]; these structures are not readily detached but dehiscence may occur when the fungus is manipulated and occurs by fracture of thin-walled adjacent hyphae. In contrast, the hyphae and arthroconidia of the *S. anamorph* of *N. mangiferae* vary greatly in width and color, and the hyphae often demonstrate conspicuous wall ornamentation [16]. Arthroconidia measure 2.5–7 μm , and may round up to 10–16 μm , with pigmentation varying from hyaline or subhyaline to brown.

The key difference between *S. lignicola* and the *Scytalidium* agents of dermatomycosis is the rapid disarticulation of hyphae and conversion into swollen, 0–1 septate arthroconidia with thick walls that occurs in the latter, and these features may be grounds for generic distinction. Indeed, Campbell & Mulder (2) noted the similarities between *S. hyalinum* and *N. mangiferae*, and their difference from *S. lignicola*. These species have been isolated from mixed infection, and antigenic similarities suggest they may be closely related [18].

Maturation of conidia of *O. canadensis* also involves swelling of intercalary and terminal regions of fertile hyphae, but in contrast to *N. mangiferae* and *S. hyalinum*, in which arthroconidia disassociate rapidly by schizolytic dehiscence, the conidia of the former persist in long chains of usually 1-septate conidia, and dehiscence appears to occur by fracture of thin-walled adjacent cells (rhexolysis). The conidial chains suggest a superficial similarity to some species of *Bispora* such as *Bispora novae-zelandiae* [13], but in *Bispora* conidia are formed in acropetal chains by blastic proliferation.

O. canadensis should be suspected when nail cultures on primary isolation media such as SAB-CCG or Littman yield a slow-growing, glabrous, raised colony that is first whitish, then turns pale-grey after 3–4 weeks at 28°C with no evidence of arthroconidial production. Its failure to produce the characteristic arthroconidia on such media may have led to its being overlooked in the past as a contaminant or sterile mold. The fungus can be differentiated from *S. hyalinum* by the persistent conidial chains, dehiscence by fracture of adjacent thin-walled cells (rhexolysis), enhanced growth on a carbohydrate-free medium, cycloheximide resistance, and presence of coiled or spiralled, knobbed brown hyphae in old cultures. Because *O. canadensis* has been isolated from clinical material on three occasions without positive evidence of tissue invasion, its pathogenic status in human disease and its occurrence in the environment merit further study.

ACKNOWLEDGEMENTS

Appreciation is extended to A. Flis for expert technical assistance, R. C. Summerbell and J. Kane, for provision of additional cultures, M. Hertwig-Jaksch, for assistance with the latin diagnosis, and the National Reference Centre for Human Mycotic Diseases, Provincial Laboratory of Public Health, Edmonton, for antifungal susceptibility testing. The following physicians provided clinical information on the cases: Case 1, N. E. J. Davies; Case 2, E. P. Skochylas and Case 3, D. Tanna. An operating grant (A3755) from the Natural Sciences and Engineering Research Council of Canada to L. Sigler is gratefully acknowledged.

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