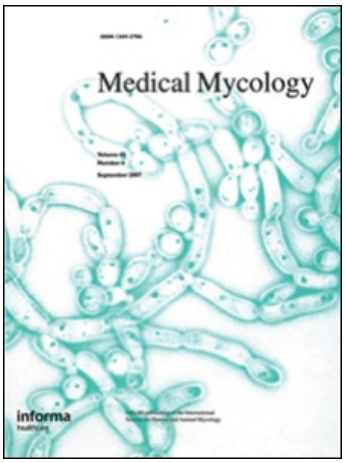

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New records of nail and skin infection due to *Onychocola canadensis* and description of its teleomorph *Arachnomyces nodosetosus* sp. nov.

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Non-dermatophytic fungi are increasingly being recognized as agents of onychomycosis. In 1990, three cases of chronic infection of the great toenail in adult female residents of Canada were attributed to *Onychocola canadensis*, a previously unknown hyphomycete. Three additional cases were suspicious but unconfirmed. This report documents seven new records, including six of toenail infection in elderly individuals and one case of glabrous skin infection. Three isolations from New Zealand represent the first report of *O. canadensis* outside Canada. Treatment with griseofulvin in one New Zealand hallux infection case was found to improve the appearance of the nail, but specimens were culture positive after 6 months. The development in culture of broad, brown, nodose, thick-walled hyphae suggested an affinity to the ascomycete genus *Arachnomyces*. Although mating experiments were attempted on several different media, ascocarps were produced in six mated pairs on sterilized rice grains or rice extract agar after 7–12 months incubation. *Arachnomyces nodosetosus* Sigler & Abbott sp. nov. is described and compared with *Arachnomyces minimus* Malloch & Cain, also rarely isolated from cutaneous specimens. The genus *Arachnomyces* is placed in the Gymnoascaceae (Onygenales).

Tinea pedis and onychomycosis are usually caused by dermatophytes, with a low frequency of infection due to non-dermatophytic molds. A Canadian study of 4000 cases of nail, sole and palm infection found that non-dermatophytic fungi accounted for 2·3% of all confirmed etiologic agents and 3·3% of agents recovered from nail [12]. In a 2-year study of 131 and 200 cases of nail lesions, Velez & Diaz [13] reported a frequency of recovery of filamentous fungi from specimens with positive direct examination of 4·5% and 9·5%, respectively. Several molds, including *Nattrassia mangiferae* (Sydow & Sydow) Sutton & Dyko (synanamorph: *Scytalidium dimidiatum* (Penz.) Sutton & Dyko) and *Scytalidium hyalinum* Campbell & Mulder, are now well-recognized as pathogens and a diagnosis of infection may be made upon isolation of the fungus. Criteria to confirm an isolated mold as an infective agent include (i) direct microscopy showing mycelial elements, especially if the filaments differ from dermatophytic hyphae, and (ii) isolation of the same mold from a repeat specimen. English [2] added two other criteria, including absence of a dermatophyte, and growth of the same mold from 5–20 pieces of inoculum. However, mixed infections by dermatophytes and non-dermatophytes, such as *Trichophyton rubrum* and *Scopulariopsis brevicaulis*, are infrequently reported [12, 13], and isolation from up to 20 pieces of inoculum may be difficult for nail specimens, which are often difficult to culture.

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Apparently unrecognized until its description in 1990 [11], *Onychocola canadensis* Sigler was shown to be the etiologic agent in three cases of infection of the hallux; three other isolates were not clinically proven. All patients were from Canada. Since then, additional cases have been observed in both Canada and New Zealand. This report summarizes the cases known to date and describes the clinical features of two cases from New Zealand. Mating tests resulted in the development of a teleomorph assignable to the ascomycete genus *Arachnomyces* Massee & Salmon.

METHODS

Review of cases

Table 1 summarizes all cases known to date and their treatment regimens, where known. Two cases from New Zealand are described in more detail.

Case 4

A 71-year-old female presented on 10 December 1990 with an infection of the left hallux. The nail showed considerable thickening on the inner margin and was discoloured yellow. It had detached from the nail bed and there was a small amount of subungual debris. Direct examination of the nail in KOH with Evans Blue showed tortuous hyphae of variable width and arthroconidia which were slightly broader than those of a dermatophyte and which had a faint yellowish-brown pigment. *O. canadensis* was isolated in culture. The patient was an avid gardener who enriched the soil with 'mushroom manure' and seaweed from a local beach. There had been no previous trauma to the involved nail, but she frequently wore open-toed shoes while gardening. She had no history of residence or travel outside New Zealand. The left hallux was the first to show signs of infection and was probably infected for at least 3 years. Two months after therapy with griseofulvin was begun, repeat specimens from both left and right hallux were positive by direct examination, but positive by culture only from the left hallux. Six months after treatment, there was an improvement in the appearance of both nails, but the distal portions were still greatly thickened and discoloured. Direct examination and culture were still positive for *O. canadensis* for the left great toenail. Therapy was discontinued after 6 months due to gastrointestinal distress.

Case 8

A 51-year-old female psychiatric outpatient presented with scaling lesions on both palms. The patient had psoriasis. The lesions were initially considered to be an extension of her psoriasis, but scrapings were taken to exclude the possibility of fungal infection. The referring laboratory reported that direct examination of scrapings was positive for both specimens, showing 'heavy hyphae' (i.e. more robust than those of a dermatophyte). The fungus grew from several pieces of inoculum on the primary isolation medium. It was referred to the Communicable Disease Centre where it was identified as *O. canadensis* and later confirmed at the University of Alberta Microfungus Collection and Herbarium (UAMH). The patient had not been overseas and listed gardening as an occasional pastime. In a follow-up study approximately 8 weeks after initial examination, culture was negative, but material was insufficient to allow both direct examination and culture. During this period, the patient had been applying Betnovate in the belief that the palm lesions were caused by her psoriasis. During the

follow-up examination, it was noted that the patient frequently clenched her fists or scratched at her palms and this may have been sufficient trauma to have initiated the original infection.

Morphological observations and mating studies

Isolates from each case were deposited in the UAMH (Table 1). Colonies and microscopic morphologies were examined as described previously [11]; isolates were grown on Pablum cereal agar (CER) [7], phytone yeast extract agar (PYE), mycosel agar, 2% phytone peptone agar (PPA) and blood agar (BA) [11].

Several attempts were made to mate available strains. In each case, the inoculum consisted of a suspension of arthroconidia in sterile distilled water, and four to five drops of suspension were applied to each plate of medium. Strains were paired in all possible combinations, in addition to self-crosses. Initial experiments involved five strains (4596, 5344, 5893, 6043, 6106) grown on BA and PPA. No teleomorph was observed after 6 months incubation at room temperature. A second experiment involved nine strains (4596, 5344, 5893, 6043, 6106, 6637, 6794, 6795, 7046) inoculated on sterile rice grains. Four grams of raw unfortified rice were placed in 6 cm glass Petri dishes with 12·5 ml of water and autoclaved at 121°C for 15 min. Plates were incubated in alternating light and dark at 25°C for 1 year. Visual examination of the plates was performed weekly for the first 8 weeks, then monthly. Since the opacity of the rice grains did not allow for a ready scrutiny of developing ascomata with a dissecting microscope, microscopic mounts were made of coloured areas or other unusual areas of the colonies at irregular intervals for the first 6 months and from all plates at 19 weeks. Because of low fertility achieved in crosses on rice grains, a third attempt was made to cross isolates in selected combinations (strains as above plus UAMH 7116, 7117 and 7139) on rice extract agar (REA) (rice filtrate was prepared by heating 25 g of unfortified rice in 500 ml water for 10 min at 121°C, then filtering and discarding rice; the rice filtrate was made up to 500 ml and combined with 7·5 g agar, autoclaved at 121°C for 15 min and dispensed into 100 ml sterile plastic petri dishes).

For scanning electron microscopy, the material was air-dried and mounted on stubs coated with a double sided adhesive. A coating of gold–paladium was applied with a Nanotech sputter coater. Specimens were examined with a Cambridge S-250 SEM (Cambridge, UK) and photographed.

The ex-type (culture derived from the type) and another strain of *Arachnomyces minimus* were obtained from the American Type Culture Collection (ATCC 18857=UAMH 7113 and ATCC 20420=UAMH 7114). Two additional isolates from clinical sources had been referred to UAMH and identified as *A. minimus*. UAMH 5590 was received from the New York State Department of Health, Albany, NY (M660-85). The isolate came from a foot scraping from a 23-year-old male. UAMH 7097 was isolated by D. Parr at Auckland and Children's Hospitals, Auckland, New Zealand from a male with nail plate infection and a yellow discoloured nail (MHI 046). All living strains were grown on the same media as was used for the study of *O. canadensis*, and on sterile rice grains as described, and other media to obtain fruiting, including Takashio agar [7] and oatmeal-salts agar [7]; however, 5590 failed to fruit on any medium. Herbarium specimens of other species were obtained on loan from the New York Botanic Garden, Bronx: *Arachnomyces nitidus*: England, Kew, Queen's Cottage, G. Massee June/August 1901, on dead grass, NY (TYPE); Canada, Ontario, York Co., Nashville, D. Malloch 20 May 1968, on chicken hay-dung compost, TRTC 45366;

TABLE 1. Summary of cases due to *O. canadensis*

Cases	UAMH[a] No.	Age (Sex)	Origin	History	Specimen	DE[b]	Clinical features	Treatment	Repeat Specimen (No.) DE	Culture
1[d]	4596	52 (F)	Canada	Prior trauma	R hallux	+	thickened, ++ subungual debris	Debridement of subungual debris, topical 4% thymol in chloroform two times daily for 2 months	+(2)	−(2)
2[d]	5344	67 (F)	Canada	Gardener	L hallux	+	thickened, friable, yellowish, ++ subungual debris	Refused	+(2)	+(2)
3[d]	5893	46 (F)	Canada	Healthy, no trauma	L hallux	+	thickened, dull greyish, onycholysis	Surgical excision. New nail discoloured after 9 months. Tolnaftate & gentian violet 6 months	ND[c]	ND
4	6794	71 (F)	NZ	Healthy, no trauma, gardener	L hallux	+	thickened, yellowish, onycholysis +subungual debris	Griseofulvin 6 months (discontinued due to GI distress)		
								L. hallux	+(3)	+(2)
								R. hallux (2 months after treatment begun)	+(2)	−(2)
5	6795	90 (M)	Canada	?	R hallux	+	?			
6	7117	76 (M)	Canada	Retired farmer, poor circulation in legs	L hallux	+	thickened, light greenish, ++subungual debris	Oral ketoconazole (10 days), discontinued due to hepatoxicity. Topical nystatin, slight improvement in nail appearance	+(1)	+(1)

TABLE 1. *Continued*

Cases	UAMH[a] No.	Age (Sex)	Origin	History	Specimen	DE[b]	Clinical features	Treatment	Repeat Specimen (No.) DE	Culture
7	7116	68 (M)	Canada	?	hallux	+	thickened, friable, yellowish + +subungual debris			
8	7139	51 (F)	NZ	Psychiatric outpatient with psoriasis	L & R palm	+	scaly lesions	Betnovate for psoriasis, no other treatment, scaling reduced after 8 weeks	ND	− (1)
9	7046	76 (F)	Canada	?	toenail	+	?			
10	7429	83 (M)	NZ	Heart failure, poor circulation, gardener	L & R hallux (pooled)	+	both nails abnormal in appearance	Surgical excision of both nails. New growth beginning	ND	
Suspicious Cases										
11[d]	6043	?	Canada	?	L hallux	?				
12[d]	6106	? (M)	Canada	?	R palm	?				
13[d]	6637	?	Canada	?	arm lesion	?				

[a]University of Alberta Microfungus Collection & Herbarium.
[b]Results of direct examination.
[c]Not determined.
[d]Previously published [11].

Arachnomyces sulphureus: England, Kew, Royal Botanic Gardens, G. Massee April 1901, on grass of old bees (*Bombus*) nest, NY (TYPE).

RESULTS

Description of the teleomorph

Arachnomyces nodosetosus Sigler & Abbott sp. nov.

Ascomycotina, Onygenales, Gymnoascaceae

status anamorphosis: *Onychocola canadensis* Sigler, 1990, apud Sigler & Congly [11].

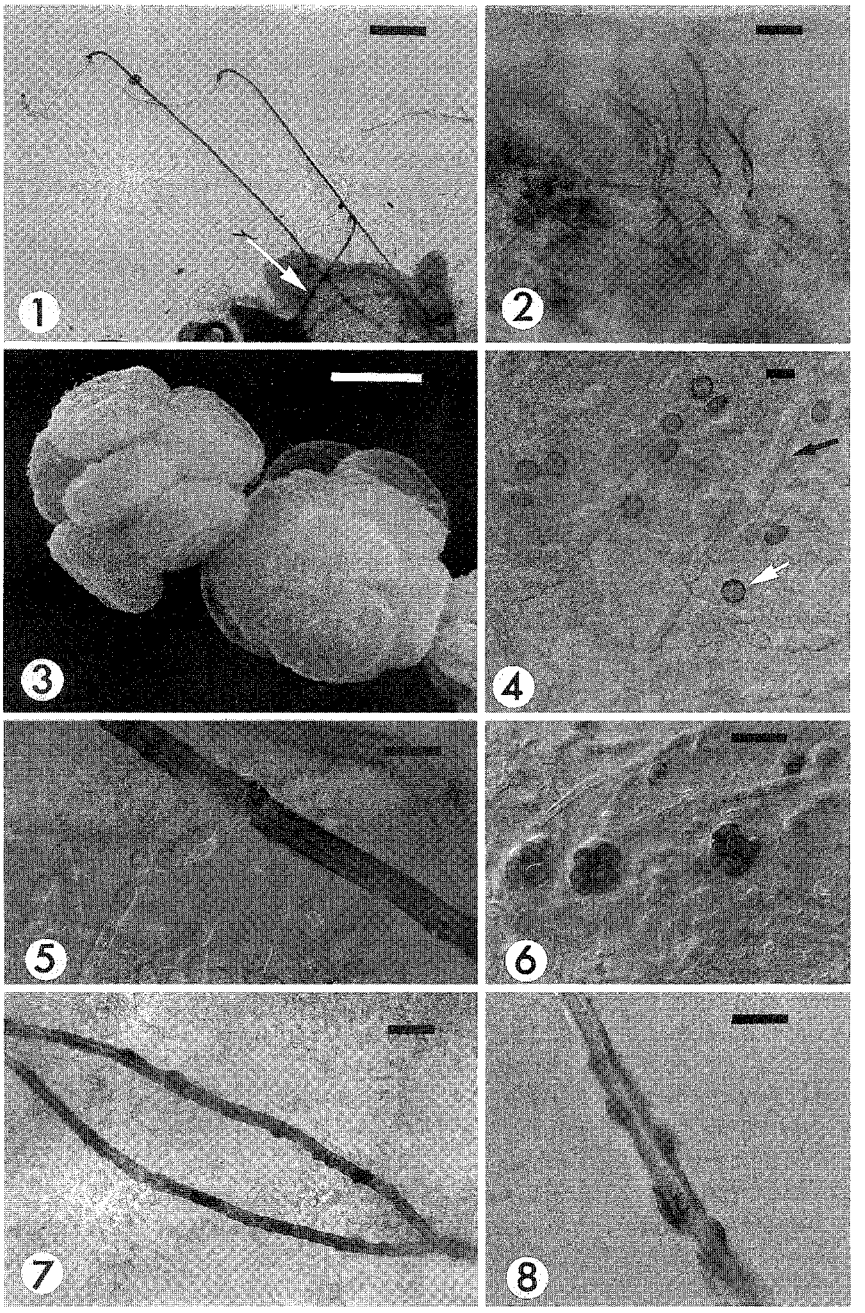
Ascocarpae globosae vel subglobosae, brunneae, 150–450 μm diam. nonostiolatae, setulosae; peridium de textura angulari; setulae brunneae vel rufo-brunneae, apice hyalinae, septatae, crasse tunicatae, nodosae, apice irregulariter contortae vel circinatae, 600–1200 μm longae, 4·5–7 μm diam., basis ad 10 μm diam; asci subglobosi vel globosi, nonstipitati, octospori, evanescentes, hyalini, 6·5–11 μm diam; ascosporae oblatae, brunneae, laeves, aguttulatae, 4–4·5 × 3–3·5 μm; mycelium hyalinum; status anamorphosis Onychocola canadensis.

Holotypus: Coloniae exsiccatae UAMH 7480, ex cruce UAMH 6043 × 6106, in granis oryzae sterilis.

Etymology: *nodosus*=knobby, nodose; *setosus*=setose

Ascocarps were produced after 19 weeks on sterile rice grains. Ascocarps measure 60–300 μm in diameter when immature and up to 450 μm when mature, and are subglobose to globose and non-ostiolate, initially hyaline or pale brown then medium or dark brown, with peridium wall of textura angularis. Typically each ascocarp bears 3–8 appendages (seta) which are 600–1200 μm long and 4·5–7 μm wide, but broader at the base where they measure up to 10 μm in diameter. Setae arise from outer cells of the peridium surface and are flexuous, often curved and bent with apices irregularly contorted or coiled and often hyaline, but as reported previously [11], the setae can also arise from the vegetative hyphae in the absence of ascomata (Fig. 2). They are thick-walled, sparsely septate and have distinct raised knobs or lumps which are irregular in shape and darker brown. Asci are 6·5–11 μm diameter, subglobose to globose, hyaline, evanescent and contain eight ascospores. Ascospores are oblate, pale brown to brown in mass, smooth, without guttules or germ pore and measure 4–4·5 × 3–3·5 μm. The vegetative mycelium is hyaline, 1·5–3 μm wide and septate. Anamorph: *O. canadensis*, arthroconidia 4–8 × 2–5 μm, if 0-septate, 8–17 × 2·5–5·5 μm, if 1-septate, ellipsoidal, barrel-shaped or subcylindrical, hyaline, without guttules, separating by fracture (rhexolysis) of thin-walled cells or by schizolysis, but often persisting in chains.

Mating strains. In crosses among nine strains, ascocarps with ascospores were produced on sterile rice grains after 7–12 months in only three mated pairs (Table 2). Two mated pairs produced infertile ascomata. No teleomorph was produced when strains were grown alone. In crosses among 12 strains on REA after 12 months, fertile ascomata formed only in one pairing (5893 × 6043), and infertile ascomata were produced in two crosses (6637 × 7139 and 6043 × 6637). Ascomata developed in old cultures often on or near the rim of the Petri dish. Although ascocarps were produced by only a few compatible strains, *A. nodosetosus* is considered to be a heterothallic ascomycete.



FIGS 1–8. *A. nodosetosus*, all from sterilized rice grains at 19 weeks: (1) Immature ascocarp (arrow) bearing appendages with contorted hyaline apices, 6043 × 4596. Bar = 100 μm; (2) Brown nodose seta-like hyphae in vegetative hyphae, 5344 × 6106. Bar = 100 μm; (3) Scanning electron micrograph of oblate ascospores, 6043 × 4596. Bar = 2 μm; (4) Oblate ascospores (white arrow) and chains of arthroconidia (black arrow), 6043 × 6106 (holotype). Bar = 5 μm; (5) Seta and arthroconidia, 6043 × 4596. Bar = 10 μm; (6) Asci and ascospores, 6043 × 5893. Bar = 10 μm; (7) Branched nodose seta, 6043 × 4596. Bar = 20 μm; (8) Thick-walled nodose seta, 6043 × 4596. Bar = 10 μm.

TABLE 2. Results of mating experiments after 12 months on sterile rice grains

	Minus mating strains			
	4596	5893	6106	6637
Plus mating strains				
5344[a]		IF[b]		
6043	+	+	+	IF
7139				IF[c]

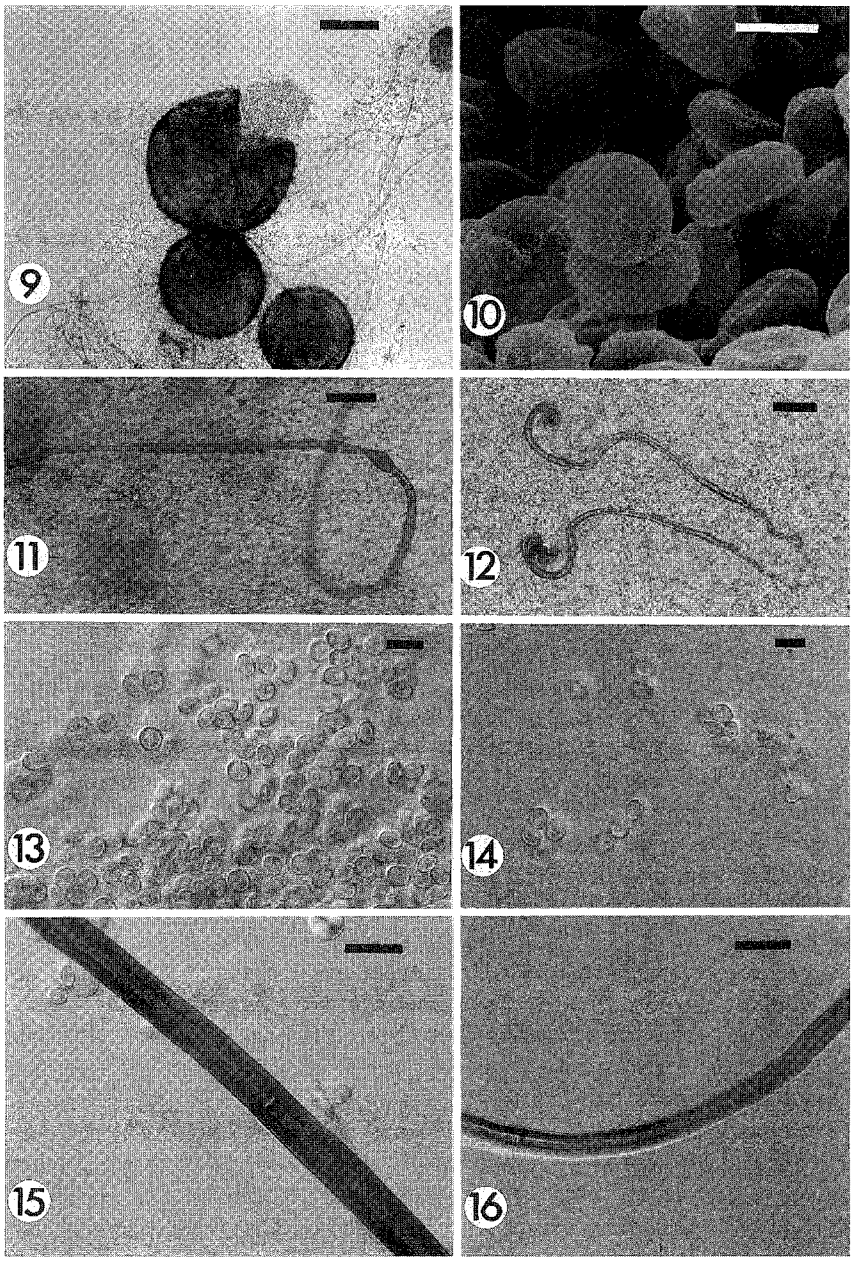
[a]Ex-type culture of *O. canadensis*.
[b]IF—infertile ascomata.
[c]After 12 months on rice extract agar only.

DISCUSSION

Two of the cases from New Zealand (8 and 10, Table 1) and two from Canada (3 and 9) could not be confirmed by repeat culture but no dermatophyte was isolated in any case, and the appearance of the hyphal elements in direct examination showed either arthroconidia and hyphae typical of *O. canadensis* [11] or hyphae dissimilar to those of a dermatophyte. The patient from New Zealand (Case 4, Table 1) described here is the first individual known to have received griseofulvin therapy for treatment of the nail infection. Previously, it was reported that *O. canadensis* had a minimum inhibitory concentration of 0·19 μg ml^{-1} to griseofulvin [11]. The *in vitro* susceptibility result, the improvement in the appearance of the nails after 6 months of therapy and failure to culture the fungus from the right nail (Table 1), suggests that this drug would be effective in eradicating the infection. However, since therapy was discontinued at 6 months with the left nail still culture positive, it is difficult to gauge the efficacy of the treatment.

The habitat of *A. nodosetosus* in nature is not known. All collections of the anamorph, *O. canadensis*, have come from clinical sources and the finding that onychomycosis occurs primarily in the older individual suggests that the fungus is able to grow only on altered keratin. The fungus is apparently not able to break down the keratin of hair as shown by *in vitro* studies, but is weakly cellulolytic [11] as are several of the other non-dermatophytic agents of onychomycosis. Other species of *Arachnomyces* have been found in association with dead grass, compost or wood, and the use of 'mushroom manure' or decomposed straw/manure mix in gardening by our hallux-infected case from New Zealand suggests that *A. nodosetosus* may have a similar habitat. Attempts to culture the organism from environmental samples were unsuccessful, however. The factors responsible for the low fertility in *A. nodosetosus* are not known. Most probably, the media chosen lacked the appropriate nutritional requirements, but the elaborate anamorph of this species may indicate a primarily asexual mode of reproduction.

A. nodosetosus fits well within the concept of the genus *Arachnomyces* as initially described [6] and as circumscribed by Malloch & Cain [5]. Three species are currently retained, including *A. nitidus* Massee & Salmon, the type species, *A. sulphureus* Massee & Salmon and *A. minimus* Malloch & Cain. *Anixiopsis peruviana* Cain, transferred to *Arachnomyces* by Malloch & Cain [5], was later redisposed in the new



FIGS 9–12. *A. minimus*: (9) Mature ascocarps bearing appendages with coiled apices, 7113 (ex-type), sterilized rice grains 13 days. Bar=100 μm; (10) Scanning electron micrograph of oblate ascospores, 7113 (ex-type). Bar=2 μm; (11) Knobby brown seta-like hypha in vegetative mycelium, 7097. Bar=20 μm; (12) Brown seta-like hyphae with coiled/contorted apices in vegetative mycelium, 5590, TAK 21 days. Bar=20 μm.

FIGS 13–16. *A. sulphureus:* (13, 15) *A. sulphureus*, holotype NY; (14, 16) *A. nitidus*, holotype NY; (13, 14) Oblate ascopores. Bar=5 μm; (15, 16) Smooth thick-walled setae. Bar=10 μm.

genus *Xanthothecium* von Arx & Samson [14] because of its roughened, rather than smooth ascospores and lack of setose ascomata. *A. nodosetosus* is easily distinguished by the distinctive nodose or knobbed setae (Figs 7, 8) and the presence of an *Onychocola* anamorph (Figs 4, 5). Ascospores of *A. nitidus, A. sulphureus* (Figs 13, 14) and *A. nodosetosus* are similar in size, but those of the latter are more darkly pigmented (Figs 4, 6). The setae of *A. nitidus* and *A. sulphureus* differ in being smooth and of even diameter throughout (Figs 15, 16). The new species also appears separable by ascocarp size, but the small number of specimens available for each species makes it difficult to obtain a true size range. Ascocarps of *A. nodosetosus* measure up to 450 μm, compared with 300 μm for *A. nitidus*, and 500–660 μm for *A. sulphureus*, which has a lighter yellow-brown peridium with yellow mycelium and broad appendages (6–7 μm diam.) (Fig. 15). However, a true concept of the biology of these two species awaits their growth in culture.

The only other species known in pure culture is *A. minimus* and it is similar to *A. nodosetosus* in being tolerant of cycloheximide, in fruiting on sterilized rice grains and in forming setae from the vegetative hyphae as well as from ascomata (Figs 9, 11, 12). It differs in having smaller ascospores, 2·5–3·5 × 1·5–2·0 μm (Fig. 10), in lacking a conidial state and in being apparently homothallic. Fruiting occurred on other media including homemade cornmeal agar, Takashio or oatmeal agar but could be induced only in three of four isolates; the sterile isolate produced only setae. Although the setae may also have slight swellings or irregularities in thickness (Fig. 11), they are narrower and more uniform in colour throughout. Colonies are slow growing on all media reaching diameters of 25–35 mm in 6 weeks on PYE or CER. Sterile isolates appear cottony and remain white or off-white, but fruiting isolates develop patches which are darker golden-yellow to pale orange or pale brown and have a diffusible violet-brown or greyish-brown pigment. Colonies are not inhibited by cycloheximide at 400 μg ml^{-1}. Isolates were shown to be slightly cellulolytic but not capable of digesting human hair as determined by methods described previously [10].

With this discovery of a connection between *O. canadensis* and *A. nodosetosus*, this is the first report of a species of *Arachnomyces* being involved in infection. *A. minimus* has been isolated on two occasions from clinical specimens, but the significance of its recovery is unknown. The most recent isolate from New Zealand (UAMH 7097) came from a toenail in which direct examination showed hyphae and 'large arthroconidia'. Two repeat specimens also showed hyphae, but neither *A. minimus* nor a dermatophyte was isolated. In intervals between repeat testing, the patient had been treated with a topical azole and griseofulvin (D. Parr, personal communication). The second clinical isolate (UAMH 5590) was identified putatively as *A. minimus* by the morphology of its setae, since no fertile ascomata could be obtained in culture. The results of direct examination are unknown. Whether *A. minimus* could also be an agent of cutaneous mycosis remains to be proven.

The most appropriate disposition of the genus *Arachnomyces* within the Ascomycotina has not been resolved. Although Malloch & Cain [5] placed it in the Gymnoascaceae where it appeared most closely related to *Aphanoascus* Zukal, a new circumscription of the Onygenales by Currah [1] led to the exclusion of *Arachnomyces* from the order. Eriksson & Hawksworth [3, 4] concurred with Currah and listed the genus with other taxa of uncertain disposition. That *Arachnomyces* is not related to *Aphanoascus* and other members of the Onygenaceae is supported by its smooth, pale brown to brown ascospores, inability to degrade keratin, and unusual arthroconidial

state. Although arthroconidia are common among members of the Onygenaceae, they are usually alternate arthroconidia of the *Malbranchea* or *Chrysosporium* type (i.e. arthroconidia separated by cells which undergo lysis) [8–10]. The arthroconidia of *O. canadensis* form in chains and dehiscence occurs by fracture of a thin-walled cell or by schizolysis. In Currah's [1] treatment of the Gymnoascaceae, he suggested that the family was phylogenetically heterogeneous. We suggest that *Arachnomyces* may be placed in the Gymnoascaceae where it may be a cleistothecial relative of some members of *Gymnascella* Peck. *Gymnascella dankaliensis* (Castellani) Currah has been shown to be a rare agent of onychomycosis [12], and other species have been isolated occasionally from clinical material as contaminants or colonizers. Recently, *Gymnascella hyalinospora*, for example, was isolated from tracheal aspirates, bronchial washings and sputum from an immunosuppressed patient (UAMH 7366).

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