Phylogeny of the genus *Arachnomyces* and its anamorphs and the establishment of *Arachnomycetales*, a new eurotiomycete order in the *Ascomycota*

C. F. C. Gibas [1, 2], L. Sigler [1*], R. C. Summerbell [3] and R. S. Currah [2]

[1]*University of Alberta Microfungus Collection and Herbarium, Edmonton, Alberta, Canada;* [2]*Department of Biological Sciences, University of Alberta, Edmonton, Alberta, Canada;* [3]*Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands*

Abstract: *Arachnomyces* is a genus of cleistothecial ascomycetes that has morphological similarities to the *Onygenaceae* and the *Gymnoascaceae* but is not accommodated well in either taxon. The phylogeny of the genus and its related anamorphs was studied using nuclear SSU rDNA gene sequences. Partial sequences were determined from ex-type cultures representing *A. minimus*, *A. nodosetosus* (anamorph *Onychocola canadensis*), *A. kanei* (anamorph *O. kanei*) and *A. gracilis* (anamorph *Malbranchea* sp.) and aligned together with published sequences of onygenalean and other ascomycetes. Phylogenetic analysis based on maximum parsimony showed that *Arachnomyces* is monophyletic, that it includes the hyphomycete *Malbranchea sclerotica*, and it forms a distinct lineage within the *Eurotiomycetes*. Based on molecular and morphological data, we propose the new order *Arachnomycetales* and a new family *Arachnomycetaceae*. All known anamorphs in this lineage are arthroconidial and have been placed either in *Onychocola* (*A. nodosetosus*, *A. kanei*) or in *Malbranchea* (*A. gracilis*). *Onychocola* is considered appropriate for disposition of the arthroconidial states of *Arachnomyces* and thus *Malbranchea sclerotica* and the unnamed anamorph of *A. gracilis* are redisposed as *Onychocola sclerotica* comb. nov. and *O. gracilis* sp. nov.

Keywords: *Eurotiomycetes, Arachnomycetales*, *Arachnomycetaceae*, *Arachnomyces*, *Onychocola*, *Malbranchea sclerotica*, SSU rDNA, *Ascomycota*, phylogeny

Introduction

The genus *Arachnomyces* comprises eight species of appendaged cleistothecial ascomycetes producing smooth, oblate, reddish brown ascospores. Massee & Salmon established the genus in 1902 to accommodate *A. nitidus* Massee & E.S. Salmon and *A. sulphureus* Massee & E.S. Salmon. Although the original authors did not select a type species, Malloch & Cain (1970) listed *A. nitidus* as lectotype. Abbott *et al*. (1996) followed this designation when they amended the genus and discussed its taxonomic placement. Although there have been two subsequent reports of *A. nitidus* from Canada and India (Malloch & Cain, 1970; Singh & Mukerji, 1978), this species has not been obtained in culture, thus limiting our ability to determine whether it has a conidial state. *Arachnomyces sulphureus* is known only from the original collection from an old bee's nest and the specimen bears only sparse ascomatal structures (Abbott *et al*., 1996). Neither cultures nor holotype material are available for the Indian species, *A. minutus* N. Singh & Mukerji and *A. validus* N. Singh & Mukerji, described from herbivore dung maintained in damp chambers (Singh & Mukerji, 1978; Mukerji, pers. comm.). The possible relationship of these fungi with species known in culture is difficult to establish.

The remaining species of *Arachnomyces* exhibit similarities in cultural features, but differences in anamorphs. Features noted for *A. minimus* Malloch & Cain were slow growth, often accompanied by a brownish diffusible pigment, and absence of an anamorph (Malloch & Cain, 1970). In 1990, an asexual fungus, *Onychocola canadensis* Sigler, was described on the basis of slow growth, cycloheximide tolerance, and chains of persistent swollen arthroconidia (Sigler & Congly, 1990). In culture, it also produced nodose, circinate to loosely coiled setae that suggested potential to produce a teleomorph. Subsequent mating experiments demonstrated the teleomorph, *A. nodosetosus* Sigler & S.P. Abbott (Sigler *et al.*, 1994). This was the first species of *Arachnomyces* linked to an anamorph. All records of *A. nodosetosus* thus far have been from human nails or skin (Sigler & Congly, 1990; Sigler *et al*., 1994; Campbell *et al*., 1997; Contet-Audonneau *et al*., 1997; Kane *et al*., 1997; Koenig *et al*., 1997; Gupta *et al*., 1998; Llovo *et al*., 2002). *Arachnomyces gracilis* was the second species described with an anamorph, but its anamorph was placed in *Malbranchea* by Udagawa &

* Corresponding author : Lynne Sigler, University of Alberta Microfungus Collection and Herbarium, Devonian Botanic Garden, Edmonton, Alberta, Canada T6G 2E1. Email: lynne.sigler@ualberta.ca

Uchiyama (1999). A third fungus, recovered occasionally from human nail specimens, demonstrated an anamorph with features intermediate between *Onychocola* and *Malbranchea.* A teleomorph identified as *A. kanei* Gibas, Sigler & Summerbell was obtained in mating experiments (Gibas *et al.*, 2002). The hyphomycete *Malbranchea sclerotica* Guarro, Gené & De Vroey (Guarro *et al*., 1993) possesses similar cultural attributes (slow growth, brown diffusible pigment, cycloheximide tolerance), and this has led to its inclusion in the present study.

The phylogenetic position of *Arachnomyces* within the *Ascomycota* is unclear and has been the subject of debate as reviewed by Abbott *et al*. (1996). The genus demonstrates morphological convergences with members of the *Onygenales*, but Currah (1985) rejected it as part of the order based on the elaborate ascomatal initials described as long cylindrical coils by Malloch & Cain (1970). Abbott *et al.* (1996) found the initials to be less elaborate, and considered the genus to be placed appropriately within the *Gymnoascaceae* (*Onygenales*) based on the smooth oblate ascospores and an inability to degrade keratin. However, the setose membranous cleistothecia and *Onychocola* arthroconidial anamorph set it apart from other members of the family.

Since the use of morphological criteria has not resolved this issue, we obtained sequences of the small subunit region (SSU) of the nuclear ribosomal RNA gene (SSU rDNA) from *Arachnomyces* species and compared these with published sequences of onygenalean and other ascomycetes to test the hypothesis that *Arachnomyces* belongs within the *Gymnoascaceae*, and to evaluate possible phylogenetic significance of the variation observed among anamorphs. Only very recently has a member of the genus been subjected to molecular scrutiny, and that study (which used sequences of the nuclear large subunit ribosomal DNA) included the single representative *A. nodosetosus* (Sugiyama & Mikawa, 2001). Our study includes all species available in culture and results provide more substantial evidence to support the appropriate disposition of the genus *Arachnomyces.*

Materials and methods

Cultures are maintained in the University of Alberta Microfungus Collection and Herbarium (UAMH). Ex-type cultures of *A. gracilis* (UAMH 9756), *A. minimus* (UAMH 7113) and *M. sclerotica* (UAMH 7183) and mating type cultures of *A. kanei* (UAMH 5908) and *A. nodosetosus* (UAMH 6106), were newly sequenced. Sequences of onygenalean taxa and other ascomycete orders and families were obtained from GenBank. *Saccharomyces cerevisiae* Meyen ex E.C. Hansen and *Taphrina deformans* (Berk.) Tulasne were used as outgroup taxa.

Cultures were grown on potato dextrose agar (PDA; Difco Laboratories, Detroit, MI) overlaid with a cellophane membrane (Carmichael, 1962). DNA extraction was done following the method of Cubero *et al.* (1999) with some modification (Gibas *et al*. 2002). Approximately 100 mg of fresh fungal mycelium was scraped from the surface of the cellophane membrane, placed in a pre-cooled sterile porcelain mortar containing a small amount of acid sterilized sand, frozen with liquid nitrogen and ground to a powder. Seven hundred fifty µl of extraction buffer [1% w/v cetyl-trimethyl ammonium bromide; 1M NaCl; 100 mM Tris; 20 mM EDTA; 1% w/v polyvinyl polypyrolidone] were added to the ground material. The mixture was transferred into a sterile 2 ml screw-capped microcentrifuge tube and incubated for 30 min at 65°C. An equal volume of chloroform:isoamyl alcohol (24:1 v/v) was added, the tube was inverted about 20 times, and the mixture centrifuged for 15 min at 10 000*g* at room temperature. The upper layer containing crude DNA was purified using the QIAquick PCR Purification Kit (QIAGEN Inc., Mississauga, ON, Canada) and stored at –20°C.

The SSU rDNA was amplified using primers NS1 and NS8 (synthesized by CyberSyn, Inc., Aston, PA) (White *et al*., 1990). PCR amplification (Perkin Elmer GeneAmp 9700 Thermal cycler, PE Applied Biosystems, Foster, CA) was performed using the following cycling parameters: denaturation at 94°C for 1 min; annealing at 55°C for 1 min and extension at 72°C for 2 min. Initial denaturation was at 94°C for 2 min, and the final extension was at 72°C for 7 min. There were 30 cycles. Cycle sequencing was done with the DYEnamic™ ET Terminator Cycle Sequencing Kit (Amersham Pharmacia Biotech, Inc., Piscataway, NJ) using forward primers NS11, NS13, and NS151 developed by K. N. Egger (University of Northern British Columbia, Prince George, B.C., Canada) and reverse primers NS2, NS4, and NS6 (White *et al.*, 1990) and run on an ABI 377 Automated sequencer (Amersham Pharmacia Biotech, Inc.). Consensus sequences were determined using Sequencher™ version 4.0.2 (Gene Codes Corp. Ann Arbor, MI) and alignment was done manually using the sequence alignment program Se-Al v1.0a1Fat (Rambaut, 1995). The data matrix was analyzed using PAUP* v. 4.0b8 (Swofford, 2001) and the robustness of the resultant phylogenetic tree or inferred clades was tested using bootstrap analysis (Felsenstein, 1985) of 500 resamplings.

Characteristics of colonies, ascomata, and anamorph were observed on oatmeal and cereal agar (Kane *et al*., 1997) and on PDA at 25°C. Microscopic

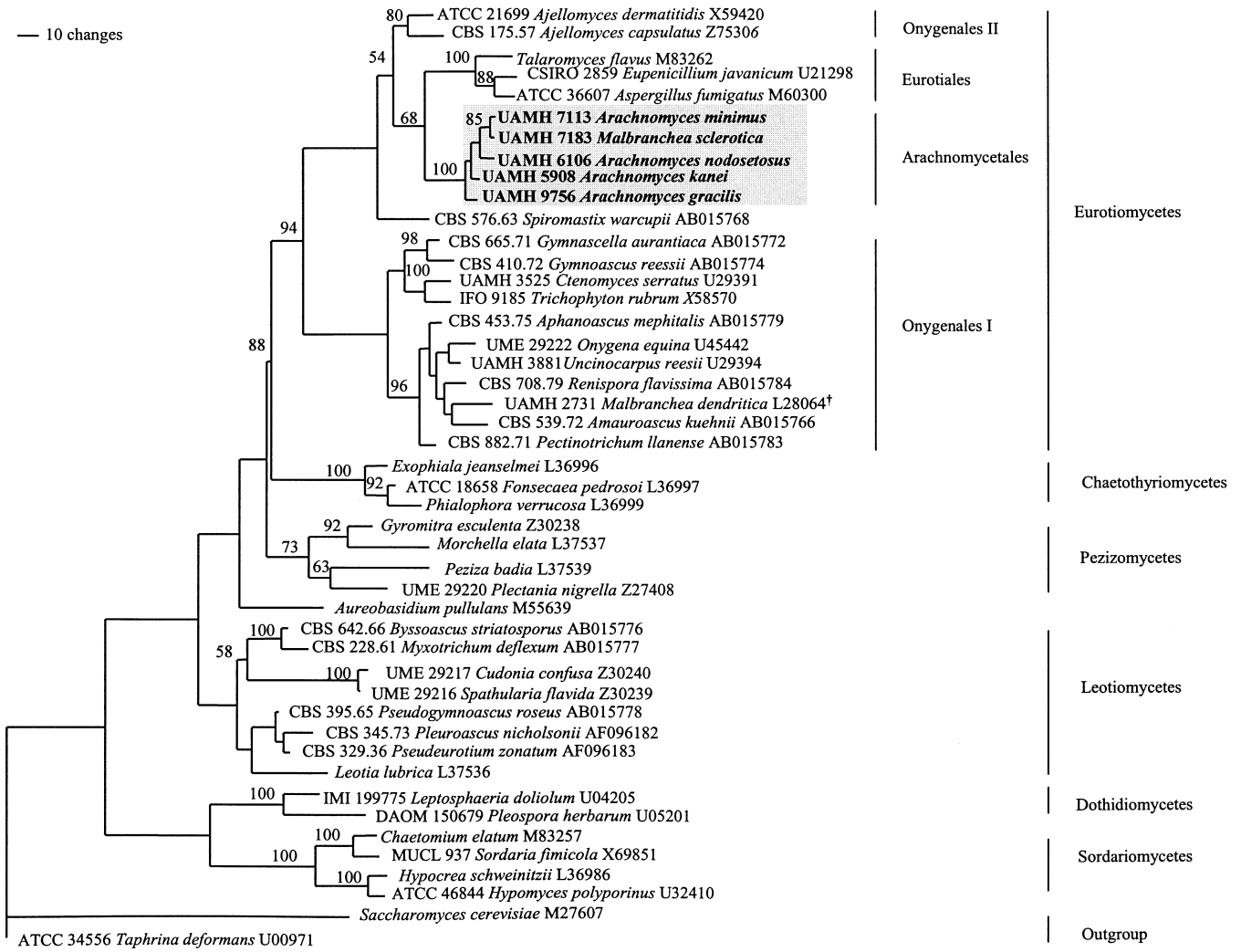

Fig. 1. One of 18 most parsimonious trees from analysis of SSU rDNA sequences for *Arachnomyces* species and other ascomycetes. The *Arachnomyces* clade is highlighted. Numbers before species names refer to accession numbers for isolates from culture collections and those after refer to GenBank accession numbers. GenBank accession numbers for the newly sequenced taxa are: *A. minimus* (UAMH 7113) = AF525311; *M. sclerotica* (UAMH 7183) = AF525310; *A. nodosetosus* (UAMH 6106) = AF525309; *A. kanei* (UAMH 5908) = AF525308; *A. gracilis* (UAMH 9756) = AF525307. Numbers above the branches are percentages of bootstrap values of 500 resamplings. Bootstrap values below 50% are not shown. † Sequence L28064 was later shown to be incorrect for *M. dendritica* UAMH 2731, see Sigler *et al*., this volume (pp. x – xx).

observations were done from mounts prepared in lactofuchsin mounting medium (Kane *et al.,* 1997). For SEM, materials were fixed in 2.5% glutaraldehyde in Millonig's buffer (Millonig, 1961), pH 7.3 and post fixed in 2% osmium tetroxide in the same buffer. After drying to the critical point, the samples were sputter coated with gold and examined with a Hitachi S-2500 (Hitachi, Ltd., Tokyo, Japan).

Results

Sequences of SSU rDNA from four *Arachnomyces* species and for *Malbranchea sclerotica* ranged from 1371 to 1700 bp. Sequences from GenBank ranged from 1095 to 1700 bp in length. Manual alignment yielded a total length of 1836 characters. Of these, 1267 were constant, 227 were variable but parsimony-uninformative, and 342 were parsimony-informative. Using the heuristic random sequence addition search option with gaps treated as missing characters, 18 most parsimonious trees were obtained; one is shown in Fig. 1. It had a consistency index of 0.520, a retention index of 0.662, a rescaled consistency of 0.345 and a homoplasy index of 0.480.

The *Eurotiomycetes* clade (bootstrap 94%) included four groups each having strong support (Fig. 1). One represented the *Eurotiales* (bootstrap 100%) and another the *Onygenales sensu stricto* (shown as Onygenales I) containing representatives of the families *Arthrodermataceae* (100%), *Onygenaceae* (96%) and *Gymnoascaceae* (98%). Species of *Ajellomyces* grouped together and apart from the other *Onygenales* (80%; shown as Onygenales II). The *Arachnomyces* species and *M. sclerotica* formed a well-supported clade (highlighted clade; 100% support) outside the clade comprising the *Onygenales sensu stricto. Malbranchea sclerotica* was shown to be

part of the *Arachnomyces* clade grouping in a subclade with *A. minimus* (bootstrap 85%).

Discussion

Some characteristics of the genus *Arachnomyces* suggest a relationship with the *Onygenales*, and this has contributed to the difficulty in classifying the genus within the Ascomycota. These characters include a membranous peridium; lightly pigmented (reddish brown) ascospores without germ pores; vegetative hyphae having ampulliform swellings (i.e. racquet hyphae) and being pale in color; anamorphs of arthroconidia or aleurioconidia having rhexolytic dehiscence, and tolerance to cycloheximide (Malloch & Cain, 1970; Currah, 1985; Sigler *et al.*, 1994; Abbott *et al.*, 1996). Sugiyama and Mikawa (2001) included *A. nodosetosus* in their study of LSU rDNA sequence relationships among onygenalean taxa; however, this species was excluded from their overall analysis of the full taxon set and was included only within a reduced taxon set assessing the phylogenetic placement of *Spiromastix* and related taxa. Their results showed that *A. nodosetosus* belongs to a lineage distinct from the clades comprising species of *Ajellomyces* and *Spiromastix,* a finding that agrees with our SSU rDNA sequence data. Our data do not support the hypothesis that *Arachnomyces* is a member of the *Gymnoascaceae* as suggested by Abbott *et al.* (1996) but instead support Currah's (1985) decision to exclude *Arachnomyces* from the *Onygenales*. The phylogenetic tree (Fig.1) shows that the genus is not closely related to either the *Onygenales sensu stricto* or the *Eurotiales* but forms an independent well-supported clade (bootstrap 100%). Although the tree shows a sister-group relationship with the *Eurotiales*, it would be difficult to justify extending the concept of the *Eurotiales* to include the *Arachnomyces* clade. The biological differences between these groups are clearly reflected in differences in phenotypic characters. *Arachnomyces* species produce arthroconidia or aleurioconidia by fragmentation of a portion of the aerial hyphae, and they are highly cycloheximide tolerant, whereas eurotialean taxa have phialidic or holoblastic anamorphs and exhibit variable, but generally relatively low, cycloheximide tolerance. Our data support the creation of a new order and family placed within the *Eurotiomycetes* (Eriksson, 2001).

Taxonomy

Arachnomycetales, ***Arachnomycetaceae*** Gibas, Sigler & Currah, *ord. et fam. nov.* **Figs**. 2-9.

Ascomata cleistothecia, nonostiolata, setulosa, peridium membranaceum de textura angulari; asci evanescentes, octospori; ascosporae oblatae; leviter pigmentatae, foramina germinalia absunt; *anamorphoses de arthroconidiis vel aleurioconidiis cum dehiscentia lytica interdum schizolytica.*

Ascomata cleistothecia, nonostiolate, with setae (**Figs**. 2, 5, 6); peridium membranous of *textura angularis* (**Fig**. 3); asci evanescent, eight spored; ascospores oblate, lightly pigmented, lacking germ pores (**Fig**. 4); anamorphs consisting of arthroconidia or aleurioconidia and having lytic dehiscence, sometimes also schizolytic dehiscence (**Figs**. 7-9).

Type genus: ***Arachnomyces*** Massee & E. S. Salmon, 1902 emend. Abbott *et al*. 1996

Table 1. Differential characteristics of *Arachnomyces* species grown in culture.

	A. gracilis	*A. kanei*	*A. minimus*	*A. nodosetosus*	*Arachnomyces* sp. I (anamorph *O. sclerotica*)
Setal wall	slightly nodose	slightly nodose	slightly nodose	strongly nodose	slightly nodose
Setal tip	straight	circinate or loosely coiled	circinate or loosely coiled	circinate or loosely coiled	circinate or loosely coiled
Ascospore size (µm)	2.8 – 3.2 x 1.5 –2[a]	3.5 – 4.5 x 2.5 – 3[b]	2.8 – 3.5 x 1.5 – 2[c]	4 – 4.5 x 3 – 3.5[d]	3 – 4 x 2.5[e]
Sclerotia	absent	absent	absent	absent	present
Anamorph	alternate cylindrical arthroconidia	stalked or sessile aleurioconidia and swollen alternate arthroconidia	absent	swollen arthroconidia in persistent chains	alternate cylindrical arthroconidia
Thallism	homothallic[f]	heterothallic	homothallic[f]	heterothallic	heterothallic[e]

[a]Udagawa & Uchiyama, 1999, [b] Gibas *et al.,* 2002, [c] Malloch & Cain, 1970, [d]Sigler *et al.,* 1994, [e]Gibas *et al.,* unpublished results, [f]Based on ex-type strains.

Eight species of *Arachnomyces* have been described, but two are considered *nomina dubia*: holotype specimens of the Indian species *A. minutus* and *A. validus* are lost. There are no living cultures of *A. nitidus* and *A. sulphureus,* and herbarium material of the latter contains few ascomata (Abbott *et al*., 1996). New collections are required to evaluate holomorph concepts for these species.

Arachnomyces minimus, *A. nodosetosus*, *A. gracilis* and *A. kanei*, and the as yet undescribed teleomorph of *Malbranchea sclerotica* (referred to hereafter as *Arachnomyces* sp. I), exhibit strongly similar cultural features, including tolerance to cycloheximide at 400-500 µg/ml, slow growth in culture with colony diameters on PDA not exceeding 2.5 cm in 21 days and the production of strong diffusible yellowish to reddish brown pigments.

Arachnomyces species grown in culture differ in their sexual systems, setal shape and ornamentation, in their production of sclerotia and in their anamorphs (Table 1). The ex-type cultures of *A. minimus* and *A. gracilis* are self-fertile and are assumed to be homothallic. However, some strains putatively identified as *A. minimus* based on growth habit, setal morphology and absence of an anamorph (Sigler *et al*. 1994), do not produce teleomorphs when grown alone, and it is not yet known whether they are conspecific. *Arachnomyces nodosetosus* and *A. kanei* are heterothallic (Sigler *et al*., 1994; Gibas *et al*., 2002), as is *Arachnomyces* sp. I (Gibas *et al.*, unpublished results). Setae of *A. gracilis* are straight and taper at the tip; those of other species are circinate to loosely or irregularly coiled (**Figs**. 2, 6). Setae are predominantly smooth, but often have intermittent, irregular wall thickenings, being strongly nodose in *A. nodosetosus* (**Fig**. 5) and slightly nodose in *A. minimus, A. gracilis, A. kanei* (**Fig**. 6) and *Arachnomyces* sp. I. In culture, setae resembling those on the ascomata may arise from the vegetative mycelium. Sclerotia are formed only in *Arachnomyces* sp. I.

No anamorph is known for *A. minimus*, and the arthroconidial anamorphs of other species differ, resulting in their disposition in either *Onychocola* or *Malbranchea. Onychocola canadensis*, the anamorph of *A. nodosetosus,* produces persistent chains of swollen arthroconidia that appear to separate by a combination of schizolytic and rhexolytic dehiscence (**Fig**. 7). *Onychocola kanei* (teleomorph, *A. kanei*) produces cylindrical to irregularly swollen, alternate arthroconidia as well as aleurioconidia that are sessile or stalked; dehiscence is purely rhexolytic (**Fig**. 8). These features initially suggested a placement within the genus *Chrysosporium* (Gibas *et al*., 2002). The similarly rhexolytically dehiscing arthroconidia of *A. gracilis* differ in being regularly cylindrical and alternate, a characteristic that led Udagawa and Uchiyama to place the anamorph in *Malbranchea* (**Fig**. 4) (Sigler & Carmichael, 1976, Udagawa & Uchiyama, 1999). Despite these morphological differences, our phylogenetic analysis showed that *Arachnomyces* is monophyletic and that inclusion of *Malbranchea sclerotica* is strongly supported. Our high bootstrap values suggest that these variations in anamorph morphology do not have significance above the species level. Thus, we are placing the anamorphs in a single genus. When Abbott *et al*. (1996) amended *Arachnomyces*, they referred the anamorph to *Onychocola* and provided the description, "conidia thallic-arthric, barrel-shaped to subcylindrical, hyaline, 0-1 septate, separating by rhexolysis of thin-walled cells or by schizolysis, often persisting in chains." We consider *Onychocola* to be appropriate for the arthroconidial states of *Arachnomyces*, and redispose the anamorph of *A. gracilis* and *M. sclerotica* within the genus. Since Udagawa and Uchiyama (1999) did not provide a binomial for the anamorph of *A. gracilis,* we propose the new species *O. gracilis*.

Onychocola gracilis Gibas, Sigler & Currah *sp. nov*.

Teleomorph: *Arachnomyces gracilis* Udagawa & Uchiyama. 1999. Mycoscience 40:286

A Latin diagnosis for the anamorph was provided by Udagawa and Uchiyama (1999) as follows:

Hyphae fertiles saepe arcuatae, repetite ramosae. Arthroconidia hyalina, cylindrica vel doliiformia, interdum curvata, 3 – 8 x 2 – 3 µm, levia vel asperulata, utrinque vulgo truncata. Arthroconidia ex hyphis principalibus hyalina, cylindrica, 2.5 – 10 x 1.5 – 3 µm, levia vel plus minusve asperulata.
Typus: UAMH 9756 colonia exsiccata ex SUM 3046, ex solo Uganda, 1996, isolata S. Uchiyama.

Fertile hyphae arising as lateral branches, often arcuate or sinuous and branching repeatedly to form dense clusters. Arthroconidia hyaline, cylindrical or barrel shaped, sometimes curved, 3 – 8 x 2 – 3 µm, smooth-walled to asperulate, truncated at both ends or rounded at one end. Arthroconidia formed on the straight primary hyphae, hyaline, cylindrical, 2.5 – 10 x 1.5 – 3 µm, smooth-walled to slightly asperulate (Udagawa & Uchiyama, 1999).
Holotype: UAMH 9756, dried colony established from the ex-type strain SUM 3046 (originally isolated from soil in an old termitarium, Uganda, 1996, S. Uchiyama).

Onychocola sclerotica (Guarro, Gené & De Vroey) Gibas, Sigler *et* Currah *comb. nov.*

Basionym: *Malbranchea sclerotica* Guarro, Gené & De Vroey. 1993. Mycotaxon 98:471.

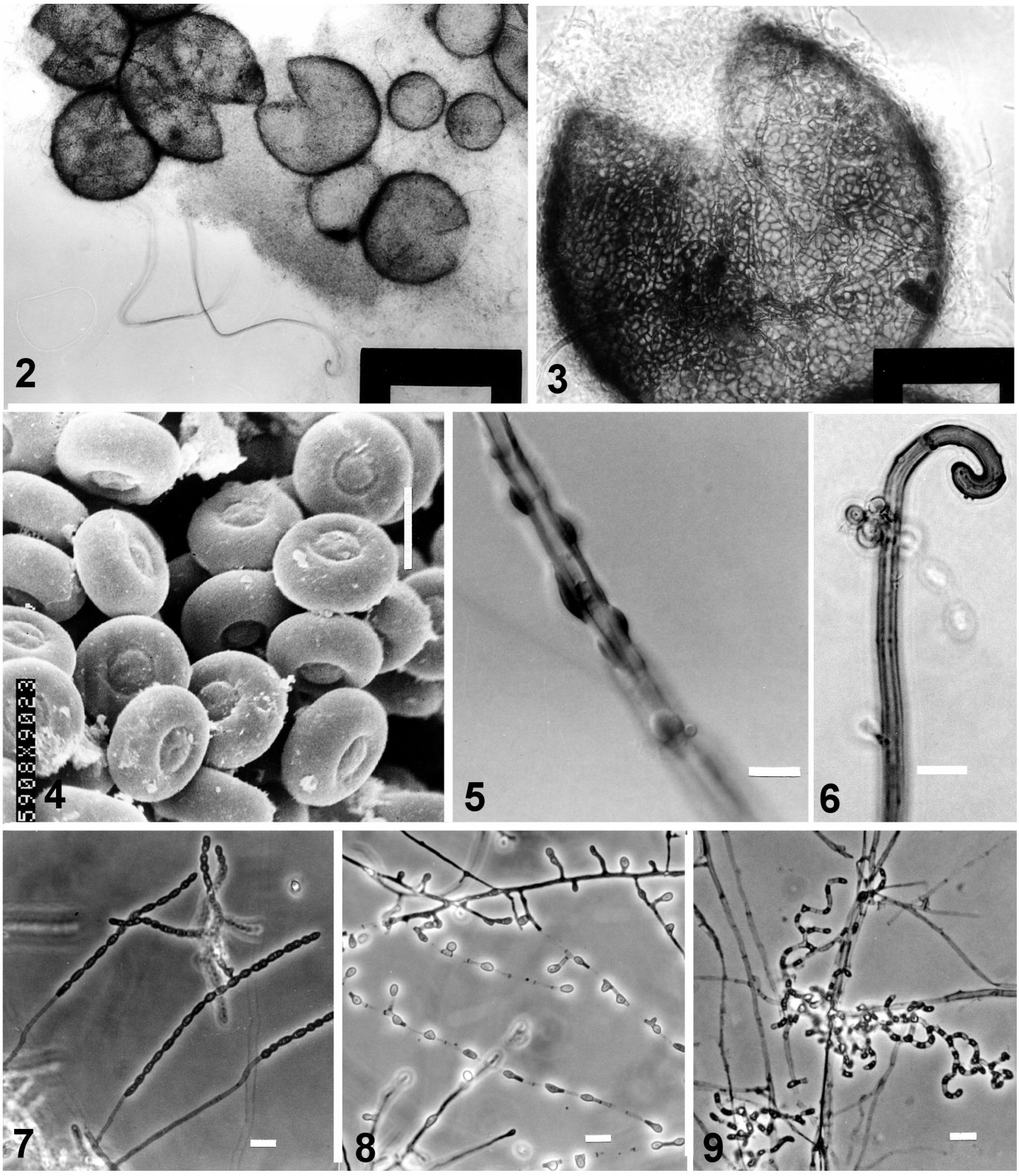

Fig. 2. *Arachnomyces minimus.* Ascomata bearing circinate setae, bar = 200 µm. **3.** *Arachnomyces minimus*. Membranous peridium of *textura angularis*, bar = 100 µm. **4.** *Arachnomyces kanei.* Smooth oblate ascospores observed by SEM (UAMH 5908 x 9023), bar = 2.5 µm. **5.** *Arachnomyces nodosetosus.* Strongly nodose seta, bar = 10 µm. **6.** *Arachnomyces kanei*. Slightly nodose seta with circinate tip, bar = 10 µm. **7.** *Onychocola canadensis.* Swollen arthroconidia in persistent chains (UAMH 5344). Bar = 10 µm **8.** *Onychocola kanei.* Sessile and stalked aleurioconidia and alternate arthroconidia (UAMH 9024), bar = 10 µm. **9.** *Onychocola gracilis.* Cylindrical alternate arthroconidia (UAMH 9756), bar = 10 µm.

The distribution and habitat of *Arachnomyces* species are not well understood. There is an association, however, with rotting grasses and with human nails. The habitat of the type species *A. nitidus* is grass stems and rat dung in the U.K. (Massee & Salmon, 1902), hay dung compost and dead grass in Canada (Malloch & Cain, 1970), and ox dung in India (Singh & Mukerji, 1978). *Arachnomyces sulphureus* was also observed on rotten straw from an old bee's nest in the U.K. (Massee & Salmon, 1902). *Arachnomyces nodosetosus* in its anamorphic form has been obtained almost exclusively from human nail samples (rarely from skin) where it usually has a role in causing onychomycosis. Cases of infection have been reported from North America, Europe, New Zealand, and Australia (Sigler & Congly, 1990; Sigler *et al*., 1994; Sigler & Flis, 1998; Campbell *et al*., 1997; Contet-Audonneau *et al*., 1997; Kane *et al*., 1997; Koenig *et al*., 1997; Gupta *et al*., 1998; Llovo *et al.*, 2002). *Arachnomyces nodosetosus* is weakly cellulolytic, as measured by its capacity to break down a cellophane membrane, and not keratinolytic as determined by its inability to degrade hairs *in vitro* (Carmichael, 1962; Sigler *et al*., 1994; Kane *et al.*, 1997). Its niche in nature is unknown, but an association with rotting plant material is suspected because many of the individuals with onychomycosis profess to be keen gardeners. One patient was known to amend her garden with a decomposed straw and manure mixture previously used for mushroom growing (Sigler *et al*., 1994). *Arachnomyces kanei*, which has been confirmed on two occasions as causing onychomycosis and is otherwise only known from nail isolations of uncertain clinical significance (Gibas *et al*., 2002), may be similar in ecology. The ex-type culture of *A. minimus* was obtained from rotting wood. Nonascocarpic isolates thought to represent this species are encountered on rare occasion from nails or skin; to date, only one of these isolates (UAMH 7097) is suspected to have been the cause of an onychomycosis (Sigler *et al.*, 1994). Similarly, *Arachnomyces* sp. I (*O. sclerotica*) is a soil species occasionally recovered from cutaneous specimens. Its isolation from at least one nail positive for fungal filaments and its isolation from nails of two other patients suggests the possibility that this species may also play a role in onychomycosis.

The inability of *Arachnomyces* species to degrade keratin does not explain their frequent isolation from nails and their role in causing nondermatophytic onychomycosis. In the case of one patient infected with *A. kanei*, infection recurred after the nail had fallen off and then regrown, suggesting that the fungus inhabits the nail bed or the paronychium (Gibas *et al*., 2002). Keratin degradation has been assessed only for some isolates of *A. nodosetosus* and *A. kanei* using the in vitro hair digestion assay (Sigler & Carmichael, 1976; Kane *et al*. 1997). Detecting keratinolytic abilities in cultures of fungi from nails or other keratin-rich materials relies on the interaction of three variable components, the fungus as it behaves in vitro, the source and condition of the native keratin used in the assay, and the individual who reads and interprets the results. Thus, reports concerning the presence of this ability in cultured fungi can differ (Scott & Untereiner, 2003). It should be noted, however, that Scott & Untereiner (2003) did not detect keratin degradation for *A. minimus* using the keratin azure test to assess dye release. The consistent and unequivocal detection of keratin degradation in vitro is useful taxonomically and especially when a strong correlation exists with other characters. Evidence of degradation that is faint or otherwise equivocal, i.e., interpreted as a doubtful or absent, is not by itself definitive for taxonomic purposes and must be weighed against the predictive value of other characters. Repeated isolation of these fungi from nails of living hosts and an inability to demonstrate keratinolysis in vitro, could indicate that the signals necessary for the regulation of the genes responsible are not being provided in culture.

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Key to species of *Arachnomyces*

1a. Ascomata produced homothallically (i.e. in single-spore culture); arthroconidial anamorph present or absent 2
1b. Ascocarps not produced in unmated cultures; arthroconidial anamorph present 3

2a. Setae coiled or circinate; anamorph absent or unknown 4
2b. Setae straight and tapered; anamorph of alternate arthroconidia .....*A. gracilis* (anamorph *O. gracilis*)

3a. Arthroconidia regularly swollen; in persistent chains.....*A. nodosetosus* (anamorph *O. canadensis*)
3b. Arthroconidia alternate; cylindrical to irregularly swollen; readily fragmenting by rhexolysis of thin walled intervening cells 6

4a. Setae slightly nodose; ascospores mostly < 3.5 µm long .....*A. minimus*
4b. Setae smooth; ascospores mostly > 3.5µm long 5

5a. Ascomata 100-300 µm diam .....*A. nitidus*
5b. Ascomata 500-700 µm diam .....*A. sulphureus*

6a. Alternate arthroconidia cylindrical or barrel shaped, stalked conidia absent; sclerotia present .....*Arachnomyces* sp. I (anamorph *O. sclerotica*)
6b. Alternate arthroconidia regularly swollen, sessile and stalked conidia also present, sclerotia absent .....*A. kanei* (anamorph *O. kanei*)

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