Rare malbranchea-like fungal isolates from clinical specimens in United States of America.

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Abstract

The fungi of the order *Onygenales* can cause important human infections; however, their taxonomy and worldwide occurrence is still little known. We have studied and identified a representative number of clinical fungi belonging to that order from a reference laboratory in the USA. A total of twenty-two strains isolated from respiratory tract (40 %) and human skin and nails (27.2 %) showed a malbranchea-like morphology. Six genera were phenotypically and molecularly identified, i.e. *Auxarthron*/*Malbranchea* (68.2 %), *Arachnomyces* (9.1 %), *Spiromastigoides* (9.1 %), and *Currahmyces* (4.5 %), and two newly proposed genera (4.5 % each). Based on the results of the phylogenetic study, we synonymised *Auxarthron* to *Malbranchea*, and erected two new genera: *Pseudoarthropsis* and *Pseudomalbranchea*. New species are proposed: *Arachnomyces bostrychodes*, *A. graciliformis*, *Currahmyces sparsispora*, *Malbranchea gymnoascoidea*, *M. multiseptata*, *M. stricta*, *Pseudoarthropsis crassisporea*, *Pseudomalbranchea gemmata* and *Spiromastigoides geomyces*, along with a new combination for *Malbranchea gypsea*. The echinocandins showed the highest *in vitro* antifungal activity against the studied isolates, followed by terbinafine and posaconazole; in contrast, amphotericin B, fluconazole, itraconazole and 5-fluorocytosine were less active or lacked *in vitro* activity against these fungi.

Taxonomic Novelties

new genera: *Pseudoarthropsis* Stchigel, Rodr.-Andr. & Cano, *Pseudomalbranchea* Rodr.-Andr., Cano & Stchigel; **new species:** *Arachnomyces bostrychodes* Rodr.-Andr., Cano & Stchigel, *Arachnomyces graciliformis* Rodr.-Andr., Stchigel and Cano, *Currahmyces sparsispora* Rodr.-Andr., Cano & Stchigel, *Malbranchea gymnoascoidea* Rodr.-Andr., Stchigel & Cano, *Malbranchea multiseptata* Rodr.-Andr., Cano & Stchigel, *Malbranchea stricta* Rodr.-Andr., Stchigel & Cano, *Pseudoarthropsis crassisporea* Rodr.-Andr., Stchigel & Cano, *Pseudomalbranchea gemmata* Rodr.-Andr., Cano & Stchigel, *Spiromastigoides geomyces* Stchigel, Rodr.-Andr. & Cano; **new combinations:** *Malbranchea californiense* (G.F. Orr & Kuehn) Rodr.-Andr., Stchigel & Cano, *Malbranchea chlamydospora* (M. Solé, Cano & Guarro) Rodr.-Andr., Cano & Stchigel, *Malbranchea compacta* (G.F. Orr & Plunkett) Rodr.-Andr., Cano & Stchigel, *Malbranchea concentrica* (M. Solé, Cano & Guarro) Rodr.-Andr., Stchigel & Cano, *Malbranchea conjugata* (Kuehn) Rodr.-Andr., Cano & Stchigel, *Malbranchea indica* (Kuehn) Rodr.-Andr., Cano & Stchigel, *Malbranchea longisporea* (Stchigel, Y. Marín, Guarro & Cano) Rodr.-Andr., Stchigel & Cano, *Malbranchea ostraviense* (Hubka, Dobiášová & M. Kolařík) Rodr.-Andr., Cano & Stchigel, *Malbranchea pseudauxarthron* (G.F. Orr & Kuehn) Rodr.-Andr., Stchigel & Cano, *Malbranchea reticulata* (Arx) Rodr.-Andr., Stchigel & Cano, *Malbranchea umbrina* (Boud.) Rodr.-Andr., Cano & Stchigel, *Malbranchea zuffiana* (Morini) Rodr.-Andr., Stchigel & Cano, *Pseudoarthropsis cirrhata* (Oorschot & de Hoog) Stchigel, Rodr.-Andr. & Cano, *Spiromastigoides gypsea* (Sigler & Carmichael) Stchigel, Rodr.-Andr. & Cano.

Introduction

The order *Onygenales* includes medically important fungi, such as the dermatophytes and the thermally dimorphic systemic pathogens (*Histoplasma*, *Coccidioides* and related fungi), which are naturally present in keratinous substrates, in soil, and in freshwater sediments (Currah 1985, 1994, Doveri *et al.* 2012, Dukik *et al.* 2017, Hubálek 2000, Hubka *et al.* 2013, Sharma & Shouche 2019). The genus *Malbranchea*, which is characterized by the production of alternate arthroconidia in branches from the vegetative hyphae, is one of the genus-form of this order; however, its pathogenic role in human infections is little known. Only a few cases of fungal infections by species of this genus have been described: *Malbranchea dendritica* has been recovered from lungs, spleen and liver of mice (Sigler & Carmichael 1976), *Malbranchea pulchella* has been suggested as a possible cause of sinusitis (Benda & Corey 1994), and *Malbranchea cinnamomea* was recovered from dystrophic nails in patients with underlying

chronic illnesses (Lyskova 2007, Salar & Aneja 2007). More recently, *Malbranchea* spp. have been proposed as one of the causative agents of Majocchi's granuloma (Govind *et al.* 2017; Durdu *et al.* 2019). In a study of 245 patients with fungal saprophytic infections of nails and skin, *Malbranchea* spp. were isolated in 1% of skin samples (Lyskova 2007). Other studies demonstrated the coexistence (0.3% of the cases) of *Malbranchea* spp. with the primary pathogen patients with tuberculosis (Benda & Corey 1994, Yahaya *et al.* 2015).

Malbranchea was erected by Saccardo in 1882 for a single species, *Malbranchea pulchella*. It is characterized by alternate arthroconidia originating in curved branches from the vegetative hyphae, which developed on the surface of wet cardboard collected by A. Malbranche in Normandy, France (Fig. 1). Cooney and Emerson reviewed the genus in 1964, providing an appropriate description for mesophilic (*M. pulchella*) and thermophilic (*Malbranchea sulfurea*) species. In a more recent revision by Sigler and Carmichael (1976) twelve species were accepted, while a close relationship with the genus *Auxarthron* (family *Onygenaceae*, order *Onygenales*) was reported, i.e. the species *Auxarthron conjugatum* forms a malbranchea-like asexual morph, and *Malbranchea albolutea* produces a sexual morph related to *Auxarthron*. Also, Sigler and co-workers (2002) connected *Malbranchea filamentosa* with *Auxarthron* based on molecular studies, and also reported the production of fertile ascomata after an *in vitro* mating of several sexually compatible strains of *M. filamentosa*. The genus *Auxarthron* produces reddish brown, appendaged gymnothecial ascomata with globose prototunicate 8-spored asci, and globose or oblate, reticulate ascospores (Solé *et al.* 2002). Some species of this genus, such as *Auxarthron ostraviense* and *A. umbrinum* have been reported as producing onychomycosis in humans (Hubka *et al.* 2013), and *Auxarthron brunneum*, *A. compactum* and *A. zuffianum* were also isolated from the lungs of kangaroo rats, *A. conjugatum* from lungs of rodents, and *A. umbrinum* from lung of dogs, bats and rodents (Orr *et al.* 1963, Kuehn *et al.* 1964).

Malbranchea-like asexual morphs are also present in other taxa of ascomycetes. The genus *Arachnomyces* (family *Arachnomycetaceae*, order *Arachnomycetales*; Malloch & Cain 1970, Guarro *et al.* 1993), characterized by the production of brightly coloured cleistothecial ascomata bearing setae, and by the production of an onychocola-like (Sigler *et al.* 1994) or a malbranchea-like (Udagawa & Uchiyama 1999) asexual morph, have been also implicated in animal and human infections. Specifically, *Arachnomyces nodosetosus* and *Arachnomyces kanei* have been reported as causing nail and skin infections in humans (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, Erbagci *et al.* 2002, Gibas *et al.* 2002, Llovo *et al.* 2002, O'Donoghue *et al.* 2003, Gibas *et al.* 2004, Stuchlík *et al.* 2011, Järv 2015, Gupta *et al.* 2016). More recently, *Arachnomyces peruvianus* has been reported to cause cutaneous infection (Brasch *et al.* 2017) and *Arachnomyces glareosus* was isolated from nail and skin samples (Gibas *et al.* 2004; Sun *et al.* 2019).

The recently described species *Spiromastigoides albida*, isolated from human lung in USA (Stchigel *et al.* 2017), also produces a malbranchea-like asexual morph. This genus (family *Spiromastigaceae*, *Onygenales*) produces orange gymnothecial ascomata with contorted to coiled appendages and pitted and lenticular ascospores (Kuehn & Orr 1962, Uchiyama *et al.* 1995, Unterwiesing *et al.* 2002, Hirooka *et al.* 2016).

Due to the limited knowledge of *Malbranchea* and their relatives on human infections, we have studied phenotypically and molecularly a set of malbranchea-like fungal strains from clinical specimens received in a fungal reference centre in the USA. Phylogenetic study and an antifungal susceptibility testing were also carried out.

Materials And Methods

Fungal strains

Twenty-two malbranchea-like fungal strains (nineteen from human specimens and three from animals) from different locations in USA were included in this study. The strain number, anatomical source, and geographic origin of the specimens are listed in Table 1. They were provided by the Fungus Testing Laboratory of the University of Texas Health Science Centre at San Antonio (UTHSC; San Antonio, Texas, USA).

Phenotypic study

For cultural characterization, suspensions of conidia were prepared in a semi-solid medium (0.2 % agar; 0.05 % Tween 80) and inoculated onto phytone yeast extract agar (PYE; Becton, Dickinson & Company, Sparks, MD, USA; Carmichael & Kraus 1959), potato dextrose agar (PDA; Pronadisa, Madrid, Spain; Hawksworth *et al.* 1995), oatmeal agar (OA; 30 g of filtered oat flakes, 15 g agar-agar, 1 L tap water; Samson *et al.* 2010), bromocresol purple-milk solids-glucose agar (BCP-MS-G; 80 g skim milk powder, 40 g glucose, 10 mL of 1.6 % of bromocresol purple in 95 % ethanol, 30 g agar-agar, 1 L tap water; Kane & Smitka 1978), and test opacity tween medium (TOTM; 10 g bacteriological peptone, 5 g NaCl, 1 g CaCl₂, 5 mL Tween, 5 mL Tween 80, 15 g agar-agar, 1 L tap water; Slifkin 2000). Colonies were characterized after 14 days at 25°C in the dark. Potato dextrose agar (PDA) was used to determine the cardinal temperatures of growth. Colour notations were taken according to Kornerup & Wanscher (1978). Christensen's urea agar (EMD Millipore SA, Darmstadt, Germany; Christensen 1946) was inoculated and incubated for 4 days at 25 °C in the dark to detect the production of urease. Cycloheximide tolerance was tested growing the fungal strains on Sabouraud dextrose agar (SDA; Pronadisa S.A., Spain) supplemented with 0.2 % cycloheximide (Sigma, USA) at 30 °C for two weeks. Fungal tolerance to NaCl was evaluated on SDA adding 3, 10 and 20 % w/w NaCl, with the same incubation conditions as previously described. The microscopic structures were characterized and measured from wet mountings of slide cultures, using water and 60% lactic acid. Photo micrographs were taken using a Zeiss Axio-Imager M1 light microscope (Oberkochen, Germany) with a DeltaPix Infinity X digital camera using Nomarski differential interference contrast. The descriptions of the taxonomical novelties were submitted to MycoBank (<https://www.mycobank.org/>; Crous *et al.* 2004).

DNA extraction, amplification and sequencing

Total DNA was extracted as previously described (Valenzuela-Lopez *et al.* 2018), and the following phylogenetic markers were amplified: the internal transcribed spacers (ITS) (ITS5/ITS4 primers; White *et al.* 1990, and a fragment of the large subunit (LSU) gene (LR0R/LR5 primers; Vilgalys & Hester 1990; Rehner & Samuels 1994) of the nrDNA. Amplicons were sequenced at Macrogen Europe (Macrogen Inc., Madrid, Spain) using the same pair of primers. Consensus sequences were obtained by SeqMan software v. 7 (DNASTar Lasergene, Madison, WI, USA). Sequences generated in this work were deposited in GenBank (Table 1).

Phylogenetic analysis

A preliminary molecular identification of the isolates was carried out with ITS and LSU nucleotide sequences using BLAST (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>), and only the sequences of ex-type or reference strains from GenBank were included for identification. A maximum level of identity (MLI) $\geq$ 98% was used for species-level and < 98% for genus-level identification. A maximum-likelihood (ML) and Bayesian-inference (BI) phylogenetic analyses of the concatenated ITS-LSU sequences were performed in order to determine the phylogenetic placement of our clinical strains. Species of the order Arachnomycetales were used as outgroup. The sequence alignments and ML / BI analyses were performed according to Valenzuela-Lopez *et al.* (2018). The final matrices used for the phylogenetic analysis were deposited in TreeBASE (www.treebase.org; accession number: 25068).

Antifungal susceptibility testing

In vitro antifungal susceptibility testing was carried out following the broth microdilution method from the Clinical and Laboratory Standards Institute (CLSI) protocol M38 (CLSI, 2017) with some modifications. The antifungal drugs tested were amphotericin B (AMB), fluconazole (FLC), voriconazole (VRC), itraconazole (ITC), posaconazole (PSC), anidulafungin (AFG), caspofungin (CFG), micafungin (MFG), terbinafine (TRB), and 5-fluorocytosine (5-FC). Briefly, incubation media, temperature and time were set to the sporulation requirements of every strain, and conidia suspensions were inoculated into the microdilution trays after being adjusted by haemocytometer counts. Incubation was set at 35 °C (without light or agitation) until the drug-free well displayed a visible fungal growth (minimum 48 h; maximum 10 days) for quantification of the Minimal Effective Concentrations (MEC) for the echinocandins and the Minimal Inhibitory Concentrations (MIC) for the other tested antifungals. The MEC value was established as the lowest drug concentration at which short, stubby and highly branched hyphae were observed, while the MIC value was defined as the lowest concentration that completely inhibited the fungal growth. *Candida parapsilosis* ATCC 22019 was used as the quality control strain in all experiments.

Results

Fungal diversity

Table 1 shows the identity of the twenty-two fungal strains studied. The highest number of strains corresponded to *Auxarthron umbrinum* (4), followed by *Auxarthron alboluteum* (2), *Auxarthron conjugatum* (2), and *Malbranchea aurantiaca* (2). *Auxarthron zuffianum*, *Currahmyces indicus* and *Malbranchea flocciformis* were represented by one strain each. Eight strains were only identified at genus-level (three belonging to *Malbranchea*, two to *Spiromastigoides*, two to *Arachnomycetes*, and one to *Arthrospis*), one strain (FMR 17684) only at family-level (*Onygenaceae*).

Molecular phylogeny

Our phylogenetic study included 92 sequences corresponding to 75 species with a total of 1,213 characters (700 ITS and 513 LSU) including gaps, of which 579 were parsimony informative (402 ITS and 177 LSU). The ML analysis was congruent with that obtained in the BI analysis, both displaying trees with similar topologies. The datasets did not show conflict with the tree topologies for the 70% reciprocal bootstrap trees, which allowed the two genes to be combined for the multi-locus analysis.

Twenty of our strains were placed into a main clade corresponding to the members of the *Onygenales* (100% BS / 1 PP), while two were placed in the *Arachnomycetales* (100% BS / 1 PP) (Fig. 2). The *Onygenales* clade was divided into eight clades corresponding to the families *Onygenaceae* (100% BS / 1 PP), *Gymnascaceae* (98% BS / 1 PP), *Nannizziopsiaceae* (100% BS / 1 PP), *Helicoarthrosporaceae* (100% BS / 1 PP), *Arthrodermataceae* (100% BS / 1 PP), *Ajellomycetaceae* (97% BS / 1 PP), *Ascosphaeraceae* (100% BS / 1 PP), and *Spiromastigaceae* (92% BS / 0.99 PP), which included a basal terminal branch for *Pseudospiromastix tentaculata*. Most of our strains (17/22) were distributed into several subclades of the *Onygenaceae*: 15/22 into *Auxarthron*/*Malbranchea* subclade (100% BS / 1 PP), one into a terminal branch (FMR 17683) together *Currahmyces indicus* (100% BS / 1 PP), and another one (FMR 17684) into a distant, independent terminal branch. One strain (FMR 17692) was placed into the *Gymnascaceae*, in a terminal branch together with *Arthrospis cirrhata* (100% BS / 1 PP). The *Spiromastigaceae* included the last two strains (FMR 17686 and FMR 17696), placed into a terminal branch together *Malbranchea gypsea* (100% BS / 1 PP).

Taxonomy

Since the strains FMR 17685 and FMR 17691 represented two species of *Arachnomyces* that were different from the other species of the genus, they are proposed as new, i.e. *Arachnomyces bostrychodes* and *Arachnomyces graciliformis*, respectively.

Arachnomyces bostrychodes Rodr.-Andr., Cano & Stchigel, *sp. nov.* Fig. 3. MycoBank MB 834921.

Etymology: From Greek *βοστρυχος*, curl, due to the appearance of the reproductive hyphae. **Diagnosis:** The phylogenetically closest species to *Arachnomyces bostrychodes* is *A. peruvianum* (Fig. 2). Nevertheless, *Arachnomyces bostrychodes* lacks of a sexual morph and racket hyphae (both present in *A. peruvianum*), and produces longer conidia than *A. peruvianum* ($4.0\text{--}8.0 \times 1.0\text{--}2.0\ \mu\text{m}$ vs. $4.0\text{--}5.0 \times 1.0\text{--}3.0\ \mu\text{m}$); also, *A. bostrychodes* grows more slowly on OA (13–14 mm diam. after 14 days at 25 °C) than *A. peruvianum* (30 mm diam.) (Cain 1957, Brasch *et al.* 2016). *Arachnomyces bostrychodes* resembles morphologically *Arachnomyces gracilis*, but the former grows faster, and produces more twisted branches and lacks sexual morph. **Type: United States of America:** Texas, from a human's scalp, 2008, *N. Wiederhold* (CBS H-24452 – holotype; CBS 146926 = FMR 17685 = UTHSCSA D118-91 – ex-type cultures; LSU sequences GenBank LR701766). **Description:** *Vegetative hyphae* hyaline, septate, branched, smooth- and thin-walled, $1.0\text{--}2.0\ \mu\text{m}$ wide. *Fertile hyphae* well-differentiated, arising as lateral branches from the vegetative hyphae, successively branching to form dense clusters, arcuate, sinuous, contorted or tightly curled, $1.0\text{--}2.0\ \mu\text{m}$ wide, forming randomly intercalary and terminally arthroconidia. *Conidia* enteroarthric, hyaline, one-celled, smooth-walled, cylindrical, barrel-shaped, and finger-like-shaped when terminal, $4.0\text{--}8.0 \times 1.0\text{--}2.0\ \mu\text{m}$, mostly curved and truncated at one or (mostly) both ends, separated from the fertile hyphae by rhexolysis. *Chlamydospores*, *racquet hyphae*, *setae*, and *sexual morph* not observed.

Culture characteristics: Colonies on PYE reaching 19–20 mm diam. after 2 weeks at 25 °C, elevated, cottony, margins regular, white (5A1), sporulation absent; reverse light orange (5A4). Colonies on PDA reaching 11–12 mm diam. after 2 weeks at 25 °C, elevated, velvety with floccose patches, margins regular, yellowish white (4A2), sporulation abundant; reverse greyish yellow (4B6). Colonies on PDA reaching 13–14 mm diam. after 2 weeks at 30 °C, slightly elevated, velvety to floccose, regular margins, white (4A1), sporulation sparse; reverse, greyish yellow (4B6). Colonies on OA researching 13–14 mm diam. after 2 weeks at 25 °C, flattened, smooth and granulose, irregular margins, yellowish white (2A2) at centre and light yellow (2A5) at edge, sporulation abundant. Exudate and diffusible pigment absent.

Minimum, optimal and maximum temperature of growth (on PDA): 10 °C, 30 °C, and 37 °C, respectively. Non-haemolytic. Casein not hydrolysed. Not inhibited by cycloheximide. Urease and esterase (TOTM) tests positive. Growth occurs at NaCl 10 % w/w, but not at 20 % w/w.

Arachnomyces graciliformis Rodr.-Andr., Stchigel & Cano, *sp. nov.* Fig. 4. MycoBank MB 834923.

Etymology: Because the morphological similarity with *Arachnomyces gracilis*. **Diagnosis:** *Arachnomyces graciliformis* is phylogenetically close to *A. glareosus* and to *A. minimus* (Fig. 2). These three species form common clade together with *A. nodosetosus* and *A. jinanicus* (84 BS / 1 PP). Unlike *A. glareosus* and *A. minimus*, *A. graciliformis* does not produces racquet hyphae nor sexual morph (Gibas *et al.* 2004) but produces longer conidia than *A. glareosus* ($4.0\text{--}10.0 \times 1.5\text{--}2.0\ \mu\text{m}$ vs. $2.5\text{--}4.5 \times 1.5\text{--}2.0\ \mu\text{m}$), which are not produced by *A. minimus*. *Arachnomyces graciliformis* resembles morphologically *Arachnomyces gracilis*, but the former grows more slowly, produces more twisted fertile branches and does not form a sexual morph (Udagawa & Uchiyama 1999). **Type:**

United States of America: Massachusetts, from an animal's bone, 2012, *N. Wiederhold* (CBS H-24453 – holotype; CBS 146927 = FMR 17691 = UTHSCSA D118-97 – ex-type cultures; LSU sequence GenBank LR743668). *Description:* *Vegetative hyphae* hyaline, septate, branched, smooth- and thin-walled, 1.0–2.0 µm wide. *Fertile hyphae* well-differentiated, arising as lateral branches from the vegetative hyphae, branching repeatedly, sinuous to arcuate or apically coiled, 1.5–2.0 µm wide, forming randomly intercalary and terminally arthroconidia. *Conidia* enteroarthric, hyaline, unicellular, smooth- and thin-walled, cylindrical or finger-like-shaped when terminal, 4.0–10.0 × 1.5–2.0 µm, mostly curved, detached from the fertile hyphae by rhexolysis. *Chlamydospores*, *racquet hyphae*, *setae*, and *sexual morph* not observed.

Culture characteristics: Colonies on PYE reaching 12–13 mm diam. after 2 weeks at 25 °C, elevated, velvety to floccose, margins regular, slightly furrowed, yellowish white (3A2), sporulation absent; reverse greyish orange (5B3). Colonies on PDA reaching 9–10 mm diam. after 2 weeks at 25 °C, slightly elevated, velvety to floccose, margins regular, slightly furrowed, yellowish white (1A2), sporulation absent; reverse greyish yellow (4B3). Colonies on PDA reaching 3–4 mm diam. after 2 weeks at 30 °C, slightly elevated, velvety to floccose, margins regular, slightly furrowed, yellowish white (1A2), sporulation absent; reverse, greyish yellow (4B3). Colonies on OA researching 6–7 mm diam. after 2 weeks at 25 °C, flattened, velvety and granulose, margins irregular, pale yellow (4A3), sporulation absent (conidia appear after 5-6 weeks incubation). Exudate and diffusible pigment absent. Minimum, optimal and maximum temperature of growth (on PDA): 10 °C, 25 °C, and 30 °C, respectively. Non-haemolytic. Casein not hydrolysed. Not inhibited by cycloheximide. Urease and esterase tests positive. Growth occurs at NaCl 10 % w/w, but not at 20 % w/w.

Dichotomous key to *Arachnomyces* (adapted from Sun *et al.* 2019).

- 1a. Homothallic; asexual morph present or not..... 2
- 1b. Heterothallic; asexual morph present 6
- 2a. Peridial setae coiled or circinate; asexual morph absent..... 3
- 2b. Peridial setae straight, tapering towards the apex; asexual morph arthroconidia..... *A. gracilis*
- 3a. Peridial setae slightly nodose; ascospores mostly < 3.5 µm diameter..... 4
- 3b. Peridial setae smooth-walled; ascospores mostly > 3.5µm diamater..... 5
- 4a. Ascospores smooth-walled..... *A. minimus*
- 4b. Ascospores echinulate *A. peruvianus*
- 5a. Ascomata 100–300 µm diameter..... *A. nitidus*
- 5b. Ascomata 500–700 µm diam..... *A. sulphureus*
- 6a. Arthroconidia alternate..... 7
- 6b. Arthroconidia in persistent chains..... 12
- 7a. Arthroconidia cylindrical or barrel-shaped; sclerotia present..... 8

- 7b. Arthroconidia distinct; sclerotia absent.....9
- 8a. Colonies becoming greyish brown, not growing at 35 °C..... *A. glareosus*
- 8b. Colonies white to pale brown, growing at 35 °C..... *A. scleroticus*
- 9a. Arthroconidia subglobose to pyriform..... 10
- 9b. Arthroconidia cylindrical to finger-like-shaped..... 11
- 10a. Arthroconidia smooth-walled to finely asperulate; setae (produced on the vegetative mycelium) smooth-walled to slightly nodose..... *A. kanei*
- 10b. Mature arthroconidia coarsely verrucose; setae (produced on the vegetative mycelium) strongly nodose..... *A. pilosus*
- 11a. Fertile hyphae successively branching to form dense clusters, arcuate, sinuous, contorted or tightly curled..... *A. bostrychodes*
- 11b. Fertile hyphae branching but not in clusters; branches only apically coiled..... *A. graciliformis*
- 12a. Setae (produced on the vegetative mycelium) strongly nodose, circinate or loosely coiled at the apex..... *A. nodosetosus*
- 12b. Setae (produced on the vegetative mycelium) strongly nodose, tip straight..... *A. jinanicus*

Since the strain FMR 17692 was placed in the same terminal clade than *Arthrospis cirrhata*, while the type species of the genus (*Arthrospis truncata*) is phylogenetically far away (into the order Sordariales; Giraldo et al. 2013), we propose the erection of the new genus *Pseudoarthropsis* for *A. cirrhata*, and the new species *Pseudoarthropsis crassispora*.

Pseudoarthropsis Stchigel, Rodr.-Andr. & Cano, **gen. nov.** MycoBank MB 834925.

Etymology: From Greek *ψευδής*-, resembling, because the morphological semblance to *Arthrospis*.

Diagnosis: *Mycelium* composed by hyaline to orange, septate hyphae. *Conidiophores* consisting in fertile lateral branches and a part of the main hyphae, which disarticulate in yellowish orange, thin-walled, cylindrical to cuboid enteroarthric conidia, or in hyaline, thick-walled, ellipsoidal, globose to barrel-shaped holarthric conidia.

Type species: *Pseudoarthropsis cirrhata* (Oorschot & de Hoog) Stchigel, Rodr.-Andr. & Cano.

Pseudoarthropsis cirrhata (Oorschot & de Hoog) Stchigel, Rodr.-Andr. & Cano, **comb. nov.** MycoBank MB 834928.

Basionym: *Arthrospis cirrhata* Oorschot & de Hoog, Mycotaxon 20: 130 (1984).

Description: *Vegetative hyphae* septate, pale yellowish orange, smooth- and thin-walled, dichotomously branched, 2–3 µm wide. *Fertile hyphae* well differentiated, arising at right angles as recurved lateral branches of the vegetative hyphae, forming septa basipetally to produce chains of enteroarthric conidia. *Arthroconidia* yellowish orange,

smooth- and thin-walled, cylindrical to cuboid, often broader than long, $2.5\text{--}4.0 \times 2\text{--}3\text{ }\mu\text{m}$, truncated at both ends, separated by trapezoid connectives, secession rhexolytic. *Colonies* on PYE reaching 4–5 mm diam. after 10 days at 25 °C, powdery, fealty, slightly raised, orange (5A7), pale orange (5A5) at centre; reverse brownish orange (7C8), diffusible pigment brown.

Type: CBS 628.83, 1984, from a wall near Schiphol, The Netherlands, collector C.A.N. van Oorschot.

Pseudoarthropsis crassispora Rodr.-Andr., Stchigel & Cano, *sp. nov.* Fig. 5. MycoBank MB 834930.

Etymology: From Latin *crassus*-, thick, and *-sporum*, spore, because of the thick wall of the conidia. *Diagnosis*: *Pseudoarthropsis crassispora* is phylogenetically close to *P. cirrhata*. Nevertheless, the former produces holoarthric conidia, while they are enterarthric in the latter. Also, the conidia of *P. crassispora* are ellipsoidal, globose or broadly barrel-shaped, while these are cylindrical to cuboid (often wider than they are long) in *P. cirrhata* (van Oorschot & de Hoog 1984). Moreover, the conidia are bigger in *P. crassispora* than in *P. cirrhata* ($4.5\text{--}5.5 \times 2.5\text{--}3.5\text{ }\mu\text{m}$ vs. $2.5\text{--}4.0 \times 2.0\text{--}3.0\text{ }\mu\text{m}$). Also, *P. crassispora* grows faster than *P. cirrhata* (on PYE at 25 °C), and the maximum temperature of growth is at 37 °C and 30 °C, respectively. *Type*: **United States of America**: Minnesota, from a human's bronchial washing, 2012, *N. Wiederhold* (CBS H-24454 – holotype; CBS 146928 = FMR 17692 = UTHSCSA DI18-98 – ex-type cultures; LSU sequence GenBank LR701763). *Description*: *Vegetative hyphae* septate, hyaline, smooth- and thin-walled, mostly straight, occasionally branched, $1.5\text{--}2.0\text{ }\mu\text{m}$ wide. *Fertile hyphae* well-differentiated, arising as lateral branches of the vegetative hyphae, hyaline, septate, smooth- and thin-walled, erect, simple or branched up to 3 times at the apex, stipe $10\text{--}20 \times 1.5\text{--}2.0\text{ }\mu\text{m}$, branches $10\text{--}70 \times 1.5\text{--}2.0\text{ }\mu\text{m}$, forming septa basipetally to produce chains of arthroconidia. *Conidia* holoarthric, unicellular, hyaline, smooth- and thick-walled, ellipsoidal, globose or barrel-shaped, transiently presents as bi-cellular conidia, $2.5\text{--}3.5 \times 4.5\text{--}5.5\text{ }\mu\text{m}$, in chains of up to 20, separate from the fertile hyphae by schizolysis, rarely by rhexolysis. *Chlamydospores*, *racquet hyphae*, *setae*, and *sexual morph* not observed.

Culture characteristics: Colonies on PYE reaching 13–14 mm diam. after 2 weeks at 25 °C, slightly elevated, velvety, margins regular, furrowed, yellowish white (3A2) and yellowish grey (4B2) at centre, sporulation abundant; reverse pale yellow (4A3). Colonies on PDA reaching 14–15 mm diam. after 2 weeks at 25 °C, flattened, velvety, margins regular, greenish white (30A2) and pastel green (30A4) at centre, sporulation abundant; reverse pastel yellow (3A4). Colonies on PDA reaching 15–16 mm diam. after 2 weeks at 30 °C, slightly elevated, velvety, margins regular, furrowed, yellowish white (3A2), sporulation sparse; reverse yellow (3A6), with a scarce production of yellowish diffusible pigment. Colonies on OA researching 10–11 mm diam. after 2 weeks at 25 °C, flattened, velvety to floccose, margins irregular, greenish white (30A2) and pale green (28A3) at centre, sporulation abundant. Exudate and diffusible pigment absent, except on PDA. Minimum, optimal and maximum temperature of growth on PDA: 10 °C, 30 °C, and 37 °C, respectively. Non-haemolytic. Casein hydrolyzed without pH change. Not inhibited by cycloheximide. Urease and esterase tests positive. The fungus grows up to NaCl 10 % w/w, but not at 20 % w/w.

Due to the strain FMR 17683 being placed into a terminal branch of the *Onygenaceae* together with *Currahmyces indicus* (Sharma & Shouche 2019), and because they differ molecularly and phenotypically, we propose the erection of the new species *Currahmyces sparsispora*.

Currahmyces sparsispora Rodr.-Andr., Cano & Stchigel, *sp. nov.* Fig. 6. MycoBank MB 835692.

Etymology: From Latin *sparsa*-, splashed, *-sporum*, spore, due to the disposition of the conidia along the hyphae.

Diagnosis: *Currahmyces sparsispora* is phylogenetically close to *Currahmyces indicus*; however, they can be differentiated because the former has broader hyphae (1.5–2.0 µm vs. 0.7–1.1 µm) and lacks a sexual morph (typical gymnothecial ascomata are produced on hair-baited soil plates by *C. indicus*).

Type: **United States of America:** Florida, from human sputum, 2007, *N. Wiederhold* (CBS H-24455 – holotype; CBS 146929 = FMR 17683 = UTHSCSA DI18-89 – ex-type cultures; LSU sequence GenBank LR723273).

Description: *Vegetative hyphae* septate, hyaline, smooth- and thin-walled, mostly straight, rarely branched, 1.5–2.0 µm wide. *Fertile hyphae* undifferentiated from the vegetative hyphae. *Conidia* enteroarthric, hyaline, unicellular, smooth- and thin-walled, disposed relatively far from each other along the fertile hyphae, separated by 1–2 evanescent connective cells, cylindrical to slightly barrel-shaped, 3.0–12.0 × 1.0–2.0 µm, separated by rhexolysis. *Chlamydospores*, *racquet hyphae*, *setae*, and *sexual morph* not observed.

Culture characteristics: Colonies on PYE reaching 27–28 mm diam. after 2 weeks at 25 °C, slightly elevated, velvety to floccose, margins regular, pale orange (5A3) at centre and white (5A1) at edge, sporulation sparse; reverse orange (5A6). Colonies on PDA reaching 23–24 mm diam. after 2 weeks at 25 °C, slightly elevated, velvety, margins regular, light orange (5A5) at centre and orange white (5A2) at edge, sporulation sparse; reverse deep orange (6A8). Colonies on PDA reaching 30–31 mm diam. after 2 weeks at 30 °C, slightly elevated, velvety, slightly furrowed, margins regular, orange (5A6), sporulation sparse; reverse brownish orange (6C8). Colonies on OA reaching 20–21 mm diam. after 2 weeks at 25 °C, slightly elevated, velvety, margins regular, orange white (5A2) at centre and white (5A1) at edge, sporulation sparse. Exudate and diffusible pigment absent in all culture media tested. Minimum, optimal and maximum temperature of growth on PDA: 10 °C, 30 °C, and 37 °C, respectively. Haemolytic. Casein not hydrolysed. Not inhibited by cycloheximide. Urease and esterase tests positive. Growth occurs at NaCl 3 % w/w and 10 % w/w, but not at 20 % w/w.

Taking into account that *Auxarthron* and *Malbranchea* are congeneric, as has been in previous studies (Sigler *et al.* 2002, Sarroco *et al.* 2015) and here (Fig. 2), and that *Malbranchea* (Saccardo 1882) has historical priority (International Code of Nomenclature for algae, fungi, and plants; Turland *et al.* 2018) on *Auxarthron* (Orr, Kuehn and Plunkett 1963), we transfer the species of *Auxarthron* (Orr *et al.* 1963) to *Malbranchea* as follows:

Malbranchea californiensis (G.F. Orr & Kuehn) Rodr.-Andr., Stchigel & Cano, ***comb. nov.***

MycoBank MB 835229.

Basionym: *Auxarthron californiense* G.F. Orr & Kuehn, *Can. J. Bot.* 41: 1442 (1963).

Synonym: *Gymnoascus californiensis* (G.F. Orr & Kuehn) Apinis, *Mycol. Pap.* 96: 12 (1964).

Malbranchea chlamydospora (M. Solé, Cano & Guarro) Rodr.-Andr., Cano & Stchigel, ***comb. nov.*** MycoBank MB 835230.

Basionym: *Auxarthron chlamydosporum* M. Solé, Cano & Guarro, *Stud. Mycol.* 47: 108 (2002).

Malbranchea compacta (G.F. Orr & Plunkett) Rodr.-Andr., Cano & Stchigel, ***comb. nov.***

MycoBank MB 835231.

Basionym: *Auxarthron compactum* G.F. Orr & Plunkett, *Can. J. Bot.* 41: 1453 (1963).

Malbranchea concentrica (M. Solé, Cano & Guarro) Rodr.-Andr., Stchigel & Cano, **comb. nov.** MycoBank MB 835232.

Basionym: *Auxarthron concentricum* M. Solé, Cano & Guarro, *Stud. Mycol.* 47: 106 (2002).

Malbranchea conjugata (Kuehn) Rodr.-Andr., Cano & Stchigel, **comb. nov.** MycoBank MB 835233.

Basionym: *Myxotrichum conjugatum* Kuehn, *Mycologia* 47: 883 (1956) [1955].

Synonym: *Auxarthron conjugatum* (Kuehn) G.F. Orr & Kuehn, *Mycotaxon* 24: 148 (1985).

Malbranchea longispora (Stchigel, Y. Marín, Guarro & Cano) Rodr.-Andr., Stchigel & Cano, **comb. nov.** MycoBank MB 835235.

Basionym: *Auxarthron longisporum* Stchigel, Y. Marín, Guarro & Cano, *Persoonia* 31: 267 (2013).

Malbranchea ostraviensis (Hubka, Dobiášová & M. Kolařík) Rodr.-Andr., Cano & Stchigel, **comb. nov.** MycoBank MB 835236.

Basionym: *Auxarthron ostraviense* Hubka, Dobiášová & M. Kolařík, *Med. Mycol.* 50: 619 (2012).

Malbranchea pseudauxarthron (G.F. Orr & Kuehn) Rodr.-Andr., Stchigel & Cano, **comb. nov.** MycoBank MB835237.

Basionym: *Auxarthron pseudauxarthron* G.F. Orr & Kuehn, *Mycologia* 64: 67 (1972).

Malbranchea umbrina (Boud.) Rodr.-Andr., Cano & Stchigel, **comb. nov.** MycoBank MB 835238.

Basionym: *Gymnoascus umbrinus* Boud., *Bull. Soc. mycol. Fr.* 8: 43 (1892).

Synonym: *Auxarthron brunneum* (Rostr.) G.F. Orr & Kuehn, *Can. J. Bot.* 41: 1446 (1963).

Auxarthron umbrinum (Boud.) G.F. Orr & Plunkett, *Can. J. Bot.* 41: 1449 (1963).

Auxarthron thaxteri (Kuehn) G.F. Orr & Kuehn, *Mycologia* 63: 200 (1971).

Gymnoascus subumbrinus A.L. Sm. & Ramsb., *Trans. Br. Mycol. Soc.* 5: 424 (1917) [1916].

Gymnoascus umbrinus var. *thaxteri* (Kuehn) Apinis, *Mycol. Pap.* 96: 14 (1964).

Myxotrichum brunneum Rostr., *Bot. Tidsskr.* 19: 216 (1895).

Myxotrichum thaxteri Kuehn, *Mycologia* 47: 878 (1956) [1955].

Malbranchea zuffiana (Morini) Rodr.-Andr., Stchigel & Cano, **comb. nov.** MycoBank MB 835239.

Basionym: *Gymnoascus zuffianus* Morini, *Mem. R. Accad. Sci. Ist. Bologna, Ser. 4* 10: 205 (1889).

Synonym: *Auxarthron zuffianum* (Morini) G.F. Orr & Kuehn, *Can. J. Bot.* 41: 1445 (1963).

We also revalidate the *Malbranchea* species listed below:

Malbranchea albolutea Sigler & J.W. Carmich., *Mycotaxon* 4(2): 416 (1976).

Synonym: Auxarthron alboluteum Sigler, Hambl. & Flis, *Stud. Mycol.* 47: 118 (2002).

Malbranchea filamentosa Sigler & J.W. Carmich., *Mycotaxon* 15: 468 (1982).

Synonym: Auxarthron filamentosum Sigler, Hambl. & Flis, *Stud. Mycol.* 47: 116 (2002).

Because in a Blast search using the ITS and LSU nucleotide sequences from the ex-type strains, *Malbranchea circinata* and *Malbranchea flavorosea* match with taxa into the family *Myxotrichaceae*, both species are excluded to the genus.

After examination of the lectotype of *Auxarthron indicum* (Patil & Pawar 1987), we concluded that this fungus must be excluded from *Malbranchea* because its sexual morph differs mainly from all species described for the former genus. Whereas *Auxarthron indicum* produces smooth-walled ellipsoidal ascospores and gymnothecial ascomata lacking of true appendages, in *Malbranchea* spp. the ascospores are globose and mostly ornamented, and the ascomata have appendages. Based on the fact that there is no type strain of this species, we consider it as invalid.

Consequently, an emended description of the genus *Malbranchea* is provided as follows:

Malbranchea Sacc. MycoBank MB 8833.

Vegetative hyphae septate, hyaline, smooth- and thin-walled, straight or branched. *Asexual morph* consisting in undifferentiated fertile hyphae, and/or well-differentiated lateral branches, curved or not, which form randomly or basipetally terminal and intercalary arthroconidia. *Conidia* enteroarthric, rarely holoarthric, unicellular, hyaline, smooth- and thin-walled, mostly cylindrical, barrel-shaped, or irregularly shaped, sometimes cylindrical, detached from the fertile hyphae by rhexolysis. *Sexual morph* (when present) consisting in ascomata formed by of an anastomosing network of orange to brown, ornamented or not thick-walled hyphae (gymnothecia), bearing elongate appendages and/or spine projections, within there are small, evanescent, inflated asci which forms eight globose to oblate ascospores, whose cell wall is ornamented with a (coarse or thin) reticulate pattern. Species homothallic or heterothallic, thermotolerant or thermophilic, keratinolytic, chitinolytic or cellulolytic.

Despite the strain FMR 17681 being placed phylogenetically close to *Malbranchea ostraviense* and *Malbranchea umbrina*, it differs genetically and phenotypically from both species, therefore we propose the new species *Malbranchea gymnoascoidea* as follows:

Malbranchea gymnoascoidea Rodr.-Andr., Stchigel & Cano, *sp. nov.* Fig. 7. MycoBank

MB 835212.

Etymology: Because to the ascomata are morphologically like to those of *Gymnoascus reessii*. *Diagnosis:* *Malbranchea gymnoascoidea* is phylogenetically close to *M. ostraviensis* and *M. umbrina* (Fig. 2). Nevertheless, *M. gymnoascoidea* produces smaller ascomata (up to 250 µm diam. in *M. gymnoascoidea* vs. up to 450 and up to 600 µm diam. in both, *M. ostraviensis* and *M. umbrina*, respectively) (Orr *et al.* 1963, Hubka *et al.* 2013). Also, the peridial appendages of *M. gymnoascoidea* are longer than those of *M. umbrina* (250–400 µm vs. 5–72 µm), but shorter than those of *M. ostraviensis* (of 350–600 µm long). The ascospores of *M. gymnoascoidea* are like to those of *M. ostraviensis* (smooth-walled under the bright field microscope, oblate to globose, 2.5–3.5 µm diam), whereas those of *M. umbrina* are lenticular and measure 2.8–4.0 × 2.1–2.6 µm. Moreover, the arthroconidia of *M. gymnoascoidea* are larger than those of *M. umbrina* (6.0–10.0 × 1.5–2.0 µm and 2.6–7.0 × 1.4 µm, respectively).

Malbranchea ostraviensis also produces a pinkish to red diffusible pigment on MEA, PDA and SDA, a feature not observed in *M. gymnoascoides* nor in *M. umbrina*. Both *Malbranchea gymnoascoides* as well as of *M. umbrina* can grow slowly at 35 °C, whereas the maximum temperature of growth for *M. ostraviensis* is of 32 °C. **Type: United State of America:** Texas, from human's bronchial washing, 2005, *N. Wiederhold* (CBS H-24456 – holotype; CBS 146930 = FMR 17681 = UTHSCSA DI18-87 – ex-type cultures; LSU sequence GenBank LR701758).

Description: *Vegetative hyphae* septate, hyaline, smooth- and thin-walled, mostly straight, rarely branched, 1.5–2.5 µm wide. *Asexual morph* consisting in undifferentiated *fertile hyphae* which form randomly intercalary and terminally arthroconidia. *Conidia* enteroarthric, unicellular, hyaline, smooth- and thin-walled, mostly barrel-shaped, sometimes cylindrical or irregularly-shaped, 6.0–10.0 × 1.5–2.0 µm, detached by rhexolysis. *Ascomata* gymnothecial, solitary or in clusters, hyaline at first, becoming orange brown with the age, globose or nearly so, 130–250 µm diam. excluding the appendages, which cover entirely the surface. *Peridial hyphae* septate, orange brown, branching and anastomosing to form a reticulate network, asperulate, very thick-walled, 3.5–5.5 µm wide, fragmenting by the septa when ageing, with lateral appendages. *Appendages* 0–1-septate, orange brown, asperulate, thick-walled, progressively tapering towards the apex, apex sinuous, 250–400 µm long, connected by basal knuckle joints. *Asci* 8-spored, globose or nearly so, 4–7 µm diam., soon deliquescent. *Ascospores* unicellular, hyaline at first, yellowish in mass when mature, smooth-walled under bright field microscope, globose, 2.5–3.5 µm diam.

Culture characteristics: Colonies on PYE reaching 46–47 mm diam. after 2 weeks at 25 °C, slightly elevated, velvety to floccose, margins regular, pale orange (5A3) at centre and white (5A1) at edge, sporulation sparse; reverse orange (5A6). Colonies on PDA reaching 36–37 mm diam. after 2 weeks at 25 °C, slightly elevated, velvety, margins regular, light orange (5A5) at centre and orange white (5A2) at edge, sporulation sparse; reverse deep orange (6A8). Colonies on PDA reaching 31–32 mm diam. after 2 weeks at 30 °C, slightly elevated, velvety, margins regular, slightly furrowed, orange (5A6), sporulation sparse; reverse brownish orange (6C8). Colonies on OA reaching 21–22 mm diam. after 2 weeks at 25 °C, slightly elevated, velvety, margins regular, orange white (5A2) at centre and white (5A1) at edge, sporulation sparse. Exudate and diffusible pigment absent in all culture media tested. Minimum, optimal and maximum temperature of growth on PDA: 10 °C, 25 °C, and 35 °C, respectively. Non-haemolytic. Casein hydrolysed without pH change. Not inhibited by cycloheximide. Urease and esterase tests positive. Growth occurs at NaCl 10 % w/w, but not at 20 % w/w.

Despite the strain FMR 17695 being phylogenetically close to *Malbranchea longispora*, it differs phylogenetically and morphologically from it. Consequently, we propose the erection of the new species *Malbranchea multiseptata*.

Malbranchea multiseptata Rodr.-Andr., Cano & Stchigel, *sp. nov.* Fig. 8. MycoBank MB 835213.

Etymology: From Latin *multi-*, many, and *-septatae*, septa, because the vegetative hyphae are multiseptate. **Diagnosis:** *Malbranchea multiseptata* is phylogenetically linked to *M. longispora*. Nevertheless, *M. multiseptata* does not form chlamydospores nor a sexual morph as in *M. longispora* (Crous *et al.* 2013). Also, *M. multiseptata* produces shorter conidia (3.0–9.0 × 1.5–2.0 µm) than those of *M. longispora* (4.0–24.0 × 1.0–5.5 µm). **Type: United States of America:** Texas, from human's bronchial washing, 2014, *N. Wiederhold* (CBS H-24457 – holotype; CBS 146931 = FMR 17695 = UTHSCSA DI18-101 – ex-type cultures; LSU sequence GenBank LR701760). **Description:** *Vegetative hyphae* hyaline, smooth- and thin-walled straight to sinuous, sparsely branched, 1.0–2.0 µm wide, becoming highly septate with the age, septa thickened. *Fertile hyphae* arising as lateral branches (sometimes arranged opposite each other) from the vegetative hyphae, unbranched, straight or slightly sinuous,

1.5–2.0 µm wide, forming randomly intercalary and terminally arthroconidia. *Conidia* enteroarthric, unicellular, hyaline, smooth- and thin-walled, separated by evanescent connective cells, cylindrical, 3.0–9.0 × 1.5–2.0 µm, rounded at the end when terminal, rhexolytic secession. *Chlamydospores*, *racquet hyphae*, *setae*, and *sexual morph* not observed.

Culture characteristics: Colonies on PYE reaching 35–36 mm diam. after 2 weeks at 25 °C, elevated, velvety to floccose, margins regular, white (5A1), sporulation sparse; reverse greyish yellow (4B4). Colonies on PDA reaching 34–35 mm diam. after 2 weeks at 25 °C, slightly elevated, velvety to floccose, margins regular, white (5A1), sporulation absent; reverse yellowish white (3A2). Colonies on PDA reaching 27–28 mm diam. after 2 weeks at 30 °C, slightly elevated, velvety to floccose, margins regular, white (5A1), sporulation absent; reverse pale yellow (3A3). Colonies on OA researching 37–38 mm diam. after 2 weeks at 25 °C, flattened, barely perceptible growth, not distinguishable colour, sporulation sparse. Exudate and diffusible pigment absent in all culture media tested. Minimum, optimal and maximum temperature of growth on PDA: 10 °C, 25 °C, and 35 °C, respectively. Haemolytic. Casein hydrolyzed without pH change. Not inhibited by cycloheximide. Urease positive. Growth occurs at NaCl 3 % w/w, but not at 10 %w/w. Neither grow on TOTM.

Because the strain FMR 17680 was placed phylogenetically close to *Malbranchea filamentosa* but in a separate terminal branch, and because both differ morphologically and genotypically, the new species *Malbranchea stricta* is proposed.

Malbranchea stricta Rodr.-Andr., Stchigel & Cano, *sp. nov.* Fig. 9. MycoBank MB 835219.

Etymology: Latin *stricta*, strict, due to the production of the typical reproductive structures of the genus. **Diagnosis:** *Malbranchea stricta* is phylogenetically close to *M. filamentosa*. Also, both species lack of a sexual morph (Sigler *et al.* 2002). However, *M. filamentosa* produces more regularly shaped conidia than *M. stricta*, and forms thick-walled brown setae, structures absent in *M. stricta*. **Type:** **United States of America:** Florida, human nail, 2003, *N. Wiederhold* (CBS H-24458 – holotype; CBS 146932 = FMR 17680 = UTHSCSA DI18-86 – ex-type cultures; LSU sequence GenBank LR701639).

Description: *Vegetative hyphae* hyaline, smooth- and thin-walled, straight to sinuous, sparsely branched, 1.5–2.0 µm wide. *Fertile hyphae* well-developed, arising as lateral branches from the vegetative hyphae, mostly unbranched, right or slightly sinuous, contorted or arcuate at the end, up to 25 µm long, 1.5–2.0 µm wide, or developing at the extremes of the vegetative hyphae, in both cases forming arthroconidia randomly intercalary and terminally. *Arthroconidia* enteroarthric, hyaline, becoming yellowish with the age, barrel-shaped, “T”-shaped, “Y”-shaped, finger-shaped or irregularly-shaped 2.0–6.0 × 1.0–2.0 µm, with rhexolytic secession. *Chlamydospores*, *racquet hyphae*, and *sexual morph* not observed.

Culture characteristics: Colonies on PYE reaching 32–33 mm diam. after 2 weeks at 25 °C, flattened, velvety, regular margins, furrowed, white (4A1), sporulation sparse; reverse pale orange (5A3). Colonies on PDA reaching 20–21 mm diam. after 2 weeks at 25 °C, slightly elevated, velvety to floccose, regular margins, white (3A1), sporulation abundant; reverse pale yellow (4A3). Colonies on PDA reaching 20–21 mm diam. after 2 weeks at 30 °C, slightly elevated, velvety to floccose, margins regular, white (3A1), sporulation abundant; reverse yellowish brown (5E8) at centre and greyish yellow (4B5) at the margins. Colonies on OA researching 16–17 mm diam. after 2 weeks at 25 °C, flattened, granulose, white (3A1), margins regular, sporulation sparse. Exudate and diffusible pigment absent. Minimum, optimum and maximum temperature of growth on PDA: 10 °C, 30 °C, and 37 °C, respectively. Colonies

haemolytic (on BA), and casein hydrolyzed without pH changes at 25 °C (on BCP-MS-G). Not inhibited by cycloheximide. Urease and esterase tests positive. Growth occurs at NaCl 10 % w/w, but not at 20 % w/w.

Dichotomous key to *Malbranchea* spp. (adapted from Sigler & Carmichael 1976, Solé *et al.* 2002, and Hubka *et al.* 2013).

- 1a. Homothallic species..... 2
- 1b. Heterothallic species..... 13
- 2a. Peridial appendages longer than 150 µm long..... 3
- 2b. Peridial appendages shorter or absent..... 8
- 3a. Appendages 350–600 µm in length; diffusible pigment pinkish to reddish; not growing at 35 °C..... *M. ostraviensis*
- 3b. Those features not combined..... 4
- 4a. Ascospores smooth-walled under bright field microscope..... *M. gymnoascoides*
- 4b. Ascospores reticulate..... 5
- 5a. Peridial cells short, 4–12 µm in length; peridial projections with truncate ends..... *M. compacta*
- 5b. Peridial cells longer; peridial projections with mostly acute ends..... 6
- 6a. Ascospores usually exceeding 4 µm diameter..... *M. californiensis*
- 6b. Ascospores ≤ 4 µm diam..... 7
- 7a. Species growing at 37 °C..... *M. conjugata*
- 7b. No growth at 37 °C..... *M. umbrina*
- 8a. Asexual morph not produced..... *M. pseudoauxarthron*
- 8b. Malbranchea-like asexual morph present..... 9
- 9a. Ascomata with spine-like peridial projections, 27–40 µm in length..... *M. zuffiana*
- 9b. Ascomata without peridial projections..... 10
- 10a. Colonies on PDA brown..... *M. kuehnii*
- 10b. Colonies on PDA otherwise..... 11
- 11a. Peridial hyphae smooth-walled..... *M. concentrica*
- 11b. Peridial hyphae strongly ornamented; chlamydospores present..... 12

12a. Arthroconidia 2–10 × 2.5–3.5 µm; growing above 30 °C.....	<i>M. chlamydospora</i>
12b. Arthroconidia 4–24 × 1.0–5.5 µm; not growing above 30 °C.....	<i>M. longispora</i>
13a. Fertile hyphae arcuate or curved.....	14
13b. Fertile hyphae straight to sinuous, branched or not.....	21
14a. Fertile hyphae coiled.....	15
14b. Fertile hyphae curved or arcuate.....	16
15a. Thermophilic; conidia 2.5–4.5 µm wide.....	<i>M. cinnamomea</i>
15b. Not thermophilic; conidia narrower.....	<i>M. pulchella</i>
16a. Colonies orange.....	17
16b. Colonies different.....	18
17a. Aleuroconidia laterally or terminally dispersed.....	<i>M. chrysosporoidea</i>
17b. Aleuroconidia absent.....	<i>M. aurantiaca</i>
18a. Colonies golden yellow, exudate brown, diffusible pigment yellow.....	<i>M. graminicola</i>
18b. Features are not combined.....	19
19a. Sexual morph produced by <i>in vitro</i> mating of compatible strains.....	<i>M. albolutea</i>
19b. Sexual morph not formed.....	20
20a. Thick-walled brown setae produced on OA from the vegetative mycelium.....	<i>M. filamentosa</i>
20b. Setae not produced.....	<i>M. arcuata</i>
21a. Fertile hyphae unbranched or scarcely branched.....	22
21b. Fertile hyphae branched.....	23
22a. Arthroconidia cylindrical; becoming many septate with the age.....	<i>M. multiseptata</i>
22b. Arthroconidia barrel-shaped, “T”-shaped, “Y”-shaped, finger-shaped or more irregular; vegetative hyphae regularly septate.....	<i>M. stricta</i>
23a. Fertile hyphae branching acutely, displaying a tree-like appearance.....	<i>M. dendritica</i>
23b. Fertile hyphae branching pattern otherwise.....	24
24a. Fertile hyphae repeatedly branched, in dense tufts.....	<i>M. flocciformis</i>

24b. Fertile hyphae more restrictedly branched.....25

25a. Colonies buff or tan..... *M. fulva*

25b. Colonies lemon yellow..... *M. flava*

Despite the strain FMR 17684 being placed phylogenetically into the *Onygenaceae*, is paraphyletic and distant from the other members of the family, therefore this fungus is proposed as the type species of the new genus *Pseudomalbranchea*.

Pseudomalbranchea Rodr.-Andr., Cano & Stchigel, **gen. nov.** MycoBank MB 835220.

Etymology: Because the morphological similarity with *Malbranchea*.

Description: *Mycelium* sparse, composed of hyaline, smooth- and thin-walled septate hyphae. *Asexual morph* consisting of mostly enteroarthric -occasionally holoarthric- conidia, intercalary disposed along unbranched vegetative hyphae, solitary or in short chains, with rhexolytic or rarely schizolysic secession. *Arthroconidia* one-celled, hyaline, smooth- and thick-walled, cylindrical but becoming globose with the age. *Chlamydospores*, *racquet hyphae* and *sexual morph* not observed.

Type species: *Pseudomalbranchea gemmata* Rodr.-Andr., Cano & Stchigel. MycoBank MB 835221.

Pseudomalbranchea gemmata Rodr.-Andr., Cano & Stchigel, **sp. nov.** Fig. 10. MycoBank MB 835221.

Etymology: From the Latin *gemmatum*, jewelled, because the swollen conidia disposed in chains.

Diagnosis: *Pseudomalbranchea gemmata* is phylogenetically close to *Uncinocarpus reesii* and *Amauroascus volatilis-patellis*. However, it does not produce a sexual morph and it differs from *U. reessi* and *A. volatilis-patellis* by the production of longer arthroconidia ($4.0\text{--}11.0 \times 2.0\text{--}3.5 \mu\text{m}$ in *P. gemmata* vs. $3.5\text{--}6.0 \times 2.5\text{--}3 \mu\text{m}$ in *U. reessi*, and $4.0\text{--}5.4 \times 2.0\text{--}3.0$ in *A. volatilis-patellis*; Orr & Kuehn 1972, Sigler & Carmichael 1976, Currah 1985). As well as *A. volatilis-patellis*, *P. gemmata* lacks appendages, which are present and similar to the asexual morph in *U. reessi* (Currah 1985). **Type:** **United States of America:** Florida, from human's bronchial washing, 2014, *N. Wiederhold* (CBS H-24459 – holotype, CBS 146933 = FMR 17684 = UTHSCSA DI18-90 – ex-type cultures; LSU sequence GenBank LR701762). **Description:** *Mycelium* sparse, composed of hyaline, smooth- and thin-walled, sparsely septate hyphae, $1.0\text{--}2.0 \mu\text{m}$ wide. *Conidia* enteroarthric (occasionally holoarthric), intercalary disposed along unbranched vegetative hyphae, one-celled, solitary or in short chains of up to 7, one-celled, hyaline, smooth- and thick-walled, cylindrical but becoming globose with the age, $4.0\text{--}11.0 \times 2.0\text{--}3.5 \mu\text{m}$, liberated from the fertile hyphae by rhexolysis (rarely by schizolysis). *Chlamydospores*, *racquet hyphae* and *sexual morph* not observed.

Culture characteristics: Colonies on PYE reaching 22–23 mm diam. after 2 weeks at 25 °C, slightly elevated, velvety, margins regular, pale yellow (3A3), sporulation sparse; reverse brown (6E6). Colonies on PDA reaching 24–25 mm diam. after 2 weeks at 25 °C, slightly elevated, velvety, margins regular, pale yellow (3A3), sporulation sparse; reverse light yellow (4A5). Colonies on PDA reaching 25–26 mm diam. after 2 weeks at 30 °C, flattened, radially folded, velvety, margins regular, pale yellow (3A3), sporulation sparse; reverse light yellow (4A5). Colonies on OA reaching 28–29 mm diam. after 2 weeks at 25 °C, flattened, velvety to granulose, irregular margins, white (6A1), sporulation sparse. Exudate and diffusible pigment lacking. Minimum, optimum and maximum temperature of growth on PDA: 10 °C, 30 °C, and 37 °C, respectively. Colonies haemolytic, casein not hydrolyzed. The fungus was

not inhibited by cycloheximide. Urease and esterase tests positive. Growth occurs at NaCl 3 % w/w, but not higher concentration.

Because the strains FMR 17686 and FMR 17696 were placed together in a terminal branch close related to the ex-type strain of *M. gypsea* into the *Spiromastigaceae* clade (Fig. 2), *M. gypsea* is renamed as *Spiromastigoides gypsea*, and the former strains are proposed as belonging to the new species *Spiromastigoides geomycoides*.

Spiromastigoides geomycoides Stchigel, Rodr.-Andr. & Cano, **sp. nov.** Fig. 11. MycoBank MB 835222.

Etymology: Because produce conidiophores morphologically similar to those of the genus *Geomyces*. **Diagnosis:** *Spiromastigoides geomycoides* is phylogenetically close to *S. gypsea*. However, *S. geomycoides* produces smaller conidia ($1.5\text{--}2.5 \times 1.0\text{--}2.0 \mu\text{m}$) than *S. gypsea* [$(2.5)3\text{--}6(9) \times 2\text{--}2.5 \mu\text{m}$] (Sigler & Carmichael 1976). Also, *S. geomycoides* grows faster than *S. gypsea* on PYE at 35 °C. **Type:** **United States of America:** Illinois, from a human foot skin, 2014, *N. Wiederhold* (CBS H-24460 – holotype, CBS 146934 = FMR 17696 = UTHSCSA DI18-102 – ex-type cultures; LSU sequence GenBank LR701768). **Description:** *Mycelium* abundant, composed of hyaline, smooth- and thin-walled, septate, branched, $1.0\text{--}2.0 \mu\text{m}$ wide hyphae, septa thickened with age. *Fertile hyphae* arising as lateral branches, straight or slightly curved, unbranched or, rarely, with a branching pattern similar to that of the conidiophores of *Geomyces*, septate, hyaline, smooth- and thin-walled, producing intercalary and terminally arthroconidia separated by 1-2 empty intermediary cells. *Conidia* enteroarthric, unicellular, hyaline, mostly barrel-shaped, less frequently “T”-shaped or cylindrical, $1.5\text{--}2.5 \times 1.0\text{--}2.0 \mu\text{m}$, rhexolytic dehiscence. *Chlamydospores*, *racquet hyphae* and *sexual morph* not observed.

Culture characteristics: Colonies on PYE reaching 24–25 mm diam. after 2 weeks at 25 °C, flattened, velvety, furrowed, regular margins, white (4A1), abundant sporulation; reverse, pale orange (5A3). Colonies on PDA reaching 26–27 mm diam. after 2 weeks at 25 °C, flattened, velvety, regular margins, white (4A1), abundant sporulation; reverse, yellowish white (4A2). Colonies on PDA reaching more than 90 mm diam. after 2 weeks at 30 °C, flattened, velvety, regular margins, yellowish white (4A2), sporulation absent; reverse, pale yellow (4A3). Colonies on OA researching 20–21 mm diam. after 2 weeks at 25 °C, flattened, granulose, regular margins, white (4A1), abundant sporulation. Exudate and diffusible pigment absent in all culture media tested. Minimum, optimum and maximum temperature of growth on PDA: 5 °C, 30 °C, and 37 °C, respectively. Colonies non-haemolytic. Casein not hydrolyzed. Resistant to cycloheximide. Urease negative and esterase positive. Growth occurs at NaCl 10 % w/w, but not at 20 % w/w.

Other specimens examined: **United States of America:** Minnesota, from blood, 2009, *N. Wiederhold* (FMR 17686).

Spiromastigoides gypsea (Sigler & Carmichael) Stchigel, Rodr.-Andr. & Cano, **comb. nov.** MycoBank MB 835228.

Basionym: *Malbranchea gypsea* Sigler & Carmichael, Mycotaxon 4: 455 (1976). MycoBank MB 317129.

Description (adapted from the original work): Arthroconidia produced intercalary or terminally along of straight primary hyphae, or on short or long lateral branches, separated each one by one or more alternate empty cells, or, rarely, formed immediately adjacent to each other. Arthroconidia unicellular, hyaline, smooth- and thin-walled, cylindrical or slightly barrel-shaped, $(2.5) 3\text{--}6 (9) \times 2\text{--}2.5 \mu\text{m}$, slightly broader than the interconnecting cells. No sexual morph obtained by matting. Colonies on PYE reaching 17–39 mm after three weeks at room temperature, chalky white to creamy white, downy to velvety, slightly raised, surface folded to convoluted, umbonated at centre,

reverse buff. Optimum temperature of growth 25–30 °C. Maximum temperature of growth 37 °C (but strain depending).

Dichotomous key to *Spiromastigoides* spp. (adapted from Hirooka *et al.* 2016).

- 1a. Homothallic..... 2
- 1b. Heterothallic..... 6
- 2a. Ascospores globose to subglobose, reticulate..... *S. sphaerospora*
- 2b. Ascospores oblate, equatorial thickening present or not..... 3
- 3a. Ascospores with equatorial thickening..... 4
- 3b. Ascospores without such equatorial thickening..... 5
- 4a. Ascomata appendages straight or slightly undulate; ascospores yellow, smooth-walled, pitted under SEM..... *S. alatospora*
- 4b. Ascomata appendages slightly undulate or wavy; ascospores pale yellowish brown, minutely punctate under SEM..... *S. saturnispora*
- 5a. Ascospores punctate, sometimes with a few fine grooves in the polar region, 2.5–2.9 × 2.0–2.5 µm..... *S. warcupii*
- 5b. Ascospores lens-shaped, regularly pitted, 3.0 × 2.0 µm..... *S. sugiyamae*
- 6a. Asexual morph chrysosporium-like; sterile ascomata present..... *S. asexualis*
- 6b. Asexual morph not so..... 7
- 7a. Asexual morph malbranchea-like..... 8
- 7b. Asexual morph more complex..... 11
- 8a. Fertile hyphae straight, branched..... *S. gypsea*
- 8b. Fertile hyphae curved..... 9
- 9a. Fertile hyphae successively branched to form sporodochia-like structures..... *S. albida*
- 9b. Fertile hyphae unbranched or scarcely branched..... 10
- 10a. Fertile hyphae unbranched or sparsely branched, curved, up to 28 µm long; chlamydospores present..... *S. curvata*
- 10b. Fertile hyphae unbranched, slightly curved, up to 15 µm long; chlamydospores absent..... *S. minimus*
- 11a. Conidiophores unbranched or scarcely branched..... *S. geomycoides*

- 11b. Conidiophores branched several times..... 12
- 12a. Conidiophores up to 300 µm in length, verticillate..... *S. kosraensis*
- 12b. Conidiophores 100–150 µm in length, with pyramidal or bush-like branching..... 13
- 13a. Conidiophores up to 150 µm long, with pyramidal branching..... *S. pyramidalis*
- 13b. Conidiophores up to 100 µm long, with bush-like branching..... *S. frutex*

***In vitro* antifungal susceptibility testing**

The results of the antifungal susceptibility test are summarized in Table 2. In general, the echinocandins (AFG, CFG and MFG) displayed the most potent *in vitro* antifungal activity, but TRB and PSC also demonstrated a good activity against these fungi. In contrast, limited to no inhibition of growth was observed with AMB, FLC, ITC and 5-FC. Antifungal activity was evaluated against all strains with the exception of FMR 17691, due to the scarce production of conidia and because this strain does not grow in RPMI medium, even after two weeks of incubation.

Discussion

To our knowledge, this is the main study on malbranchea-like fungi from a clinical origin. We have shown that several of these fungi have not been reported previously from human specimens, and although the pathologic role remains uncertain, their diversity is of interest since some represent new species.

Morphological and physiological characterization and phylogenetic analysis has allowed us to identify fifteen strains as belonging to the genus *Malbranchea* (syn. *Auxarthron*), of which three of them are proposed as new species. These results indicate a high diversity of onygenalean fungi in these sorts of substrates, which may be difficult to differentiate only by using phenotypic characteristics.

All strains belonging to *Malbranchea* displayed thermotolerance, suggesting the potential pathogenicity of this genus in animals, including humans, as has been previously noted by others (Saccardo 1908, Saccardo & Trotter 1913, Cooney & Emerson 1964, Sigler & Carmichael 1976). In fact, all them were able to grow at 30 °C, and most of them at 35-37 °C.

Malbranchea-like fungi were most commonly isolated from the respiratory tract (40 %) followed by nails and skin (27.2 %). *Currahmyces sparsispora*, *Malbranchea albolutea*, *M. conjugata*, *M. gymnoascoides*, *M. multiseptata*, *Pseudoarthropsis crassispora* and *Pseudomalbranchea gemmata* were all recovered from respiratory tract specimens (mostly obtained by bronchial-alveolar washing), while those of *M. umbrina* were isolated from the widest variety of anatomical sites. The rest of the taxa isolated were mostly from skin and annexes.

Regarding to the antifungal susceptibility of malbranchea-like fungi, limited data are available. However, in a previous study on onychomycosis-causing strains of *Auxarthron ostraviense* and *Auxarthron umbrinum* (transferred to the genus *Malbranchea* in the present study) reduced susceptibility to AMB, ITC and PSC was reported, but a high susceptibility to TRB was observed (Hubka *et al.* 2013). Another study (Gupta & Kohli 2003) showed that strains of *Arachnomyces nodosetosus* (syn. *Onychocola canadensis*) were highly susceptible to cicciclopirox and TRB. Our results are consistent with such previous studies, but we also demonstrated the enhanced susceptibility of the malbranchea-like fungi to the echinocandins.

List Of Abbreviations

5-FC = 5-fluorocytosine

AFG = anidulafungin

AMB = amphotericin B

BCP-MS-G = bromocresol purple milk solids glucose agar

BI = Bayesian-inference

BLAST = Basic Local Alignment Search Tool

BS = bootstrap support

CFG = caspofungin

CLSI = Clinical and Laboratory Standards Institute

DNA = deoxyribonucleic acid

FLC = fluconazole

ITC = itraconazole

ITS = ribosomal internal transcribed spacers

LSU = large sub unit of the ribosomal genes

MEC = Minimal Effective Concentrations

MFG = micafungin

MIC = Minimal Inhibitory Concentrations

ML = maximum-likelihood

MLI = maximum level of identity

OA = oatmeal agar

PDA = potato dextrose agar

PP = posterios probability

PSC = posaconazole

PYE = phytone yeast extract agar

SDA = Sabouraud dextrose agar

SEM = scanning electron microscopy

TOTM = test opacity tween medium

TRB = terbinafine

TreeBASE = a repository of user-submitted phylogenetic trees and data used to build them

USA = United States of America

UTHSCA = University of Texas Health Science Centre at San Antonio

VRC = voriconazole

Declarations

Ethics approval and consent to participate

Not applicable.

Adherence to national and international regulations

The authors confirm that this manuscript respects the Nagoya Protocol to the Convention on Biological Diversity.

Consent for publication

Not applicable.

Availability of data and material

All data generated or analysed during this study are included in this published article.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

ER-A performed all the experimental work, performing their phenotypic characterization, as well as the DNA extraction and purification, gene sequencing and data processing for phylogenetic analysis, being one of the major contributors of this manuscript. PC-A, performed and supervised with ER-A all the Antifungal susceptibility testing, reviewed the draft and writing part of "Materials and methods". AMS, because their experience on fungi belonging to Onygenales, supervised all steps of the experimental work by ER-A, collaborating in the description of the novel

fungi and in the writing of chapters “Introduction” and “Discussion”, reviewing of the draft several times. WN, carried out the collection and morphological identification of the analysed strains, and reviewing the draft. JG contributed actively in the identification and taxonomy of the fungal strains, and reviewed the draft several times. JFC-L supervised the nucleotide sequence alignment and phylogenetic reconstruction, took the pictures that appear in the figures, contributed actively in the identification and taxonomy of the fungal strains, gave useful suggestions to write the manuscript and reviewed several times the draft. All authors read and approved the final manuscript.

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References

- Benda TJ, Corey JP (1994). *Malbranchea pulchella* fungal sinusitis. Otolaryngology – Head and Neck Surgery 110:501-504.
- Brasch J, Beck-Jendroscheck V, Voos K, Y Andrey, Stchigel AM *et al* (2017). *Xanthothecium peruvianum* isolated from human stratum corneum: A case report, characterization and short review that suggest emendation of *Arachnomyces peruvianus*. Mycoses 60:469-476.
- Cain RF (1957). Studies of coprophilous ascomycetes. IV. Species from the Hudson bay area. Canadian Journal of Botany 35:255-268.
- Campbell CK, Johnson EM, Warnock DW (1997). Nail infection caused by *Onychocola canadensis*: report of the first four British cases. Journal of Medical and Veterinary Mycology 35:423-425.
- Carmichael JW, Kraus HJ (1959). The cattle ringworm fungus, *Trichophyton verrucosum*, in Alberta. Alberta Medical Bulletin 24:201-203.
- Christensen WB (1946). Urea decomposition as means of differentiating *Proteus* and *Paracolon* cultures from each other and from *Salmonella* and *Shigella* types. Journal of Bacteriology 52:461-466.
- CLSI (2017). *Reference method for broth dilution antifungal susceptibility testing of filamentous fungi*, 3rd ed. CLSI standard M38. Clinical and Laboratory Standards Institute, Wayne, PA, USA.
- Contet-Audonneau N, Schmutz JL, Basile AM, De Bièvre C (1997). A new agent of onychomycosis in the elderly: *Onychocola canadensis*. European Journal of Dermatology 7:115–117.
- Cooney DG, Emerson R (1964) Thermophilic fungi. Freeman WH and Co., San Francisco, USA.
- Crous PW, Gams W, Stalpers JA, Robert V, Stegehuis G (2004). MycoBank: an online initiative launch mycology into the 21st century. Studies in Mycology 50:19-22.
- Crous PW, Wingfield MJ, Guarro J, Cheewangkoon R, van der Bank M *et al* (2013). Fungal Planet description sheets: 154-213. Persoonia 31:188-296.

- Currah RS (1985). Taxonomy of the Onygenales: *Arthrodermataceae*, *Gymnoascaceae*, *Myxotrichaceae* and *Onygenaceae*. *Mycotaxon* 24:1-216.
- Currah RS (1994). *Peridial morphology and evolution in the protunicate ascomycetes*. In *Ascomycetes systematics*. Hawksworth DL (ed.). Plenum Press, New York, USA.
- de Hoog GS, Dukik K, Monod M, Packeu A, Stubbe D *et al* (2017). Toward a novel multilocus phylogenetic taxonomy for the dermatophytes. *Mycopathologia* 182:5–31.
- Doveri F, Pecchia S, Vergara M, Sarrocco S, Vannacci G (2012). A comparative study of *Neogymnomyces virgineus*, a new keratinolytic species from dung, and its relationships with the Onygenales. *Fungal Diversity* 52:13–34.
- Dukik K, Muñoz JF, Jiang Y, Feng P, Sigler L *et al* (2017). Novel taxa of thermally dimorphic systemic pathogens in the *Ajellomycetaceae* (Onygenales). *Mycoses* 60:296-309.
- Durdu M, Kandemir H, Ilkit M, de Hoog S (2019). Changing concepts and current definition of Majocchi's Granuloma. *Mycopathologia* 185:187-192.
- Erbagci Z, Balci I, Erkiliç S, Zer Y, Inci R (2002). Cutaneous hyalohyphomycosis and onychomycosis caused by *Onychocola canadensis*: Report of the first case from Turkey. *The Journal of Dermatology* 29:522–528.
- Gibas CF, Sigler L, Currah RS (2004). Mating patterns and ITS sequences distinguish the sclerotial species *Arachnomycetes glareosus* sp. nov. and *Onychocola sclerotica*. *Studies in Mycology* 50:525–531.
- Gibas CF, Sigler L, Summerbell R, Hofstader S, Gupta A (2002). *Arachnomycetes kanei* (anamorph *Onychocola kanei*) sp. nov., from human nails. *Medical Mycology* 40:573–580.
- Giraldo A, Sutton D, Gené J *et al*. (2013). Rare arthroconidial fungi in clinical samples: *Scytalidium cuboideum* and *Arthrospira hispanica*. *Mycopathologia* 175:115–121.
- Govind A, L'Etoile N, Vasquez G (2017). The first reported case of Majocchi's granuloma with *Malbranchea* sp. in a immunocompetent patient. *Case Reports in Infectious Diseases* ID 9196528.
- Guarro J, Gené J, De Vroey CH (1993). Studies on keratinophilic fungi. I. A new *Malbranchea* from Sulawesi. *Mycotaxon* 48:471–476.
- Gupta AK, Gupta G, Jain HC, Lynde CW, Foley K *et al* (2016). The prevalence of unsuspected onychomycosis and its causative organism in a multicenter canadian sample of 30 000 patients visiting physicians's offices. *Journal of the European Academy of Dermatology and Venereology* 30:1567–1572.
- Gupta AK, Horgan-Bell CB, Summerbell RC (1998). Onychomycosis associated with *Onychocola canadensis*: ten case reports and a review of the literature. *Journal of the Academy of Dermatology* 39:410–417.
- Gupta AK, Kohli Y (2003). *In vitro* susceptibility testing of ciclopirox, terbinafine, ketoconazole and itraconazole against dermatophytes and non-dermatophytes, and *in vitro* evaluation of combination antifungal activity. *British Journal of Dermatology* 149:296–305.
- Hawksworth DL, Kirk PM, Sutton BC, Pegler DN (1995). *Ainsworth & Bisby's Dictionary of the Fungi*. 8th edition. CAB International, Wallingford.

- Hirooka Y, Tanney JB, Nguyen H, Seifert K (2016). Xerotolerant fungi in house dust: taxonomy of *Spiromastix*, *Pseudospiromastix* and *Sigleria* gen nov. in *Spiromastigaceae* (Onygenales, Eurotiomycetes). *Mycologia* 108:135–156.
- Hubka V, Dobiasova S, Lyskova P, Mallátová N, Chlebkova J *et al* (2013). *Auxarthron ostraviense* sp. nov., and *A. umbrinum* associated with non-dermatophytic onychomycosis. *Medical Mycology* 51:614–624.
- Hubálek Z (2000). Keratinophilic fungi associated with free-living mammals and birds. *Revista Iberoamericana de Micología* 17:93-103.
- Järv H (2015). Onychomycosis caused by *Onychocola canadensis*: the first report in Estonia and lessons to learn. *Mycoses* 58:113-117.
- Jiang Y, Dukik K, Muñoz JF, Sigler L, Schwartz I *et al* (2018). Phylogeny, ecology and taxonomy of systemic pathogens and their relatives in *Ajellomycetaceae* (Onygenales): *Blastomyces*, *Emergomyces*, *Emmonsia*, *Emmonsiiellopsis*. *Fungal Diversity* 90:245–291.
- Kane J, Smitka C (1978). Early detection and identification of *Trichophyton verrucosum*. *Journal of Clinical Microbiology* 8:740–747.
- Kane J, Summerbell RC, Sigler L, Krajden S, Land G (1997). *Laboratory handbook of dermatophytes: A clinical guide and laboratory manual of dermatophytes and other filamentous fungi from skin, hair and nails*. Belmont CA: Star Publishing Company. 344 pp.
- Koenig H, Ball C, de Bievre C (1997). First european cases of onychomycosis caused by *Onychocola canadensis*. *Journal of Medical and Veterinary Mycology* 35:71–72.
- Kornerup A, Wanscher JH (1978). *Methuen handbook of colour*. 3rd edition. Methuen, London.
- Kuehn HH, Orr GF (1962). A new genus of Gymnoascaceae. *Mycologia* 54:160–167.
- Kuehn HH, Orr GF, Ghosh GR (1964). Pathological implications of the *Gymnoascaceae*. *Mycopathologia et Mycologia Applicata* 24:35–46.
- Llovo J, Prieto E, Vazquez H, Muñoz A (2002). Onychomycosis due to *Onychocola canadensis*: report of the first two Spanish cases. *Medical Mycology* 40:209–212.
- Lyskova P (2007). Saprophytic microscopic fungi and dermatophytes accompanying infections of the skin and nails of patients in the Moravian-Silesian region. *Czech Mycology* 59:125–137.
- Malloch D, Cain RF (1970) The genus *Arachnomycetes*. *Canadian Journal of Botany* 48:839-845.
- O'Donoghue NB, Moore MK, Creamer D (2003). Onychomycosis due to *Onychocola canadensis*. *Clinical and Experimental Dermatology* 28:283-284.
- Orr GF, Kuehn HH, Plunkett OA (1963). A new genus of the *Gymnoascaceae* with swollen peridial septa. *Canadian Journal of Botany* 42:1439-1456.

- Orr GF, Kuehn HH (1972). Notes on *Gymnoascaceae* II. Some *Gymnoascaceae* and keratinophilic fungi from Utah. *Mycologia* 64:55-72.
- Pan S, Sigler L, Cole GT (1994). Evidence for a phylogenetic connection between *Coccidioides immitis* and *Uncinocarpus reesii* (Onygenaceae). *Microbiology* 140:1481-1494.
- Patil MS, Pawar AB (1987). Plectomycetes from Maharashtra II. *Geobios new Reports* 6:194-196.
- Rehner SA, Samuels GJ (1994). Taxonomy and phylogeny of *Gliocladium* analysed from nuclear large subunit ribosomal DNA sequences. *Mycological Research* 98:625–634.
- Rizzo L, Sutton DA, Wiederhold NP, Thompson EH, Friedman R *et al* (2014). Isolation and characterisation of the fungus *Spiromastix asexualis* sp. nov. from discospondylitis in German Shepherd dog, and review of *Spiromastix* with the proposal of the new order Spiromastixales (Ascomycota). *Mycoses* 57:419–428.
- Saccardo PA (1882). Fungi Gallici. Series IV. *Michelia* 2:583–648.
- Saccardo PA (1908). *Notae mycologicae Ser. X. Annales Mycologici* 6:553–516.
- Saccardo PA, Trotter A (1913). *Sylloge fungorum omnium hucusque cognitorum* 22:1240.
- Samson RA, Houbraken J, Thrane U, Frisvad JC, Andersen B (2010) Food and indoor fungi. CBS-KNAW Fungal Biodiversity Centre, Utrecht.
- Sarrocchio S, Diquattro S, Baroncelli R, Cimmino A, Evidente A *et al* (2015). A polyphasic contribution to the knowledge of *Auxarthron* (Onygenaceae). *Mycological Progress* 14:112.
- Sharma R, Shouche YS (2019). Diversity of onygenalean fungi in keratin-rich habitats of Maharashtra (India) and description of three novel taxa. *Mycopathologia* 185:67-85.
- Sigler L, Abbott SP, Woodgyer AJ (1994). New records of nail and skin infection due to *Onychocola canadensis* and description of its teleomorph *Arachnomyces nodosetosus* sp. nov. *Journal of Medical and Veterinary Mycology* 32:275-285.
- Sigler L, Carmichael JW (1976). Taxonomy of *Malbranchea* and some other Hyphomycetes with arthroconidia. *Mycotaxon* 4:349-488.
- Sigler L, Congly H (1990). Toenail infection caused by *Onychocola canadensis* gen. et sp. nov. *Journal of Medical and Veterinary Mycology* 28:405–417.
- Sigler L, Hambleton S, Flis AL, Paré JA (2002). *Auxarthron* teleomorphs for *Malbranchea filamentosa* and *Malbranchea albolutea* and relationships within *Auxarthron*. *Studies in Mycology* 47:111–122.
- Slifkin M (2000). Tween 80 opacity test responses of various *Candida* species. *Journal of Clinical Microbiology* 38:4626-4628.
- Solé M, Cano J, Guarro J (2002). Molecular phylogeny of *Amauroascus*, *Auxarthron*, and morphologically similar onygenalean fungi. *Mycological Research* 106:388-396.

- Stchigel AM, Sutton DA, Cano-Lira JF, Wiederhold N, Guarro J (2017). New species *Spiromastigoides albida* from a lung biopsy. *Mycopathologia* 182:967-978.
- Stuchlík D, Mencl K, Hubka V, Skorepova M (2011). Fungal melanonychia caused by *Onychocola canadensis*: first record of nail infections due to *Onychocola* in the Czech Republic. *Czech Mycology* 63:83–91
- Sugiyama M, Mikawa T (2001). Phylogenetic analysis of the non-pathogenic genus *Spiromatix* (*Onygenaceae*) and related onygenalean taxa based on large subunit ribosomal DNA sequences. *Mycoscience* 42:413–421.
- Sugiyama M, Okawa A, Mikawa T (1999). Molecular phylogeny of onygenalean fungi based on small subunit ribosomal DNA (SSU rDNA) sequences. *Mycoscience* 40:251-258.
- Sugiyama M, Summerbell RC, Mikawa T (2002). Molecular phylogeny of onygenalean fungi based on small subunit (SSU) and large subunit (LSU) ribosomal DNA sequences. *Studies in Mycology* 47:5-23.
- Sun B, Zhou Y, Chen AJ, Houbraken J (2019). Phylogeny and a new species of the genus *Arachnomycetes* (*Arachnomycetaceae*). *Phytotaxa* 394:89-97.
- Turland NJ, Wiersema JH, Barrie FR, Greuter W, Hawksworth DL *et al* (eds.) (2018). International Code of Nomenclature for algae, fungi, and plants (Shenzhen Code) adopted by the Nineteenth International Botanical Congress Shenzhen, China, July 2017. *Regnum Vegetabile* 159. Glashütten: Koeltz Botanical Books.
- Uchiyama S, Kamiya S, Udagawa S (1995). *Spiromastix saturnispora*, a new species from Indonesian soil. *Mycoscience* 36:353-357.
- Udagawa S, Uchiyama S (1999). Taxonomic studies on new or critical fungi of non-pathogenic Onygenales 1. *Mycoscience* 40:277-290.
- Untereiner WA, Scott JA, Naveae FA, Currah S, Bachewich J (2002). Phylogeny of *Ajellomyces*, *Polytolypa* and *Spiromastix* (*Onygenaceae*) inferred from rDNA sequence and non-molecular data. *Studies in Mycology* 47:25-35.
- Valenzuela-Lopez N, Cano-Lira JF, Guarro J, Sutton D, Wiederhold N *et al* (2018). Coelomycetous *Dothideomycetes* with emphasis on the families *Cucurbitariaceae* and *Didymellaceae*. *Studies in Mycology* 90:1-69.
- van Oorschot CA, de Hoog GS (1984). Some hyphomycetes with thallic conidia. *Mycotaxon* 20:129-132.
- Vilgalys R, Hester M (1990). Rapid genetic identification and mapping of enzymatically amplified ribosomal DNA from several *Cryptococcus* species. *Journal of Bacteriology* 172:4238-4246.
- White T, Bruns T, Lee S, Taylor J (1990). Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis M, Gelfand D, Shinsky J, White T (eds) *PCR Protocols: a guide to methods and applications*, Academic Press, San Diego.
- Yahaya H, Taura D, Aliyu IA, Bala JA, Yunusa I *et al* (2015). Spectrum of opportunistic mould infections in suspected pulmonary tuberculosis (TB) patients. *International Journal of Microbiology and Application* 2:6–11.
- Zhan P, Dukik K, Li D, Sun J, Stielow B *et al* (2018). Phylogeny of dermatophytes with genomic character evaluation of clinically distinct *Trichophyton rubrum* and *T. violaceum*. *Studies in Mycology* 89:153–175.

Tables

Table 1. DNA barcodes used to build the phylogenetic tree.

Species	Strains ¹	GenBank accession # ²		Geographic origin and source
		ITS ³	LSU ³	
<i>Ajellomyces capsulatus</i>	UAMH 3536 ^T	AF038354	AF038354	Alberta, Canada; woman, 25-years-old, biopsy of right middle lobe lung
<i>Amauroascus niger</i>	ATCC 22339	MH869547	AY176706	California, U.S.A.; soil
<i>Amauroascus purpureus</i>	IFO 32622 ^T	AJ271564	AY176707	Japan; soil
<i>Amauroascus volatilis-patellis</i>	CBS 249.72 ^T	MH860467	MH872189	Utah, U.S.A.; soil
<i>Aphanoascus mephitalis</i>	ATCC 22144	MH859941	AY176725	Ontario, Canada; wolf dung
<i>Arachniotus verruculosus</i>	CBS 655.71	NR_145221	AB040684	Utah, U.S.A.; soil
<i>Arachnomyces bostrychodes</i> sp. nov.	UTHSCSA DI18-91 = FMR 17685 = CBS 146926 ^T	LR701765	LR701766	Texas, U.S.A.; human scalp
<i>Arachnomyces glareosus</i>	CBS 116129 ^T	AY624316	FJ358273	Alberta, Canada; man, 30-years-old, thumb nail
<i>Arachnomyces graciliformis</i> sp. nov.	UTHSCSA DI18-97 = FMR 17691 = CBS 146927 ^T	LR743667	LR743668	Massachusetts, U.S.A.; animal bone
<i>Arachnomyces gracilis</i>	UAMH 9756 ^T	AY123779	-	Uganda; termitarium soil
<i>Arachnomyces jinanicus</i>	CGMCC3.14173 ^T	KY440749	KY440752	Jinan, China; pig farm soil
<i>Arachnomyces kanei</i>	UAMH 5908 ^T	AY123780	-	Toronto, Canada; human nail
<i>Arachnomyces minimus</i>	CBS 324.70 ^T	AY123783	FJ358274	Ontario, Canada; decaying wood
<i>Arachnomyces nitidus</i>	UAMH 10536	-	AB075351	Israel; twigs
<i>Arachnomyces nodosetosus</i>	CBS 313.90 ^T	AY123784	AB053452	Saskatchewan, Canada; woman, 67-years-old, onychomycosis
<i>Arachnomyces peruvianus</i>	CBS 112.54 ^T	MF572315	MH868792	Peru; <i>Globodera rostochiensis</i> cyst
<i>Arachnomyces pilosus</i>	CBS 250.93 ^T	MF572320	MF572325	Catalonia, Spain; river sediment
<i>Arachnomyces scleroticus</i>	UAMH 7183 ^T	AY123785	-	Sulawesi, Indonesia; poultry farm soil
<i>Arthroderma curreyi</i>	CBS 353.66 ^T	MH858822	MH870459	UK; unknown
<i>Arthroderma onychocola</i>	CBS 132920 ^T	KT155794	KT155124	Prague, Czech

				Republic; human nail
<i>Ascosphaera apis</i>	CBS 252.32	-	AY004344	København, Denmark; <i>Apis mellifera</i>
<i>Ascosphaera subglobosa</i>	A.A. Wynns 5004 (C) ^T	NR_137060	HQ540517	Utah, U.S.A.; pollen provisions of <i>Megachile rotundata</i>
<i>Auxarthronopsis bandhavgarhensis</i>	NFCCI 2185 ^T	HQ164436	NG_057012	Bandhavgarh, India; soil
<i>Auxarthronopsis guizhouensis</i>	CGMCC3.17910 ^T	KU746668	KU746714	Guizhou, China; air
<i>Blastomyces percusus</i>	CBS 139878 ^T	NR_153647	KY195971	Israel; human granulomatous lesions
<i>Canomyces reticulatus</i>	MCC 1486 ^T	MK340501	MK340502	Maharashtra, India; soil
<i>Chrysosporium keratinophilum</i>	CBS 392.67	MH859002	AY176730	New Zealand; soil
<i>Chrysosporium tropicum</i>	MUCL 10068 ^T	MH858134	AY176731	Guadalcanal, Solomon islands; woollen overcoat
<i>Currahmyces indicus</i>	MCC 1548 ^T	MK340498	MK340499	Maharashtra, India; hen resting area
<i>Currahmyces sparsispora</i> sp. nov.	UTHSCSA DI18-89 = FMR 17683 = CBS 146929 ^T	LR723272	LR723273	Florida, U.S.A.; human sputum
<i>Gymnoascus reesii</i>	CBS 410.72	MH860507	MH872224	California, U.S.A.; soil
<i>Helicoarthrosporum mellicola</i>	CBS 143838 ^T	LR761645	LT906535	Granada, Spain; honey
<i>Helicoarthrosporum mellicola</i>	FMR 15673	LR761646	LT978462	Valencia, Spain; honey
<i>Malbranchea albolutea</i>	UTHSCSA DI18-85 = FMR 17679	LR701834	LR701835	Texas, U.S.A.; human BAL
<i>Malbranchea albolutea</i>	UTHSCSA DI18-95 = FMR 17689	LR701836	LR701837	Texas, U.S.A.; human BAL
<i>Malbranchea albolutea</i>	CBS 125.77 ^T	MH861039	MH872808	Utah, U.S.A.; soil
<i>Malbranchea aurantiaca</i>	UTHSCSA DI18-94 = FMR 17688	LR701824	LR701825	California, U.S.A.; animal
<i>Malbranchea aurantiaca</i>	UTHSCSA DI18-88 = FMR 17682	LR701826	LR701827	Texas, U.S.A.; animal skin lesion
<i>Malbranchea aurantiaca</i>	CBS 127.77 ^T	NR_157447	AB040704	Utah, U.S.A.; culture contaminant
<i>Malbranchea californiensis</i>	ATCC 15600 ^T	MH858121	NG_056947	California, U.S.A.; dung of pack rat
<i>Malbranchea chrysosporioidea</i>	CBS 128.77 ^T	AB361632	AB359413	Arizona, U.S.A.; soil

<i>Malbranchea circinata</i>	ATCC 34526 ^T	MN627784	MN627782	Utah, U.S.A.; soil
<i>Malbranchea conjugata</i>	UTHSCSA DI18-105 = FMR 17699	LR701828	LR701829	Florida, U.S.A.; human lung tissue
<i>Malbranchea conjugata</i>	UTHSCSA DI18-103 = FMR 17697	LR701830	LR701831	Texas, U.S.A.; human BAL
<i>Malbranchea conjugata</i>	CBS 247.58	NR_121475	HF545313	Arizona, U.S.A.; soil
<i>Malbranchea dendritica</i>	CBS 131.77 ^T	AY177310	AB359416	Utah, U.S.A.; soil
<i>Malbranchea filamentosa</i>	CBS 581.82 ^T	NR_111136	AB359417	Argentina; soil
<i>Malbranchea flava</i>	CBS 132.77 ^T	AB361633	AB359418	California, U.S.A.; soil
<i>Malbranchea flavorosea</i>	ATCC 34529 ^T	NR 158362	AB359419	California, U.S.A.; soil
<i>Malbranchea flocciformis</i>	UTHSCSA DI18-104 = FMR 17698	LR701822	LR701823	Texas, U.S.A.; human skin
<i>Malbranchea flocciformis</i>	CBS 133.77 ^T	AB361634	AB359420	France; saline soil
<i>Malbranchea fulva</i>	CBS 135.77 ^T	NR_157444	AB359422	Utah, U.S.A.; air
<i>Malbranchea gymnoascoides</i> sp. nov.	UTHSCSA DI18-87 = FMR 17681 = CBS 146930 ^T	LR701757	LR701758	Texas, U.S.A.; human BAL
<i>Malbranchea kuehnii</i>	CBS 539.72 ^T	NR_103573	NG_056928	Unkown; dung
<i>Malbranchea longispora</i>	FMR 12768 ^T	HG326873	HG326874	Beija, Portugal; soil
<i>Malbranchea multiseptata</i> sp. nov.	UTHSCSA DI18-101 = FMR 17695 = CBS 146931 ^T	LR701759	LR701760	Texas, U.S.A.; human BAL
<i>Malbranchea ostraviense</i>	CBS 132919 ^T	NR_121474	-	Ostrava, Czech Republic; fingernail sample
<i>Malbranchea pulchella</i>	CBS 202.38	AB361638	AB359426	Italy; unknown
<i>Malbranchea stricta</i> sp. nov.	UTHSCSA DI18-86 = FMR 17680 = CBS 146932 ^T	LR701638	LR701639	Florida, U.S.A.; human nail
<i>Malbranchea</i> sp.*	CBS 319.61	MH858065	MH869635	California, U.S.A.; soil
<i>Malbranchea umbrina</i>	UTHSCSA DI18-106 = FMR 17700	LR701814	LR701815	Colorado, U.S.A.; human BAL
<i>Malbranchea umbrina</i>	UTHSCSA DI18-107 = FMR 17701	LR701816	LR701817	Colorado, U.S.A.; human sinus
<i>Malbranchea umbrina</i>	UTHSCSA DI18-100 = FMR 17694	LR701818	LR701819	Baltimore, U.S.A.; human wound
<i>Malbranchea umbrina</i>	UTHSCSA DI18-99 = FMR 17693	LR701820	LR701821	Washington DC, U.S.A.; human nail
<i>Malbranchea umbrina</i>	CBS 105.09 ^T	MH854591	MH866116	UK; soil

<i>Malbranchea umbrina</i>	CBS 226.58	MH857765	MH869296	Unknown
<i>Malbranchea umbrina</i>	CBS 261.52	MH857026	MH868556	UK; soil
<i>Malbranchea zuffiana</i>	UTHSCSA DI18-96 = FMR 17690	LR701832	LR701833	Washington DC, U.S.A.; human wound
<i>Malbranchea zuffiana</i>	CBS 219.58 ^T	MH869293	AY176712	Texas, U.S.A.; prairie dog lung
<i>Nannizziopsis guarroi</i>	CBS 124553 ^T	MH863384	MH874904	Barcelona, Spain; iguana skin
<i>Nannizziopsis vriesii</i>	ATCC 22444 ^T	AJ131687	AY176715	The Netherlands; Ameiva (lizard) skin and lung
<i>Neogymnomyces demonbreunii</i>	CBS 427.70	AJ315842	AY176716	Missouri, U.S.A.; unknown
<i>Onychocola canadensis</i>	CBS 109438	-	KT154998	Italy; nail and skin scrapings
<i>Paracoccidioides brasiliensis</i>	UAMH 8037 ^T	AF038360	AF038360	Alberta, Canada; man, 59-years-old, lung biopsy
<i>Pseudoarthropsis cirrhata</i>	CBS 628.83 ^T	-	NG_060792	Schiphol, The Netherlands; wall sample
<i>Pseudoarthropsis crassispora</i> sp. nov.	UTHSCSA DI18-98 = FMR 17692 = CBS 146928 ^T	LR701763	LR701764	Minnesota, U.S.A.; human BAL
<i>Pseudomalbranchea gemmata</i> gen. nov. et sp. nov.	UTHSCSA DI18-90 = FMR 17684 = CBS 146933 ^T	LR701761	LR701762	Florida, U.S.A.; human BAL
<i>Pseudospiromastix tentaculata</i>	CBS 184.9210536	AY527406	LN867603	Hiram, Somalia; soil
<i>Renispora flavissima</i>	CBS 708.79 ^T	AF299348	AY176719	Kansas, U.S.A.; soil in barn housing <i>Myotis velifer</i>
<i>Spiromastigoides alatosporus</i>	CBS 457.73 ^T	MH860740	AB075342	Madras, India; <i>Vigna sinensis</i> rhizosphere
<i>Spiromastigoides albina</i>	CBS 139510 ^T	LN867606	LN867602	Texas, U.S.A.; human lung biopsy
<i>Spiromastigoides asexualis</i>	CBS 136728 ^T	KJ880032	LN867603	Phoenix, U.S.A.; discospondylitis material from a German shepherd dog
<i>Spiromastigoides curvata</i>	JCM 11275 ^T	KP119631	KP119644	México; contaminant of a strain of <i>Histoplasma capsulatum</i>
<i>Spiromastigoides frutex</i>	CBS 138266 ^T	KP119632	KP119645	Nayarit, Mexico; house dust, rental studio

<i>Spiromastigoides geomycoides</i> sp. nov.	UTHSCSA DI18-92 = FMR 17686	LR701769	LR701770	Minnesota, U.S.A.; human blood
<i>Spiromastigoides geomycoides</i> sp. nov.	UTHSCSA DI18-102 = FMR 17696 = CBS 146934 ^T	LR701767	LR701768	Illinois, U.S.A.; human skin foot
<i>Spiromastigoides gypsea</i>	CBS 134.77 ^T	KT155798	NG_063935	California, U.S.A.; soil
<i>Spiromastigoides kosraensis</i>	CBS 138267 ^T	KP119633	KP119646	Kosrae, Micronesia; house dust
<i>Spiromastigoides pyramidalis</i>	CBS 138269 ^T	KP119636	KP119649	Australia; house dust
<i>Spiromastigoides sugiyamae</i>	JCM 11276 ^T	LN867608	AB040680	Japan; soil
<i>Spiromastigoides warcupii</i>	CBS 576.63 ^T	LN867609	AB040679	Australia; soil
<i>Strongyloarthrosporum capsulatus</i>	CBS 143841 ^T	LR760230	LT906534	Toledo, Spain; honey
<i>Trichophyton bullosum</i>	CBS 363.35 ^T	NR_144895	NG_058191	Unkown
<i>Uncinocarpus reesii</i>	ATCC 34533	MH861035	AY176724	Australia; feather

¹**ATCC**: American Type Culture Collection, Virginia, USA; **BCCM/MUCL**: Mycothèque de l'Université Catholique de Louvain, Louvain-la-Neuve, Belgium; **CBS**: Culture collection of the Westerdijk Biodiversity Institute, Utrecht, The Netherlands; **CGMCC**: China General Microbiological Culture Collection Center, Beijing, China; **FMR**: Facultat de Medicina, Reus, Spain; **IFO**: Institute for Fermentation Culture Collection, Osaka, Japan; **JCM**: Japan Collection of Microorganisms, Tsukuba, Japan; **MCC**: Microbial Culture Collection, Universite of Pune Campus Ganeshkhind, India; **NFCCI**: National Fungal Culture Collection of India, Maharastra, India; **UAMH**: University of Alberta Microfungus Collection and Herbarium, Alberta, Canada; **UTHSC**: Fungus Testing Laboratory, University of Texas Health Science Center at San Antonio, San Antonio, Texas, United States.

²Strains studied by us are indicated in **bold**.

³ITS: internal transcribed spacer region 1 and 2 including 5.8S nrDNA; LSU: large subunit of the nrRNA gene.

^TEx-type strain.

*Strain formerly assigned to *Auxarthron thaxteri* (a species synonymized with *Malbranchea umbrina*).

Table 2. Antifungal susceptibility of malbranchea-like strains studied.

Taxon	Strain	MIC/MEC (µg/mL)									
		AMB	FLC	VRC	ITC	PSC	AFG	CFG	MFG	TRB	5-FC
<i>Arachnomyces bostrychodes</i>	FMR 17685	>16	>16	2	>16	>16	0.03	0.06	0.06	0.5	>16
<i>Currahmyces sparsispora</i>	FMR 17683	>16	>16	4	>16	2	>16	8	>16	≤0.03	>16
<i>Malbranchea albolutea</i>	FMR 17679	8	>16	1	1	0.25	0.03	0.06	0.06	0.25	>16
	FMR 17689	8	>16	2	>16	1	0.12	0.06	0.25	0.25	>16
<i>M. aurantiaca</i>	FMR 17682	>16	>16	1	>16	0.25	0.12	1	0.12	4	>16
	FMR 17688	>16	>16	2	>16	0.5	0.5	0.06	1	2	>16
<i>M. conjugata</i>	FMR 17697	8	>16	0.5	0.25	≤0.03	0.06	0.25	0.25	1	>16
	FMR 17699	>16	>16	0.5	2	0.5	0.12	0.25	0.25	1	>16
<i>M. flocciformis</i>	FMR 17698	>16	>16	1	>16	0.5	0.12	0.03	0.12	0,5	>16
<i>M. gymnoascoidea</i>	FMR 17681	>16	>16	8	>16	1	0.03	0.03	0.12	0,5	>16
<i>M. multiseptata</i>	FMR 17695	16	>16	0.12	0.5	0.25	0.03	0.5	2	1	>16
<i>M. stricta</i>	FMR 17680	8	>16	0.25	0.12	0.12	0.03	0.25	0.25	0,12	>16
<i>M. umbrina</i>	FMR 17693	4	>16	2	>16	0.5	0.06	0.06	0.12	0.25	>16
	FMR 17694	>16	>16	4	>16	0.5	0.06	1	0.12	0.25	>16
	FMR 17700	>16	>16	>16	>16	>16	0.5	1	0.5	>16	>16
	FMR 17701	>16	>16	4	>16	0.12	0.03	0.03	0.03	0.12	>16
<i>M. zuffiana</i>	FMR 17690	>16	>16	1	>16	0.5	0.05	1	4	0.25	>16
<i>Pseudomalbranchea gemmata</i>	FMR 17684	2	>16	0.25	0.25	0.25	16	1	16	≤0.03	>16
<i>Spiromastigoides geomyces</i>	FMR 17686	>16	>16	2	1	1	>16	2	>16	0.12	>16
	FMR 17696	>16	>16	2	0.5	0.5	2	16	>16	0.06	>16

AMB, amphotericin B; FLC, fluconazole; VRC, voriconazole; ITC, itraconazole; PSC, posaconazole; AFG, anidulafungin; CFG, caspofungin; MFG, micafungin; TRB, terbinafine; 5-FC, 5-fluorocytosine. ND*: Non-determined due to no fungal growth under the conditions established by the CLSI protocol.

Figures

Figure 1

Malbranchea pulchella Sacc. & Penzig. Holotype and lectotype. Black ink drawings by A. Malbranche, and pencil drawings by P. A. Saccardo (credits: Rosella Marcucci, erbario micologico di Pier Andrea Saccardo, Università di Padova, Italy).

Figure 2

ML phylogenetic tree based on the analysis of ITS-LSU nucleotide sequences for the twenty-two clinical fungi from the USA. Bootstrap support values/Bayesian posterior probability scores of 70/0.95 and higher are indicated on the nodes. T = ex type. Fully supported branched (100% BS / 1 PP) are indicated in bold. Strains identified by us are in bold. *Arachnomycetes* spp. were chosen as out-group. The sequences used in this analysis are in Table 1

Figure 3

Arachnomyces bostrychodes CBS 146926 T. A. Colonies on PYE, PDA and OA after 14 d at 25°C, from left to right (top row, surface; bottom row, reverse). B. Detail of the colony on OA. C, D. Sinuous, contorted to coiled fertile hyphae. E. Arthroconidia. Scale bar = 10 µm.

Figure 4

Arachnomyces graciliformis CBS 146927 T. A. Colonies on PYE, PDA and OA after 14 d at 25°C, from left to right (top row, surface; bottom row, reverse). B. Detail of the colony on OA. C–E. Contorted, apically coiled fertile hyphae bearing arthroconidia. Scale bar = 10 µm.

Figure 5

Pseudoarthropsis crassispora CBS 146928 T. A. Colonies on PYE, PDA and OA after 14 d at 25°C, from left to right (top row, surface; bottom row, reverse). B. Detail of the colony on OA. C–E. Bi- to trichotomously-branched fertile hyphae. F. A large chain of holoarthric conidia. Scale bar = 10 µm.

Figure 6

Currahmyces sparsispora CBS 146929 T. A. Colonies on PYE, PDA and OA after 14 d at 25°C, from left to right (top row, surface; bottom row, reverse). B. Detail of the colony on OA. C–D. Intercalary arthroconidia along the fertile hyphae. Scale bar = 10 μ m.

Figure 7

Malbranchea gymnoascoides CBS 146930 T. A. Colonies on PYE, PDA and OA after 14 d at 25°C, from left to right (top row, surface; bottom row, reverse). B. Detail of the colony on OA. C–D. Young and mature ascomata. E. Young ascus on fertile hyphae. F. Peridial spine-like appendage. G. Intercalary arthroconidia along the fertile hyphae. Scale bar = 10 µm.

Figure 8

Malbranchea multisepitata CBS 146931 T. A. Colonies on PYE, PDA and OA after 14 d at 25°C, from left to right (top row, surface; bottom row, reverse). B. Detail of the colony on PDA. C–D. Highly septate fertile hyphae and arthroconidia. Scale bar = 10 µm.

Figure 9

Malbranchea stricta CBS 146932 T. A. Colonies on PYE, PDA and OA after 14 d at 25°C, from left to right (top row, surface; bottom row, reverse). B. Detail of the colony on OA. C–E. Alternate arthroconidia on primary hyphae and lateral branches. Scale bar = 10 µm.

Figure 10

Pseudomalbranchea gemmata CBS 146933 T. A. Colonies on PYE, PDA and OA after 14 d at 25°C, from left to right (top row, surface; bottom row, reverse). B. Detail of the colony on OA. C–D. Large, intercalary, irregularly-shaped arthroconidia disposed singly or in chains along the fertile hyphae. Scale bar = 10 µm.

Figure 11

Spiromastigoides geomyces CBS 146934 T. A. Colonies on PYE, PDA and OA after 14 d at 25°C, from left to right (top row, surface; bottom row, reverse). B. Detail of the colony on OA. C. Fertile lateral branches mimicking *Geomyces* spp. conidiophores. D–E. Fertile hyphae with intercalary, barrel-shaped arthroconidia. F. Morphological diversity of arthroconidia. Scale bar = 10 μ m.