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

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Research

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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 synonymysed *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 crassispora*, *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, uconazole, 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 crassispora* 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 longispora* (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, it’s 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 originit 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 appropriated 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. zuillianum 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, Stuchlik 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, Unterainer 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) ≥ 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 ºC (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 *Arthropsis*), 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 *Arthropsis 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 botrychodes* lacks of a sexual morph and racket hyphae (both present in *A. peruvianum*), and produces longer conidia than *A. peruvianum* (4.0–8.0 × 1.0–2.0 μm vs. 4.0–5.0 × 1.0–3.0 μm); 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 DI18-91 – ex-type cultures; LSU sequences GenBank LR701766). *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, successively branching to form dense clusters, arcuate, sinuous, contorted or tightly curled, 1.0–2.0 μm wide, forming randomly intercalary and terminally arthroconidia. Conidia enteroarthric, hyaline, one-celled, smooth-walled, cylindrical, barrel-shaped, and finger-like-shaped when terminal, 4.0–8.0 × 1.0–2.0 μm, 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* (4.0–10.0 × 1.5–2.0 μm vs. 2.5–4.5 × 1.5–2.0 μm), 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 DI18-97 – 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 > 3.5 μm diameter ...................... 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 Arthropsis cirrhata, while the type species of the genus (Arthropsis 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 Pseudarthropsis crassispora.

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

Etymology: From Greek ψευδός, resembling, because the morphological semblance to Arthropsis.

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 holoarthric 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: Arthropsis 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, 2.5–4.0 × 2–3 μm, 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 *-sporarum*, 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 enteroarthric 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* (4.5–5.5 × 2.5–3.5 μm vs. 2.5–4.0 × 2.0–3.0 μm). 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, 1.5–2.0 μm 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 10–20 × 1.5–2.0 μm, branches 10–70 × 1.5–2.0 μm, 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, 2.5–3.5 × 4.5–5.5 μm, 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, *-sporarum*, 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 MB 835237.

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

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

*Synonym:* *Auxarthron zuanum* (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 filamentos**a 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 cinnat**a* 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 oblale 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, oblale 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 reaching 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 reaching 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. zuana*

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. concentrata*

11b. Peridial hyphae strongly ornamented; chlamydospores present................................... 12
12a. Arthroconidia 2–10 × 2.5–3.5 µm; growing above 30 °C .......................... *M. chlamydospora*

12b. Arthroconidia 4–24 × 1.0–5.5 µm; not growing above 30 °C .......................... *M. longispora*

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.................................................. *M. cinnamomea*

15b. Not thermophilic; conidia narrower....................................................... *M. pulchella*

16a. Colonies orange...................................................................................... 17

16b. Colonies different................................................................................... 18

17a. Aleuroconidia laterally or terminally dispersed................................. *M. chrysosporoidea*

17b. Aleuroconidia absent........................................................................... *M. aurantiaca*

18a. Colonies golden yellow, exudate brown, diffusible pigment yellow........ *M. graminicola*

18b. Features are not combined...................................................................... 19

19a. Sexual morph produced by *in vitro* mating of compatible strains........ *M. albolutea*

19b. Sexual morph not formed....................................................................... 20

20a. Thick-walled brown setae produced on OA from the vegetative mycelium.............................................................. *M. filamentosa*

20b. Setae not produced.............................................................................. *M. arcuata*

21a. Fertile hyphae unbranched or scarcely branched......................................... 22

21b. Fertile hyphae branched........................................................................ 23

22a. Arthroconidia cylindrical; becoming many septate with the age........ *M. multisepatata*

22b. Arthroconidia barrel-shaped, “T”-shaped, “Y”-shaped, finger-shaped or more irregular; vegetative hyphae regularly septate.................................................. *M. stricta*

23a. Fertile hyphae branching acutely, displaying a tree-like appearance......... *M. dendritica*

23b. Fertile hyphae branching pattern otherwise........................................... 24

24a. Fertile hyphae repeatedly branched, in dense tufts.............................. *M. flocciformis*
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 rheolytic or rarely schizolytic 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 reessii 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 (4.0–11.0 × 2.0–3.5 μm in *P. gemmata* vs. 3.5–6.0 × 2.5–3 μm in *U. reessi*, and 4.0–5.4 × 2.0–3.0 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, 1.0–2.0 μm 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, 4.0–11.0 × 2.0–3.5 μm, liberated from the fertile hyphae by rheolyis (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 geomyces*.

### *Spiromastigoides geomyces* 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 geomyces* is phylogenetically close to *S. gypsea*. However, *S. geomyces* produces smaller conidia (1.5–2.5 × 1.0–2.0 μm) than *S. gypsea* [(2.5)3–6(9) × 2–2.5 μm] (Sigler & Carmichael 1976). Also, *S. geomyces* grows faster than *S. gypsea* on PYE at 35 ºC.

**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, 1.0–2.0 μm 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 enteroarthic, unicellular, hyaline, mostly barrel-shaped, less frequently “T”-shaped or cylindrical, 1.5–2.5 × 1.0–2.0 μm, 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 ºC, 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 ºC, 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 ºC, 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 ºC, 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 ºC, 30 ºC, and 37 ºC, 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) 3–6 (9) × 2–2.5 μm, 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; chlamydosporates present............................................................................................... *S. curvata*

10b. Fertile hyphae unbranched, slightly curved, up to 15 μm long; chlamydosporates 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 ºC, and most of them at 35-37 ºC.

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. *Onychocha canadensis*) where highly susceptible to ciciclopirox 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 = posterior 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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Tables

Table 1. DNA barcodes used to build the phylogenetic tree.
| Species                          | Strains\(^1\)     | GenBank accession #\(^2\) | Geographic origin and source                                                                 |
|---------------------------------|-------------------|--------------------------|-----------------------------------------------------------------------------------------------|
|                                 | ITS\(^3\)    | LSU\(^3\)    |                                                                                               |
| *Ajellomyces capsulatus*        | UAMH 3536 \(^T\) | AF038354 AF038354 | Alberta, Canada; woman, 25-years-old, biopsy of right middle lobe lung                           |
| *Amauroascus niger*             | ATCC 22339       | MH869547 AY176706 | California, U.S.A.; soil                                                                       |
| *Amauroascus purpureus*         | IFO 32622 \(^T\) | AJ271564 AY176707 | Japan; soil                                                                                    |
| *Amauroascus volatilis-patellis*| CBS 249.72 \(^T\) | MH860467 MH872189 | Utah, U.S.A.; soil                                                                             |
| *Aphanoascus mephitalis*        | ATCC 22144       | MH859941 AY176725 | Ontario, Canada; wolf dung                                                                     |
| *Arachniotus verruculosus*      | CBS 655.71       | NR_145221 AB040684 | Utah, U.S.A.; soil                                                                             |
| *Arachnomyces bostrychodes* sp. nov. | UTHSCSA DI18-91 = FMR 17685 = CBS 146926 \(^T\) | LR701765 LR701766 | Texas, U.S.A.; human scalp                                                                     |
| *Arachnomyces glareosus*        | CBS 116129 \(^T\) | AY624316 FJ358273 | Alberta, Canada; man, 30-years-old, thumb nail                                                |
| *Arachnomyces graciliformis* sp. nov. | UTHSCSA DI18-97 = FMR 17691 = CBS 146927 \(^T\) | LR743667 LR743668 | Massachusetts, U.S.A.; animal bone                                                             |
| *Arachnomyces gracilis*         | UAMH 9756 \(^T\) | AY123779 - | Uganda; termitarium soil                                                                        |
| *Arachnomyces jinanicus*        | CGMCC3.14173 \(^T\) | KY440749 KY440752 | Jinan, China; pig farm soil                                                                      |
| *Arachnomyces kanei*            | UAMH 5908 \(^T\) | AY123780 - | Toronto, Canada; human nail                                                                     |
| *Arachnomyces minimus*          | CBS 324.70 \(^T\) | AY123783 FJ358274 | Ontario, Canada; decaying wood                                                                  |
| *Arachnomyces nitidus*          | UAMH 10536       | - AB075351 | Israel; twigs                                                                                  |
| *Arachnomyces nodosetosus*      | CBS 313.90 \(^T\) | AY123784 AB053452 | Saskatchewan, Canada; woman, 67-years-old, onychomycosis                                         |
| *Arachnomyces peruvianus*       | CBS 112.54 \(^T\) | MF572315 MH868792 | Peru; *Globodera rostochiensis* cyst                                                           |
| *Arachnomyces pilosus*          | CBS 250.93 \(^T\) | MF572320 MF572325 | Catalonia, Spain; river sediment                                                                |
| *Arachnomyces scleroticus*      | UAMH 7183 \(^T\) | AY123785 - | Sulawesi, Indonesia; poultry farm soil                                                          |
| *Arthrodema curreyi*            | CBS 353.66 \(^T\) | MH858822 MH870459 | UK; unknown                                                                                    |
| *Arthrodema onychocola*         | CBS 132920 \(^T\) | KT155794 KT155124 | Prague, Czech                                                                                  |
| Species                        | CBS/MCC/UTHSCSA | T/A   | GenBank Accession Numbers | Location and Additional Information |
|-------------------------------|-----------------|-------|---------------------------|---------------------------------------|
| Ascosphaera apis              | CBS 252.32      | -     | AY004344                  | København, Denmark; *Apis mellifera*   |
| Ascosphaera subglobosa        | A.A. Wynns 5004 (C) T | NR_137060 | HQ540517                  | Utah, U.S.A.; pollen provisions of *Megachile rotundata* |
| Auxarthronopsis bandhavgarhensis | NFCC 2185 T      | HQ164436 | NG_057012                 | Bandhavgarh, India; soil              |
| Auxarthronopsis guizhouensis  | CGMCC3.17910 T   | KU746668 | KU746714                  | Guizhou, China; air                   |
| Blastomyces perclus           | CBS 139878 T     | NR_153647 | KY195971                  | Israel; human granulomatous lesions   |
| Canomyces reticulatus         | MCC 1486 T       | MK340501 | MK340502                  | Maharashtra, India; soil              |
| Chrysosporium keratinophilum  | CBS 392.67       | MH859002 | AY176730                  | New Zealand; soil                     |
| Chrysosporium tropicum        | MUCL 10068 T     | MH858134 | AY176731                  | Guadalcanal, Solomon islands; woollen overcoat |
| Currahmyces indicus           | MCC 1548 T       | MK340498 | MK340499                  | Maharashtra, India; hen resting area  |
| Currahmyces sparsispora sp. nov. | UTHSCSA DI18-89 = FMR 17683 = CBS 146929 T | LR723272 | LR723273                  | Florida, U.S.A.; human sputum         |
| Gymnoascus reessi             | CBS 410.72       | MH860507 | MH872224                  | California, U.S.A.; soil              |
| Helicoarthrosporum melicola   | CBS 143838 T     | LR761645 | LT906535                  | Granada, Spain; honey                 |
| Helicoarthrosporum melicola   | FMR 15673        | LR761646 | LT978462                  | Valencia, Spain; honey                |
| Malbranchea albolutea         | UTHSCSA DI18-85 = FMR 17679 | LR701834 | LR701835                  | Texas, U.S.A.; human BAL              |
| Malbranchea albolutea         | UTHSCSA DI18-95 = FMR 17689 | LR701836 | LR701837                  | Texas, U.S.A.; human BAL              |
| Malbranchea albolutea         | CBS 125.77 T     | MH861039 | MH872808                  | Utah, U.S.A.; soil                    |
| Malbranchea aurantiaca        | UTHSCSA DI18-94 = FMR 17688 | LR701824 | LR701825                  | California, U.S.A.; animal            |
| Malbranchea aurantiaca        | UTHSCSA DI18-88 = FMR 17682 | LR701826 | LR701827                  | Texas, U.S.A.; animal skin lesion     |
| Malbranchea aurantiaca        | CBS 127.77 T     | NR_157447 | AB040704                  | Utah, U.S.A.; culture contaminant     |
| Malbranchea californiensis    | ATCC 15600 T     | MH858121 | NG_056947                 | California, U.S.A.; dung of pack rat  |
| Malbranchea chrysosporioidea  | CBS 128.77 T     | AB361632 | AB359413                  | Arizona, U.S.A.; soil                 |
| **Malbranchea circinata** | ATCC 34526<sup>T</sup> | MN627784 | MN627782 | Utah, U.S.A.; soil |
|--------------------------|------------------------|----------|----------|------------------|
| **Malbranchea conjugata** | UTHSCSA DI18-105 = FMR 17699 | LR701828 | LR701829 | Florida, U.S.A.; human lung tissue |
| **Malbranchea conjugata** | UTHSCSA DI18-103 = FMR 17697 | LR701830 | LR701831 | Texas, U.S.A.; human BAL |
| Malbranchea conjugata | CBS 247.58 | NR_121475 | HF545313 | Arizona, U.S.A.; soil |
| Malbranchea dendritica | CBS 131.77<sup>T</sup> | AY177310 | AB359416 | Utah, U.S.A.; soil |
| Malbranchea filamentosa | CBS 581.82<sup>T</sup> | NR_111136 | AB359417 | Argentina; soil |
| Malbranchea flavida | CBS 132.77<sup>T</sup> | AB361633 | AB359418 | California, U.S.A.; soil |
| Malbranchea flavorosea | ATCC 34529<sup>T</sup> | NR 158362 | AB359419 | California, U.S.A.; soil |
| **Malbranchea flocciformis** | UTHSCSA DI18-104 = FMR 17698 | LR701822 | LR701823 | Texas, U.S.A.; human skin |
| Malbranchea flocciformis | CBS 133.77<sup>T</sup> | AB361634 | AB359420 | France; saline soil |
| Malbranchea fulva | CBS 135.77<sup>T</sup> | NR_157444 | AB359422 | Utah, U.S.A.; air |
| **Malbranchea gymnoascoides sp. nov.** | UTHSCSA DI18-87 = FMR 17681 = CBS 146930<sup>T</sup> | LR701757 | LR701758 | Texas, U.S.A.; human BAL |
| Malbranchea kuehnii | CBS 539.72<sup>T</sup> | NR_103573 | NG_056928 | Unknown; dung |
| Malbranchea longispora | FMR 12768<sup>T</sup> | HG326873 | HG326874 | Beija, Portugal; soil |
| **Malbranchea multiseptata sp. nov.** | UTHSCSA DI18-101 = FMR 17695 = CBS 146931<sup>T</sup> | LR701759 | LR701760 | Texas, U.S.A.; human BAL |
| Malbranchea ostraviense | CBS 132919<sup>T</sup> | NR_121474 | - | Ostrava, Czech Republic; fingernail sample |
| Malbranchea pulchella | CBS 202.38 | AB361638 | AB359426 | Italy; unknown |
| **Malbranchea stricta sp. nov.** | UTHSCSA DI18-86 = FMR 17680 = CBS 146932<sup>T</sup> | LR701638 | LR701639 | Florida, U.S.A.; human nail |
| Malbranchea sp.* | CBS 319.61 | MH858065 | MH869635 | California, U.S.A.; soil |
| **Malbranchea umbrina** | UTHSCSA DI18-106 = FMR 17700 | LR701814 | LR701815 | Colorado, U.S.A.; human BAL |
| **Malbranchea umbrina** | UTHSCSA DI18-107 = FMR 17701 | LR701816 | LR701817 | Colorado, U.S.A.; human sinus |
| **Malbranchea umbrina** | UTHSCSA DI18-100 = FMR 17694 | LR701818 | LR701819 | Baltimore, U.S.A.; human wound |
| **Malbranchea umbrina** | UTHSCSA DI18-99 = FMR 17693 | LR701820 | LR701821 | Washington DC, U.S.A.; human nail |
| **Malbranchea umbrina** | CBS 105.09<sup>T</sup> | MH854591 | MH866116 | UK; soil |
| **Malbranchea umbrina** | CBS 226.58 | MH857765 | MH869296 | Unknown |
|-------------------------|-------------|----------|----------|---------|
| **Malbranchea umbrina** | CBS 261.52  | MH857026 | MH868556 | UK; soil |
| **Malbranchea zuffiana** | UTHSCSA DI18-96 = FMR 17690 | LR701832 | LR701833 | Washington DC, U.S.A.; human wound |
| **Malbranchea zuffiana** | CBS 219.58 T | MH869293 | AY176712 | Texas, U.S.A.; prairie dog lung |
| **Nannizziopsis guarroi** | CBS 124553 T | MH863384 | MH874904 | Barcelona, Spain; iguana skin |
| **Nannizziopsis vriesii** | ATCC 22444 T | AJ131687 | AY176715 | The Netherlands; Ameiva (lizard) skin and lung |
| **Neogymnomyces demonbreunii** | CBS 427.70 | AJ315842 | AY176716 | Missouri, U.S.A.; unknown |
| **Onychocola canadensis** | CBS 109438 | - | KT154998 | Italy; nail and skin scrapings |
| **Paracoccidioides brasiensis** | UAMH 8037 T | AF038360 | AF038360 | Alberta, Canada; man, 59-years-old, lung biopsy |
| **Pseudoarthropsis cirrhata** | CBS 628.83 T | - | NG_060792 | Schiphol, The Netherlands; wall sample |
| **Pseudoarthropsis crassispora sp. nov.** | UTHSCSA DI18-98 = FMR 17692 = CBS 146928 T | LR701763 | LR701764 | Minnesota, U.S.A.; human BAL |
| **Pseudomalbranchea gemmata gen. nov. et sp. nov.** | UTHSCSA DI18-90 = FMR 17684 = CBS 146933 T | LR701761 | LR701762 | Florida, U.S.A.; human BAL |
| **Pseudospiromastix tentaculata** | CBS 184.9210536 | AY527406 | LN867603 | Hiram, Somalia; soil |
| **Renispora flavissima** | CBS 708.79 T | AF299348 | AY176719 | Kansas, U.S.A.; soil in barn housing *Myotis velifer* |
| **Spiromastigoides alatosporus** | CBS 457.73 T | MH860740 | AB075342 | Madras, India; *Vigna sinensis* rhizosphere |
| **Spiromastigoides albina** | CBS 139510 T | LN867606 | LN867602 | Texas, U.S.A.; human lung biopsy |
| **Spiromastigoides asexualis** | CBS 136728 T | KJ880032 | LN867603 | Phoenix, U.S.A.; discospondylitis material from a German shepherd dog |
| **Spiromastigoides curvata** | JCM 11275 T | KP119631 | KP119644 | México; contaminant of a strain of *Histoplasma capsulatum* |
| **Spiromastigoides frutex** | CBS 138266 T | KP119632 | KP119645 | Nayarit, Mexico; house dust, rental studio |
| **Spiromastigoides**
| **geomycoides** sp. nov. | UTHSCSA DI18-92 = FMR 17686 | LR701769 | LR701770 | Minnesota, U.S.A.; human blood |
| **Spiromastigoides**
| **geomycoides** sp. nov. | UTHSCSA DI18-102 = FMR 17696 = CBS 146934 T | LR701767 | LR701768 | Illinois, U.S.A.; human skin foot |
| **Spiromastigoides**
| gypseae | CBS 134.77 T | KT155798 | NG_063935 | California, U.S.A.; soil |
| **Spiromastigoides**
| kosraensis | CBS 138267 T | KP119633 | KP119646 | Kosrae, Micronesia; house dust |
| **Spiromastigoides**
| pyramidalis | CBS 138269 T | KP119636 | KP119649 | Australia; house dust |
| **Spiromastigoides**
| sugiyamae | JCM 11276 T | LN867608 | AB040680 | Japan; soil |
| **Spiromastigoides**
| warcupii | CBS 576.63 T | LN867609 | AB040679 | Australia; soil |
| **Strongyloarthrosporum**
| capsulatus | CBS 143841 T | LR760230 | LT906534 | Toledo, Spain; honey |
| **Trichophyton**
| bullosum | CBS 363.35 T | NR_144895 | NG_058191 | Unkown |
| **Uncinocarpus**
| reesii | 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, Utrech, 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, Univesite 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.

2 Strains studied by us are indicated in bold.

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

T Ex-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 |
|-----------------------------|--------------|-----------------|-----|-----|-----|-----|-----|-----|-----|-----|-----|------|
| *Arachnomyces bostrychodes* | FMR 17685    | >16 >16 2 >16 >16 | 0.03 | 0.06 | 0.06 | 0.5 | >16 |
| *Currahmyces sparsispora*   | FMR 17683    | >16 >16 4 >16 2 >16 8 >16 | ≤0.03 | >16 |
| *Malbranchea albolutea*     | 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 |
| *M. aurantiaca*             | 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 |
| *M. conjugata*              | 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 |
| *M. flocciformis*           | FMR 17698    | >16 >16 1 >16 0.5 | 0.12 | 0.03 | 0.12 | 0.5 | >16 |
| *M. gymnoascoidea*          | FMR 17681    | >16 >16 8 >16 1 | 0.03 | 0.03 | 0.12 | 0.5 | >16 |
| *M. multiseptata*           | FMR 17695    | 16 >16 0.12 0.5 0.25 | 0.03 | 0.5 | 2 | 1 | >16 |
| *M. stricta*                | FMR 17680    | 8 >16 0.25 0.12 0.12 | 0.03 | 0.25 | 0.25 | 0.12 | >16 |
| *M. umbrina*                | 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 |
| *M. zuflana*                | FMR 17690    | >16 >16 1 >16 0.5 | 0.05 | 1 | 4 | 0.25 | >16 |
| *Pseudomalbranchea gemmata* | FMR 17684    | 2 >16 0.25 0.25 0.25 | 16 | 1 | 16 | ≤0.03 | >16 |
| *Spiromastigoides geomyces* | 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.