A revision of malbranchea-like fungi from clinical specimens in the United States of America reveals unexpected novelty

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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 22 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 synonymized Auxarthron with Malbranchea, and erected two new genera: Pseudoarthropsis and Pseudomalbranchea. New species proposed are: Arachnomyces bostrychodes, A. graciliformis, Currahmyces sparsispora, Malbranchea gymnoascoides, M. multiseptata, M. stricta, Pseudoarthropsis crassispora, Pseudomalbranchea gemmata, and Spiromastigoides geomycoides, 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.

KEYWORDS: Antifungals, Arachnomycetales, Auxarthron, Clinical fungi, Malbranchea, Onygenales, New taxa

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 and 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 and Carmichael 1976), Malbranchea pulchella has been suggested as a possible cause of sinusitis (Benda and Corey 1994), and M. cinnamomea was recovered from dystrophic nails in patients with underlying chronic illnesses (Lyskova 2007, Salar and 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 and 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 appropriated description for mesophilic (*M. pulchella*) and thermophilic (*Malbranchea sulfurea*) species. In a more recent revision by Sigler and Carmichael (1976) 12 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).

![Fig. 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)](image)
Malbranchea-like asexual morphs are also present in other taxa of ascomycetes. The genus *Arachnomyces* (family *Arachnomycetaceae*, order Arachnomyctales; Malloch and Cain 1970; Guarro et al. 1993), characterized by the production of brightly coloured cleistothecial ascomata bearing setae, and by the production of an onychocolla-like (Sigler et al. 1994) or a malbranchea-like (Udagawa and 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 and 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. 1994; Campbell et al. 1997; Contet-Audonneau 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 also implicated in animal and human infections. (Udagawa and Uchiyama 1999) asexual morph, have been isolated from nail and skin samples (Gibas et al. 2004; Sun et al. 2019).

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

Due to the limited knowledge of *Malbranchea* and their relatives in 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 (19 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 and 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 and Smitska 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 Kornerek and Wanscher (1978). Christensen’s urea agar (EMD Millipore, 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, Spain) supplemented with 0.2% cycloheximide (Sigma, USA) at 30 °C for two wk. 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 and Hester 1990; Rehner and Samuels 1994) of the nrrDNA. 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
| Species                        | Strains*                      | GenBank accession numbers | Geographic origin and source                          |
|-------------------------------|-------------------------------|---------------------------|-------------------------------------------------------|
| **Ajellomyces capsulatus**    | UAMH 3536 d                   | 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 d                   | AJ271564 AJ271567         | Japan; soil                                           |
| **Amauroascus volantis-pellatis** | CBS 249.72 d               | MH860467 MH872189         | Utah, U.S.A.; soil                                   |
| **Aphanoascus mephitidis**    | ATCC 22144                    | MH859941 AY176725         | Ontario, Canada; wolf dung                            |
| **Arachniotus verruculosus**  | CBS 655.71                    | AB040684                  | Utah, U.S.A.; soil                                   |
| **Arachniotus bostrychodes**  | UTHSCSA DI18-91 = FMR 17685 = CBS 146926 d | LR701765 LR701766 | Texas, U.S.A.; human scalp                           |
| **Arachniotus glareosus**     | CBS 116129 d                  | AY624316 FJ358273         | Alberta, Canada; man, 30-years-old, thumb nail       |
| **Arachniotus graciliformis** | UTHSCSA DI18-97 = FMR 17691 = CBS 146927 d | LR743667 LR743668 | Massachusetts, U.S.A.; animal bone                   |
| **Arachniotus gracilis**      | UAMH 9756 d                   | AY123779 –                | Uganda; termitarium soil                             |
| **Arachniotus jonianicus**    | CGMMCC3.14173 d              | KY440749 KY440752         | Jinan, China; pig farm soil                          |
| **Arachniotus kanei**         | UAMH 5908 d                   | AY123780 –                | Toronto; Canada; human nail                          |
| **Arachniotus minimus**       | CBS 324.70 d                  | AY123783 FJ358274         | Ontario; Canada; decaying wood                       |
| **Arachniotus nitsus**        | UAMH 10536                    | – AB075351                | Israel; twigs                                        |
| **Arachniotus nodosetosus**   | CBS 313.90 d                  | AY123784 AB053452         | Saskatchewan, Canada; woman, 67-years-old, onychomycosis |
| **Arachniotus peruvianus**    | CBS 112.54 d                  | MF572315 MH868792         | Peru; Globodera rostochiensis cyst                    |
| **Arachniotus pilosus**       | CBS 250.93 d                  | MF572320 MF572335         | Catalonia, Spain; river sediment                      |
| **Arachniotus scleroticus**   | UAMH 7183 d                   | AY123785 –                | Sulawesi, Indonesia; poultry farm soil               |
| **Arthroderma cureyi**        | CBS 353.66 d                  | MH858822 MH870459 UK; unknown |
| **Arthroderma onychochola**   | CBS 132920 d                  | KT155794 KT155124         | Prague, Czech Republic; human nail                   |
| **Ascosphaera apis**          | CBS 252.32                    | – AY004344                | Kopenhagen, Denmark; A. mellifera                    |
| **Ascosphaera subglobosa**    | A.A. Wynns 5004 (C) d        | NR_137060 HQ540517        | Utah, U.S.A.; pollen provisions of M. rotundata      |
| **Auxarthronopsis bandhavgarhensis** | NFCCI 2185 d               | HQ164436 NG_057012        | Bandhavgarh, India; soil                             |
| **Auxarthronopsis guizhouensis** | CGMCC3.17910 d              | KU746668 KU746714         | Guizhou, China; air                                  |
| **Blastomyces percsus**       | CBS 139878 d                  | NR_153647 KY195971        | Israel; human granulomatous lesions                   |
| **Canomyces reticulatus**     | MCC 1486 d                    | MK340501 MK340502         | Maharashtra, India; soil                             |
| **Chrysosporium keratinophilum** | CBS 392.67                  | MH859002 AY176730 New Zealand; soil |
| **Chrysosporium tropicum**    | MUCL 10068 d                  | MH858134 AY176731 Guadalcanal, Solomon islands; woollen overcoat |
| **Currahmyces indicus**       | MCC 1548 d                    | MK340498 MK340499         | Maharashtra, India; hen nesting area                  |
| **Currahmyces sparsispora**   | UTHSCSA DI18-89 = FMR 17683 = CBS 146929 d | LR723272 LR723273 | Florida, U.S.A.; human sputum                        |
| **Gymnoascus reesi**          | CBS 410.72                    | MHB0507 MH872224          | California, U.S.A.; soil                             |
| **Helicoarthrosporum mellicola** | CBS 143838 d                 | LR761645 LT006535         | Granada, Spain; honey                                |
| **Helicoarthrosporum mellicola** | FMR 15673                    | LR761646 LT987462         | 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 californiensis** | CBS 125.77 d                  | MHB61359 MHB72808         | 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 d                  | – AB040704                | Utah, U.S.A.; culture contaminant                     |
| **Malbranchea aurantiaca**    | ATCC 15600 d                  | MHB58121 NG_              | California, U.S.A.; dung of pack rat                 |
| Species                  | Strains* | GenBank accession | Geographic origin and source                        |
|-------------------------|----------|-------------------|-----------------------------------------------------|
| Malbranchea chiniense   | CGMCC3.19572 | MK329076 MK328981 | Guangxi, Luotian Cave, China; Soil                   |
| Malbranchea chrysosporioidea | CBS 128.77 | AB361632 AB359413 | Arizona, U.S.A.; soil                               |
| Malbranchea cincinnata  | ATCC 34526 | 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         | Arizona, U.S.A.; soil                               |
| Malbranchea densitica    | CBS 131.77 | AY177310 AB359416 | Utah, U.S.A.; soil                                  |
| Malbranchea filamentosa  | CBS 581.82 | AB359417 Mn627782 | Argentina; soil                                    |
| Malbranchea flavus       | CBS 137.77 | AY177310 AB359416 | California, U.S.A.; soil                           |
| Malbranchea flavus       | CBS 135.77 | AB359417 MN627782 | California, U.S.A.; soil                           |
| Malbranchea floccifera   | UTHSCSA DI18-104 = FMR 17698 | LR701822 LR701823 | Texas, U.S.A.; human skin                         |
| Malbranchea floccifera   | CBS 133.77 | AB359417 MN627782 | France; saline soil                                |
| Malbranchea fulva        | CBS 137.77 | AB359417 MN627782 | Utah, U.S.A.; air                                  |
| Malbranchea gymanosoides | UTHSCSA DI18-87 = FMR 17681 = CBS 146930 | LR701757 LR701758 | Texas, U.S.A.; human BAL                          |
| Malbranchea guanxiense   | CGMCC3.19634 | MK329076 MK328985 | Guangxi, E’gu Cave, China; Soil                     |
| Malbranchea kuehnii      | CBS 539.72 | NR_103573         | Unknown; dung                                      |
| Malbranchea longispora   | FMR 12768 | HG326873 HG326874 | Beja, Portugal; soil                               |
| Malbranchea multiseptata | UTHSCSA DI18-101 = FMR 17695 = CBS 146931 | LR701757 LR701760 | Texas, U.S.A.; human BAL                          |
| Malbranchea ostraviense  | CBS 132919 | NR_121474         | Ostrava, Czech Republic; fingernail sample         |
| Malbranchea pseudoauxarthron | IFO 31701 = CBS 657.71 = ATCC 22158 = NRRL 5132 | MHB60293 KY014424 | Utah, U.S.A.; domestic rabbit dung                  |
| Malbranchea pulchella    | CBS 202.38 | AB359417 MN627782 | Utah, U.S.A.; air                                  |
| Malbranchea stricta      | UTHSCSA DI18-86 = FMR 17680 = CBS 146931 | LR701638 LR701639 | Florida, U.S.A.; human nail                       |
| Malbranchea sp.*         | CBS 319.61 | MHB58065 MHB59635 | 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 | MHB54591 MHB66116 | UK; soil                                           |
| Malbranchea umbrina      | CBS 226.58 | MHB57765 MHB59296 | Unknown                                            |
| Malbranchea umbrina      | CBS 261.52 | MHB5752 MHB68655 | UK; soil                                           |
| Malbranchea zuffiana     | UTHSCSA DI18-96 = FMR 17690 | LR701832 LR701833 | Washington DC, U.S.A.; human wound                |
| Malbranchea zuffiana     | CBS 219.58 | MHB69293 AY176712 | Texas, U.S.A.; prairie dog lung                     |
| Nannizziopsis guarroi    | CBS 124553 | MHB63384 MHB74904 | Barcelona, Spain; iguana skin                     |
| Nannizziopsis vriesii    | ATCC 22444 | AJ131687 AJ176715 | The Netherlands; Ameiva (lizard) skin and lung     |
| Neogymnomyces dembonreunii | CBS 427.70 | AJ131584 AY176716 | Missouri, U.S.A.; unknown                          |
| Onychocola canadensis    | CBS 109438 | – KT154998       | Italy; nail and skin scrapings                     |
| Paracoccidioides brasiliensis | UAMH 8037 | AF038360 AF038360 | Alberta, Canada; man, 59-years-old, lung biopsy    |
| Pseudoarthrops cinhata  | CBS 628.83 | – NG_060792     | Schiphol, The Netherlands; wall sample              |
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

### Table 1 DNA barcodes used to build the phylogenetic tree (Continued)

| Species | Strains* | GenBank accession | Geographic origin and source |
|---------|----------|-------------------|-----------------------------|
| | | ITS* | LSU* |
| **Pseudoarthropsis crassispora** sp. nov. | UTHSCSA DI18-98 = FMR 17692 = CBS 146928 | LR701763 | LR701764 | Minnesota, U.S.A.; human BAL |
| **Pseudomalbranchea gemmata** gen. nov. et sp. nov. | UTHSCSA DI18-90 = FMR 17684 = CBS 146933 | LR701761 | LR701762 | Florida, U.S.A.; human BAL |
| Pseudosporiomastigites tantaculata | CBS 184.921053 | AYS27406 | LN867503 | Hiram, Somalia; soil |
| Reniispora flavissima | CBS 708.79 | AF299348 | AY176719 | Kansas, U.S.A.; soil in barn housing M. velifer |
| Spiromastigoiades alatosporus | CBS 457.73 | MH860740 | AB075342 | Madras, India; V. sinensis rhizosphere |
| Spiromastigoiades albina | CBS 139510 | LN867606 | LN867602 | Texas, U.S.A.; human lung biopsy |
| Spiromastigoiades asexualis | CBS 136728 | KJ880032 | LN867603 | Phoenix, U.S.A.; discospondylitis material from a German shepherd dog |
| Spiromastigoiades curvata | JCM 11275 | KI119631 | KI119644 | Mexico; contaminant of a strain of Histoplasma capsulatum |
| Spiromastigoiades frutex | CBS 138266 | KI119632 | KI119645 | Nayarit, Mexico; house dust, rental studio |
| Spiromastigoiades geomycoides** sp. nov. | UTHSCSA DI18-92 = FMR 17686 | LR701767 | LR701768 | Minnesota, U.S.A.; human blood |
| Spiromastigoiades geomycoides** sp. nov. | UTHSCSA DI18-102 = FMR 17696 = CBS 146934 | LR701767 | LR701768 | Illinois, U.S.A.; human skin foot |
| Spiromastigoiades gypseus | CBS 134.77 | KT155798 | NG_063935 | California, U.S.A.; soil |
| Spiromastigoiades kosraensis | CBS 138267 | KI119633 | KI119646 | Kosrae, Micronesia; house dust |
| Spiromastigoiades pyramidalis | CBS 138269 | KI119636 | KI119649 | Australia; house dust |
| Spiromastigoiades sugiyamae | JCM 11276 | LN867608 | AB040680 | Japan; soil |
| Spiromastigoiades varcupii | CBS 576.63 | LN867609 | AB040679 | Australia; soil |
| Strongyloarthrosporum capsulatus | CBS 143841 | LR760230 | LT906534 | Toledo, Spain; honey |
| Trichophyton butois | CBS 363.35 | NR_ | Unknown |
| Uncinocarpus reesii | ATCC 34533 | MH861035 | AY176724 | Australia; feather |

*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 Facultad de Medicina, Reus, Spain, IFI Institute for Fermentation Culture Collection, Osaka, Japan, JCM Japan Collection of Microorganisms, Tsukuba, Japan, MCC Microbial Culture Collection, University of Pune Campus Ganeshkhind, India, NFCCI National Fungal Culture Collection of India, Maharashtra, 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 |

*Strains studied by us are indicated in bold |

*ITS internal transcribed spacer region 1 and 2 including 5.8S nrDNA, LSU large subunit of the nrRNA gene |

*Ex-type strain |

*Strain formerly assigned to Auxarthron thaxteri (a species synonymized with Malbranchea umbrina)
highly branched hyphae were observed, while the MIC value was defined as the lowest concentration that completely inhibited the fungal growth. *C. parapsilosis* ATCC 22019 was used as the quality control strain in all experiments.

**RESULTS**

Fungal diversity

Table 1 shows the identity of the 22 fungal strains studied. The highest number of strains corresponded to *Auxarthron umbrinum* (4), followed by *A. alboluteum* (2), *A. conjugatum* (2), and *Malbranchea aurantiaca* (2). *Auxarthron zuffianum*, *Curtarolomyces indicus* and *M. flocciformis* were represented by one strain each. Eight strains were only identified at genus-level (three belonging to *Malbranchea*, two to *Spiromastigoides*, two to *Arthrodermataceae* and one to *Arthropodales*), 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 1213 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-gene tree. 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-gene phylogenies. 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-gene phylogenies. 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-gene phylogenies.

**TAXONOMY**

*Arachnomyces*

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 described as new, here.

*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, *A. bostrychodes* lacks 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 2 wk. at 25 °C) than *A. peruvianum* (30 mm diam) (Cain 1957; Brash et al. 2017). *Arachnomyces bostrychodes* morphologically resembles *Arachnomyces gracilis*, but the former grows faster, produces more strongly contorted branches and lacks of a sexual morph.

Type: USA: Texas: from a human scalp, 2008, N. Wiederhold (CBS H-24452 – holotype; CBS 146926 = FMR 17685 = UTHSCSA DI118–91 – ex-type cultures; LSU/ITS sequences GenBank LR701766/LR701765).

Description: Vegetative hyphae hyaline, septate, branching to form dense clusters, arcuate, sinuous, contorted or tightly curled, 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 wk. at 25 °C, elevated, cottony, margins regular, white (5A1), sporation absent; reverse light orange (5A4). Colonies on PDA reaching 11–12 mm diam after 2 wk. at 25 °C, elevated, velvety with floccose patches, margins regular, yellowish white (4A2), sporation abundant; reverse greyish yellow (4B6). Colonies on PDA reaching 13–14 mm diam after 2 wk. at 30 °C, slightly elevated, velvety to floccose, regular margins, white (4A1), sporation sparse; reverse, greyish yellow (4B6). Colonies
on OA researching 13–14 mm diam after 2 wk. 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: Recalling 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 a 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 morphologically resembles A. gracilis, but the former grows more slowly, produces more twisted fertile branches and does not form a sexual morph (Udagawa and Uchiyama 1999).

Type: USA: Massachusetts: from an animal’s bone, 2012, N. Wiederhold (CBS H-24453 – holotype; CBS 146927 = FMR 17691 = UTHSCSA DI18-97 – ex-type cultures; LSU/ITS sequences GenBank LR743668/ LR743667).

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. Coconidia 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 wk. at 25 °C, elevated, velvety to floccose, margins regular, slightly furrowed, yellowish white (3A2), sporulation absent; reverse greyish orange.
Colonies on PDA reaching 9–10 mm diam after 2 wk. 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 wk. 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 wk. at 25 °C, flattened, velvety and granulose, margins irregular, pale yellow (4A3), sporulation absent (conidia appear after 5–6 wk. 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.

Currahmyces

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

Currahmyces sparsispora Rodríguez-Andr., Cano & Stchigel, sp. nov.

(Fig. 5)

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 *C. 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: **USA**: Florida: from human sputum, 2007, *N. Wiederhold* (CBS H-24455 – holotype; CBS 146929 = FMR 17683 = UTHSCSA DI18-89 – ex-type cultures; LSU/ITS sequences GenBank LR723273/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 wk. 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 wk. 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 wk. 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 wk. 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.

**Malbranchea**

An emended description of the genus Malbranchea is provided as follows:

**Malbranchea** Sacc., *Michelia* 2 (no. 8): 639 (1882).

*Malbranchea* was originally described by Saccardo (1882) as a genus of the family Malbrancheaceae. However, with the advancement of molecular phylogenetics and the recent reclassification efforts, the family has undergone significant changes. The genus *Malbranchea* is now recognized as a member of the family Tremellaceae, and its species are grouped within the subfamily Tremellaceae, as per the most recent classification.

**Description**: 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, 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, thermostolerant or thermophilic, chitinolytic. Species homothallic or heterothallic, whose cell wall is ornamented with a (coarse or thin) reticulate pattern. Species homothallic or heterothallic, thermostolerant or thermophilic, chitinolytic, chitinolytic pattern. Species homothallic or heterothallic, whose cell wall is ornamented with a (coarse or thin) reticulate pattern. Species homothallic or heterothallic, thermostolerant or thermophilic, chitinolytic, chitinolytic pattern.

Taking into account that Auxarthron and Malbranchea are congeneric, as has been shown in previous studies (Sigler et al. 2002; Sarrocco et al. 2015) and here (Fig. 2), and that Malbranchea (Saccardo 1882) has historical priority (Turland et al. 2018), we transfer the species of Auxarthron to Malbranchea as follows:

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

MycoBank MB 835229

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

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

**Malbranchea chinensis** (Z.F. Zhang & L. Cai) Rodr.-Andr., Cano & Stchigel, **comb. nov.** MycoBank MB 839604

*Basionym*: Auxarthron chinense Z.F. Zhang & L. Cai, *Fungal Divers.* 106: 55 (2020).

**Malbranchea chlamydospora** (M. Solé et al.) Rodr.-Andr., Cano & Stchigel, **comb. nov.**

MycoBank MB 835230

*Basionym*: Auxarthron chlamydosporum M. Solé, et al., *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 et al.) Rodr.-Andr., Stchigel & Cano, **comb. nov.**

MycoBank MB 835232

*Basionym*: Auxarthron concentricum M. Solé et al., *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"*].

**Malbranchea guangxiensis** (Z.F. Zhang & L. Cai) Rodr.-Andr., Cano & Stchigel, **comb. nov.**

MycoBank MB 839605

*Basionym*: Auxarthron guangxiense Z.F. Zhang & L. Cai, *Fungal Divers.* 106: 57 (2020).

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

**Malbranchea longispora** (Stchigel et al.) Rodr.-Andr., Stchigel & Cano, **comb. nov.**

MycoBank MB 835235

*Basionym*: Auxarthron longisporum Stchigel et al., *Persoonia* 31: 267 (2013).

**Malbranchea ostraviensis** (Hubka et al.) Rodr.-Andr., Cano & Stchigel, **comb. nov.**

MycoBank MB 835236

*Basionym*: Auxarthron ostraviensis Hubka et al., *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).

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

*Auxarthron umbrinus* (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) Apiinis, *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 update the Malbranchea species names listed below:

Malbranchea albolutea Sigler & J.W. Carmich., Mycotaxon 4: 416 (1976).
Synonym: Auxarthron albuloteum Sigler et al., Stud. Mycol. 47: 118 (2002).

Malbranchea filamentososa Sigler & J.W. Carmich., Mycotaxon 15: 468 (1982).
Synonym: Auxarthron filamentosum Sigler et al., Stud. Mycol. 47: 116 (2002).

Because in a BLAST search using the ITS and LSU nucleotide sequences from the ex-type strains, Malbranchea crinicipa and M. flavorosea, both those species are excluded to the genus.

After examination of the lectotype of Auxarthron indicum (Patil and Pawar 1987, as "indica"), 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 available we consider it as of uncertain application.

Despite the strain FMR 17681 being placed phylogenetically close to Malbranchea ostraviensis and M. umbrina, it differs genetically and phenotypically from both species, therefore we describe the new species Malbranchea gymnoascoideae as follows:

Malbranchea gymnoascoideae Rodr.-Andr., Stchigel & Cano, sp. nov.
(MycoBank MB 835212

Etymology: As the ascomata are morphologically like those of Gymnascus reessii.

Diagnosis: Malbranchea gymnoascoideae is phylogenetically close to M. ostraviensis and M. umbrina (Fig. 2). Nevertheless, M. gymnoascoideae produces smaller ascomata (to 250 μm diam in M. gymnoascoideae vs. to 450 and 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. gymnoascoideae are longer than those of M. umbrina (250–400 μm vs. 5–72 μm), but shorter than those of M. ostraviensis (350–600 μm long). The ascospores of M. gymnoascoideae are like 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. gymnoascoideae 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. gymnoascoideae nor in M. umbrina. Both Malbranchea gymnoascoideae 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: USA: Texas: from human bronchial washing specimen, 2005, N. Wiederhold (CBS H-24456 – holotype; CBS 146930 = FMR 17681 = UTHSCSA DI18-87 – ex-type cultures; LSU/ITS sequences GenBank LR701758/LR701757).

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 enterothric, 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. Ascospore 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 wk. 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 wk. 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 wk. 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 wk. 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 describe the new species *Malbranchea multiseptata*.

**Malbranchea multiseptata** Rodr.-Andr., Cano & Stchigel, sp. nov.

(Fig. 7)

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:** **USA**: Texas: from human bronchial washing specimen, 2014, N. Wiederhold (CBS H-24457 – holotype; CBS 146931 = FMR 17695 = UTHSCSA DI18-101 – ex-type cultures; LSU/ITS sequences GenBank LR701760/LR701759).

**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 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 wk. 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 wk. 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 wk. 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 wk. 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 also described.

**Malbranchea stricta** Rodr.-Andr., Stchigel & Cano, sp. nov.

(Fig. 8)

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 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:** **USA**: Florida: human nail, 2003, N. Wiederhold (CBS H-24458 – holotype; CBS 146932 = FMR 17680 = UTHSCSA DI18-86 – ex-type cultures; LSU/ITS sequences GenBank LR701639/LR701638).

**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 wk. 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 wk. 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 wk. at 30°C, slightly elevated, velvety to floccose, margins regular, white (3A1), sporulation abundant; reverse pale yellow (4A3). Colonies on PDA reaching 16–17 mm diam after 2 wk. 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.

Pseudoarthropsis

Since the strain FMR 17692 was placed in the same terminal clade as *Arthropsis cirrhata*, while the type species of the genus (*Arthropsis truncata*) is phylogenetically distant (in *Sordariales*; Giraldo et al. 2013), we erect 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 ἑμβής-, resembling, because the morphological semblance to *Arthropsis*.

Diagnosis: Mycelium composed by hyaline to orange, septate hyphae. Conidiophores consisting of fertile lateral branches and a portion of the main subtending hypha, with all these structures disintegrating into yellowish orange, thin-walled, cylindrical to cuboid enteroarthric conidia, or into hyaline, thick-walled, ellipsoidal, globose to barrel-shaped holoarthric conidia.

Type species: *Pseudoarthropsis cirrhata* (Oorschot & de Hoog) Stchigel, Rodr.-Andr. & Cano 2021.

*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 d at 25 °C, powdery, fealty, slightly raised, orange (5A7), pale orange (5A5) at centre; reverse brownish orange (7C8), diffusible pigment brown.

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

Pseudoarthropsis crassispora Rodr.-Andr., Stchigel & Cano, sp. nov.

(Fig. 9)

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 and 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: USA: Minnesota: from a human bronchial washing specimen, 2012, N. Wiederhold (CBS H-24454 – holotype; CBS 146928 = FMR 17692 = UTHSCSA DI18-98 – ex-type cultures; LSU/ITS sequences GenBank LR701763/LR701764).

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

Fig. 8 Malbranchea stricta CBS 146932T. 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
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 wk. 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 wk. 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 wk. 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 wk. 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.

**Pseudomalbranchea**

Despite the strain FMR 17684 being placed phylogenetically in *Onygenaceae*, it is paraphyletic described as the type species of the new genus *Pseudomalbranchea*.

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

MycoBank MB 835220

**Etymology:** Recalling the morphological similarity with *Malbranchea*.

**Diagnosis:** Arthroconidia one-celled, intercalary disposed along unbranched vegetative hyphae, mostly enteroarthric, occasionally holoarthric, cylindrical but becoming globose with the age.

**Type species:** *Pseudomalbranchea gemmata* Rodr.-Andr., Cano & Stchigel 2021
Description: Mycelium sparse, composed of hyaline, smooth- and thin-walled septate hyphae. Asexual morph consisting of mostly enterothecaric, occasionally holoarthric, conidia, intercalary disposed along unbranched vegetative hyphae, solitary or in short chains, with rhexolytic 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.

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 Anauroascus 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 and Kuehn 1972, Sigler and 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: USA: Florida: from human bronchial washing specimen, 2014, N. Wiederhold (CBS H-24459 – holotype, CBS 146933 = FMR 17684 = UTHSCSA DI18-90 – ex-type cultures; LSU/ITS sequences GenBank LR701762/LR701761).

Description: Mycelium sparse, composed of hyaline, smooth- and thin-walled, sparsely septate hyphae, 1.0–2.0 μm wide. Conidia enterothecaric (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 rhexolysis (rarely by schizolysis). Chlamydospores, racquet hyphae and sexual morph not observed.

Culture characteristics: Colonies on PYE reaching 22–23 mm diam after 2 wk. 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 wk. 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 wk. 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 wk. 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.

Spiromastigoides

Because strains FMR 17686 and FMR 17696 were placed together in a terminal branch close to the ex-type strain of M. gypsea in the Spiromastigaceae clade (Fig. 2). M. gypsea is combined into Spiromastigoides and these two strains are described as the new species S. geoncoides.

Spiromastigoides geoncoides Stchigel, Rodr.-Andr. & Cano, sp. nov.

(Fig. 11)
Mycobank MB 835222

Etymology: From the production of conidiophores morphologically similar to those of the genus Geoncyes.

Diagnosis: Spiromastigoides geoncoides is phylogenetically close to S. gypsea. However, it 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 and Carmichael 1976]. Also, S. geoncoides grows faster than S. gypsea on PYE at 35°C.

Type: USA: Illinois: from a human foot skin, 2014, N. Wiederhold (CBS H-24460 – holotype, CBS 146934 = FMR 17696 = UTHSCSA DI18-102 – ex-type cultures; LSU/ITS sequences GenBank LR701768/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 Geoncyes, septate, hyaline, smooth- and thin-walled, producing intercalary and terminally arthroconidia separated by 1–2 empty intermediate cells. Conidia enterothecaric, 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 wk. 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 wk. 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 wk. at 30°C, flattened, velvety, regular margins, yellowish white (4A2), sporulation absent; reverse, pale yellow (4A3). Colonies on OA reaching 20–21 mm diam after 2 wk. 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 specimen examined: USA: 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).

Description (adapted from the original description): Arthroconidia produced intercalary or terminally along 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 wk. 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).
Fig. 11 *Spiromastigoides geomyoides* 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 μm

**KEYS**

*Key to Arachnomyces species*

Adapted from Sun et al. (2019).

1 Homothallic; asexual morph present or not.................................................................................................................. 2  
Heterothallic; asexual morph present .................................................................................................................................. 6

2(1) Peridial setae coiled or circinate; asexual morph absent........................................................................................... 3  
Peridial setae straight, tapering towards the apex; asexual morph arthroconidia ................................................................................................................................. gracilis

3(2) Peridial setae slightly nodose; ascospores mostly < 3.5 μm diam .................................................................................. 4  
Peridial setae smooth-walled; ascospores mostly > 3.5 μm diam............................................................................................ 5

4(3) Ascospores smooth-walled................................................................................................................................................ minimus  
Ascospores echinulate.......................................................................................................................................................... peruvianus

5(3) Ascomata 100–300 μm diam........................................................................................................................................ nitidus  
Ascomata 500–700 μm diam............................................................................................................................................... sulphureus

6(1) Arthroconidia alternate....................................................................................................................................................... 7  
Arthroconidia in persistent chains...................................................................................................................................... 12

7(6) Arthroconidia cylindrical or barrel-shaped; sclerotia present......................................................................................... 8  
Arthroconidia distinct; sclerotia absent................................................................................................................................ 9

8(7) Colonies becoming greyish brown, not growing at 35 °C.............................................................................................. glareosus  
Colonies white to pale brown, growing at 35 °C.................................................................................................................. scleroticus

9(7) Arthroconidia subglobose to pyriform.......................................................................................................................... 10
Arthroconidia cylindrical to finger-like-shaped.................................................................................................................. 11

10(9) Arthroconidia smooth-walled to finely asperulate; setae (produced on the vegetative mycelium) smooth-walled to slightly nodose........................................................................................................... kanei
Mature arthroconidia coarsely verrucose; setae (produced on the vegetative mycelium) strongly nodose.................................................................................................................. pilosus

11(9) Fertile hyphae successively branching to form dense clusters, arcuate, sinuous, contorted or tightly curled........................................................................................................ bostrychodes
   Fertile hyphae branching but not in clusters; branches only apically coiled.................................................................................................................. graciliformis
12(6) Setae (produced on the vegetative mycelium) strongly nodose, circinate or loosely coiled at the apex.................................................................................................................. nodosotetosus
   Setae (produced on the vegetative mycelium) strongly nodose, tip straight.................................................................................................................. jinanicus

Key to Malbranchea species
Adapted from Sigler and Carnichael (1976), Solé et al. (2002), and Hubka et al. (2013).

1 Homothallic species................................................................. 2

2(1) Peridial appendages longer than 150 μm long................................. 3
   Peridial appendages shorter or absent.................................................. 8

3(2) Appendages 350–600 μm in length; diffusible pigment pinkish to reddish; not growing at 35 °C ............... ostraviensis
   Above features not combined............................................................... 4

4(3) Ascospores smooth-walled under bright field microscope.......................................................... gymnoascomoides
   Ascospores reticulate............................................................................. 5

5(4) Peridial cells short, 4–12 μm in length; peridial projections with truncate ends............................... compacta
   Peridial cells longer; peridial projections with mostly acute ends........... 6

6(5) Ascospores usually exceeding 4 μm diam................................................................. californiensis
   Ascospores ≤4 μm diam........................................................................ 7

7(6) Species growing at 37 °C................................................................. 8
   No growth at 37 °C............................................................................. umbrina

8(2) Asexual morph not produced........................................................................... guangxiensis / pseudeuxarthron
   Malbranchea-like asexual morph present.............................................. 9

9(8) Ascomata with spine-like peridial projections, 27–40 μm in length....................... zuffiana
   Ascomata without peridial projections................................................... 10

10(9) Colonies on PDA brown........................................................................... kuehnii
   Colonies on PDA otherwise.................................................................. 11

11(10) Peridial hyphae smooth-walled............................................................... concentrica
   Peridial hyphae strongly ornamented; chlamydospores present ........... 12

12(11) Arthroconidia 2–10 × 2.5–3.5 μm; growing above 30 °C .................... chlamydospora
   Arthroconidia 4–24 × 1.0–5.5 μm; not growing above 30 °C................. longispora
13(1) Fertile hyphae arcuate or curved............................................................. 14
   Fertile hyphae straight to sinuous, branched or not................................. 21
14(13) Fertile hyphae coiled............................................................................. 15
   Fertile hyphae curved or arcuate............................................................. 16
15(14) Thermophilic; conidia 2.5–4.5 μm wide........................................... cinnamomea
   Not thermophilic; conidia narrower...................................................... pulchella
16(14) Colonies orange................................................................................... 17
   Colonies different.................................................................................. 18

17(16) Aleuroconidia laterally or terminally dispersed........................................ chrysosphoroides
Aleoconidia absent.................................................................................. aurantiaca
18(16) Colonies golden yellow, exudate brown, diffusible pigment yellow........... graminicola
   Above features not combined............................................................... 19
19(18) Sexual morph produced by in vitro mating of compatible strains................................. albolutea
   Sexual morph not formed..................................................................... 20
20(19) Thick-walled brown setae produced on OA from the vegetative mycelium.................... filamentosa
   Setae not produced................................................................................ arcuata
21(13) Fertile hyphae unbranched or scarcely branched....................................... 22
   Fertile hyphae branched....................................................................... 24
22(21) Arthroconidia cylindrical; becoming many septate with the age.................. multiseptata
   Arthroconidia barrel-shaped, “I”-shaped, “Y”-shaped, finger-shaped or more irregular, mostly unicellular...... 23
23(22) Arthroconidia barrel-shaped, 4–8 × 2–3.5 μm; racquet hyphae present.................................................................................. **chinensis**
Arthroconidia barrel-shaped, “T”-shaped, “Y”-shaped, finger-shaped or more irregular, 2–6 × 1–2 μm;
racquet hyphae absent.................................................................................................................................................. **stricta**
24(21) Fertile hyphae branching acutely, displaying a tree-like appearance.......................................................... **dendritica**
Fertile hyphae branching pattern otherwise................................................................................................................. 25
25(24) Fertile hyphae repeatedly branched, in dense tufts.................................................................................... **flocciformis**
Fertile hyphae more restrictedly branched.................................................................................................................. 26
26(25) Colonies buff or tan........................................................................................................................... **fulva**
Colonies lemon yellow............................................................................................................................................... 26

**Key to Spiromastigoides species**
Adapted from Hirooka et al. (2016).

1 Homothallic.................................................................................................................................................................. 2
Heterothallic..................................................................................................................................................................... 6
2(1) Ascospores globose to subglobose, reticulate.................................................................................. **sphaerospora**
Ascospores oblate, equatorial thickening present or not......................................................................................... 3
3(2) Ascospores with equatorial thickening.......................................................................................... **asefusis**
Ascospores without such equatorial thickening........................................................................................................ 5
4(3) Ascomata appendages straight or slightly undulate; ascospores yellow, smooth-walled under LM,
pitted under SEM.................................................................................................................................................. **alatospora**
Ascomata appendages slightly undulate or wavy; ascospores pale yellowish brown,
minutely punctate under SEM............................................................................................................................... **saturnispora**
5(3) Ascospores punctate, sometimes with a few fine grooves in the polar region,
2.5–2.9 × 2.0–2.5 μm............................................................................................................................................... **warcupii**
Ascospores lens-shaped, regularly pitted, 3.0 × 2.0 μm.................................................................................. **sugiyamae**
6(1) Asexual morph chrysosporium-like; sterile ascomata present........................................................................... **asefusis**
Asexual morph distinct.............................................................................................................................................. 7
7(6) Asexual morph malbranchea-like.................................................................................................................. **gypesea**
Conidiophores well-developed............................................................................................................................... 11
8(7) Fertile hyphae straight, branched..................................................................................................................... **gypesea**
Fertile hyphae curved............................................................................................................................................... 9
9(8) Fertile hyphae successively branched to form sporodochia-like structures.................................................... **albida**
Fertile hyphae unbranched or scarcely branched............................................................................................. 10
10(9) Fertile hyphae unbranched or sparsely branched, curved, > to 28 μm long; chlamydospores present........... **curvata**
Fertile hyphae unbranched, slightly curved, > to 15 μm long; chlamydospores absent........................................ **minimus**
11(7) Conidiophores unbranched or scarcely branched........................................................................................... **geomycoides**
Conidiophores branched several times................................................................................................................... 12
12(11) Conidiophores > to 300 μm in length, verticillate.................................................................................. **kosraensis**
Conidiophores 100–150 μm in length, with pyramidal or bush-like branching..................................................... 13
13(12) Conidiophores > to 150 μm long, with pyramidal branching........................................................................... **pyramidalis**
Conidiophores > to 100 μm long, with bush-like branching.............................................................................. **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 wk. of incubation.

DISCUSSION
To our knowledge, this is the main study to be produced on malbranchea-like fungi from a clinical origin to date. 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 15 strains as belonging to the genus Malbranchea (syn. Auxarthron), of which three of them are described as new species. These results indicate a high diversity of onyenalean fungi in these sorts of substrates, which may be difficult to differentiate using only 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 and Trotter 1913; Cooney and Emerson 1964; Sigler and Carmichael 1976). All 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. multisep-tata, Pseudoarthropsis crassisspora, 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 the antifungal susceptibility of malbranchea-like fungi, limited data are available. However, in a previous study on onychomycosis-causing strains of

| 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 17679 | >16 | >16 | 4 | >16 | 2 | >16 | 8 | >16 | 0.03 | >16 |
| Malbranchea albolutea    | FMR 17689 | >16 | >16 | 2 | >16 | 1 | 0.12 | 0.06 | 0.25 | >16 |
| M. aurantiaca            | FMR 17682 | >16 | >16 | 1 | >16 | 0.25 | 0.12 | 1 | 0.12 | 4 | >16 |
| M. conjugata             | FMR 17688 | >16 | >16 | 2 | >16 | 0.5 | 0.06 | 1 | 2 | >16 |
| M. gymnoascoides         | FMR 17697 | >16 | >16 | 1 | >16 | 0.5 | 0.12 | 0.03 | 0.12 | 0.5 | >16 |
| M. stricta               | FMR 17699 | >16 | >16 | 0.5 | 2 | 0.5 | 0.12 | 0.25 | 0.25 | 1 | >16 |
| M. umbrina               | FMR 17698 | >16 | >16 | 1 | >16 | 0.5 | 0.12 | 0.03 | 0.12 | 0.5 | >16 |
| M. umbrina               | FMR 17694 | >16 | >16 | 4 | >16 | 0.5 | 0.06 | 1 | 0.12 | 0.25 | >16 |
| M. umbrina               | FMR 17700 | >16 | >16 | >16 | >16 | >16 | 0.5 | 1 | 0.5 | >16 | >16 |
| M. umbrina               | FMR 17701 | >16 | >16 | 4 | >16 | 0.12 | 0.03 | 0.03 | 0.12 | 0.12 | >16 |
| M. zuffiana              | FMR 17690 | >16 | >16 | 1 | >16 | 0.5 | 0.06 | 0.12 | 0.25 | >16 |
| Pseudomalbranchea gemmata| FMR 17684 | >16 | >16 | 1 | >16 | 0.5 | 0.05 | 1 | 4 | 0.25 | >16 |
| Spiromastigoides geomyoides| FMR 17686 | >16 | >16 | 2 | >16 | 1 | 0.12 | 0.25 | >16 |
| Spiromastigoides geomyoides| FMR 17696 | >16 | >16 | 2 | >16 | 1 | >16 | >16 | >16 | >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
**CONCLUSIONS**

From all malbranchea-like strains from clinical specimens (mostly human) in the USA that we studied, only 13 out of 22 could be identified at the species level, three of them belonging to the genus *Malbranchea*. With the exception of one strain initially identified as *Currahomyces indicus*, the others were identified as species of *Auxarthron*, a genus synonymized with *Malbranchea* during the course of the present work. Eight of the remaining strains have been assimilated to the genera *Arachnomycyes* (2), *Arthropsis* (1), *Malbranchea* (3), and *Spiromastigoides* (2), the latter only located at family level (*Onygenaceae*). This is an extraordinary finding, because nearly half of the fungal strains presumed to belong to the genus *Malbranchea* resulted in becoming new taxa for science. Finally, despite the lack of histopathological data, which could have undoubtedly proven that these strains were the causative agents of the infections that led to the request for sample collection, we would highlight their poor sensitivity to first-line drugs such as AMB, FLC, and ITC, but better sensitivity to echinocandins and PSC.

**ABBREVIATIONS**

- S-FC: 5-Fluorocytosine
- AFG: Anidulafungin
- AMB: Amphotericin B
- BCP-MS: Bromocresol purple milk solids glucose agar
- BI: Bayesian-Inference
- BLAST: Basic Local Alignment Search Tool
- BS: Bootstrap support
- CFG: Caspofungin
- CLSI: Clinical Laboratory Standards Institute
- DNA: Deoxyribonucleic acid
- FLC: Fluconazole
- ITC: Itraconazole
- ITS: Ribosomal internal transcribed spacers
- LM: Light microscope
- LSU: Large subunit 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
- PDA: Potato Dextrose Agar
- PDA: Potato dextrose agar
- PDI: Oatmeal agar
- PSC: Posaconazole
- PYE: Phytone dextrose agar
- SEM: Scanning electron microscopy
- T = ex t ype
- TOTM: Test opacity tween medium
- TRB: Terbinafine
- UTHSCA: University of Texas Health Science Centre at San Antonio
- VRC: Voriconazole

**Supplementary Information**

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**Adherence to national and international regulations**

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

**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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**Availability of data and materials**

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

**DECLARATIONS**

**Ethics approval and consent to participate**
Not applicable.

**Consent for publication**
Not applicable.

**Competing interests**

The authors declare that they have no competing interests.

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**Additional file 1** Fig. S1. ML phylogenetic tree based on the analysis of ITS nucleotide sequences for the 22 clinical fungi from the USA.
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