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TAXONOMIC DESCRIPTION
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Paucimyces polynucleatus gen. nov, sp. nov., a novel polycentric genus of anaerobic gut fungi from the faeces of a wild blackbuck antelope

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Abstract

The anaerobic gut fungi (AGF; phylum *Neocallimastigomycota*) reside in the alimentary tracts of herbivores. Multiple novel, yet-uncultured AGF taxa have recently been identified in culture-independent diversity surveys. Here, we report on the isolation and characterization of the first representative of the RH5 lineage from faecal samples of a wild blackbuck (Indian Antelope, *Antilope cervicapra*) from Sutton County, Texas, USA. The isolates displayed medium sized (2–4 mm) compact circular colonies on agar roll tubes and thin loose biofilm-like growth in liquid medium. Microscopic examination revealed monoflagellated zoospores and polycentric thalli with highly branched nucleated filamentous rhizomycelium, a growth pattern encountered in a minority of described AGF genera so far. The obtained isolates are characterized by formation of spherical vesicles at the hyphal tips from which multiple sporangia formed either directly on the spherical vesicles or at the end of sporangiophores. Phylogenetic analysis using the D1/D2 regions of the large ribosomal subunit (D1/D2 LSU) and the ribosomal internal transcribed spacer 1 (ITS1) revealed sequence similarities of 93.5 and 81.3%, respectively, to the closest cultured relatives (*Orpinomyces joyonii* strain D3A (D1/D2 LSU) and *Joblinomyces apicalis* strain GFH681 (ITS1). Substrate utilization experiments using the type strain (BB-3^T) demonstrated growth capabilities on a wide range of mono-, oligo- and polysaccharides, including glucose, xylose, mannose, fructose, cellobiose, sucrose, maltose, trehalose, lactose, cellulose, xylan, starch and raffinose. We propose accommodating these novel isolates in a new genus and species, for which the name *Paucimyces polynucleatus* gen. nov., sp. nov. is proposed.

INTRODUCTION

In the herbivorous gut, a diverse community of bacterial, archaeal, protozoan and fungal species synergistically mediate the breakdown of plant biomass [1]. Fungi in the herbivorous gut belong to a distinct fungal phylum (*Neocallimastigomycota*) and play a pivotal role in this process through mechanical and enzymatic means [2]. Nineteen anaerobic gut fungal (AGF) genera have been characterized so far [3–14]. However, culture-independent diversity surveys have identified representatives of multiple yet-uncultured AGF genera [15–17]. The amenability of such lineages to isolation is uncertain. The lack of cultured representatives could be a reflection of the complexity and difficulty in isolation and maintenance of these AGF lineages. Alternatively, it is possible that some yet-uncultured AGF taxa have complex nutritional requirements that are not satisfied in current media and isolation procedures.

Based on prior evidence [15], we hypothesize that success in isolating a fungal taxon is directly proportional to its relative abundance within a specific sample. As such, targeting samples assessed to harbour a relatively large fraction of yet-uncultured taxa using culture-independent approaches should be prioritized in culture-based diversity efforts. During a recent culture-independent diversity survey of the AGF community in wild, zoo-housed and domesticated herbivores in the US states of Oklahoma and Texas, we encountered several samples that harboured a high proportion of yet-uncultured genus-level clades of AGF [15]. We here report on the targeted isolation and detailed characterization of multiple strains belonging to one of these clades (lineage RH5). Morphological, microscopic and phylogenetic characterization justifies proposing a novel genus and species to accommodate these isolates, for which the name *Paucimyces polynucleatus* is proposed.

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Keywords: fungi; *Neocallimastigomycota*; herbivores.
Abbreviations: AGF, anaerobic gut fungi; ITS1, internal transcribed spaces 1.
Ribosomal operon sequences encompassing ITS1–5.8S–ITS2–D1/D2 LSU regions for type strain BB-3^T have been deposited in GenBank under accession numbers MW694896–MW694898. Genus and species information has been deposited in Mycobank under the ID numbers MB838953 and MB838954.

Table 1. Substrate utilization pattern of strain BB-3^T

Substrate	Growth*
Polysaccharides:	
Cellulose	+
Xylan	+
Starch	+
Raffinose	+
Inulin	–
Poly-galacturonate	–
Chitin	–
Alginate	–
Pectin	–
Disaccharides:	
Cellobiose	+
Sucrose	+
Maltose	+
Trehalose	+
Lactose	+
Monosaccharides:	
Glucose	+
Xylose	+
Mannose	+
Fructose	+
Glucuronic acid	–
Arabinose	–
Ribose	–
Galactose	–
Peptides:	
Peptone	–
Tryptone	–

*+, Growth was observed following three consecutive subcultures; –, no growth was observed with the carbon source.

METHODS

Samples

Fresh faecal samples were collected from a wild blackbuck antelope (*Antilope cervicapra*) during a hunting expedition in Sutton County, Texas, USA in April 2018. All hunters had the appropriate licenses and animals were shot either on a private land with the owner's approval or on public land during the hunting season. Samples were collected in 50 ml sterile plastic tubes, stored on ice and promptly (within 24 h) transferred to the laboratory. Upon arrival, a portion of the sample was stored at −20 °C. Isolation was conducted on −20 °C stored samples. No special preservation procedures or chemicals were added to theses samples.

Isolation

Enrichments targeting AGF were conducted on faecal samples from a wild blackbuck antelope (*Antilope cervicapra*) for 24 h at 39 °C in rumen fluid (RF) media [18] amended with 0.5% cellobiose as a substrate. Enriched tubes were serially diluted in RF media supplemented with a (1:1) mixture of cellobiose and switchgrass. Antibiotics mixture (50 µg ml^{-1} penicillin, 20 µg ml^{-1} streptomycin and 50 µg ml^{-1} chloramphenicol) was added to inhibit bacterial growth. Dilutions showing visible signs of fungal growth such as clumping and floating of the switchgrass and/or production of gas bubbles were used for colony isolation using the roll tube procedure [19]. Purity of the obtained cultures was ensured by conducting three rounds of roll tubing and colony picking. Isolates were maintained by bi-weekly sub-culturing into cellobiose containing RF media. Long-term storage of the obtained isolates was conducted by surface inoculation on RF–cellobiose agar medium as previously described in [18].

Morphological and microscopic characterization

Three-day-old colonies and liquid cultures were examined to describe the isolate's growth pattern on solid and liquid media, respectively.

Both light and scanning electron microscopy were utilized to examine different fungal structures at various stages of growth. For light microscopy, fungal biomass was stained with lactophenol cotton blue and examined using an Olympus BX51 microscope equipped with a DP71 digital camera (Olympus). Nuclei localization was examined by staining the samples with 4,6′-diamidino-2-phenylindole (DAPI; at final concentration of 10 µg ml^{-1}), followed by incubation in the dark for 10 min at room temperature. Treated samples were examined with LSM 980 confocal microscope with Airyscan 2 (Carl Zeiss AG). Sample preparation and fixation for scanning electron microscopy was conducted as previously described [8]. Samples were examined on an FEI Quanta 600 scanning electron microscope.

Substrate utilization

The substrate utilization capabilities of strain BB-3^T were assessed in an RF basal medium with no carbon source as previously described [8]. Twenty-four different substrates were tested by adding them to the basal media at a final concentration of 0.5% w/v (Table 1). Growth and viability of a 10% inoculum was compared to a substrate-free medium. The ability of strain BB-3^T to utilize a specific substrate was considered positive when the tested substrate supports the culture viability after three successive sub-culturing events.

Phylogenetic analysis and ecological distribution

Fungal biomass was harvested from actively growing 3-day-old cultures and ground in liquid nitrogen. DNA was

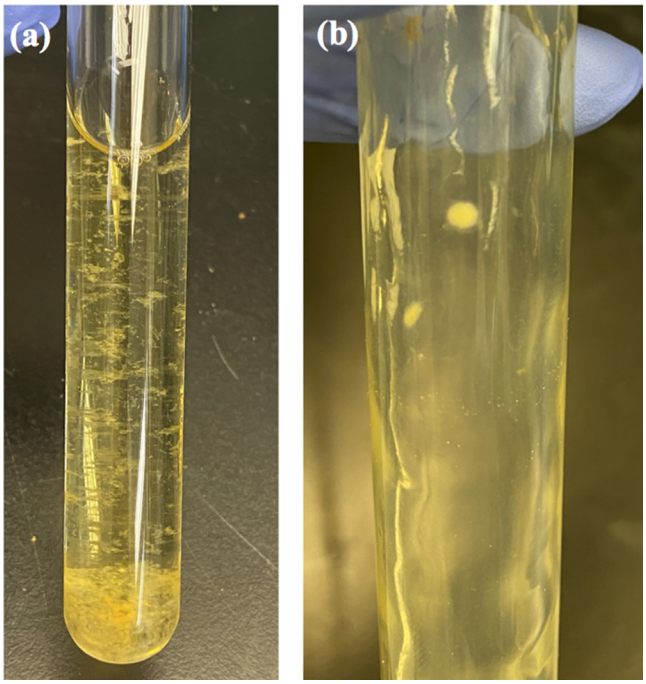

Fig. 1. Macroscopic features of *Paucimyces polynucleatus* strain BB-3^T. (a) Thin and loose fungal biofilm-like growth in liquid cellobiose rumen fluid medium. (b) White, circular compact colony on cellobiose agar roll tube.

extracted from the ground biomass using DNeasy PowerPlant Pro Kit (Qiagen) according to the manufacturer's instructions. The ITS1, 5.8S rRNA, ITS2 and D1/D2 region of the LSU rRNA was amplified using the primers ITS5 (5′ -GGAAGTAAAAGTCGTAACAAGG-3′) and NL4 (5′-TCAACATCCTAAGCGTAGGTA-3′) as described previously [20]. Amplicons were purified using PureLink PCR Purification Kit (ThermoFisher Scientific) and cloned using a TOPO-TA cloning vector according to the manufacturer's instructions (Life Technologies). Three clones were Sanger-sequenced at the Oklahoma State University DNA sequencing core facility. Regions corresponding to the ITS1 and D1/D2 LSU regions from the obtained amplicons were aligned to reference ITS1 and D1/D2 LSU sequences using the MAFFT aligner [21]. Maximum-likelihood phylogenetic trees were reconstructed in FastTree using *Chytriomyces* sp. WB235A isolate AFTOL-ID 1536 as an outgroup. Bootstrap values were calculated on the basis of 1000 replicates.

To evaluate the ecological distribution of this novel lineage, we queried the ITS1 sequences of isolates obtained from this study against GenBank nr (non-redundant) database using BLASTn and modified the output to display 5000 instead of the default 100 aligned sequences. Sequence similarity cutoff of 95% was used to filter the BLASTn output. The phylogenetic position of sequences with significant similarity (≥95%) was evaluated by inserting into ITS1 reference phylogenetic trees.

Data and culture accession

Sequences generated in this study are deposited in GenBank under accession numbers MW694896–MW694898. Cultures are available at Oklahoma State University, Department of Microbiology and Molecular Genetics culture collection (accession code PauciBB-3). Genus and species information has been deposited in Mycobank under the ID numbers MB838953 and MB838954, respectively.

RESULTS

Isolation

Multiple colony morphologies were obtained in roll tubes derived from enrichments of the faeces of a wild blackbuck antelope. One colony type showed little morphological resemblance to currently described taxa and its distinctness was confirmed by microscopic and phylogenetic analysis (see below). Four isolates (BB-12, BB-14, BB-2, BB-3^T) were examined, and all showed identical morphological and microscopic attributes. One isolate (strain BB-3^T) was chosen as the type strain for detailed characterization.

Morphology

On solid media, strain BB-3^T formed white compact circular uniform colonies that lacked a darker central core of sporangial structures, often observed with monocentric AGF genera (Fig. 1a). Colony size ranged from 2 to 4 mm. In liquid media, strain BB-3^T forms a loose thin white biofilm-like growth (Fig. 1b).

Microscopic features

Strain BB-3^T produces globose zoospores (Fig. 2a, b), with an average diameter of 7.5 µm (range, 6–10 µm). The majority of zoospores were monoflagllated (Fig. 2a), although biflagellated zoospores were occasionally observed (Fig. 2b). Flagellum length ranged between 15–30 µm. Upon germination, zoospores contents migrated into the germ tube and the remaining empty zoospore cyst had no further function in the thallus development. This is in contrast to the zoospore cyst of monocentric genera that either enlarges into sporangia or develops a sporangiophore with a sporangium at the end [22]. The germ tube eventually germinated to produce extensively branched polycentric thalli with nucleated filamentous rhizomycelium. The nucleated rhizomycelium produced multiple sporangia, giving rise to a polycentric thallus of indeterminate length (Fig. 2c–f). During early thallus development, the hyphal tips started to swell forming spherical vesicles (Fig. 2g), from which multiple sporangiophores arose (Fig. 2h, i). Each sporangiophore had a single sporangium at its end (Fig. 2c, d, i–k). In many cases, sporangia developed directly on the spherical vesicles without sporangiophores (Fig. 2l, m). In rare occasions, strain BB-3^T produced thalli with single sporangia (Fig. 2n, o). Sporangia were mainly ovoid and ranged in size between 15–90×10–55 µm (Fig. 2i–o). Upon maturity, basal walls were formed to separate the mature sporangia from the sporangiophores (Fig. 2j–k). Old cultures appeared to progressively lose the ability to produce sporangia and

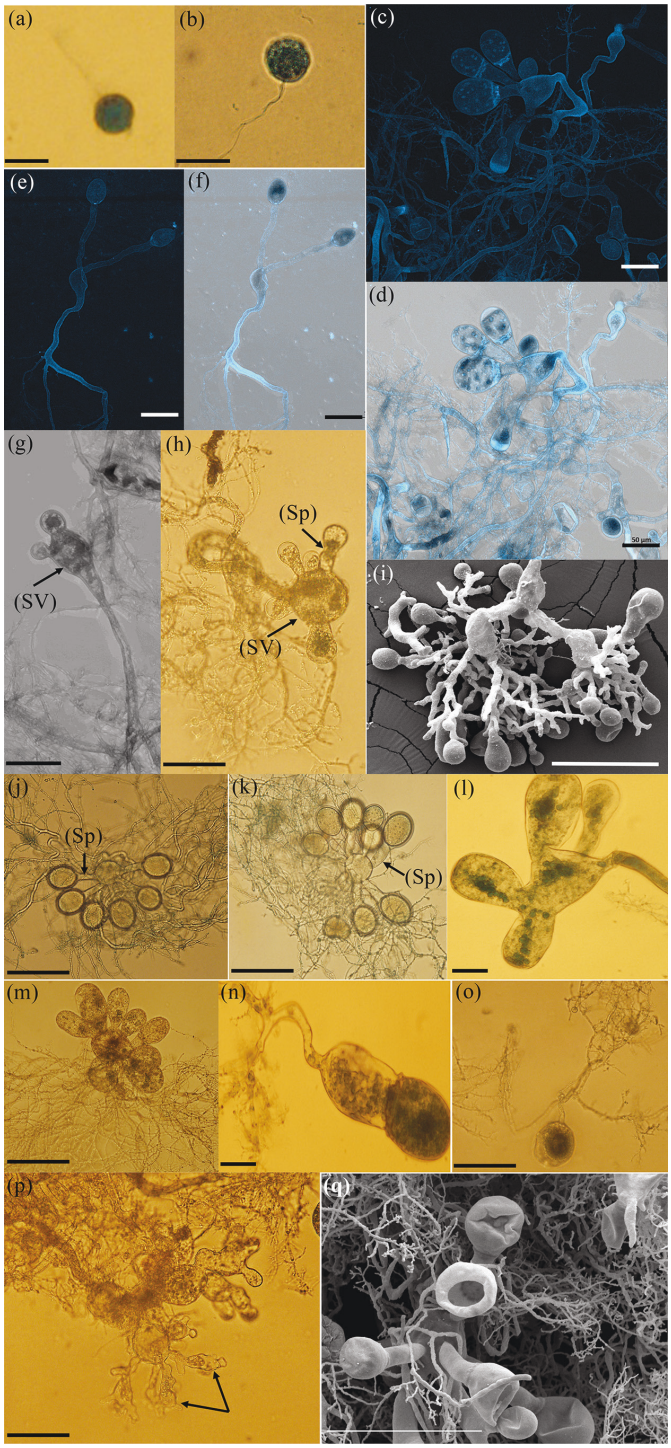

Fig. 2. Microscopic features of *Paucimyces polynucleatus* strain BB-3^T. Light (a, b, h and j–p), confocal (c–g) and scanning electron (i and q) microscopy images are shown. Overlay images are shown in d and f. (a) A monoflagellated zoospore. (b) A biflagellated zoospore. (c–f) Polycentric thalli, with nuclei present in the rhizomycelium. Note the nucleated zoospores inside the mature sporangia (arrows). (g) Early thallus development stage starts by swelling of the hyphal tip forming a spherical vesicle (SV) and developing immature sporangia (s) (arrows). (h) Multiple sporangiophores (Sp) develop on the spherical vesicle (SV). (i) A mature thallus with multiple sporangia. (j–k) Ovoid sporangia developing at apices of sporangiophores (Sp), note the basal wall separating mature sporangia from the sporangiophores (arrows). (l–m) Sporangia developing directly on the spherical vesicles (SV). (n–o) Mature thalli with single ovoid sporangium. (p) An old culture producing empty sporangiophore initials (arrows). (q) An empty sporangium after zoospores release through a wide apical pore, with sporangial wall staying intact. Bar: a, b and l, 20 µm; c–h, j, k, m–p, 50 µm; i and q, 100 µm.

only produced sporangiophores initials (Fig. 2l), a distinct feature that was observed in old *Orpinomyces* cultures [22]. Zoospores were released through a wide apical pore at the top of the sporangia, with the sporangial wall staying intact after the discharge (Fig. 2r). Similar to the majority of polycentric AGF genera, strain BB-3^T culture lost its zoosporogenesis ability due to frequent sub-culturing and started to produce sterile sporangia that did not differentiate into zoospores.

Substrate utilization

Strain BB-3^T was able to utilize a wide range of substrates as the sole carbon and energy source (Table 1). The monosaccharides glucose, xylose, mannose and fructose all supported growth, whereas glucuronic acid, arabinose, ribose and galactose failed to sustain the viability of strain BB-3^T cultures. Strain BB-3^T was able to utilize all disaccharides tested including cellobiose, sucrose, maltose, trehalose and lactose. Out of the polysaccharides tested, strain BB-3^T was able to grow on cellulose, xylan, starch and raffinose, but not inulin, poly-galacturonate, chitin, alginate and pectin. Strain BB-3^T also did not grow on peptone or tryptone (Table 1).

Phylogenetic analysis and ecological distribution

The D1/D2 regions of strain BB-3^T showed very low intra-strain sequence divergence (0–0.25%) and length (778–780 bp) heterogenicity. Similarly, the ITS1 region of strain BB-3^T showed low intra strain sequence divergences (0–0.38%) and length (263–264 bp) heterogenicity. The ITS1 and D1/D2-LSU regions from strain BB-3^T were 100% similar to sequences assigned to the uncultured lineage RH5 obtained in a previous culture-independent diversity survey from the same sample on which isolation was conducted (blackbuck deer), as well as few other samples (aoudad sheep, domesticated sheep and axis deer), demonstrating that these newly obtained isolates are cultured representatives of the RH5 lineage [15].

In D1/D2 LSU trees, strain BB-3^T formed a distinct cluster, within a broader supra-genus clade comprising the genera *Orpinomyces*, *Pecoramyces*, *Ghazallomyces*, *Neocallimastix*, *Feramyces* and *Aestipascuomyces* (Fig. 3a). D1/D2 LSU sequence divergences between strain BB-3^T and its closest relatives in these lineages were 93.5% to *Orpinomyces joyonii* strain D3A, 91.05% to *Pecoramyces ruminantium* strain S4B, 92.32% to *Ghazallomyces constrictus* strain AXS31, 92.6% to *Neocallimastix cameroonii* strain G3, 91.2% to *Feramyces austinii* strain DS10 and 89.43% to *Aestipascuomyces dupliciliberans* strain A252. In ITS1 trees, the closest relatives were members of the genera *Orpinomyces*, *Pecoramyces*, *Ghazallomyces*, *Neocallimastix*, *Feramyces*, *Aestipascuomyces*, *Joblinomyces* and *Agriosomyces* (Fig. 3b). The closest cultured representative based on ITS1 sequence similarity was *Joblinomyces apicalis* strains GFH681 and SFH683 (81.25% similarity). Interestingly, he strain BB-3^T ITS1 sequence showed 95.2% similarity to an isolate described as *Anaeromyces* sp. strain W-98 (GenBank accession number AY091485), but no publication or documentation on the fate of that isolate is available.

DISCUSSION

Strain BB-3^T represents the first cultured representative of the RH5 lineage and would constitute the twentieth described genus within the phylum *Neocallimastigomycota*. In addition to its distinct phylogenetic position in AGF D1/D2 LSU and ITS1 trees (Fig. 3a, b), strain BB-3^T possesses multiple unique morphological and microscopic characteristics that differentiate it from all described AGF genera. Strain BB-3^T exhibits a polycentric thallus growth pattern, in which the zoospore contents completely migrate into the germ tube that eventually develops into a nucleated rhizomycelium capable of producing multiple sporangia per thallus. This thallus development pattern has been encountered only in the AGF genera *Anaeromyces* [4], *Orpinomyces* [3] and *Cyllamyces* [13]. However, there are several key morphological and microscopic features that clearly differentiate strain BB-3^T from other polycentric AGF genera. For example, strain BB-3^T has a filamentous rhizomycelium, distinguishing it from the characteristic bulbous rhizomycelium of the genus *Cyllamyces*.

Compared to *Orpinomyces* species, strain BB-3^T exhibits a thin and loose biofilm-like growth in liquid media, and produces small compact colonies (2–4 mm), unlike the cottony growth pattern and the large (usually>1 cm diam.) colonies characteristic of *Orpinomyces* species. Microscopically, strain BB-3^T produces monoflagellated, occasionally biflagellated, zoospores in contrast to the polyflagellated *Orpinomyces* zoospores. Compared to *Anaeromyces* species, strain BB-3^T produces a non-constricted hyphae and ovoid sporangia, unlike members of *Anaeromyces* species that are known to produce constricted sausage-shaped hyphae and mucronate sporangia. Further, members of the genus *Anaeromyces* produce uniflagellated spores (i.e. all spores always have only a single flagellum), as opposed to the monoflagellated (occasionally biflagellated) zoospores of strain BB-3^T.

A diagnostic characteristic of strain BB-3^T is the formation of spherical vesicles (swellings at the hyphal tips; Fig. 2g, h) from which multiple sporangia are formed either directly on the spherical vesicles (Fig. 2l, m) or at the end of a sporangiophore (Fig. 2j, k). Such a feature has rarely been observed in previously reported taxa. Notably, a single isolate designated *Piromyces polycephalus* and isolated from the rumen fluid of water buffalo was found to display a similar sporangial development pattern (Figs 3 and 4 in [23]). The proposed affiliation with the genus *Piromyces* implies a monocentric growth pattern, although the pictures do not clearly show the growth pattern and nuclear localization. Unfortunately, the absence of extant culture of *P. polycephalus* prevents further investigation into this issue. Also, lack of sequence data for *P. polycephalus* precluded our full understanding of the phylogenetic relationship between *P. polycephalus* and strain BB-3^T.

D1/D2 LSU sequences representing lineage RH5 were identified in one recent culture-independent diversity survey, where it was encountered in 10/31 faecal animal samples and constituted >10% in four samples (a domesticated sheep, a wild blackbuck antelope, an aoudad sheep and an axis deer) [15]. RH5 sequences were identified in foregut fermenters (nine out of ten

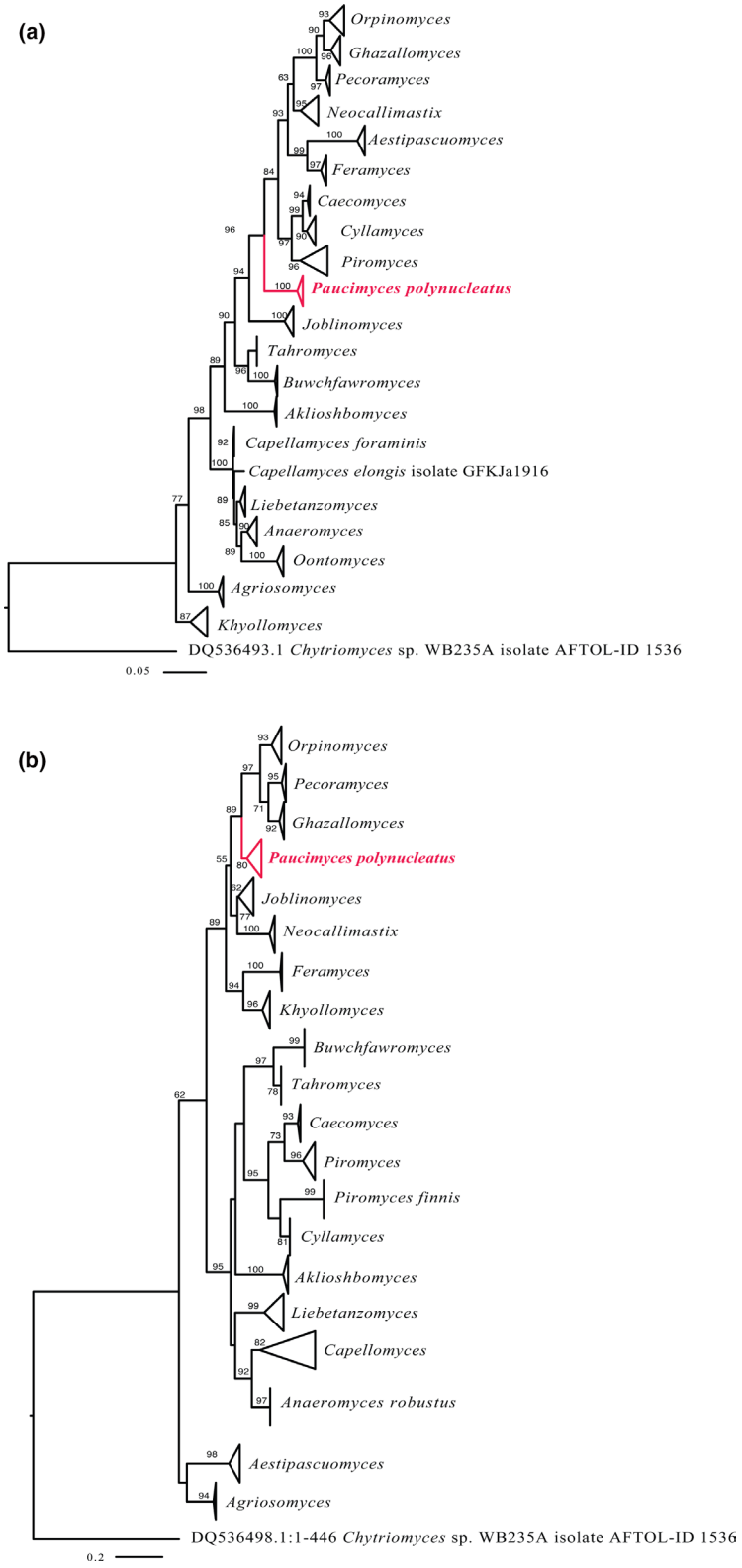

Fig. 3. Phylogenetic affiliation of the *Paucimyces* clade to other AGF genera based on the sequences of (a) D1–D2 LSU and (b) ITS1 sequences. Sequences were aligned in MAFFT [21] and the alignments were used to reconstruct maximum-likelihood trees in FastTree using the GTR model with *Chytriomyces* sp. WB235A isolate AFTOL-ID 1536 as the outgroup. Bootstrap values (from 1000 replicates) are shown for nodes with more than 50% bootstrap support.

samples), with a 0.2% relative abundance in miniature donkey samples, the sole hindgut animal that harboured this lineage. ITS1 sequences similar to the RH5 lineage were also identified in four zoo-housed animals including American bison, llama, sable antelope and western tufted deer with a relative abundance of 0.03, 1.2, 18.93 and 0.03% respectively. These sequences originated from a previous culture-independent survey conducted on zoo-housed animals [17]. Collectively, this pattern suggests a limited global distribution of lineage RH5 in the herbivorous gut and a clear preference to ruminants over hindgut fermenters. However, studies on anaerobic gut fungal diversity are relatively sparse, localized and lack spatiotemporal dimensions. As such, these observations should be regarded as preliminary and more in-depth sampling and diversity assessment efforts are needed to confirm, disprove or identify additional patterns governing the distribution of this lineage.

Thallus development pattern is a key feature used in the classification of the basal fungal lineages including the *Neocallimastigomycota*. Strain BB-3^T exhibited a classical polycentric thallus growth, i.e. multiple sporangia per thallus. This growth pattern is associated with migration of the nucleus out of the zoospore into the germ tube, which elongates and branches into rhizomycelium. Within the rhizomycelium, repeated nuclear divisions occur and nuclei migrate into individual hyphae, resulting in a fungal thallus of unlimited extent and with multiple sporangia. Such pattern is in contrast to the monocentric thallus growth (single sporangium per thallus), where the rhizoid is devoid of nuclei and the thallus is of determinate extent with a single sporangium [2, 22]. It is worth noting that the presence of multiple sporangia per thallus is a hallmark of polycentric growth. However, some monocentric genera such as *Caecomyces communis*, *Piromyces polycephalus* and *Khyollomyces ramosus* produce branched sporangiophores with two or more sporangia resulting in a multi-sporangiate thallus [10, 22, 23]. In addition to the *Neocallimastigomycota* [22], polycentric growth pattern is known to occur in several basal fungal lineages, e.g. the genera *Nowakowskiella* and *Cladochytrium* in the phylum *Chytridiomycota* [24]. Phylogenetic analysis shows that polycentric genera are polyphyletic within the *Neocallimastigomycota*, suggesting that multiple events of acquisition/loss of this trait has occurred throughout *Neocallimastigomycota* evolution and obscuring the nature of the AGF last common ancestor. The genetic and epigenetic determinants of this phenotypic pattern is yet unclear, hindered by the absence of genome representatives from most of the currently described AGF genera [25, 26]. Similarly, the niche preference of polycentric versus monocentric taxa, and correlation between such growth pattern and ecological distribution is murky. It is notable that prior studies have suggested that all previously described polycentric genera (*Anaeromyces*, *Orpinomyces* and *Cyllamyces*) appear to exhibit a distribution pattern where they are present in the majority of examined animals, but often in low relative abundance. In contrast, RH5 appear to have a much more limited distribution, but could represent a majority of the community in rare cases [15, 17].

Based on morphological, physiological, microscopic and phylogenetic characteristics, we propose accommodating these new isolates into a new genus, for which the name *Paucimyces* is proposed. The type strain is *Paucimyces polynucleatus* strain BB-3^T.

DESCRIPTION OF *PAUCIMYCES* GEN. NOV.

Paucimyces (Pau.ci.my'ces. L. masc. adj. *paucus* few, little; Gr. masc. n. *mykes* fungus; N.L. masc. n. *Paucimyces* a little-distributed fungus).

Mycobank accession number: MB838953

Typification: *Paucimyces polynucleatus* Radwa A. Hanafy, Noha H. Youssef and Mostafa Elshahed

Obligate anaerobic fungus that produces polycentric thallus with highly branched nucleated rhizomycelium of indeterminate length. The fungus is characterized by formation of spherical vesicles at the hyphal tips. Multiple sporangia are developed either directly on the spherical vesicles or the end of sporangiophores. Mature sporangia are separated from the sporangiophores by basal walls. Old cultures produce sporangiophore initials with no sporangia. Zoospores are mainly monoflagellated. Bi-flagellated zoospores are occasionally encountered. Frequent sub-culturing results in cultures that lose the zoosporogenesis ability and produce sterile sporangia. The clade is defined by the sequence MW694896 (for ITS1, 5.8S rRNA, ITS2, D1–D2 28S rRNA). The most genetically similar genera are *Orpinomyces*, which is characterized by its polyflagellated zoospores and polycentric thallus that produce sporangia that are either terminal or intercalary, and *Joblinomyces*, which is defined as producing monocentric thalli and monoflagellated zoospores.

DESCRIPTION OF *PAUCIMYCES POLYNUCLEATUS* SP. NOV.

Paucimyces polynucleatus (po.ly.nu.cle.a'tus. Gr. masc. adj. *polys* many; L. masc. adj. *nucleatus* having a kernel or stone, intended to mean nucleated; N.L. masc. adj. *polynucleatus* having many nuclei).

Mycobank accession number: MB838954.

Typification: The holotype (Fig. 2c) was derived from the following: USA Oklahoma: Stillwater, 36.12° N, 97.06° W, ~300 m above sea level, live 3-day-old liquid culture, air-dried and stained by DAPI (4′,6-diamidino-2-phenylindole, 10 µg ml^{-1}). Isolated from frozen faecal samples of a wild blackbuck antelope (*Antilope cervicapra*) in December 2020 by Radwa Hanafy. Ex-type culture BB-3^T is stored on solid agar media at 39 °C at Oklahoma State University. GenBank accession number MW694896 (for ITS1, 5.8S rRNA, ITS2, D1-D2 28S rRNA). The holotype specimen is deposited at Oklahoma State University, Department of Microbiology and Molecular Genetics culture collection (accession code PauciBB-3).

Etymology: the species epithet (*polynucleatus*) reflects the polynucleated filamentous rhizomycelium produced during growth.

An obligate anaerobic fungus that produces globose (6–10 µm in diameter) monoflagellated zoospores. Biflagellated zoospores are occasionally observed. Flagellum length ranges from 15 to 30 µm. Zoospores germinate into polycentric thalli with extensively branched nucleated rhizomycelium of indeterminate extent. Spherical vesicles are developed at the hyphal tips, and multiple sporangia are developed directly on the spherical vesicles or at the end of sporangiophores. Sporangia are mainly ovoid and ranged in size (15–90×10–55 µm). Old cultures produce empty sporangiophores initials. Also, prolonged subculturing results in sterile sporangia that fail to differentiate into zoospores. Cultures grown in cellobiose liquid media exhibit a thin loose biofilm-like growth and form white compact circular filamentous colonies (2–4 mm diameter) on agar roll tubes. The clade is defined by the sequence MW694896 (for ITS1, 5.8S rRNA, ITS2, D1-D2 28S rRNA).

Additional specimens examined: USA Oklahoma: Stillwater, 36.12° N, 97.06° W at ~300 m above sea level, isolated from frozen faecal samples of a wild blackbuck antelope (*Antilope cervicapra*), in December 2020 by Radwa Hanafy. These cultures are named BB-2, BB-12 and BB-14.

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Author contributions
R. A. H., Conceptualization, methodology, formal analysis, writing: original draft, visualization. N. H. Y., Conceptualization, formal analysis, writing: review and editing, funding. M. S. E., Conceptualization, review and editing, funding, project administration.

Conflicts of interest
The authors declare that there are no conflicts of interest.

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