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Melanodevriesia, a new genus of endolichenic oleaginous black yeast recovered from the Inner Mongolia Region of China

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Abstract: Black yeasts are a phylogenetically diverse group of ascomycetous fungi that may exist in both unicellular and mycelial morphs. This group of fungi contains numerous commercially significant species as well as others whose precise roles are unknown, such as endolichenic species. There is currently a paucity of data about endolichenic black yeast species. To bridge this gap, we surveyed China's Inner Mongolia Autonomous Region in July 2019. Several fungal species associated with diverse lichens were isolated during this survey. Among these were two isolates of a previously unknown species of oleaginous black yeast from *Mycosphaerellales*. Analyses of morphological and molecular data revealed that these two isolates were closely related to *Xenodevriesia strelitziicola* (*Xenodevriesiaceae*), although with significant differences. As a result, we established the genus *Melanodevriesia* *gen. nov.* to describe this previously unknown species, *Melanodevriesia melanelixiae* *sp. nov.* In addition, we used Transmission Electron Microscopy to visualise the intracellular oil bodies metabolised by this fungus in its unicellular state. The black yeast species identified in this study may have a wide range of commercial applications. More research is needed to determine the chemical composition of the microbial oil synthesized by this fungus and whether it has commercial value.

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INTRODUCTION

Fungi and algae (or cyanobacteria) form a symbiotic relationship known as lichen (Lutzoni & Miadlikowska 2009). *Ascomycota* makes up the bulk of lichenised fungi, whereas the remaining fungi are from the *Basidiomycota* (He & Zhang 2012). In addition to these symbiotic fungi, lichen thalli also house a variety of other fungi such as endolichenic fungi (Kellogg & Raja 2017). The ecological role of these non-symbiotic fungi is still largely unknown (Singh *et al.* 2017). It is estimated that more than 18 000 endolichenic fungi colonise lichen thalli (Nash 2008); this includes a group of fungi often referred to as “black yeasts” (also known as “black fungi”) (Cañete-Gibas & Wiederhold 2018).

Black yeasts are melanised, non-lichenised and dematiaceous fungi that can concurrently exist in both unicellular and mycelial forms (Zalar *et al.* 1999). The group is phylogenetically diverse, although it mostly consists of fungi from *Ascomycota* (Selbmann *et al.* 2014b). Most of these black yeasts are from the classes *Dothideomycetes* and *Eurotiomycetes* (Egidi *et al.* 2014, Selbmann *et al.* 2014a). Black yeasts from *Dothideomycetes* concentrate in the order *Mycosphaerellales* (Abdollahzadeh *et al.* 2020), whereas in *Eurotiomycetes* they exclusively represent *Chaetothyriales* (Selbmann *et al.* 2005, Isola *et al.* 2016, Selbmann

et al. 2014b, Sun *et al.* 2020). Melanisation and meristematic growth amongst these fungi evolved in response to extreme environments, in which they thrive (de Hoog 1993, Haase *et al.* 1999, Prenafeta-Boldú *et al.* 2006), such as high temperature, UV radiation, toxic chemicals, oligotrophic environments and many more (Jacobson 2000, Langfelder *et al.* 2003, Lian *et al.* 2005, Selbmann *et al.* 2005, Dadachova *et al.* 2007, Dadachova & Casadevall 2008, Zhao *et al.* 2010).

Apart from melanin, black yeasts also metabolise various other compounds that allow them to thrive in these extreme habitats such as betaine, carotenoids, mycosporines, trehalose and polyalcohols (Moreno *et al.* 2018). Furthermore, while growing on a carbohydrate-rich substrate, some black yeast species accumulate microbial oils (Lamers *et al.* 2016). Single-cell oils or microbial oils are intracellularly stored lipids produced by a variety of oleaginous microorganisms, such as fungi, bacteria, and algae (Li *et al.* 2008, Bellou *et al.* 2016). Single-cell oils are composed of triacylglycerols (TAGs), free fatty acids, polar lipids, sterols, hydrocarbons, and pigments (Ratledge 2004). Microbial oils are preferred over plant- and animal-derived oils because they can be readily scaled up through the application of biotechnology. Furthermore, seasonal fluctuations, geographic location, harvest time, and transportation, which are obstacles in the production

of plant and animal oils, do not influence on the production of single-cell oil (Ward & Singh 2005, Thiru *et al.* 2011).

Oleaginous yeasts are a favoured source of microbial oils because they may accumulate more lipids than other microorganisms. Furthermore, the oil synthesised by bacteria is stored on the external membrane, making it difficult to extract, whereas those produced intracellularly by algae and yeasts have a high concentration of unsaturated fatty acids (Vasconcelos *et al.* 2019). So far, oleaginous yeast such as *Yarrowia lipolytica*, *Rhodotorula glutinis*, *Cryptococcus curvatus*, and *Lipomyces starkeyi* have all been widely studied (Qiao *et al.* 2017).

The majority of black yeast research in China is focused on species that cause human diseases, such as *Exophiala asiatica*, *Aureobasidium* spp., and others (Li *et al.* 2009, Wang *et al.* 2019). The knowledge on endolichenic black yeast species from China and globally is currently scarce. In an attempt to overcome this gap, we surveyed China's Inner Mongolia Autonomous Region in July 2019. Several fungal species associated with diverse lichens were isolated during this survey. Among them were two isolates of a previously unknown species of black yeast from the order *Mycosphaerellales*. In this study, we described this black yeast species using both morphological and genetic data. In addition, we used transmission electron microscopy to visualize the intracellular oil bodies associated with this newly discovered species.

MATERIALS AND METHODS

Collections of lichens

Several *Melanelixia subargentifera* thalli were collected in July 2019 from Mt. Qingyangcheng, Balin Right Banner, Chifeng City, Inner Mongolia Autonomous Region (14 98.8m a.s.l., 44°13'45"N, 118°44'57"E). An individual lichen thallus was scraped off the substrate and kept separately in paper bags. Fungal isolations were made from lichen thalli in the laboratory.

Isolation of fungi from lichen thalli

An individual lichen thallus was cleaned with tap water and then repeatedly rinsed with sterile deionised water. The upper cortex of the thallus was scraped off using a Leica Zoom 2000 dissecting microscope. Pieces of medullary tissues were put on the surface of potato dextrose agar medium (PDA; 46 g PDA powder (Qingdao Hope Bio-Technology Co., Ltd., Shandong, China), and 1 L distilled water, pH 5.6 ± 0.2) amended with 0.05 % streptomycin (Cao *et al.* 2002). All Petri dishes were incubated at 25 °C for 14 d. Mycelia emerging from medullary tissues were sub-cultured onto new PDA plates.

DNA extraction, amplification and sequencing

Using the modified CTAB technique (Doyle & Doyle 1990), genomic DNA was extracted from 14-d-old fungal cultures growing on PDA. For all fungal isolates, the complete internal transcribed spacer (ITS) and partial nuclear large subunit ribosomal DNA (LSU) regions were amplified using primers ITS1/ITS4 (White *et al.* 1990) and LR0R/LR5 (Vilgalys & Hester 1990), respectively.

Each 50 µL of PCR amplification reaction included 19 µL of PCR grade water, 25 µL of 1-5™ 2× High-Fidelity Master Mix

(Tsingke Biotech Co., China), 2 µL of each primer (10 µM), and 1 µL DNA template. For both gene regions, PCR amplifications were conducted with an initial denaturation at 94 °C for 3 min, followed by 30 cycles of 94 °C for 30 s, 56 °C for 1 min, 72 °C for 1 min; and final extension at 72 °C for 10 min. Positive amplifications were verified using agarose gel electrophoresis and stained using ethidium bromide. Sangon Biotech Company (Shanghai, China) cleaned and sequenced the PCR products.

The BLAST algorithm (Altschul *et al.* 1990) available through NCBI GenBank was used for the preliminary identification of the fungal DNA sequences. All DNA sequences generated in this study were deposited in NCBI GenBank's nucleotide database (Table 1).

Sequence alignment and phylogenetic analyses

During the preliminary identification of the ITS and LSU sequence data, two of our isolates appeared as a potentially new taxon, closely linked to *Xenodevriesia* and *Paradevriesia* (*Mycosphaerellales*). As a result, two separate datasets for the ITS and LSU gene regions were constructed for phylogenetic analyses. The sequences of the supposedly new taxon identified in this study were included in this data set, as well as selected taxa from the order *Mycosphaerellales* retrieved from GenBank. For phylogenetic taxon sampling, the neighbour-joining trees generated during BLAST searches and previously published phylogenetic by Crous *et al.* (2020) were used. Both datasets were aligned separately with MAFFT v. 7 (Katoh *et al.* 2019) and manually adjusted with MEGA v. 10.2.0 (Kumar *et al.* 2018).

Phylogenetic analyses of single-gene and concatenated datasets were done using maximum likelihood (ML), Bayesian inference (BI) and maximum parsimony (MP) approaches. Software required for ML and BI analyses were accessed through the CIPRES Science Gateway platform (<https://www.phylo.org>) (Miller *et al.* 2010). The best models of nucleotide substitution were determined by using jModelTest v. 2.1.6 (Darriba *et al.* 2012). RAxML v. 8.2.12 was used for ML analyses with GTR+GAMMA as the substitution model and 1 000 bootstrap replications (Stamatakis *et al.* 2008). For BI analyses, MrBayes v. 3.2.7 (Ronquist & Huelsenbeck 2003) with four MCMC chains were run from a random starting tree for 5 M generations with the stop value set at 0.01, the temperature set at 0.2, with trees sampled every 100 generations. We discarded 25 % of trees sampled as burn-in and the remaining trees (37 500) were used to construct majority rule consensus trees. The MP analyses were performed using MEGA v. 10.2.0 with 1 000 bootstrap replicates, gaps were treated as a fifth state character. The phylogenetic trees from the ML, MP and BI analyses were viewed using FigTree v. 1.4.4 (<http://tree.bio.ed.ac.uk/software/figtree/>). All the alignments and phylogenetic trees were submitted to TreeBASE under accession number 28863.

Colony morphology and light microscopy

The two isolates (CGMCC3.20308 and CGMCC3.20309) of the potentially new taxon from *Mycosphaerellales* were used for recording culture morphology and microscopic structures. For this purpose, both isolates were sub-cultured onto PDA and oatmeal agar (OA; 30 g oatmeal, 15 g agar, 1 L distilled water, pH 7.2 ± 0.2). All the Petri plates were incubated at 25 °C for 40 d. Microscopic morphological characters such as hyphae, conidia, and conidiophores were photographed and measured (n =50 /

Table 1. GenBank accession numbers of selected taxa from *Mycosphaerellales* used for phylogenetic analyses. The new species is shown in boldface.

Species	Strain/Voucher	LSU	ITS
<i>Batcheloromyces alistairii</i>	CPC 18251	JX556237	JX556227
<i>Batcheloromyces leucadendri</i>	CPC 18277	JF499852	JF499832
<i>Batcheloromyces proteae</i>	CBS 110696	EU019247	JF746163
<i>Capnodium coffeae</i>	CBS 147.52	GU214400	MH856967
<i>Capnodium coffeicola</i>	MFLUCC15-206	KU358920	KU358921
<i>Cladosporium cladosporioides</i>	CBS 129108	MH876646	MH865207
<i>Cladosporium herbarum</i>	CBS 129088	MH876640	MH865203
<i>Cladosporium myrtacearum</i>	CBS 126349	MH875385	MH863925
<i>Cladosporium phyllactiniicola</i>	CBS 126354	MH875390	MH863930
<i>Cladosporium pseudocladosporioides</i>	CBS 125993	MH875333	MH863872
<i>Cladosporium scabrellum</i>	CBS 126358	MH875394	MH863934
<i>Cladosporium tenuissimum</i>	CBS 125995	MH876286	MH864840
<i>Cladosporium varians</i>	CBS 126361	MH875397	MH863937
<i>Devriesia shelburniensis</i>	CBS 115876	KF442544	KF442505
<i>Juncomyces californiensis</i>	CPC 37989	MT373351	NR_170828
<i>Lecanosticta pini</i>	CBS 871.95	GQ852598	GU214663
<i>Leptoxyphium madagascariense</i>	CBS 124766	MH874923	MH863407
<i>Melanodevriesia melanelixia sp.nov.</i>	CGMCC3.20308	MW528742	MW528736
	CGMCC3.20309	MW580586	MW580587
<i>Meristemomyces frigidus</i>	CCFEE5457	GU250389	KF309967
<i>Microcyclosporella mali</i>	CBS 126135	MH875501	MH864044
<i>Microxiphium theae</i>	CBS 202.30	MH866561	MH855113
<i>Montagnula cylindrospora</i>	UTHSC-DI16-208	LN907351	LT796834
<i>Muriphila oklahomaensis</i>	CCF5751	LR736041	LR736040
<i>Mycosphaerelloides madeirae</i>	CBS 116066	KX286989	AY853188
<i>Neocatenulostroma germanicum</i>	CBS 539.88	EU019253	MH862143
<i>Neocatenulostroma microsporium</i>	CBS 110890	EU019255	AY260097
<i>Neodevriesia cladophorae</i>	OUCMBI110119	KU578114	KP269029
<i>Neodevriesia grateloupiae</i>	OUCMBI101249	KU578120	KU578118
<i>Neodevriesia modesta</i>	CCFEE5672	KF310026	KF309984
<i>Neodevriesia simplex</i>	CCFEE5681	KF310027	KF309985
<i>Neodevriesia strelitziae</i>	CBS 122379	GU301810	MH863206
<i>Paramycosphaerella watsoniae</i>	CPC 37392	MN567653	MN562146
<i>Paradevriesia compacta</i>	CBS 118294	NG_059089	NR_144955
<i>Paradevriesia pseudoamericana</i>	CPC 16174	GU570544	GU570527
<i>Paradevriesia americana</i>	CBS 117726	NG_059077	NR_159866
<i>Phyllachora pomigena</i>	CBS 195.33	MH866862	MH855411
<i>Polychaeton citri</i>	CBS 116435	GU214469	GU214649
<i>Pseudotaeniolina globosa</i>	CBS 109889	MH874434	MH862844
<i>Ramularia acris</i>	CBS 109794	KX287010	KX287311
<i>Ramularia acroptili</i>	CBS 120253	EU019257	EU019257
<i>Ramularia helminthiae</i>	CPC 11504	KX287183	KX287481
<i>Ramularia lethalis</i>	CPC 25910	KX287174	KX287472
<i>Ramularia tovarae</i>	CBS 113305	KJ504764	KJ504807
<i>Stenella araguata</i>	CBS 105.75	EU019250	MH860897
<i>Teratosphaeria dimorpha</i>	CPC 14132	FJ493215	FJ023537
<i>Teratosphaeria ovata</i>	CPC 14632	FJ493218	FJ023538
<i>Teratosphaeria profusa</i>	CPC 12821	FJ493220	FJ493196

Table 1. (Continued).

Species	Strain/Voucher	LSU	ITS
<i>Xenodevriesia strelitziicola</i>	CBS 122480	NG_059085	MH863214
	X1045	GU214635	GU214635
<i>Xenopenidiella nigrescens</i>	DOC356	KU216335	KT833169
<i>Xenoramularia arxii</i>	CBS 342.49	NG_058254	KX287552
<i>Xenoteratosphaeria jonkershoekensis</i>	CBS 122897	MH874777	MH863253

structure) using a Leica DFC495 camera attached to a Leica DM6 microscope. ImageJ was used for measuring the taxonomically relevant structures (Collins 2007).

The ex-holotype cultures were deposited in Beijing, China General Microbiological Culture Collection Center (CGMCC). The type specimen was deposited in the Institute of Microbiology's (HMAS) Fungarium in Beijing, China.

Electron microscopy for visualising intracellular oil bodies

For visualising intracellular oil bodies using transmission electron microscopy (TEM), isolates of the unknown fungus were sub-cultured onto PDA for 14 d. Thereafter, the yeast-like cells were fixed using 2.5 % glutaraldehyde at 4 °C for 2–3 h (Brisson *et al.* 1996). The fixed cells were rinsed repeatedly using 0.1 M phosphate buffer saline (PBS; pH 7.2). Cells were post-fixed using 1 % osmium tetroxide for 1.5 h in darkness. These post-fixed cells were rinsed twice with PBS followed by ultrapure water (three to four times). The cells were gradually dehydrated with 50, 70, 80, and 90 % ethanol, then 90 % acetone and absolute acetone. The dehydrated tissues were embedded in Epon 812 and sliced into 70 nm ultra-thin sections using a Leica UC7 ultramicrotome. Sections were stained using 2 % uranyl acetate for 15 min followed by lead citrate for 8 min (Reynolds 1963). Stained sections were dried under infrared light for 10 min. The structure of oil bodies in the cells were observed using a Hitachi HT-7800 transmission electron microscope at 80 kV.

RESULTS

Phylogenetic analyses

In the phylogeny of selected taxa from the *Mycosphaerellales*, *Cladosporiales* and *Capnodiales*, the ML tree topologies were largely consistent between the datasets (Fig. 1). However, compared to the LSU and concatenated ITS+LSU phylogeny, the placement of the novel species differed in the ITS phylogeny. In both the LSU and ITS+LSU phylogenies the new species is sister to *Xenodevriesia strelitziicola* in the *Xenodevriesiaceae*. However, posterior probability and maximum-likelihood bootstrap values supporting this clustering were highly significant for the LSU tree only (Fig. 1). In the ITS tree, the new species emerged as a basal lineage to a clade that included species of *Neodevriesia*, *Paradevriesia*, and *X. strelitziicola* with poor statistical support. The parsimony analyses did not provide any support for the associations in the ITS+LSU and ITS phylogenies, but moderate support in the LSU phylogeny (Fig. 1). The strange placement of our isolates in the ITS phylogeny could be an artefact of the divergent ITS sequences spanning different families used in the analysis.

The tree topologies from both the LSU and ITS+LSU datasets, as well as the accompanying statistical support values, revealed that our two isolates of the previously undescribed species represents a new genus. Below, we establish the new genus *Melanodevriesia* to accommodate this unknown species as *Melanodevriesia melanelixiae* *sp. nov.*

Taxonomy

Melanodevriesia H.L. Si, W.Q. Cao, & T. Bose, *gen. nov.*
MycoBank MB 839404.

Etymology: The name refers to the black colony formed by the fungus when growing on PDA and OA.

Slow-growing colonies on PDA and OA are black to brownish black in colour. The fungus grows in a yeast-like unicellular state on PDA, producing pseudohyphae by continuous budding. These yeast-like cells have several conspicuous intracellular oil bodies. The thallus on OA and other oligotrophic media is made up of septate straight or corrugated branching hyphae.

Type species: *Melanodevriesia melanelixiae* H.L. Si, W.Q. Cao & T. Bose

Notes: *Melanodevriesia* is currently a monotypic genus that includes *M. melanelixiae*, which is described below. Despite being a sister genus of *Xenodevriesia* (*Xenodevriesiaceae*), *Melanodevriesia* has distinct morphological characteristics. *Melanodevriesia* has two thallus morphologies: yeast-like and mycelial, both of which are black to brownish black in colour, but *Xenodevriesia* possesses a brown mycelial thallus (Crous *et al.* 2019). *Melanodevriesia* produces chlamydospores which are lacking in *Xenodevriesia*.

Melanodevriesia melanelixiae H.L. Si, W.Q. Cao, T. Bose, *sp. nov.*
MycoBank MB 840429. Figs 2, 3.

Etymology: The name is derived from the lichen *Melanelixia subargentifera*, from which both isolates of this fungus were obtained.

This fungus can exist in both a yeast-like and a mycelial state. The yeast-like thallus produces pseudohyphae through budding. These *pseudohyphae* are branched, septate, constricted at the septa, composed of oval to urceiform cells, hyaline to brown in colour, smooth-walled, guttulate, measuring 1.4–3 × 2.3–4.6 μm (Fig. 2). In the *mycelial state*, hyphae grow into the substrate. *Hyphae* branched, septate, smooth-walled, smooth or corrugated, cylindrical, hyaline to pale brown in colour, measuring 1.3–2 μm wide (Fig. 2). *Chlamydospores* spherical to ovoid in shape, solitary often moniloid forming radiating clusters, smooth-walled, pale

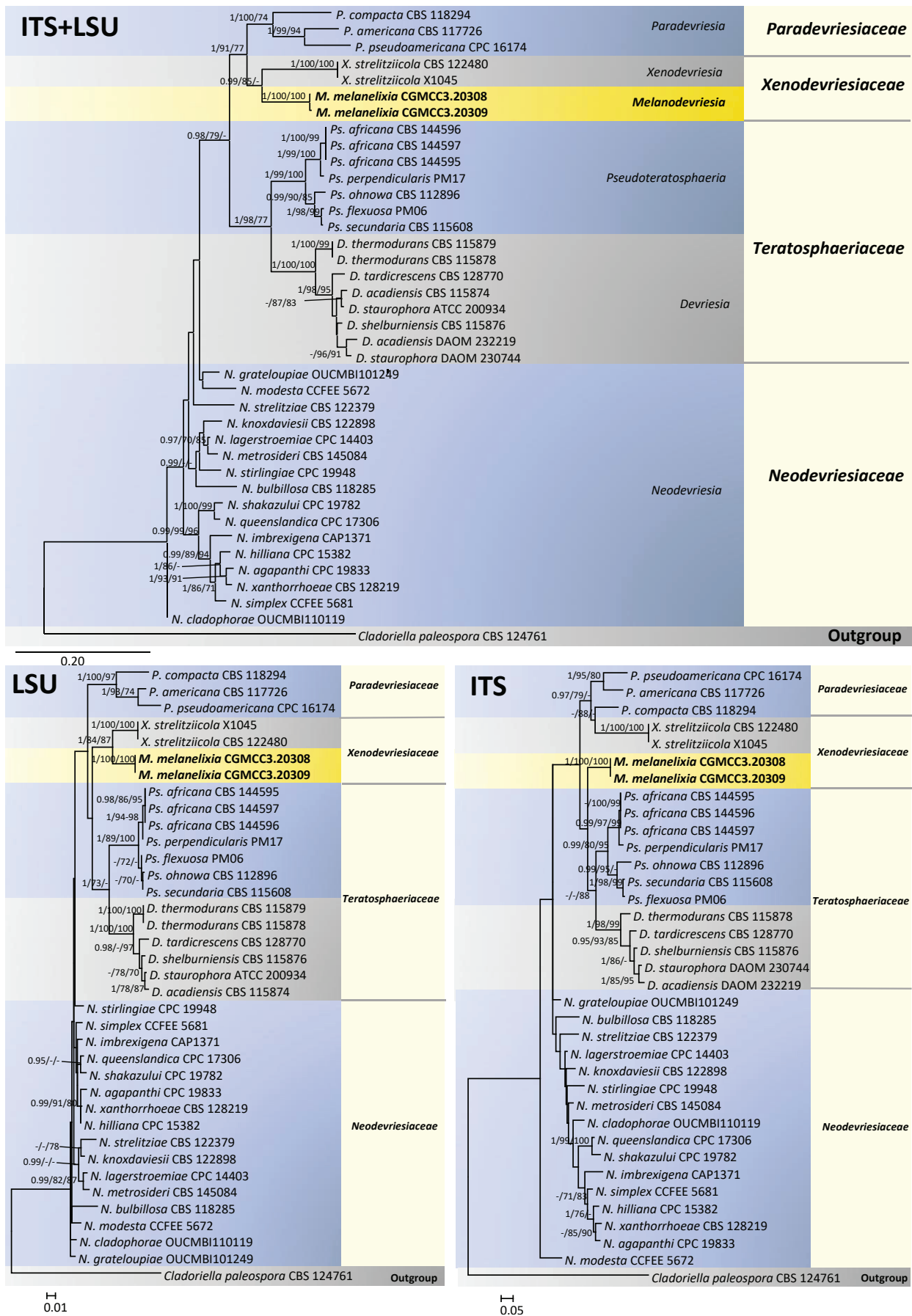


Fig. 1. Maximum likelihood trees were constructed using single gene (ITS and LSU) and concatenated (ITS+LSU) datasets. In the ITS+LSU and LSU trees, both isolates of *Melanodevriesia melanelixiae* sp. nov. formed a monophyletic clade and were sisters to *Xenodevriesia strelitziicola*. However, this clustering was highly significant for the LSU tree only. In the ITS tree, *M. melanelixiae* emerged as a basal diverging taxon within a clade that includes species of *Neodevriesia*, *Paradevriesia*, and *Xenodevriesia strelitziicola*, but with poor statistical support. The numbers on the branches are statistical support values, Bootstrap values ($< 75\%$) from maximum likelihood and maximum parsimony analyses, respectively. Thickened branches indicate the posterior probability values ≥ 0.90 .

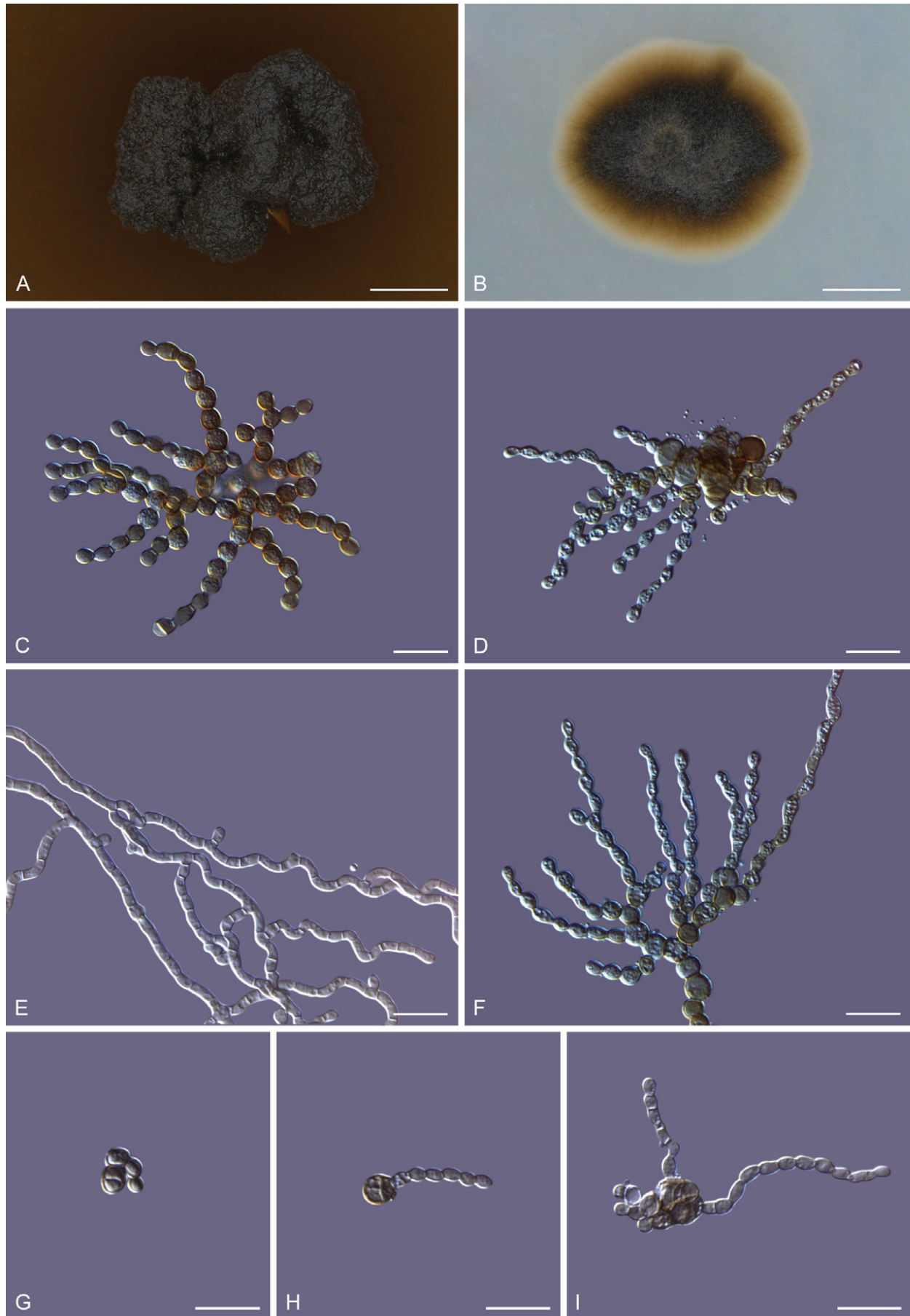


Fig. 2. Morphology of *Melanodevriesia melanelixiae* sp. nov. (ex-type CGMCC3.20309). Colony morphology on potato dextrose agar (**A**) and oatmeal agar (**B**). **C, D.** Microscopic structures of 14-d-old culture growing on PDA medium with yeast-like unicellular morph forming pseudohyphae through budding. **E.** Straight and corrugated septate hyphae produced by the mycelial state of the fungus. **F.** A cluster of monilioid chlamydo spores. **G–I.** Single chlamydo spores germinating into unicellular cells that multiply through budding, forming a multicellular structure from which pseudohyphae emerge. Scale bars: A, B = 2 mm; C–I = 10 μ m.

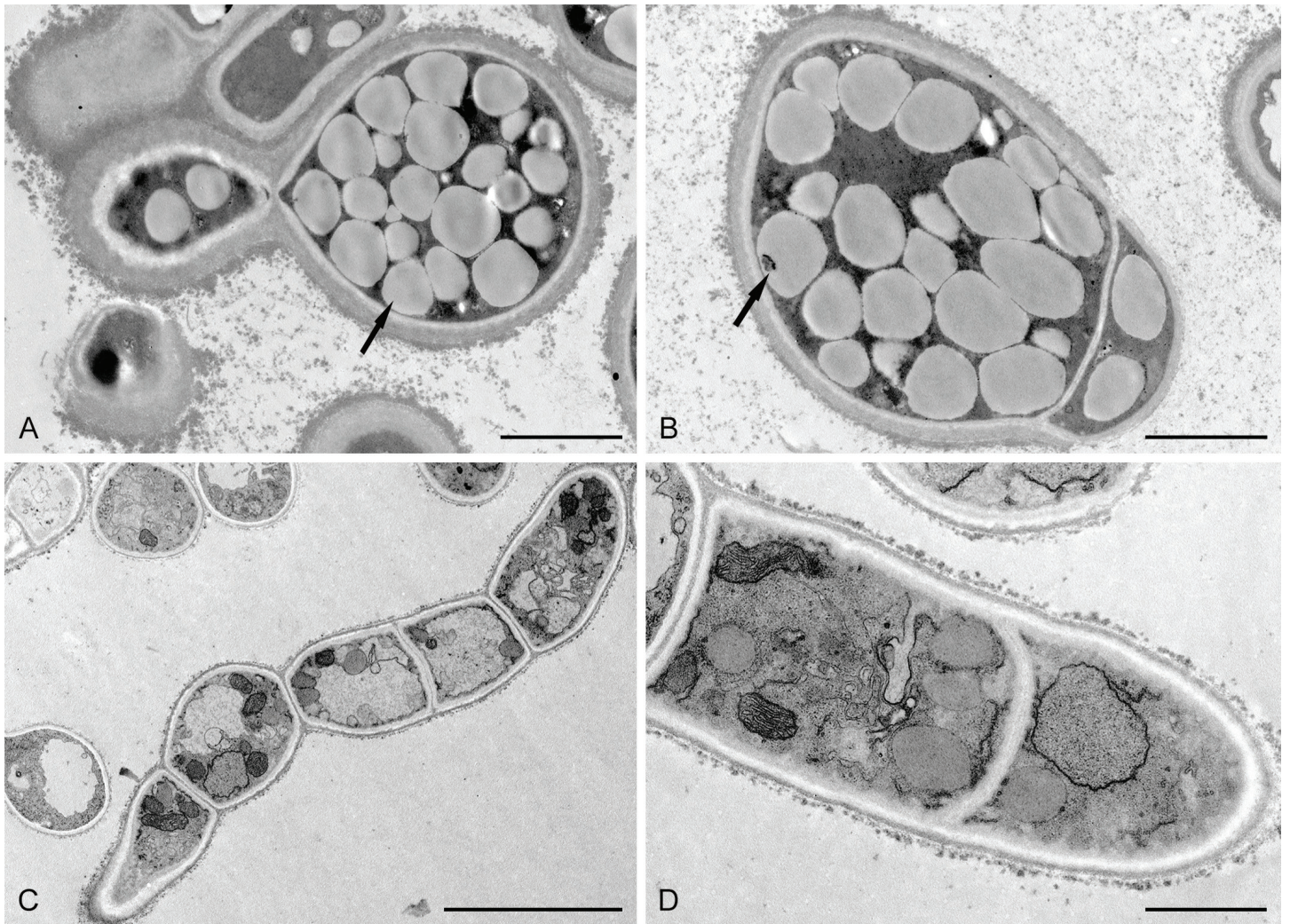


Fig. 3. Transmission electron microscopic images of pseudohyphae and mycelium of *Melanodevriesia melanelixiae* sp. nov. (ex-type CGMCC3.20309). **A, B.** Budding yeast-like unicellular cell with thick cell walls. Multiple intracellular oil bodies concealing the cell organelles (indicated with arrows). **C, D.** Septate hyphae with a thin cell wall that is devoid of intracellular oil bodies. Due to the lack of intracellular oil bodies, various cell organelles are visible. Scale bars: A, B = 2 μ m; C = 10 μ m; D = 1 μ m.

brown to dark brown in colour, usually aseptate, rarely septate, guttulate, measuring 2.8–4.2 \times 2.8–4.8 μ m (Fig. 2). Chlamydospores geminate into yeast-like *unicellular conidia* that are globose to sub-globose in shape, pale brown to dark brown in colour, thick-walled, measuring 4–7.3 \times 3.6–6.2 μ m (Fig. 2). These unicellular cells multiply through budding (Fig. 2) forming multicellular structures from which pseudohyphae emerge randomly (Fig. 2). No sexual reproductive structures were observed.

Culture characteristics: After 12 wk on PDA, the surfaces of the colonies were dark brown to black with the reverse dull brown in colour, erumpent, hollow, with irregular margins, rarely with a few aerial mycelia. After a few weeks after subculturing, the colony stains the PDA brown. Colonies slow-growing, reaching 3.1 \pm 0.1 mm diam after incubating at 25 $^{\circ}$ C for 12 wk (Fig. 2).

After 8 wk on OA, the colonies are round to oval in shape, with smooth margins, surface taupe brown to olive-brown with the reverse taupe brown in colour. Colonies are slow-growing on OA yet faster than on PDA, reaching 5.42 \pm 0.2 mm diam after incubating at 25 $^{\circ}$ C for 8 wk (Fig. 2).

Intracellular oil bodies: The TEM of yeast-like cells grown on PDA revealed thick cell walls with many inconspicuous oil bodies

concealing the other cell organelles. Hyphae grown on OA lacked thick cell walls and intracellular oil bodies (Fig. 3).

Typus: China, Inner Mongolia Autonomous Region, Chifeng, Balin Right Banner, Mt. Qingyangcheng, 44 $^{\circ}$ 13'46"N, 118 $^{\circ}$ 44'57"E, 1 498.8 m alt, isolated from the medullary tissue of *Melanelixia subargentifera*, 7 Jul. 2019, H.L. Si (**holotype** HMAS 350275; ex-type culture CGMCC3.20308).

Notes: *Melanodevriesia melanelixiae* differs from *X. strelitzicola* in that it contains at least two thallus morphologies and chlamydospores. Besides this, we did not observe any sexual reproductive structures (Crous *et al.* 2009, 2019).

DISCUSSION

In the present study, two isolates of a black yeast species were isolated from two separate thalli of *Melanelixia subargentifera* collected at the same coordinates. Analyses of morphological and molecular data revealed that these two isolates represent an undescribed genus. As a result, we established *Melanodevriesia* gen. nov. to describe this fungus as *Melanodevriesia melanelixiae*

sp. nov. The TEM images revealed that during the unicellular phase of its life cycle, this fungus accumulates multiple prominent intracellular oil bodies.

In our LSU and ITS+LSU phylogenies, *Melanodevriesia melanelixiae sp. nov.* emerged as a sister taxon of *X. strelitzicola*, a mycelial fungus isolated from a *Strelitzia sp.* in South Africa (Crous et al. 2009, 2019). This clustering, however, was only significant in the LSU tree. Future discoveries of new species from *Xenodevriesiaceae* and the availability of sequences from additional gene regions may aid in further delimiting this family.

Melanodevriesia melanelixiae sp. nov. was isolated from the medullary tissue of the lichen *Melanelixia subargentifera*. The slow growth and melanisation of this fungus, like that of other black yeasts, allow it to flourish in harsh conditions like the one where we collected our samples in China. We were unable to determine the particular ecological role of *M. melanelixiae*. However, we believe that this fungus increases the overall fitness of the lichen, allowing it to flourish in harsh environments. This is not an unreasonable hypothesis because *Phaeotheca*, an early-diverging capnodiaceous black yeast encapsulates the algae *Trentepohlia* when proliferating within the thallus of *Racodium rupestre* (Crous et al. 2009). This loose association of black yeast and algae might be the early stages of lichen development because the fungus increases the carbon supply to the algae (Gostinčar et al. 2012).

Transmission electron microscopy images of our newly discovered fungus, *M. melanelixiae*, revealed that in its yeast-like form, this organism accumulates a copious number of intracellular oil bodies. Similar to several other black yeast species, the microbial oil metabolised by *M. melanelixiae* might have a wide range of commercial applications. However, more research is needed to determine the chemical composition of the microbial oil metabolised by *M. melanelixiae* and if this fungus can be commercially exploited for the production of microbial oils.

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Conflict of interest: The authors declare that there is no conflict of interest.

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