UNIVERSIDADE FEDERAL DO PARANÁ

MORGANA FERREIRA VOIDALESKI

MOLECULAR EPIDEMIOLOGY OF CHAETOTHYRIALES FUNGI ASSOCIATED WITH HUMAN AND ANIMAL INFECTION

CURITIBA

2023

MORGANA FERREIRA VOIDALESKI

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Orientador: Prof. Dra. Vania Aparecida Vicente

Co-Orientador: Prof. Dr. G. Sybren de Hoog

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Os membros da Banca Examinadora designada pelo Colegiado do Programa de Pós-Graduação MICROBIOLOGIA, PARASITOLOGIA E PATOLOGIA da Universidade Federal do Paraná foram convocados para realizar a arguição da tese de Doutorado de **MORGANA FERREIRA VOIDALESKI** intitulada: **Molecular epidemiology of Chaetothyriales fungi associated with human and animal infection**, sob orientação da Profa. Dra. VANIA APARECIDA VICENTE, que após terem inquirido a aluna e realizada a avaliação do trabalho, são de parecer pela sua APROVAÇÃO no rito de defesa.

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Dedico

Aos meus pais Alexandrina e Fernando

Aos meus irmãos Amanda e João

Obrigada por tudo!

I dedicate

To my parentes Alexandrina and Fernando;

To my sibling Amanda and João

Thank you so much for everything!

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To God for the life and for guiding my way with so many adversities.

To my parents Fernando and Alexandrina for the love and all support during this journey.

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RESUMO

As leveduras negras pertencentes a ordem Chaetothyriales são organismos de natureza oligotrófica, que compartilham características específicas, como a presença de melanina na parede celular, um ciclo de vida polimórfico, com uma ecologia significativamente variável. A ordem engloba 5 famílias, Chaetothyriaceae, Cyphellophoraceae, Epibryaceae, Herpotrichiellaceae e Trichomeriaceae, que podem estar presentes em uma ampla variedade de nichos, tais como solo, plantas, matéria orgânica em decomposição, além de microrganismos patógenos e oportunistas associados a doenças em hospedeiros humanos e animais. As espécies relacionadas as infecções como cromoblastomicose, micetoma e feohifomicose, pertencem aos gêneros *Exophiala*, *Cladophialophora*, *Capronia*, *Fonsecaea*, *Phialophora*, *Rhinocladiella*, *Veronea* e *Cyphellophora*. Neste contexto, o objetivo principal deste trabalho foi explorar a presença de membros da ordem Chaetothyriales em dados de metagenômica e isolamento de países endêmicos e países com baixa incidência de casos das doenças relatadas, com foco no entendimento da ecoepidemiologia. Sequencias de marcadores moleculares, sondas e barcodes foram selecionados na literatura e aplicados como ferramentas de identificação das espécies da ordem Chaetothyriales, aplicados a identificação de espécies em dados públicos de metagenômica disponíveis no banco de dados SRA (NCBI), de amostras ambientais de 27 países (endêmicos e não endêmicos) para cromoblastomicose e feohifomicose (Capítulo II). Nesta análise, foram identificadas 54 espécies sendo 12 estritamente ambientais e 42 agentes de cromoblastomicose ou feohifomicose. *Exophiala* foi o gênero mais prevalente e *Exophiala equina* a espécie mais abundante (501588 reads). Novos nichos ambientais foram associados ao grupo, incluindo espécies vegetais (*Zea mays*, *Pinus nigra*, *Oryza sativa*,etc.), animais (ostra, caranguejo, *Ochotona curzoniae* e *Sus scrofa*) e insetos (*Aedes albopictus*, vespa, abelhas e borboletas). A metodologia de identificação e os marcadores selecionados foram utilizados para explorar a presença de fungos chaetothyrialean em dados metagenômicos da pele (Capítulo III). Os dados analisados foram identificados 24 espécies de fungos negros, sendo *Exophiala* o gênero mais prevalente e *Cyphellophora europaea* a espécie mais abundante. Os agentes causais freqüentemente associados a doenças não foram encontrados nos dados avaliados, enquanto espécies da família Cyphellophoraceae foram dominantes dentre as espécies analisadas. Além deste estudo, a diversidade de leveduras pretas em amostras ambientais de solo, planta e material em decomposições provenientes de uma região endêmica para cromoblastomicose no Brasil, foram avaliadas pelo método de isolamento seletivo de flotação em óleo mineral (Capítulo IV). Espécies dos gêneros *Fonsecaea*, *Exophiala*, e *Cyphellophora* foram recuperadas do material analisado. A identificação preliminar baseada no sequenciamento da região ITS mostrou que uma espécie ainda não descrita estava filogeneticamente relacionada a ordem Chaetothyriales com posição taxonômica indefinida. A análise filogenética confirmou se tratar de uma nova espécie, classificada na clade *Incertae sedis*, sem gênero definido, de acordo com a atual classificação taxonômica proposta. Os resultados deste estudo nos levaram a elucidar alguns aspectos da ecologia das leveduras negras, bem como desvendar o potencial biotecnológico dos microrganismos de procedência ambiental.

Palavras-chave: Chaetothyriales; metagenômica; marcadores moleculares; isolamento ambiental; fungos negros.

ABSTRACT

Black yeasts belonging to the order Chaetothyriales are organisms of an oligotrophic nature, with specific characteristics, such as the presence of melanin in the cell wall, a polymorphic life cycle, and a significantly variable ecology. The order encompasses 5 families, Chaetothyriaceae, Cyphellophoraceae, Epibryaceae, Herpotrichiellaceae and Trichomeriaceae, which may be present in a wide variety of niches, such as soil, plants, decaying organic matter, as well as pathogenic and opportunistic microorganisms associated with diseases in humans. and animals hosts. Species involved in infections such as chromoblastomycosis, mycetoma and phaeohyphomycosis belong to the genera *Exophiala*, *Cladophialophora*, *Capronia*, *Fonsecaea*, *Phialophora, Rhinocladiella*, *Veronaea* and *Cyphellophora*. In this context, the main objective of this work was to explore the presence of members of the order Chaetothyriales in metagenomic and isolation data from several endemic countries and regions with low incidence of cited mycosis, with a focus on understanding ecoepidemiology of this group. Sequences of molecular markers, probes, and barcodes were selected in the literature and applied as identification tools for species of the order Chaetothyriales, in public metagenomic data available in the SRA database (NCBI). Data with environments link from 27 countries (endemic and non-endemic) for chromoblastomycosis and phaeohyphomycosis (Chapter II) was included in the database. In this analysis, 54 species were identified, 12 environmental and 42 agents of chromoblastomycosis or phaeohyphomycosis infection. *Exophiala* was the most prevalent genus and *Exophiala equina* the most abundant species (501588 reads). Environmental niches never reported before, were associated with the group, including plant species (*Zea mays*, *Pinus nigra*, *Oryza sativa*, etc.), animals (oyster, crab, *Ochotona curzoniae* and *Sus scrofa*) and insects (*Aedes albopictus*, wasp, bees and butterflies). The methodology applied in the first chapter and selected markers were used to explore the presence of chaetothyrialean fungi in skin metagenomic data (Chapter III). The analyzed data identified 24 species of black fungi, with *Exophiala* being the most prevalent genus and *Cyphellophora europaea* the most abundant species. The causal agents associated with diseases were not found in the data found, while species of the Cyphellophoraceae family were dominant among the remaining species. In addition to this study, the diversity of black yeasts in environmental samples of soil, plant, and decomposing plant material from an endemic region for chromoblastomycosis in Brazil, were evaluated by the method of selective isolation of flotation in mineral oil (Chapter IV). Species of the genera *Fonsecaea*, *Exophiala* and *Cyphellophora* were recovered from the analyzed material. A preliminary identification based on the sequencing of the ITS region showed that a species not yet described was phylogenetically related to the order Chaetothyriales with an undefined taxonomic position. The phylogenetic analysis confirmed that it is a new species, classified in the clade Incertae sedis, without defined genus, according to the current proposed taxonomic classification. The results of this study led us to elucidate some aspects of black yeast ecology, as well as to develop the biotechnological potential of microorganisms of environmental origin.

Keywords: Chaetothyriales; metagenomics; molecular markers; environmental isolation; black fungi.

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SUMARY

PREFACE

This study is presented in five chapters.

Chapter I exhibited contextual items, that is, a general introduction and the objectives followed by a literary review, which addresses the main aspects of black yeast, chromoblastomycosis and epidemiology of the disease, molecular study, and techniques to the characterization of fungi in the environment.

In Chapter II, the Sequence of molecular markers was applied as a technique to characterize the pathogenic species in environmental sources from metagenomic studies from endemic and non-endemic areas around the world, was contemplating to identify the environmental sources of pathogenic chaetotrialean species involved in disease.

In Chapter III, the methodology applied in chapter I was used to reveal chromoblastomycosis and phaeohyphomycosis agents in metagenomic data from the human skin.

In Chapter IV the selective isolation in environmental sources from the Maranhão state region showed a rich diversity of species and the description of new specie from the Chaetothyriales order.

A general conclusion and final consideration highlighting the main scientific findings are presented in Chapter V

CHAPTER I

Outline of the thesis

1 **GENERAL INTRODUCTION**

Black yeasts are microorganisms belonging to the order Chaetothyriales, and the main characteristics of the group are slow growth and black coloration due to the presence of melanin in cells (DE HOOG et al., 2014; TEIXEIRA et al., 2017; SEYEDMOUSAVI et al., 2014). The order can be divided according to ecological characteristics (SEYEDMOUSAVI et al., 2013), being saprobes associated with asymptomatic disorders in vertebrates, etiological agents of disease isolated from environmental sources; highly virulent pathogens found only in human hosts (HORRÉ; DE HOOG, 1999; KANTARCIOGLU et al., 2017)

In the environment the elucidate niche involve substrates such as rock, soil, wood, and decaying plants (NIMRICHTER et al., 2005; SANTOS et al.; 2007; TORRES-GUERRERO et al., 2012; SEYEDMOUSAVI et al., 2013; WALKER, MCGINNIS; 2014; VOIDALESKI et al., 2020), in addition to arid and hot climates, toxic niches or even as opportunistic pathogens (SEYEDMOUSAVI et al., 2014; TEIXEIRA et al., 2017). Few cases reported black yeast in association with insects, an unexplored niche associated to this group (LIMA et al., 2020; KONTOYIANNIS et al., 2019; CHEN et al., 2016).

Chaetothyrialean yeast associated with animal and human infections are mainly classified in the Herpotrichiellaceae family, which comprises organisms involved in persistent infections that affect animals and humans (QUEIROZ-TELLES et al., 2017). The pathogenesis of species is multifactorial, however the virulence factors shared by the group are the presence of carotene, thick walls, differentiation into muriform cells, yeast phases, osmotolerance, adhesion, hydrophobicity and melanin (QUEIROZ-TELLES et al., 2017; SANTOS et al., 2007) presents during infection in parasitic cells (NIMRICHTER et al., 2005; SANTOS et al., 2007).

Chromoblastomycosis is an implantation mycosis, classified as a rare, chronic, neglected tropical disease, cutaneous or subcutaneous, with nodular appearance, characterized by the presence of muriform bodies in the tissue (WALKER; MCGINNIS; 2014; QUEIROZ-TELLES et al., 2017). The major etiological agents of the disease are species of the genera *Cladophialophora* and *Fonsecaea*, the first are predominate agent related in infection from regions with arid climate and the second are prevalent in infections reported to hot and humid tropical areas, however there is no relationship between a single agent and the clinical pattern observed in the infection (SANTOS et al., 2007; QUEIROZ-TELLES et al., 2012; QUEIROZ-TELLES et al., 2017). Immunocompetent patients who carry out agricultural activities without protection are more affected by this disease, with the lower and upper limbs being the most susceptible anatomical regions (MARQUES et al., 2006; QUEIROZ-TELLES et al., 2017).

Endemic regions are established in tropical and subtropical climates such as Latin America (NAJAFZADEH et al., 2011; DENG et al., 2015; GOMES et al., 2016), countries in Africa and Asia (YANG et al., 2013; QUEIROZ- TELLES et al., 2017). The environmental occurrence of chromoblastomycosis agents is still little explored due to the large sampling effort required to characterize the environmental substrates (SUDHADHAM et al., 2010; DE HOOG et al., 2007; VICENTE et al., 2008, 2014).

Molecular markers such as probes and barcodes are essential in characterizing the environmental occurrence of pathogens, since they have high specificity, reproducibility, and sensitivity (SUN et al., 2010; TEHRANI, 2014; BRUMFIELD et al., 2003; HEINRICHS et al. 2012; SINGH; GUPTA, 2017). Barcodes are described as short sequences (25–41 bp) DNA identifiers, which for fungi were described based on the sequence of the ITS region, to characterize and distinguish closely related species by variable regions (HEINRICHS et al, 2012). Padlock probes are oligonucleotides capable of recognizing target and amplified nucleotide sequences based on regions of SNPs (Single Nucleotide Polymorphism) (ANTSON et al., 2000; DAVARI et al., 2012; LACKNER; et al., 2012; HAMZEHEI et al., 2013; NAJAFZADEH et al., 2011; ZOU et al., 2012). The method has application in the genotyping of large populations and has been used to identify chromoblastomycosis agents (NAJAFZADEH et al., 2013; NAJAFZADEH et al., 2011; FENG et al., 2013; DENG et al., 2014; HAMZEHEI et al., 2013; NAJAFZADEH et al., 2018) including in environmental samples (VOIDALESKI, 2020).

Metagenomics involves sequencing billions of DNA fragments simultaneously in order to characterize microbial systems through genomic comparison with sequence datasets (GU et al., 2019; HEINRICHS et al, 2012; MITCHELL et al., 2015; MEYER et al., 2008). Tools such as barcodes (MITCHELL et al., 2012), and padlock probe sequences (NAJAFZADEH et al., 2013; NAJAFZADEH et al., 2011; FENG et al., 2013; DENG et al., 2014; HAMZEHEI et al., 2013; NAJAFZADEH et al., 2018; SCHNEIDER et al., 2019) contribute to making the identification of rare pathogens such as chromoblastomycosis agents more accurate (CUADROS-ORELLANA et al. 2013; COSTA et al., 2020). Studies that approach sample characterization through metagenomic data are effective in the taxonomic identification of orders and families that are more abundant in these habitats, but less abundant organisms and taxonomic identification at the species level is often unfeasible due to the length and quality of the sequence fragments obtained (CUADROS-ORELLANA et al., 2013; de SOUZA et al., 2016). Costa et al., (2020) proposes the use of sequences of specific markers (probes and barcodes) as tools for the identification of black yeasts of the Herpotrichiellaceae family in

environmental samples from Brazilian studies, making it possible to identify previously unidentified species, expanding the scope of the study. ecological niche of black yeasts in Brazil.

In this context, the understanding of the epidemiological aspects of chromoblastomycosis involves the mapping of endemic regions in the world, through high sensitivity techniques capable of demonstrating the presence of disease agents in environmental substrates (TSUI et al., 2011; HEINRICHS et al., 2012). Thus, the purpose of this study is to infer data eco epidemiology of Chaetothyrialean species, with investigation of the presence pf this group in human skin microbiome, and in environmental sources in endemic and non-endemic areas for the chromoblastomycosis and phaeohyphomycosis disease by analyzing metagenomic data and explore the diversity by selective isolation, with description of new species belong to Chaetothyriales order.

2 OBJECTIVES

2.1 GENERAL

To infer insights on eco-epidemiology and diversity of black yeast-like-fungi in focusing Chaetothyriales by isolation and non-isolation techniques, in order to clarify ecological trends among this group of fungi.

2.2 SPECIFIC

To elucidate the world ecology of chromoblastomycosis and phaeohyphomycosis agents in public data of metagenomics using molecular markers.
To characterize chromoblastomycosis and phaeohyphomycosis agents from skin in metagenomics data by padlock probes and barcodes.
To perform isolation of Chaetothyriales in endemic areas based on molecular data.
To characterize the Chaetothyriales-Like-fungi in environmental sources, include description of new specie.

3 LITERATURE REVIEW

3.1 TAXONOMY AND DIVERSITY OF BLACK YEASTS

Black yeasts comprise a phylogenetically heterogeneous group of melanized fungi cycle, belonging to different orders Chaetothyriales and Dothideales in the phylum Ascomycota (DE HOOG, MCGINNIS, 1987; VICENTE et al., 2012; HUBKA et al., 2014). They are also known as dematiaceous or melanized fungi due to melanin deposition in their cell walls during a part of their life cycle (HOOG et al., 2014). Macro and microscopically the group were recognized by brown, olivaceous, or black tinge (SEYEDMOUSAVI et al., 2014; TEIXEIRA et al., 2017). The group shares some morphological and physiological characters and presents diverse ecologies and morphology being a mycelial to a yeast-like growth as well (DE HOOG, MCGINNIS, 1987; STERFLINGER, 2006; ISOLA et al., 2016). The species showing a slow growing with yeast like appearance and with time they become velvety dark colonies with mononuclear hyphae showing uniform pigmentation (DIXON, POLAK-WISS, 1991).

Dothideales is a high diversity of species with a variable ecology found in environments as decaying woody materials to in the marine and freshwater environment (SUETRONG et al., 2009; MAGAÑA-DUEÑAS; CANO-LIRA; STCHIGEL, 2021). Some genera like *Cladosporium* and *Hortea* belonging to saprobe ecology (HOOG et al., 2000; STERFLINGER, 2006) besides other genus as *Alternaria* and *Curvularia* can be associated to clinical infections as keratitis (KUMAR et al. 2019, KISS et al., 2019) or phytopathogens (KWON et al., 2021, LIANG et al., 2018)

The order Chaetothyriales containing black yeasts and their filamentous relatives which are numerous species with different ecology (QUAN et al., 2020; TEIXEIRA et al., 2017). Chaetothyriales black yeasts was isolated in environment from a variety of niches such as soil, tree bark, thorns, fruits, plants, and decomposing organic matter (VICENTE, 2000; SALGADO et al., 2004; VICENTE et al., 2008; NASCIMENTO et al., 2017). The oligotrophic metabolism of species allows the isolation of black yeast from adverse and extreme conditions, such as rocks (SUN et al., 2020), in hot and arid climates (DE HOOG, 2014), soils contaminated with hydrocarbons (SATOW et al., 2008), soft drinks (CROUS et al., 2007) and domestic environments as dishwashers to kitchens (ZUPANČIČ et al., 2016). The order was re-classified by QUAN et al. 2020 and the number of species and families has been increasing continuously (DE HOOG, 2014). The order accepts nine clades Herpotrichiellaceae, Cyphellophoraceae,

Phaeosaccardinulaceae, Domatia, Melanina, Trichomeriaceae, Chaetothyriaceae, Epibryaceae (QUAN et al., 2020).

The Cyphellophoraceae comprises a monophyletic well-supported clade by the genus *Cyphellophora* and its relatives in *Phialophora*, associated with homogeneous clinical manifestations as superficial colonizers or as causes of local invasive disease of human skin and nails (GOMES et al., 2016; FENG et al. 2013). Plant-associated species like *Cyphellophora phyllostachysdis*, *Cyphellophora artocarpi* and *Cyphellophora musae* are present in this family **(**GAO et al., 2015). The family Trichomeriaceae is composed by species epiphytic, isolates from rock-inhabiting (CHOMNUNTI et al. 2012; ISOLA et al. 2016; TEIXEIRA et al., 2017) and *Arthrocladium* strain is the representative pathogen causing a fatal disseminated human infection (NASCIMENTO et al. 2017). Epibryaceae family was proposed by Gueidan et al.2014 to cover the genus *Epibryon* and three *Cladophialophora* species *C. sylvestris*, *C. humicola* and *C. minutissima* (BADALI et al. 2008, DE HOOG et al. 2011, GUEIDAN et al. 2014).

The Herpotrichiellaceae family is the most relevant family in the Chaetothyriales order because this family belonging important saprobes and opportunistic agents that have highly diversified lifestyle and are associated to disease in humans and cold-blooded vertebrates, such as chromoblastomycosis, mycetoma, and phaeohyphomycosis (MCGINNIS, 1992, TEIXEIRA et al., 2017; HOOG et al., 2014; SEYEDMOUSAVI et al., 2014). Saprobic species commonly isolated from the environmental samples are also included in this family (VICENTE, 2000; VICENTE et al., 2008). Fifteen genera are belonging to Herpotrichiellaceae group being them *Exophiala*, *Rhinocladiella*, *Thysanorea*, *Phaeoannellomyces*, *Minimelanolocus*, *Melanoctona*, *Capronia*, *Cladophialophora*, *Atrokylindriopsis*, *Fonsecaea*, *Phialophora*, *Uncispora*, *Marinophialophora*, *Aculeata*, *Veronaea* (HOOG et al., 2000; TEIXEIRA et al. 2017 QUAN et al., 2021) (Figure 1). Due to physiologic characteristics the recovering of these species is restricted, also from environmental sources or clinical cases, therefore, it becomes important to develop specific methods of flotation in mineral oil (SATOW et al. 2008; VICENTE, 2000; VICENTE et al., 2008). The bantiana-clade and carrionii-clade comprises chromoblastomycosis and phaeohyphomycosis agents (DE HOOG et al., 2007; AZEVEDO et al., 2015; TEIXEIRA et al. 2017), and the clades jeanselmei, salmonis have pathogenic species which occasionally affect animals (DE HOOG et al., 2011).

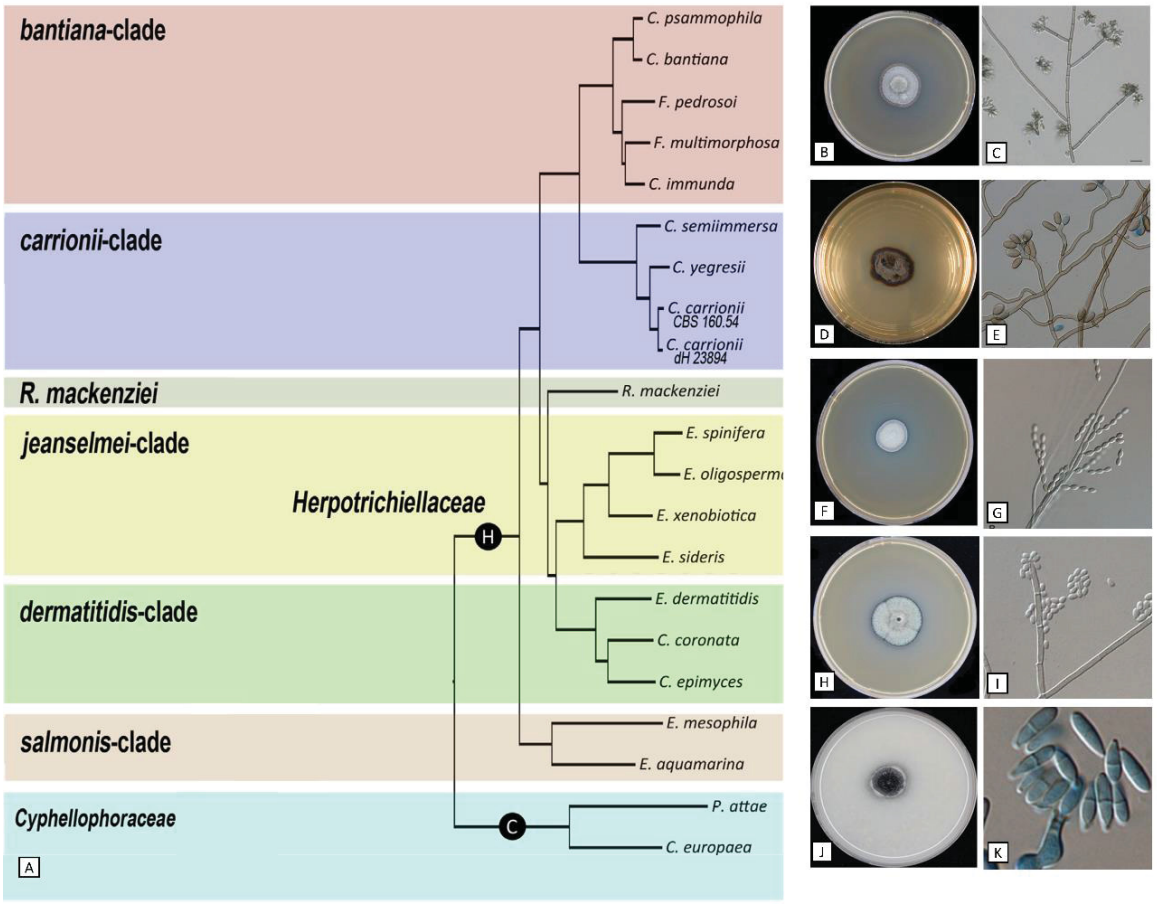


Figure 1- The saprobes and opportunistic agents of Chaetothyriales order. A. Phylogenomic distribution Herpotrichiellaceae and Cyphellophoraceae families. B and C *Fonsecaea pedrosoi*. D and E. *Rhinocladiella mackenziei*. F and G. *Cladophialophora carrionii*. H and I. *Phialophora verrucosa*. J and K. *Cyphellophora pluriseptata*. Adapted from TEIXEIRA et al., 2017 and De Hoog et al. (2014).

The genus *Fonsecaea* presents seven species morphological characterized by colonies of slow growth, velvety appearance and black or dark brown color, and micromorphological conidia widely clavate and the hyphae are regular, melanized and branched in the apical part (WALKER, MCGINNIS; 2014; TORRES- GUERRERO et al., 2012; SANTOS et al.; 2007; NIMRICHTER et al., 2005). *Fonsecaea* species are morphologically indistinguishable (AZEVEDO et al., 2015; VICENTE et al., 2014; NAJAFZADEH et al. 2010). *Fonsecaea pedrosoi* is the type strain of genus and the main agent related to chromoblastomycosis (WALKER; MCGINNIS; 2014; FRANZEN et al., 2008; DE HOOG et al. 2004), therefore *F. nubica* and *F. monophora* as involving in disease (QUEIROZ-TELLES et al., 2017). *F. pugnacius* are considered an exclusively human pathogen capable of colonizing the cutaneous region and causing brain infection at the same time in the same patient (AZEVEDO et al., 2015). Moreover, two species are related to animal infections, which are *F. multimorphosa* feline cerebral abscess (NAJAFZADEH et al., 2011) and *F. brasiliensis* associated lethargic

disease in crabs (VICENTE et al., 2012). Environmental species as represents by *F. erecta* and *F. minima* (VICENTE et al. 2014*)*, however studies demonstrated that this species are potential to infection, reported by edema in animal model after infection by *F. erecta* (VICENTE et al., 2017).

Cladophialophora carrionii is the type of strain of a polyphyletic genus *Cladophialophora* (BADALI et al., 2008). In the environment the genus is frequently isolated from decomposing organic matter, plants, and rocks (BADALI et al., 2008; VICENTE et al., 2012; DE HOOG et al. 2007). *C. yegresii* are reported in living plants of Cactaceae (DE HOOG et al., 2007) and *C. exuberans* was described from babassu coconut (NASCIMENTO et al., 2017). *Cladophialophora bantiana* is a fatal pathogen involving in cerebral infections in immunocompromised and immunocompetent patients (HORRÉ; DE HOOG, 1999; KANTARCIOGLU et al., 2017) while *C. carrionii* are an important agent of Chromoblastomycosis (DE HOOG et al., 2007; BADALI et al., 2008).

The genus *Exophiala* comprising polyphyletic genus distributed in different clades and the majority species number in members of Herpotrichiellaceae family with of more than 40 species described (TEIXEIRA et al., 2017; BORMAN et al., 2017; QUAN et al., 2021). They can be isolated from several environmental sources as soil, biological crusts, polluted materials with hydrocarbons, rock, air, natural water masses, rhizosphere, and plant tissues (ADDY et al. 2005; JULOU et al. 2005; BATES et al. 2006; NEUBERT et al. 2006; BUKOVSKÁ et al. 2010; DE HOOG et al. 2011; FERRARI et al. 2011). Several species are described as pathogen in animals (OVERY et al., 2015; HOPF et al., 2020; DE HOOG et al., 2011; LI et al., 2011). Clinical species as *E. jeanselmei*, *E. asiatica*, *E. spinifera* and *E. dermatitidis* are involved in cases of human infection as phaeohyphomycosis and chromoblastomycosis (PINHEIRO et al., 2019; ZENG et al., 2007; IWAHASHI et al., 2020; PEREZ et al., 2020).

Rhinocladiella genus comprises few species with clinical and environmental spectrum (BADALI et al., 2009). *Rhinocladiella mackenziei* is a neurotropic fungus of severe infections related only in human infections with almost 100% fatality being considered geographically restricted and never isolated from the environment (RAPNOUIL et al., 2021; MUSHTAQ et al., 2020). *Rhinocladiella similis*, *R. tropicalis* and *Rhinocladiella aquaspersa* are described involving in chromoblastomycosis (GONZÁLEZ et al., 2013; GOMES et al., 2016; HEIDRICH et al., 2017).

Black yeasts are microorganism with a high capacity to adapt in hostile to host environmental conditions; the presence of carotene, thick walls, differentiation into muriform cells, yeast phase, UV light absorption, osmotolerance, adhesion, hydrophobicity, assimilation

of aromatic hydrocarbons, protection against enzymatic lysis, tolerate conditions like variations in pH and salt concentration production of siderophores and melanin are important virulence factors shared by the group (WALKER; MCGINNIS; 2014; HOOG, 1993; GOSTINCAR, 2011; SEYEDMOUSAVI et al., 2014; FRANZEN et al. ., 2008).

The presence of melanin in the vegetative cells are indicate as an important virulence factor of these fungi to adaptation of human hosts (Figure 3). The presence of melanin was related to activity against antifungal agents, photoprotection, antioxidant, energy harvesting, thermoregulation, metal binding (RIBEIRO et al., 2006; SUN et al., 2012; GOSTICAR, GRUBEB, GUNDE-CIMERMAN, 2011). In addition, of the pigmentation function melanin enhance the survival and competitive abilities of fungi against adverse conditions, environmental stresses contribute to degradation of organic pollutants such as lignin (GOSTICAR, GRUBEB, GUNDE-CIMERMAN, 2011; CORDERO; CASADEVALL, 2017).

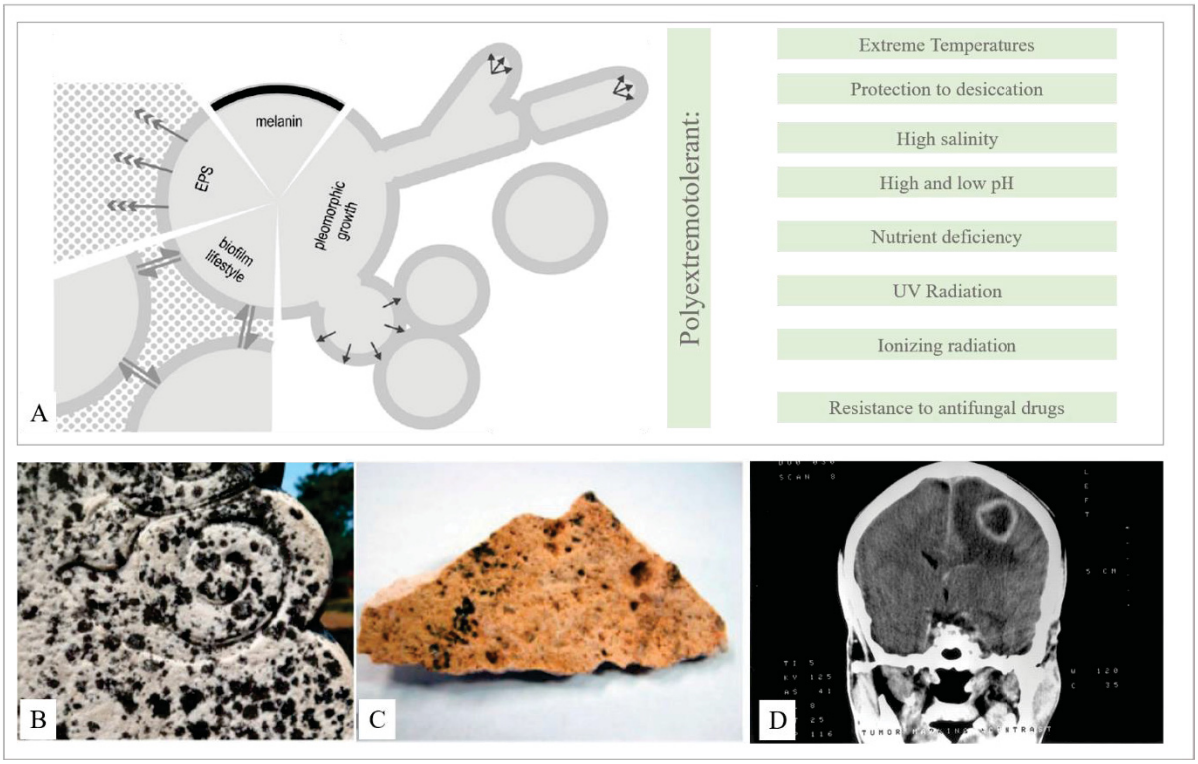


Figure 2- Polyextremotolerant character of black yeast. A. The wide variety of stressful conditions, due to virulence factor as presence of melanin, the ability to grow meristematically, yeast-like and filamentous forms, production of extracellular polysaccharides (EPS), and biofilms enable black yeasts. B. and C. Rock colonization for black fungi species; D. Cerebral colonization by black fungi. Adapted from Sutton et al.,1998; Sun et al., 2020; Gosticar, Grubeb, Gunde-Cimerman, 2011.

The thermotolerance is a virulence factor suggests to determines the choice of the host or host predilection and fundamental to their high ability to colonize a wide variety of environments and to withstand different ecological conditions (GOSTINCAR et al., 2011). The ability to grow at 37 °C are reported in species involved in systemic human infection, like *C.*

bantiana, *Exophiala dermatitidis*, and *Exophiala jeanselmei* (HORRÉ and DE HOOG, 1999; SEYEDMOUSAVI; GUILLOT; HOOG, 2013). Saprobe's species organisms, like the *C. carrionii* and the *P. europaea* while those with maximum growth temperatures of around 35 to 37°C cause (sub)cutaneous and superficial infections (HOOG, et al., 2011; BADALI et al., 2008; SUDHADHAM et al., 2008). The waterborne species of salmonis clade growth temperatures of 27 to 33°C are restricted to disease in cold-blooded animals (HOOG et al., 2011; VICENTE et al., 2012; SEYEDMOUSAVI; GUILLOT; HOOG, 2013; VICENTE, et al., 2014).

3.2 CHROMOBLASTOMYCOSIS AND PHAEOHYPHOMYCOSIS

Many species of black yeasts have a dual ecology. Some species exhibiting a biotechnological profile to assimilation of hydrocarbons compounds when hostile environments, natural saprobes in ecosystems, while in host tissue showing a pathogenic profile (BLASI et al., 2016). Although little reported in human pathology, black yeasts are the only group of pathogens involved in several persistent infections that affect immunocompetent and immunosuppressed patients (SEYEDMOUSAVI et al., 2014).

The diseases caused by these organisms are mycetoma, a deep tissue infection characterized by the presence of mycotic granules; phaeohyphomycosis, presence of septate hyphae and dark colored reproductive structures in the host tissue and chromoblastomycosis a rare and chronic subcutaneous infection characterized by the presence of muriform cells (TEIXEIRA et al., 2017; SEYEDMOUSAVI et al., 2014; WALKER; MCGINNIS, 2014).

Chromoblastomycosis (CBM) is a chronic, granulomatous mycosis of the skin and subcutaneous tissue produced by transcutaneous trauma of various dematiaceous fungi of the order Chaetothyriales and family Herpotrichiellaceae The first reported case of CBM were observed by Pedroso and Gomes in 1911 (PEDROSO; GOMES, 1920) and in the current days the disease presents worldline distribution and commonly misdiagnosed as various other infectious and noninfectious diseases. The mycosis is characterized by (I) traumatic inoculation with environmental source with an initial cutaneous lesion at the inoculation site; (ii) progressive and chronic involvement of cutaneous and subcutaneous tissular structures, a fibrous granulomatous often with tissue proliferation; (iii) a nonprotective T helper type 2 (Th2) immune response with ineffective humoral involvement; and (iv) the presence of muriform (sclerotic) cells in the affected tissue (QUEIROZ-TELLES et al, 2017). The muriform cells

constitute fungal cells with transverse and longitudinal septation (Figure 2) (QUEIROZ-TELLES, SANTOS, PEDROSO, 2015).

Phaeohyphomycosis is a cluster of infrequent infectious syndromes in human caused by dematiaceous, or darkly pigmented, fungi in immunocompromised and immunocompetent individuals (REVANKAR et.al., 2017; CAVIEDES et al., 2017). The term phaeohyphomycosis was designed to reference to tissue invasion by pigmented septate hyphae (THOMAS et al., 2018) presents in a large variety of clinical syndromes, including allergic disease, keratitis, superficial cutaneous or subcutaneous disease, deep local infections and disseminated disease (ARCOBELLO, REVANKAR, 2020).

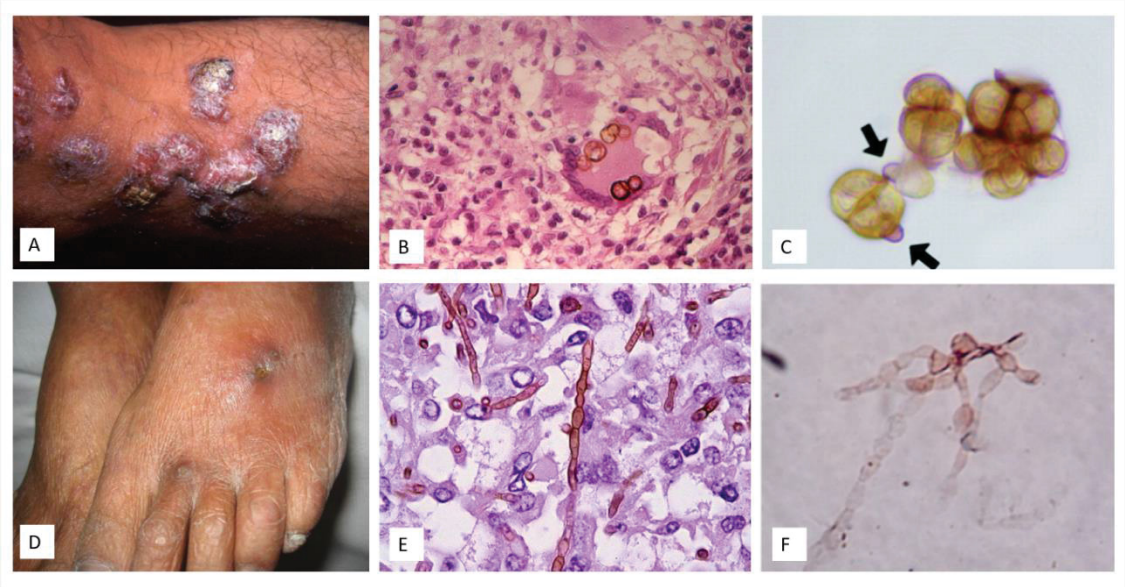


Figure 3- Clinical aspects of chromoblastomycosis (A to C) and phaeohyphomycosis (D to F). A: Nodular cutaneous lesions; B: Histopathology of cutaneous tissue with cells brownish; C: muriform cells; D: Oval-shaped, painful, erythematous nodules arranged in a linear configuration on the dorsum of the left foot; E: histology consistent phaeohyphomycosis; F: Dematiaceous septate hyphae in the aspirate (KOH 20%). Adapted from: KANTARCIOGLU et al., 2017; QUEIROZ-TELLES et al., 2017; HOWLETT et al., 2019, MARTINEZ, TOVAR et al2007; FERNANDES et al.2007, CAVIEDES et al., 2017

3.2.1 **Etiology and diagnosis**

Concerning the fungal infections caused by Chaetothyriales members, chromoblastomycosis and phaeohyphomycosis are the infections more associated to the order (QUEIROZ-TELLES, 2015; QUEIROZ-TELLES et al., 2017). Pathogenic species are polyphyletic with dispersion all over the order, but some clades are clustered like species involved in CBM and waterborne species of *Exophiala*. *Fonsecaea* and *Cladophialophora* are the main genus associated to CBM while the phaeohyphomycosis agents are not restricted to a

single genus (FERNANDEZ-PITTOL et al., 2019; HOWLETT et al., 2019; KIRCHHOFF et al., 2019).

In the *Fonsecaea* cluster, which is included in the “bantiana clade", the prevalent agents of CBM namely *F. pedrosoi*, *F. nubica*, *F. monophora* and *F. pugnacius* (HOOG et al., 2000; SUN, et al., 2012; NAJAFZADEH, et al., 2010; DE AZEVEDO et al., 2015). The species *F. pedrosoi* is the most frequently related to chromoblastomycosis (WALKER; MCGINNIS; 2014; FRANZEN et al., 2008; DE HOOG et al. 2004), however the species of the genus *Fonsecaea* are morphologically indistinguishable, not all cases show growth of the pathogen and the species differ from each other because they are not equally efficient in developing the disease (AZEVEDO et al., 2015; VICENTE et al., 2014; NAJAFZADEH et al. 2010). The species *F. pedrosoi* and *F. nubica* are pathogens related solely to chromoblastomycosis while *F. monophora* has a diversified clinical spectrum (QUEIROZ-TELLES et al., 2017). *F. pugnacius* proved to be a species capable of colonizing the cutaneous region and causing brain infection at the same time in the same patient, considered an exclusively human pathogen (AZEVEDO et al., 2015). Studies based on genomic analyzes and tests on larval models have demonstrated the potential for adaptation and induction of an inflammatory process in animal tissue by *F. erecta*, while *F. pedrosoi* presents a characteristic of chronicity with the development of dark plaques (VICENTE et al., 2017; FORNARI et al., 2018).

Additionally, pathogenic species of *Cladophialophora* are grouped in a clade named “carrionii clade” which includes *Cladophialophora carrionii* and some saprobes and opportunistic species involved in human disease along with the agents of primary brain infection like *C. bantiana* (HOOG, et al., 2004; BADALI, et al., 2008; SURASH, et al., 2005). Furthermore, the species *Rhinocladiella aquaspersa* belong to separate clade one of the CBM agents while includes the species of *Exophiala* that cause human infection, such as *Exophiala jeanselme*i, *E. dermatitidis* and *E. spinifera* (NAKA, et al., 1986; QUEIROZ-TELLES, et al., 2003; TOMSOM, et al., 2006; BORELLI, 1972; GONZÁLES, et al., 2013). The groups of other species that are associated with the environmental occurrence and animal disease (ZENG, et al., 2007; VICENTE, et al., 2012) belong to this clade.

The infective form of CBM and phaeohyphomycosis remains unclear, besides hyphae and muriform cells of the fungus can establish chromoblastomycosis in a murine model, which does not happen with conidia (SIQUEIRA et al., 2017). The favorable prognosis are dependents to the causative agents, severity of the lesions and therapy, with *F. pedrosoi* being the least sensitive species to fungal therapy, among the chromoblastomycosis agents (AGARWAL et al., 2017).

The clinical aspect of the chromoblastomycosis disease is highly diversified (GONZÁLEZ et al., 2013; QUEIROZ-TELLES et al., 2009). Lesions are formed by polymorphic verrucous plaques, suppurative, hyperkeratotic, cicatricial granulomas, micro abscesses and rarely tuberculoid granulomas and hyperplasia, chronic and persistent, with no tendency to spontaneous healing (BRUN et al., 2015; PIRES et al., 2013; SANTOS et al., 2013; SANTOS et al., 2007; NIMRICHTER et al., 2005). According to the severity, the clinical manifestations are classified as mild, moderate and severe, each with a distinct response to treatment (QUEIROZ-TELLES et al., 2009). The disease is not contagious and despite being painless, some patients report itching, pain and burning sensation (DENG et al, 2015; MARQUES et al., 2015). Fibrotic and lymphatic changes cause secondary complications such as lymphoedema, symptoms similar to elephantiasis and self-inoculation occurs due to intense itching, often associated with bacterial infections (QUEIROZ-TELLES et al., 2017; SANTOS et al., 2007).

Phaeohyphomycosis is associated with a variety of syndromes due to dematiaceous fungi. Clinical manifestations are classified into, (i) superficial: including infection of superficial layers (“black Piedra” and “tinea nigra”); (ii) cutaneous and corneal form (dermatomycosis, onychomycosis and mycotic keratitis); (iii) subcutaneous form comprising of cystic lesions which usually follow after a traumatic event; (iv) systemic phaeohyphomycosis, in which there is dissemination to the nervous system and organs of the abdominal cavity; this form occurs in immunocompromised patients (ISA-ISA et al., 2012; ARCOBELLO; REVANKAR, 2020; MADKE; KHOPKAR, 2015). The most aggressive form of phaeohyphomycosis is the cerebral infection, a fatal disease caused by neurotropic species, characterized by black necrotic tissue, black pus, and black cerebrospinal fluid (WANG et al, 2019)

The diagnosis of both diseases are performed by direct microscopy, culture, histopathological examination, showing the presence of muriform bodies of brown color, size of 5-12 mm and rounded shape, thick walls with the formation of biplanar septa for CBM (QUEIROZ-TELLES et al., 2017; SANTOS et al., 2007), and the presence of dark hyphae invading tissue of diagnosis and should be obtained alongside culture whenever possible for diagnosis of the Phaeohyphomycosis (ARCOBELLO; REVANKAR, 2020). Radiological examination and biopsy sites are an alternative to diagnosis to cerebral and dissemination cases (WANG et al., 2019; ARCOBELLO; REVANKAR, 2020).

Serological techniques, intradermal assays and molecular methods of species identification are not usual in clinical routine, but they are accurate methods of laboratory

diagnosis (WALKER, MCGINNIS, 2014; TORRES-GUERRERO et al., 2012). Species-level identification is clinically important due to differences in infection prognosis and variation in the susceptibility profile of antifungal agents according to the species in question (GOMES et al., 2016; HARRIS et al., 2009). The clinical signs and characteristics of the disease lesions can be confused with other fungal, bacterial infections, parasitic infections and non-infectious diseases such as carcinoma or lupus erythematosus (QUEIROZ-TELLES; SANTOS, 2012).

3.3 EPIDEMIOLOGY OF BLACK YEAST INFECTIONS

3.3.1 **Chromoblastomycosis**

The CBM disease is considered ubiquitous, besides the highest incidence is related to the tropic's regions with hot and humid climate such as Latin America (BRUN, et al., 2015; CHAABANE et al., 2015; TORRES-GUERRERO et al., 2012). Endemic countries for the disease are Madagascar, Japan, China, Mexico, Brazil, Costa Rica, Puerto Rico, Cuba and Venezuela (CHAABANE et al., 2015; TORRES-GUERRERO et al., 2012; DE HOOG et al., 2004; SANTOS et al., 2021) humid and hot regions, the clinical cases are most associated to *Fonsecaea* species while in arid vegetation of Central and South America and Australia the genus related is *Cladophialophora (*LAVELLE, 1980; TREJOS, 1954; DE HOOG et al., 2007).

Chromoblastomycosis is not common on the European continent (QUEIROZ-TELLES, 2012). According to Santos et al, 2021 most of the cases were reported from South America (2,619 cases), followed by Africa (1,875 cases), Central America and Mexico (1,628 cases), Asia (1,390 cases), Oceania (168 cases), Europe (35 cases), and USA and Canada (25 cases) (Figure 3). Japan has 10 cases per year with a probability of 01 case/46,000 people, and together with China they are the areas studied in the endemic area of the continent (Figure 3). In Latin America, the disease has not yet been reported in Chile, and in countries such as Venezuela, the frequency of cases of chromoblastomycosis is 16 cases/1,000 people (DENG et al, 2015) (Figure 3). In the Maranhão state, Brazil, the incidence of cases is 5.9/year; there are 872 cases in the southern region, in the state of Paraná the annual average is 6.4/year and 2.6/year for Rio Grande do Sul (QUEIROZ-TELLES et al., 2017).

The current hypothesis is that chromoblastomycosis is a traumatic infection that has a higher prevalence among male farm workers, who acquire the disease by contaminated plant thorns or wood cortex. This seems to be the main route of the infection and is supported by clinical reports of infected patients (RIPPON, 1988; SAYAL, et al., 2002; SALGADO, et al.,

2004; VICENTE et al., 2008; MENEZES et al., 2008; KHAN et al., 2015). The prevalence in rural workers, male (81.7%) (SANTOS et al, 2021), with an average age of 52.5 years (SANTOS et al, 2021), living in rural areas (MARQUES et al., 2015). The anatomical regions most likely to be affected are lower and upper limbs, while trunk, nose, ears, shoulders are less common anatomical regions (QUEIROZ-TELLES et al., 2017).

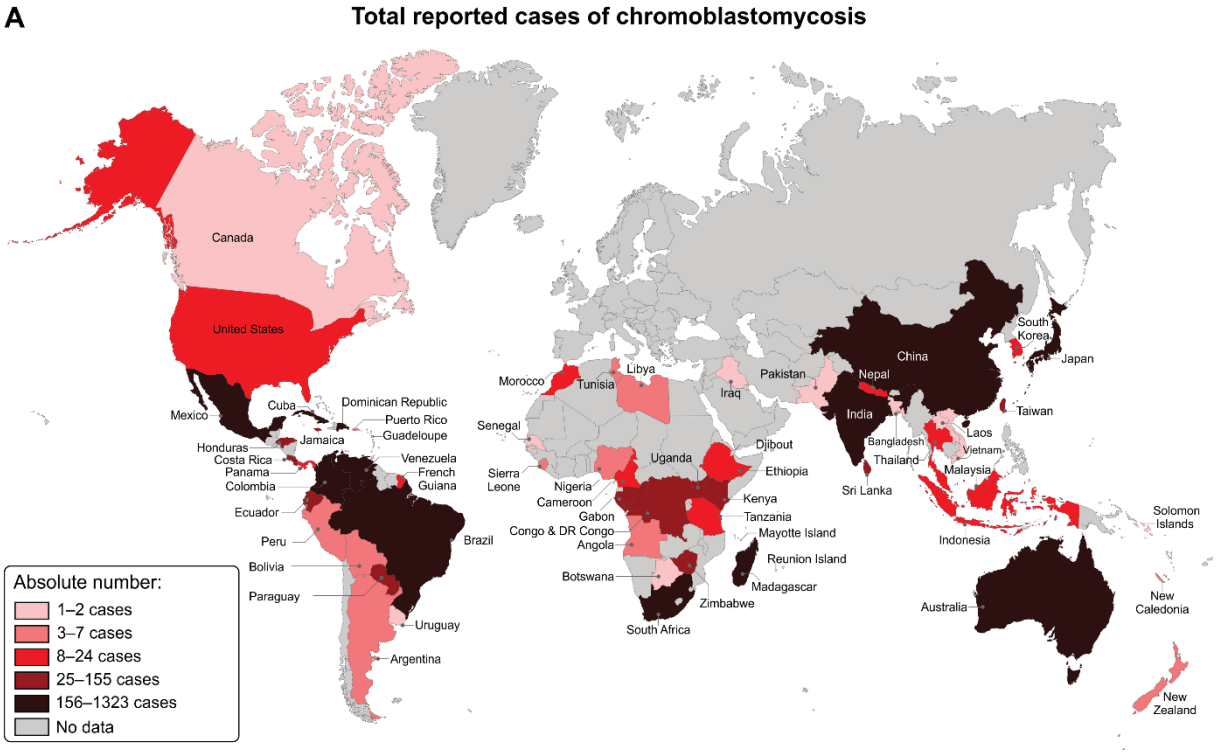


Figure 4- Prevalence and absolute number of reported cases of chromoblastomycosis. From: Santos et al., 2021.

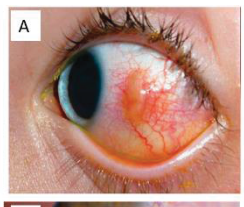
3.3.2 **Phaeohyphomycosis**

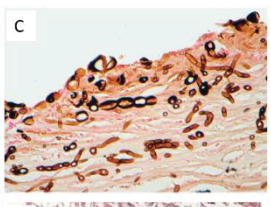
Phaeohyphomycosis is a general term designed by an infection caused by dematiaceous or darkly fungi that are being increasingly seen in a variety of clinical syndromes in both immunocompromised and normal individuals. Although rare, the most common are superficial infections as tinea nigra and keratitis, frequently associated with minor trauma and rarely lead to life-threatening disease. In Chaetothyriales order more than a single genus are related as phaeohyphomycosis agents (FERNANDEZ-PITTOL et al., 2019), however most life-threatening infections due to these unusual fungi are seen in immunocompromised hosts, associated with strict pathogens as *C. bantiana, R. mackenziei* and *F. pugnacius* with the possible exception of central nervous system (CNS) (HOWLETT et al., 2019), while species of

Exophiala genus, like *E. dermatitis*, are related in cases less invasive (KIRCHHOFF et al., 2019).

The infection is worldwide spread; however, studies have suggested these infections are more common in warmer climates and the species not appear to be geographically restricted it occurs with higher prevalence in tropical climatic regions such as Madagascar and northern Venezuela (REVANKAR et al., 2017). In Brazil, it has been described in the Amazon in the northeast (GOMES et al., 2016) and also in other regions (MINOTTO et al., 2014; OLIVEIRA et al., 2016). This is a sporadic infection which apparently affects immunocompetent as well as immunosuppressed individuals (REVANKAR et al., 2017). For the diversity of spectrum disease, the epidemiology of the disease remains unknown (Figure 4).

This suggests that most if not all individuals are exposed to them, presumably from inhalation and the commonest mechanism of acquiring the infection is via implantation by organic matter (MAUDGIL et al., 2016). Exposure is thought to be from inhalation or minor trauma, which may not even be noticed by the patient. Since these are widespread in the environment, individuals are constantly exposed to them, though they rarely cause disease. Fungal infections are frequently associated to hospital environments the highest risk is seen in small bowel transplants (11.6%), followed by lung (8.6%), and liver (4.7%), with renal transplants having the lowest risk (1.3%).





Clinical syndrome	Associated melanized fungi E
Allergic fungal sinusitis	*Curvularia*
Allergic bronchopulmonary mycosis	*Curvularia*
Subcutaneous nodules	*Alternaria, Exophiala, Phialophora*
Invasive sinusitis	*Curvularia, Alternaria, Exserohilum*
Bone and joint infections	*Lomentospora, Alternaria, Exophiala, Phialophora*
Catheter-related peritonitis	*Curvularia, Exophiala, Alternaria*
Pneumonia	*Verruconis, Exophiala, Chaetomium, Alternaria*
CNS disease	*Cladophialophora, Curvularia, Rhinocladiella, Verruconis, Exophiala, Fonsecaea*
Disseminated disease	*Lomentospora, Exophiala, Curvularia, Alternaria*

Figure 5- Clinical manifestations of Phaeohyphomycosis A, B. Clinical presentation of ocular Phaeohyphomycosis; C, D. Hyphae in host tissue; E. Clinical syndrome and associated fungi involved in Phaeohyphomycosis, Adapted from: Maudgil et al., 2016.

3.3.4 **Environmental occurrence of black yeast pathogens**

The epidemiology of black yeast diseases suggests that the etiologic agents are present in the environment and the infection is accidental. In addition, CBM is assumed to be a human

disease due to the scarce reports of this infection in other animals. The most clinical cases of this skin lesion reported in animals have been considered as phaeohyphomycosis (PHM), because typical muriform cells were lacking (QUEIROZ–TELLES, 2017). Some studies reported the ability of pathogens development in live plant isolates as *Cladophialophora yegresii* are very close to the pathogenic species *C. carrionii*, and both have a distinct pathogenicity profile and ability to develop muriform cells in plant models (De Hoog et al., 2007). The results of Vicente et al. (2014) suggest that species closely related to the pathogenic lineages of the *Fonsecaea* genus, namely *F. erecta* and *F. minima*, are associated with plants, but the relationship of mycosis agents with this substrate remains unknown.

In Brazil, where cases involving the four species of the genus *Fonsecaea* (*F. pedrosoi*, *F. nubica*, *F. monophora* and *F. pugnacius*) have already been reported (GOMES et al., 2016). However, the environmental isolation of *Fonsecaea pedrosoi* was reported only in babassu coconut (*Orbygnia phalerata*) and from thorns of the native plant of the northern region, *Mimosa pudica*, after a report of infection by a patient (SALGADO et al., 2004), through morphological characterization of the strains, demonstrating its environmental epidemiology. However, the similarity between clinical and environmental species confirms the insufficiency of data in establishing the relationship of the etiological agents of chromoblastomycosis with live plants (VICENTE et al., 2014; VICENTE et al., 2008; DE HOOG et al., 2007).Few studies had been focused in the isolation of black fungi involved in disease (VICENTE 2001; 2012, 2014; SALGADO et al., MARQUES et al.,2006; NASCIMENTO et al., 2017; LIMA et al., 2020; IWATSU et al., 1981)

Molecular tools are able to identified *F. pedrosoi* in live plants as *S. paniculatum* (Jurubeba), in decomposing material, soil, and shells of babassu coconut (*Orbygnia phalerata*) and *Fonsecaea monophora* in stems of *M. paniculate* (Murta tree), leaves and stems of *A. vulgare* (Tucum tree), leaves and stems of *S. dulcis* (Vassourinha tree), and leaves of *P. insignis* (Bacuri tree), decomposing material, soil, and shell of babassu coconut (*O. phalerata*). Costa et al., 2020, reported several chromoblastomycosis and phaeohyphomycosis agents in environmental sources using molecular markers in metagenomic datasets, like *Exophiala* species*, E. angulospora, E. pisciphila, E. equina, E. cancerae* associated to soils, roots and plants and in sugar filter cake and *E. castellanii* water, *E. brunnea* from litter and *E. sideris* from the hydrocarbon-polluted environments; *Cladophialophora* agents, *C. arxii and C. immunda, C. arxii* and *C. immunda* in soils contaminated with crude oils and *C. chaetospira* found it in the mangrove and contaminated soil with crude oil data; genus *Rhinocladiella*, *R.*

similis and *R. atrovirens* from dialysis water and babassu and *R. similis* was observed in root-associated of maize and *R. atrovirens* was identified in plant and soil-associated.

The natural niche of black yeast species is not yet fully elucidated (DE HOOG et al., 2004; VICENTE et al., 2014), as well as the way in which symptomatic patients acquire the infection, which is assumed to result from traumatic inoculation (QUEIROZ-TELLES et al., 2017).

3.4 NON-ISOLATION TECHNIQUES FOR ENVIRONEMNTAL STUDIES

Studies for the characterization of substrates and microorganisms are generally carried out by methods of selective and non-selective isolation, by culture of colonies following by morphological and molecular characterization, restricting the data to cultivable microorganisms (TEHRANI et al., 2014; VAN ELSAS, BOERSMA, 2011). The direct detection of fungi in environmental samples allows evaluating the different genotypes in a substrate, interactions between species and nutritional demand, or present themselves in very low concentrations that allow its growth by known cultivation techniques (HAMZEHEI et al., 2013; TSUI et al., 2011; SUN et al., 2010; ANDRADE et al., 2007). Fungi are an interesting group with features intrinsic that required evolutionary changes and adaptations in the environments and the omics sciences including genomics, metagenomics, phylogenomic, transcriptomics, metabolomics, and proteomics have advanced the way to understand fungal diversity at diverse taxonomic levels their potential applications in bioremediation, and as new sources of natural products of therapeutic value (MIYAUCHI et al., 2020; MUGGIA et al., 2020).

Next-generation sequencing (NGS) is a new technological tool that allows the sequencing of thousands to billions of DNA fragments simultaneously and independently (GU et al., 2019). Genomic was firstly applied to provides the information about the genes and respectively functions, after other analysis was developed to explore this technique. Comparative genomics analyses focus on the similarity and differences between the annotation or between the sequence of two or more genomes (HERRERO et. al., 20216). Metagenomics encompasses studies that use this next-generation sequencing to characterize microbial genomic and transcriptomic systems material from any ecological samples designated as metagenomics has immense potential, targeting specific marker genes (HEINRICHS et al, 2012; MITCHELL et al., 2015; BAHRAM et al., 2021; HANDELSMAN et al., 1998; WOOLEY; GODZIK; FRIEDBERG, 2010). The samples that generate the metagenomic data are very diverse, and can be environmental metagenomes (water, air, soil, ice) (KING et al.,

2016; YANG et al., 2019; MA et al., 2017), host-associated metagenomes (plants, invertebrates or non-human mammals) (HASIÓW-JAROSZEWSKA, BOEZEN, ZWART, 2021; Yang et al, 2018) and humans (skin, gut, oral and other mucosal organs) (WILSON et al 2019) (EDWARDS et al., 2020; CUADROS-ORELLANA et al., 2013). However, actually the data generated have emerged as a new challenge for organization and analysis of the data (CARVALHO; SILVA, 2010). With the recent advancements in functional genomic research, the NGS technologies presents are faster, cost-effective, and can sequence the samples on a large-scale in one shot (KIM et al., 2013), and an essential and powerful tool for understanding various molecular aspects of a biological process.

The application of culture-independent methods (e.g., DNA sequencing) has provided additional insights in the study of black yeast group (TEIEXEIRA, et al., 2017; VICENTE et al., 2017; MORENO et al., 2017; COSTA et al., 2021). The genome and comparative genomic analysis of Herpotrichiellaceae members was performed to understood phylogenomic relationships, transposable elements, sex-related genes, protein family evolution, genes related to protein degradation (MEROPS), carbohydrate-active enzymes (CAZymes), melanin synthesis, secondary metabolism (TEIXEIRA et al., 2017; VICENTE et al., 2017), identification of specific gene, as laccases (MORENO et al., 2017) and transcriptome analysis of pathogens black yeasts (LI et al., 2016; POYNTNER et al., 2016; BLASI et al., 2017). Costa et al., (2020) provides new insights environmental niches of black yeast pathogens in Brazil, using metagenomic data.

The isolation method by flotation in mineral oil (IWATSU et al., 1981; VICENTE et al, 2008) as considered the standard selective method for the recovery of chromoblastomycosis and phaeohyphomycosis agents, due to the affinity of the species to assimilate aromatic hydrocarbons present in mineral oil, considered an important virulence factor (SEYEDMOUSAVI et al., 2014; VICENTE et al., 2011; 2014). However, despite being selective, few chromoblastomycosis agents have been isolated from environmental substrates (VICENTE et al. 2001, 2008, 2014; MARQUES et al., 2006; LIMA et al., 2020). The limitations of conventional methods do not allow the complete characterization of microorganisms and respectively particular niches, while metagenomic techniques that evaluate nucleic acids or biomarking are presented as tools of greater sensitivity and specificity in environmental studies, allowing the approach of the niche as a whole, regardless of the nutritional or cultivation conditions of the species (HAMZEHEI et al., 2013; LACKNER et al., 2012; TSUI et al., 2011; BAHRAM et al., 2021; YANG et al., 2018). After the sequencing approach, data analysis involves the use of sequence datasets, such as GenBank, for

comparison, taxonomic annotations and data mining (MEYER et al., 2008), for this reason the challenge of the metagenomic is in annotation of microbial genome, for this reason the selection of molecular markers is essential for this analysis and characterization.

3.5 MOLECULAR MARKERS

The identification of species is mostly carried out by amplifying highly variable regions using standard molecular biology techniques like extraction of DNA, PCR amplification, and identification by DNA sequencing (THOMPSON et al., 2013). The applications of many molecular markers have been reported in various DNA marker protocols useful for telling the individual genotypic, these markers are mainly nucleic acids that are polymorphic among individuals or populations applied for taxonomic application (AMITEYE et al., 2021; THOMPSON et al., 2013). Single Nucleotide Polymorphisms (SNPs) are regions characterized by a unique difference in nucleotides of the DNA sequences of the individuals of a population, observed due to transversions (C/G. A/T, C/A and T/G), transitions (C/T) or G/A), insertions and deletions (SINGH, GUPTA, et al., 2003; AMITEYE et al., 2021) commonly located in coding and non-coding as well as intergenic regions of genomes (AMITEYE et al., 2021). SNPs genotyping assays are currently available for genetic diversity detection and identification of organisms and species (NAJAFZADEH et al., 2011; CONZEMIUS, HAUNOLD, BARIŠI, 2021; AMITEYE et al., 2021).

Barcodes are short nucleotide sequences designed in regions of high variability that provides a robust strategy with low intraspecific and high interspecific distinction to identify and discover unknown species based on a reference sequence library (HEINRICHS et al., 2012; VU et al., 2017; GUAN et al., 2020; YANG et al., 2018). The tool emerges as a complement to traditional taxonomy by facilitating the identification species, including cryptic species (WANG et al. 2018; LÜCKING et al., 2020). The DNA barcoding sequence has been a molecular marker not influenced by the morphological diversity of species, growth phases and environmental factors, contributing to field misidentification with applicability in studies about living organisms, include metagenomic (KENNEDY, et al. 2020; LÜCKING et al., 2020).

Accuracy of the molecular markers depends on the extent validation including the choice of the gene, the intraspecific variation and interspecific divergence (Ahmed et al., 2022; VĚTROVSKÝ et al., 2020; LÜCKING et al., 2020). After the design, the selected DNA marker barcode, should be compared on the basis of in public datasets, which provide a large suite of online resources for biological information and data for subsequent investigation. The in silico

analysis include, alignment using software such as BLAST algorithm (NCBI) and Clustal W (THOMPSON et al., 1994) in order to comparing their DNA sequences to known sequences in reference libraries (AHMED, et al. 2022); software to check the efficiency and specificity of sequence (CHUKWUEMEKA et al., 2020); and in silico PCR method able to use newly designed primers and identify potential mismatches in the primer binding sites and avoid the amplification of unwanted amplicons (KUMAR, CHORDIA, 2015; YU, ZHANG, 2011; CHUKWUEMEKA et al., 2020). The final step is called Wet Lab Validation, that's provides the specificity, efficiency, sensitivity and background signal in negative controls of molecular marker in laboratory testing procedures (HEINRICHS et al., 2012; NAJAFZADEH et al., 2011; 2013; VOIDALESKI et al., 2020;)

Internal transcribed spacer (ITS) region was established as fungal DNA barcode by Schoch et al. (2012) due to main characteristics, the composition of gene (two non-coding and variable regions, ITS1 and ITS2, flanking the highly conserved 5.8S gene), easily amplified from most fungal taxa, using universal primers. In 2012 the ITS region (internal transcribed spacer) was chosen as the main barcode of a fungus by the International Fungal Barcoding Consortium (SCHOCH et al. 2012; XU, 2016; LÜCKING et al., 2020). For the black yeast group, this gene appears as a hyper-variable region, especially the ITS2 domain, which in the Herpotrichiellaceae family is between 27 and 50 bp in length, capable of taxonomically assigning the species level to black yeast isolates (HEINRICHS et al, 2012). Heinrichs et al., (2012) developed barcode identifiers to black yeasts, with approximately 27-50bp located in the ITS2 region, considered the region with the greatest variation that allows differentiation at the species level of this group, based on SNPs regions.

Padlock probe sequences designed for black yeast species demonstrate 100% specificity and no cross-reactions between closely related species (IRINYI et al., 2015; NAJAFZADEH et al., 2011; ATKINS, CLARK, 2004) in addition to representing a tool effective for recognition and identification of adjacent sequences in the target DNA (NILSSON et al., 1994), applied in genotyping in large populations (>500 individuals) (TSUI et al., 2011; ATKINS, CLARK, 2004; RODRIGUES et al., 2015). The probe specificity is determined by the recognition in regions of single nucleotide polymorphisms (SNPs) (HAMZEHEI et al., 2013; LACKNER et al., 2012; ANTSON et al., 2000; CONZEMIUS, HAUNOLD, BARIŠI, 2021), that is, gene regions where differences are observed. unique in nucleotides of DNA sequences due to transversions, transitions, insertions and deletions (SINGH, GUPTA, 2017; BRUMFIELD et al., 2003).

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Chapter II

Global eco-epidemiological assessment of chaetothyrialean diversity reveals unexplored habitats

Global eco-epidemiological assessment of chaetothyrialean diversity reveals unexplored habitats

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1 **ABSTRACT**

The technology of non-culture high-throughput DNA sequencing and metagenomics have deeply transformed the research on fungal diversity. The chaetothrialean fungi, especially the opportunistic pathogens have poorly been explored, mainly using isolation and culturing methods. Several of these fungi are associated with superficial and systemic infections, known as chromoblastomycosis and phaeohyphomycosis. Metagenomic data have been collected in large publicly available data bases Across the public metagenomic data available in GenBank's Sequence Read Archive (SRA), >1,211,579 Terabytes of sequencing studies published until 2021 and based on the nuclear ribosomal internal transcribed spacer (ITS), were solved with the aid of 105 barcodes and 34 padlock sequences. The analysis showed the presence of black yeasts in substrates that have never been reported, and in poorly studies regions around the world. *Exophiala* was the most prevalent genus reported, species being found in soil, air, and insects. *Exophiala equina* was the most abundant species (501588 reads). of the neurotropic species *Cladophialophora bantiana* was characterized repeatedly in soil. *Fonsecaea* agents of chromoblastomycosis were detected in low abundance in variable habitats such as soil, dust, freshwater, rhizosphere, sludge air and roots. High-throughput sequencing data can complement predictions based on methods of traditional mycology and increase our understanding of epidemiological data, substrates and niches associated to chaetothrialean black yeasts.

Key Words: metagenomic; black yeast; environmental analysis; metabarcoding

2 **INTRODUCTION**

The order Chaetothyriales comprises melanized fungi, known as black yeasts and relatives, some of which are important agents of human and animal diseases, such as chromoblastomycosis (CBM) and phaeohyphomycosis (PMC). These organisms are able to survive at low prevalence in common as well as adverse environments, enhanced by the oligotrophic nature of these fungi. As the diseases are neglected, and there is no mandatory reporting of cases, the epidemiology of CBM and PMC remains largely unclear. The limited number of environmental isolation studies recovered only few pathogenic species,

Chromoblastomycosis (CBM) is a cutaneous infection, caused by implantation of environmental debris such as plant thorns and/or wood fragments (MARQUES et al., 2006; SALGADO et al., 2004). The infection is characterized by the presence of muriform cells in host tissue (SEYEDMOUSAVI et al.,2014; AZEVEDO et al., 2015; QUEIROZ-TELLES et al., 2017). An environmental origin of the disease is confirmed by the studies using methods of selective isolation (VICENTE et al., 2001; 2008; SATOW et al., 2008; GUERRA et al., 2013). The disease is worldline distributed in the (sub)tropical climate zone, with endemic zones in the American and Asiatic continents (QUEIROS-TELLES et al, 3017; SANTOS et al., 2021).

Phaeohyphomycosis (PMC) is a general term designed for infection by melanized fungi. Agents are found in numerous genera in particularly Chaetothyriales and Pleosporales (FERNANDEZ-PITTOL et al., 2019). Due to the diverse spectrum of disease, the endemic areas and the epidemiology of PMC remain unknown. The current hypothesis is that the hosts are exposed to agents in the environments, presumably from inhalation in case of disseminated infection, or by acquiring the infection via implantation in case of localized infection (MAUDGIL et al., 2016). Infections can be severe to mild, and in the latter case are not even reported by the patient. The mycosis is frequently associated to hospital environments (REVANKAR et al., 2015; PINHEIRO et al. 2020).

While CBM agents are frequently recognized in environmental samples, those causing PMC, such as *Fonsecaea pugnacius*, *Rhinocladiella mackenziei* or *Cladophialophora bantiana* are almost exclusively reported from clinical cases; the origin of infection by these species has not been elucidated. Metagenomic studies provide a powerful tool for the study of fungi (IHRMARK et al., 2012) included black yeasts (VOIDALESKI et al., 2020). High-throughput sequencing offers new possibilities to recognize fungi in the environment based on molecular markers that are not widely applied in traditional approaches (DAVISON et al., 2015; BALDRIAN et al., 2021). Mining of publicly available metagenomic databases using

molecular markers, barcodes, and padlock probes (COSTA et al., 2020) presents a high potential for the exploration of global black yeast diversity (TEDERSOO et al. 2020; TALBOT et al. 2014).

The aim of this paper is to explore the global black yeast diversity in environmental sources based on public metagenomic database dataset, captured by molecular markers as barcode and padlock probe sequences to identify habitats that harbour black yeast-like opportunistic pathogens.

3 **MATERIALS AND METHODS**

3.1 DATABASE CONSTRUCTION

The database of the Sequence Read Archive (SRA) (https://www.ncbi.nlm.nih.gov/sra) was consulted for the present study. The database comprises metagenomic studies available until 2021. Briefly, the selection criteria for the inclusion of samples are: (1) samples are environmental (air, deadwood, arthropods and other animals, rock, lichen, litter, soil, rhizosphere soil, root, aquatic bodies); (2) the country location of each sample has been reported in cases of phaeohyphomycosis and/or chromoblastomycosis; (3) data from sequences of the internal transcribed spacer regions (ITS1, ITS2, or both) were subject to amplification; and (4) the sequencing data are publicly available. In total, 1211579 Terabytes were analyzed. From the selected countries, we used literature reviews of clinical cases and other descriptions of fungal disease (QUEIROZ-TELLES et al., 2017; SANTOS et al, 2021), and classified the areas according to the prevalence of report cases of chromoblastomycosis disease. We used the terms “metagenomic” plus the name of country i.e., “Chile”. The projects and the countries are listed in Table 1. A previous selection of data was performed manually, and studies of non-environmental samples were excluded.

Table 1. Areas selected to comprise the dataset included in this study.

Continent		Country	Prevalence of report cases *	Total Size Analyzed (TB)
Asia		China	High	365 145
		India	High	5 942
		Japan	High	5 822
		Pakistan	Low	275
		Iran	Low	684
America	North America	Low	Low	98 561
		United States	Low	584 873
		Mexico	High	18 140
	Central America	Puerto Rico	High	19 054
		Costa Rica	High	2 973
		Cuba	High	484
		Panama	High	6 659
		Dominican Republic	High	0,000024
		Honduras	High	8
		Haiti	High	0
		Jamaica	High	16
	South America	Chile	Low	9 270
		Argentina	Low	1 302
		Colombia	High	2 046
Europe		Germany	Low	26 267
		France	Low	13 953
		Spain	Low	12 265
		Portugal	Low	1 078
		Italy	Low	16 234
		Netherlands	Low	14 821
Oceania		Australia	High	4 014
Africa		Madagascar	High	1 693
		Angola	Low	0
Total Size Analyzed (TB)				**1 211 579**

*Low – Total reported cases between 1-24; High- Total reported cases greater than 25; The classification was based on Queiroz-Telles et al, 2017 and Santos et al., 2021

3.2 IDENTIFCATION TOOLS

Molecular markers were used as a tool for the identification of species, according to methods described by Costa et al. (2020). For the order Chaetothyriales, 119 markers (Table S-2) were applied (HEINRICHS et al., 2012; NAJAFZADEH et al., 2013; 2011; 2018; FENG et al., 2013; DENG et al., 2014; SCHNEIDER et al., 2019; HAMZEHEI et al., 2013). In addition, 41 padlock probes containing 28–42 bp, and 105 barcode identifiers comprising 25–41 bp were used. These represented 72 species of chaetothyrialean fungi, including 50 species that were potential causative agents of phaeohyphomycosis and/or chromoblastomycosis (Table S-2). Padlock probes and barcodes were designed in regions with SNPs in one of the rDNA internal transcribed spacers (ITS1, ITS2), or in both.

3.3 IDENTIFCATION *in silico*

The analysis of fungal identification was conducted by clustering sequences into operational taxonomic units at 100% similarity using local BLASTn search (v2.6.0.+). The analysis only comprised sequences fulfilling the quality criteria with coverage and identity cutoff of 100% and matches with values below the cutoff were excluded. Visual inspections were randomly conducted to verify sequence alignments. Because of the specific identification of sequence marker padlock and barcode probes, cases of slight misalignment and non-perfect sequence identity were considered as not characterizing the target fungus. Metagenome reads from double strand sequencing were considered once in the final read count.

3.4 CLUSTERING DATA

The data obtained were visually inspected and separated into 14 different substrates (soil; decomposing process; root; rhizosphere; plant association; air; water; ice; marine sediment; dust; animal association; insects association; cave and rock) according to the project and/or the analyzed environmental sample. Since the number of projects evaluated in each country were different, , the relative abundance was normalized, normalizing dividing the total reads of each genus by the total number of reads according to each country.

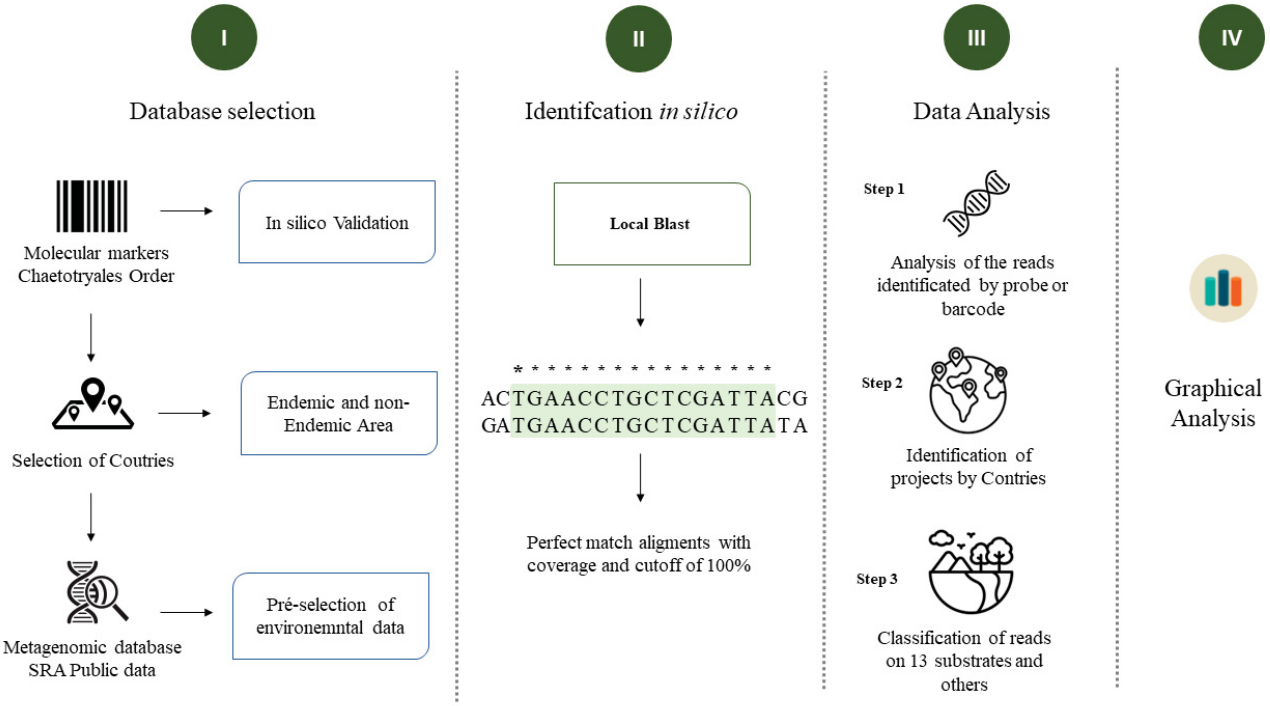

Figure 1- Workflow of data analysis.

4. **RESULTS**

The dataset consisted of 1,211,579 Terabytes of ITS sequences. Clustering at a 100% sequence similarity threshold produced a total of 960,147 reads of black yeast-like fungi from environmental metagenomics from 27 countries in 5 continents. A total of 54 species was identified (Figure 2), of which 12 species were associated with environmental sources and 42 species with agents of phaeohyphomycosis and/or chromoblastomycosis in 20 countries (Figure 3). 19 species did not match with any project and 7 countries did not return any data.

The generated data was dependent on available metagenome projects in the database and resulted in a high variation in size in each country. China and USA had the highest representation in the analysis and were the countries with highest numbers of species identified. Habitat sampling is largely dominated by soil studies, followed by living plants and plant debris, respectively (Figure 2).

The genus *Exophiala* was the prevalent genus in American, European, and Asian continents (Figure 2 and 3) and predominant species were *Exophiala equina*, *E. xenobiotica* and *E. pisciphila* (Table S-2). The second largest group was the genus *Cladophialophora* (Figure 2) represented by 10 species including *C. bantiana*. The latter species, an agent of cerebral phaeohyphomycosis, was reported in soil, water, and animal association in data from Porto Rico, Canada and China (Table S-2). Geographically restricted opportunists like *Rhinocladiella mackenziei* and *Fonsecaea pugnacius* were not identified in data analyzed. The major agent of chromoblastomycosis, *F. pedrosoi*, as well as *F. monophora* and *F. nubica* were detected at low incidence, while *Cladophialophora carrionii* was not found.

The soil substrate was positive for the major number of species in this study (n=50), with species belonging to *Exophiala*, *Cladophialophora*, *Cyphellophora*, *Phialophora*, *Rhinocladiella*, *Fonsecaea*, *Veronaea* and *Knufia* (Figure 2). The second highest diversity was found in plant association (n=36), species being reported from leaf, wood, and grain. Of 42 plant species, association with black fungal opportunists had never been reported (Table S-3). Strictly environmental species as *Fonsecaea erecta* and *Cladophialophora yegresii* were not encountered in this study. *Cladophialophora chaetospira* was the major species reported in association with living plants (reads=37668), i.e., from seven countries (Chile, The Netherlands, France, Spain, Italy, USA, and China).

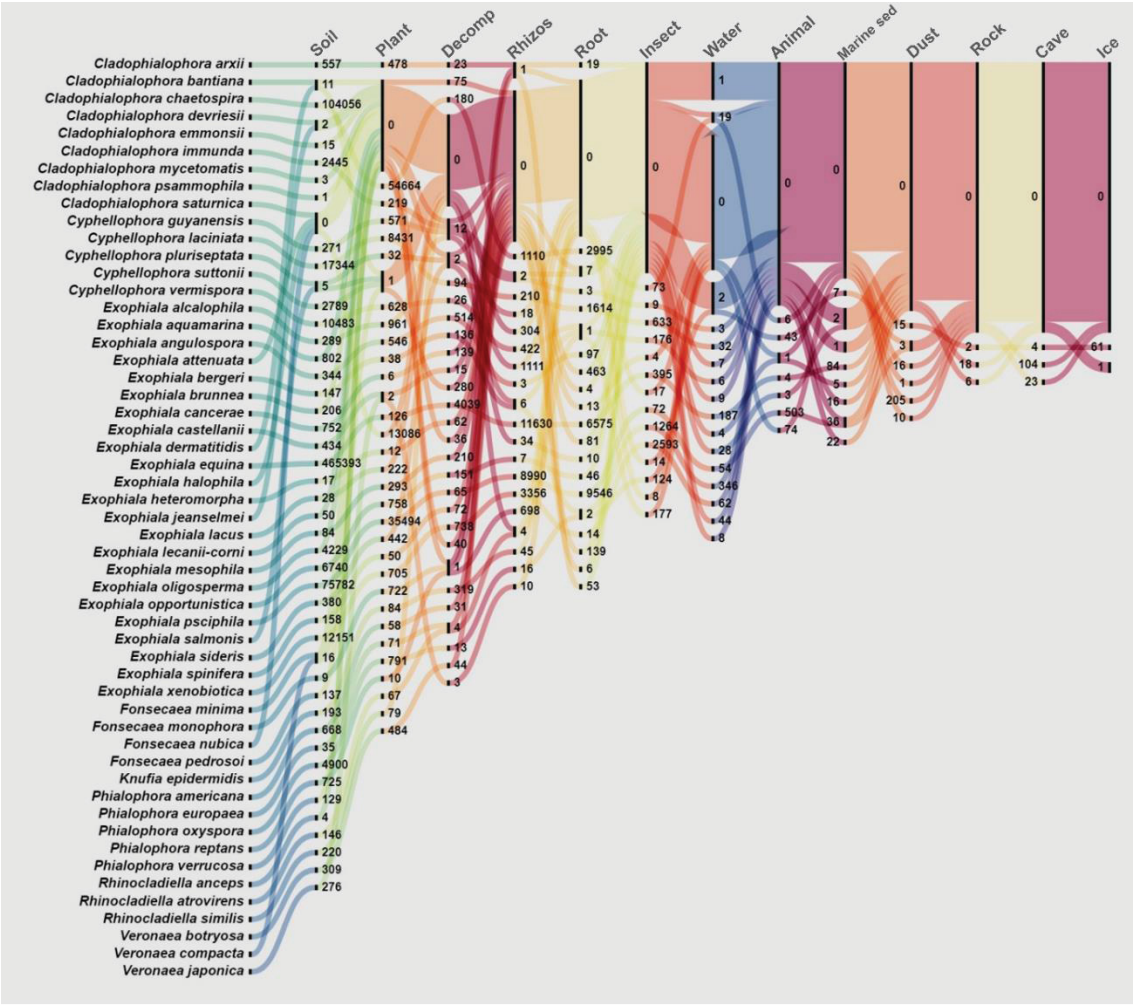

Figure 2- Environmental distribution of Chaetothyriales species in the substrates analyzed.

Chaetothyrialean species were only rarely reported of in association with mammals. i.e., *Cladophialophora chaetospira* (reads =6), *C. immunda* (reads = 43), *Cyphellophora oxyspora* (reads=74), *Cy. vermispora* (reads=1), *Exophiala oligosperma* (reads = 7), *E. equina* (reads = 4), *E. lecanii-corni* (reads = 1) and *E. pisciphila* (reads = 503) (Table 2). The purportedly waterborne salmonis-clade was represented by *E. equina*, was encountered in mangroves and meiofauna, while freshwater contained *E. pisciphila* (France, China and USA), *E. equina* (Colombia, Japan, Portugal, France, USA and China), *E. cancerae* and *E. opportunistica* (China). *Cladophialophora bantiana* was reported in Porto Rico, *Fonsecaea monophora* in China and *F. pedrosoi* in the U.S.A., in all cases from freshwater sources.

Table 2- Species associated with animals and crustaceous.

Specie	Country	Nº of Sequence	Project	Source
Cladophialophora chaetospira	China	6	PRJNA268045	Sika Deer *Cervus nippon*
Cladophialophora immunda	India	43	PRJNA509093	Mangroves
Cyphellophora oxyspora	India	74	PRJNA509093	Mangroves
Cyphellophora vermispora	France	1	PRJNA419907	Oyster
Exophiala equina	Portugal	2	PRJNA611064	Meiofauna diversity in estuarine ecosystems
	China	1	PRJEB13006	*Ochotona curzoniae*
	China	1	PRJEB13006	*Ochotona curzoniae*
Exophiala lecanii-corni	China	1	PRJEB13006	*Ochotona curzoniae*
Exophiala oligosperma	India	7	PRJNA509093	Mangroves
Exophiala pisciphila	Spain	503	PRJNA608629	Gut of pig, *Sus scrofa*

Nº. Number.

Even though there are no systematic field studies, our data reveal an association of black yeast-like fungi with insects in Italy, U.S.A., France, Madagascar, China and Costa Rica. *Exophiala* (reads= 4521) and *Cyphellophora* (reads = 824) were found in association with mosquitoes, wasps, beetles, honeybees and butterflies, *Phialophora* (reads = 8) and *Rhinocladiella* (reads = 133) in wasps. *Cladophialophora* (reads =73) was associated with butterflies and moth larvae (Table 3).

Table 3- Chaetothyriales species associated to insect source.

Species	Country	Nº of Sequence	Source
Cladophialophora chaetospira	Costa Rica	4	Gut Neotropical butterflies
	China	26	Larvae (Dong Chong Xia Cao)
	China	28	insect, soil and plant
	China	15	insect
Cyphellophora americana	Italy	14	Gut of wasp
Cyphellophora atrovirens	USA	13	Insects/ambrosia beetles
	China	31	*Dendroctonus valens* - beetle
Cyphellophora guyanensis	Costa Rica	6	Gut of *Neotropical butterflies*
	China	3	insect, soil and plant
Cyphellophora laciniata	Madagascar	627	*Aedes albopictus*
	France	5	*Aedes albopictus*
	USA	1	Insects/ambrosia beetles
Cyphellophora oxyspora	Costa Rica	124	Gut of *Neotropical butterflies*
Exophiala bergeri	Italy	176	Gut of wasp
Exophiala castellanii	France	4	*Aedes albopictus*
Exophiala equina	Madagascar	8	*Aedes albopictus*
	France	136	*Aedes albopictus*
	Italy	193	*Xyleborinus saxesenii* and *Xylosandrus germanus*
	France	26	*Aedes albopictus*
	China	9	Larvae (Dong Chong Xia Cao)
	China	23	*Ophiocordyceps sinensis*
Exophiala jeanselmei	Madagascar	5	*Aedes albopictus*
	France	2	*Aedes albopictus*
	Madagascar	8	*Aedes albopictus*
	France	2	*Aedes albopictus*
Exophiala pisciphila	Costa Rica	5	Gut of neotropical Butterflies
	China	51	insect, soil and plant
	China	16	insect
Exophiala sideris	France	49	*Aedes albopictus*
	Italy	1215	Gut of wasp
Exophiala xenobiotica	Madagascar	837	*Aedes albopictus*
	France	190	*Aedes albopictus*
	Italy	593	Gut of wasp
	Italy	8	Scolytid beetles
	Italy	52	*Xyleborinus saxesenii* and *Xylosandrus germanus*
	USA	53	Honeybee
	Madagascar	2	*Aedes albopictus*
	France	198	*Aedes albopictus*
	Italy	595	Gut of wasp
	Italy	10	Scolytid beetles
	Italy	1	Gut of wasp
	USA	54	Honeybee
Phialophora verrucosa	Italy	8	Gut of wasp
Rhinocladiella atrovirens	Italy	133	Gut of wasp

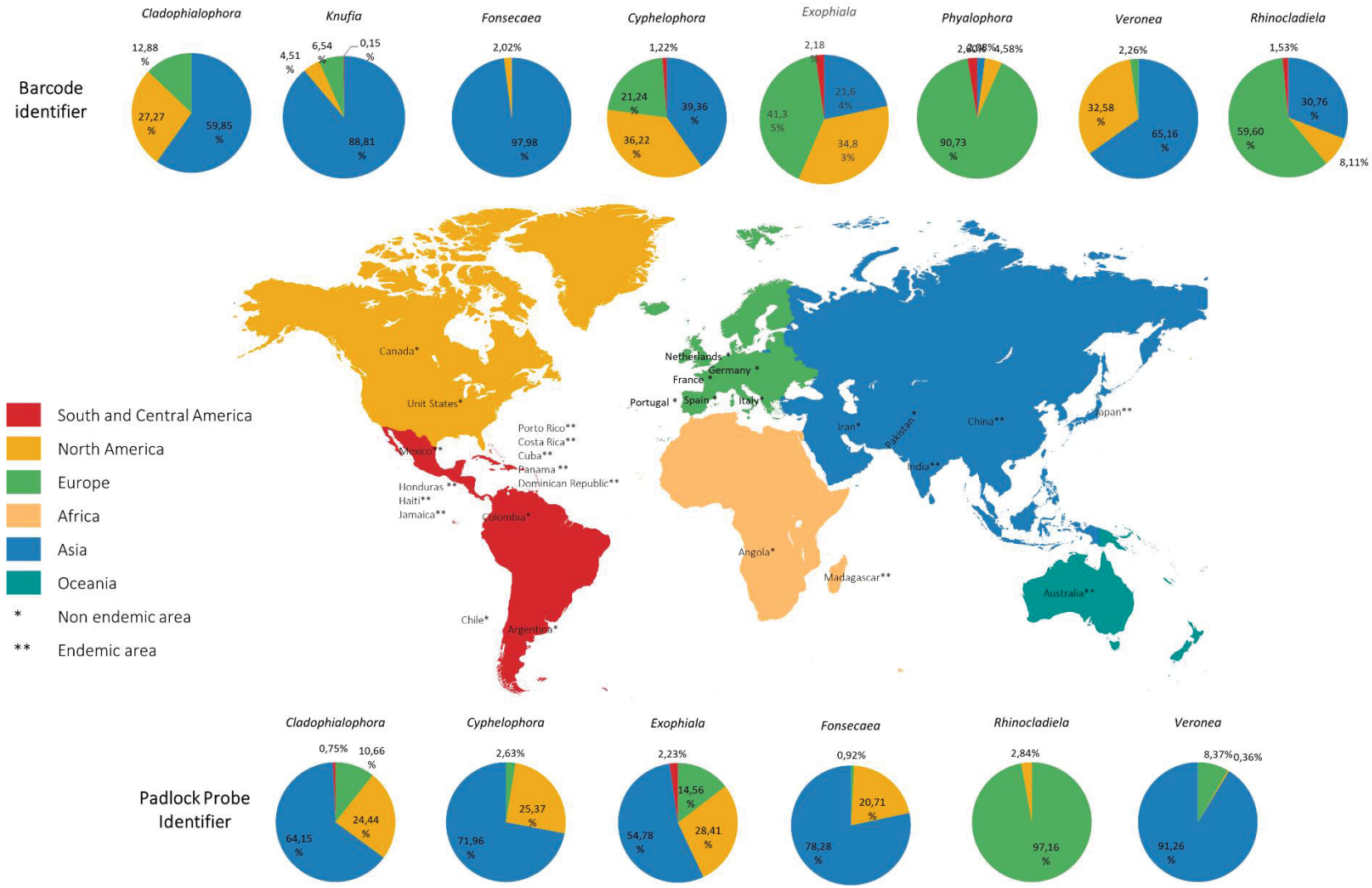

Figure 3- Worldwide distribution of Chaetothyriales genus. The countries selected to compose the data set are identified in the figure in each of the evaluated continents; The graphs represent the frequency of each genus in the continents evaluated, in the upper part the frequency was determined only with the data identified by sequences of barcodes, whereas in the lower part those identified by padlock probes.

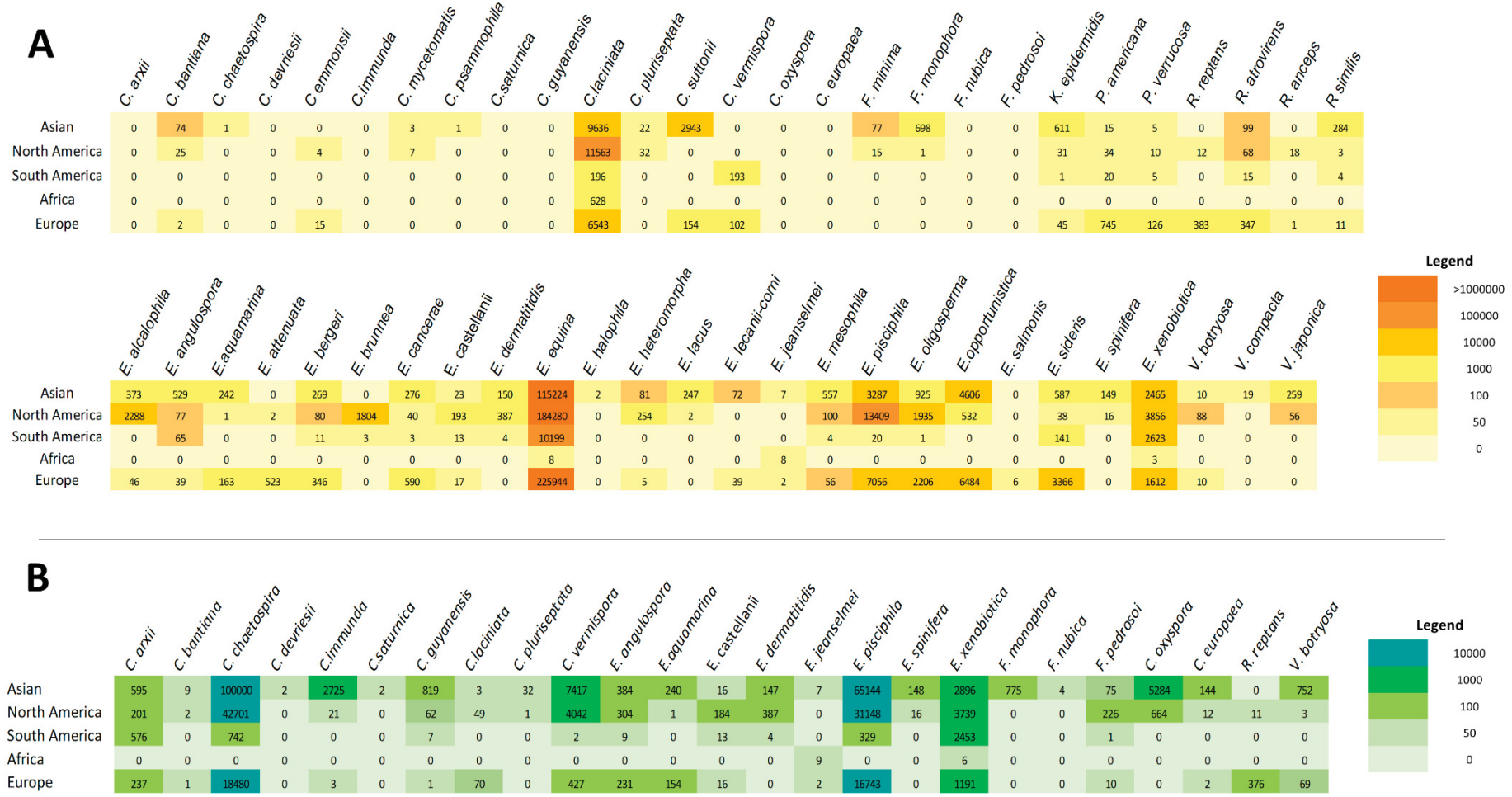

Figure 4- Heatmap of number species sequence identified in the data set analyzed. A- Barcode identifiers; B- Padlock probe identifiers

5. **DISCUSSION**

The metagenomic data indicate that chaetothyrialean fungi, mainly belonging to the family *Herpotrichiellaceae*, are omnipresent in subtropical as well as temperate climate zones. Earlier ecological studies appear inadequate, probably due to the fact that black fungi are poor competitors against neighboring microbes and therefore are difficult to isolate in culture. Technologies based on high-throughput DNA sequencing circumvents this problem and allows the investigation of big data with high accuracy of species identification. Costa et al., (2020) proposed this novel methodology to understand the environmental origin of black yeast-like fungi from Brazil. In the present study, we expand this analysis to investigate the presence of sequences of Chaetothyriales in environmental from 27 countries. The study was focused of environmental habitats of agents of chromoblastomycosis and phaeohyphomycosis agents, the latter being unexplored by conventional methods. The dataset represented the global diversity of black yeast-like fungi that have been characterized.

Some species of black yeast-like fungi appear to be quite common in a variety of substrates besides soil and decomposing material that were the subject of earlier environmental isolation studies (VICENTE et al., 2014; MARQUES et al., 2016). Some species as *Cladophialophora immunda*, *Exophiala lecani-corni*, *E. alcalophila*, *E. spinifera* and *Fonsecaea monophora* have been reported from soils before. In our study we also recovered the waterborne species *Exophiala aquamarina*. The enigmatic agent of cerebral phaeohyphomycosis, *Cladophialophora bantiana*, was found in soil in Spain and China. This fungus has rarely been isolated from natural sources other than human brain, where it causes (RATHOD et al, 2020; SLÁDEKOVA et al., 2014; DENG et al., 2016). The diversity of the of *Chaetothyriales* in soil was evidenced by a large number of species recognized (n=50) belonging to the genera *Exophiala*, *Cladophialophora*, *Cyphellophora*, *Phialophora*, *Rhinocladiella*, *Fonsecaea*, *Veronaea* and *Knufia*. Metagenome studies focusing on forest soils already initiated the high total pathogen diversity (VĚTROVSKÝ et al. 2020; BALDRIAN et al., 2020), but the black yeast group had remained unexplored in these studies.

Cladophialophora chaetospira was isolated from *Phyllostachys bambusoides* (Gramineae), decaying bamboo (CROUS et al. 2007). The species was described as an endophyte applied in disease suppression of fusariosis of strawberries (HARSONOWATI et al., 2020). *Exophiala dermatitidis* was reported in *Bothriochloa bladhii*, a perennial grass from the U.S.A., where cases have been reported in patients of nosocomial common-source outbreaks (WOOLLONS et al. 1996, ENGEMANN et al. 2002). Sudhadham et al. (2008) suggested

possible translocation from tropical fruits to the human environment. In metagenome data from China, the major agent of chromoblastomycosis, *F. pedrosoi* was identified in a leaf of *Coix lacryma-jobi*, and in addition, *F. monophora* was identified in wood. According to Santos et al. (2021), *Fonsecaea* species are the most frequent etiologic agents of chromoblastomycosis in Asia. The current hypothesis of CBM infection by trauma from plant material seems to be supported by these studies, and by the proven ability of the opportunists to survive in and colonize plant tissue (FORNARI et al, 2018). In addition, genome analysis demonstrated the presence genes that indicate a plant-associated lifestyle (VICENTE et al. 2017; MORENO et al., 2018).

The ability of black yeast in participate in plant decomposition recorded in several studies (VICENTE et al, 2014; COSTA et al, 2020; VOIDALESKI et al., 2020). In that sense the chaetothyrialean black yeasts are similar to moribund plant-inhabiting members of Pleosporales and Capnodiales such as *Alternaria* and *Cladosporium* (TEIXEIRA et al, 2017; SCHOCH et al. 2009). However, the Chaetothyriales, although present on the same substrate, may inhabit another micro niche. Species were abundant in the presence of hydrocarbon and terpene compounds, e.g., on scales of babassu coconuts (NASCIMENTO et al. 2017). *Cladophialophora bantiana* was reported in plant litter in France (PRJNA314749) and in Canada (PRJNA347436).). Though never related to that niche *F. monophora* was identified in fermentation study from China and *E. sideris* from wine fermentation from Portugal.

The rhizosphere and plan roots were reported by Costa et al. (2020) as important substrates for members of Herpotrichiellaceae, showing high diversity in rhizosphere (n=26) and root (n=23) including main CBM agents, *Fonsecaea monophora* and *F. nubica*. The strictly environmental species *F. minima* was present in litter material from China. Also, members of *Exophiala* and *Cladophialophora* were abundant in rhizosphere (XIAOLONG et al., 2022; LI et al.,2020; REN et al., 2021). The frequent association of Herpotrichiellaceae, including the opportunistic pathogens, in plant material is a new insight compared to conventional studies (VICENTE et al., 2001; 2008; 2014; NASCIMENTO et al, 2017; MARQUES et al, 2006; LIMA et al., 2020; SALGADO et al, 2006).

The ‘waterborne clade’ is a delimited group of *Exophiala* species in Herpotrichiellaceae associated with phaeohyphomycosis infections in cold-blooded animals, mainly fish, frogs, toads, turtles or crabs (DE HOOG et al., 2011; ORÉLIS-RIBEIRO et al., 2017; SEYEDMOUSAVI, GUILLOT, DE HOOG, 2013; GROFF et al., 2021). The group comprises *E. psychrophila*, *E. opportunistica*, *E. equina*, *E. salmonis*, *E. pisciphila*, *E. canceaea*, *E. aquamarina*, *E. salmonis*, *E. botryosa* and *E. brunnea* (DE HOOG et al., 2011). *Exophiala*

cancerae and *Fonsecaea brasiliensis* have been described as causing lethargic crab disease (LCD) in populations of the mangrove land crab *Ucides cordatus* disease (DE HOOG et al., 2011; VICENTE et al., 2012; ORÉLIS-RIBEIRO et al., 2017; ARMWOOD et al. 2021). Instead *Cladophialophora immunda*, *Cyphellophora oxyspora* and *E. oligosperma* were detected in mangrove data from India (Table 2). Four species of the salmonis-clade was reported in water sources analyzed in this study, *E. pisciphila, E. cancerae* and *E. opportunistica*. Some species was identified in freshwater as *C. bantiana* (Porto Rico), *F. monophora* (China) and *F. pedrosoi* (USA).

Rather pronounced association of black yeast with insects is a new finding. Chen et al. (2016) reported a possible insect route of transmission for *F. nubica* in a chromoblastomycosis case from China. Ancestral black yeasts are known to be associated with tropical social insects like ants (VOJVODIC et al., 2009; MORENO et al., 2019; QUAN et al., 2022; Mayr et al. 2022), and ant-related strains are scattered in the phylogeny of Herpotrichiellaceae (Y. Quan, unpublished data).

Several environmental species which were expected to be rather common, such as *Fonsecaea erecta* were no detected in this study, and neither *Cladophialophora carrionii* the main *Cladophialophora* species causing chromoblastomycosis (ROJAS et al., 2015; WEI; YU, 2021). *C. carrionii* is associated with hot, arid climates and occurs in Madagascar, a country subject of the present study.

In conclusion, metagenomic studies provide an entirely different view on the distribution of chaetothyrialean black fungi than acquired by conventional studies. These data are still preliminary and required careful consideration. For example, the high-throughput sequencing papers published so far show a significant geographical bias, with 243 samples per one million square km reported from Europe but only 5 samples per one million square km from Africa (BALDRIAN et al., 2021; DAVISON et al., 2021; VĚTROVSKÝ et al., 2020). The number of available projects is directly related to the number of species characterized. Developed countries that concentrate large research centers exploit new technologies such as metagenomics more abundantly, while developing or underdeveloped countries have low resources and technology (BALDRIAN et al., 2021; DAVISON et al., 2021; VĚTROVSKÝ et al., 2020; XU et al., 2020; NAYFACH et al., 2021). The increase of metagenomic studies and a sharing of this data with the scientific community will improve epidemiological studies, particularly with underexplored groups such as black yeasts.

The publicly available data were generated for different goals than our study and important information about the geography and substrate are insufficient for understanding the

ecology and risk assessment of black yeasts. Is crucial knowledge on micro niches inhabited by the black yeasts, their behavior in tissue and infection-related symptoms of the host are required; that demonstrate the conventional studies of isolation is essential to the ecology approaches studies. The unexpected global epidemiology of black yeasts as revealed by our metagenomic study will stimulate further research about the functional diversity of black yeasts, their life cycles, and survival strategies.

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SUPPLEMENTARY MATERIAL

Table S-1. Molecular markers described in the literature of Chaetothyriales.

Organism	Strain	Sequence (3' 5')	Reference
Cladophialophora arxii	CBS306.94	TAAACAAAGGGTTGGGAGGTCAGGCCTAGAAGACCCTAACTC	Hamzehei et al., 2013; Heinrichs, De Hoog, Haase, 2012
		ACGGTTTGGTGGAAGGGTGACACCCTTCCGCCCGT	
	CBS409.96	ACGGTTCGGTGGGGAAGGGGTACACCCTTCCACCCGT	
Cladophialophora bantiana	CBS173.52	ACGCTGGCCAGAGGACGCCGAGTCGGCGGTCCTCCGGCGGT	Hamzehei et al., 2013; Heinrichs, De Hoog, Haase, 2012
		ACGGTCTGGCGGAAGTGTCGTGCACCCCGCCCCT	
Cladophialophora carrionii	CBS160.54	AGAGTTTGGGGTTGGCTGTCGGCGGACACGGGCCCAGAG	Hamzehei et al., 2013; Heinrichs, De Hoog, Haase 2012; Deng et al., 2014
		GTCTTTAGGAGGGGTCGAGAACACTCGACCAAAACCGTCCAA	
		ACGGTTTTGGTCGAGTGTTCTCGACCCCT	
	CBS163.54	ACGGTCTTGGTCGAGTGTTCTCGACCCCT	
	CBS260.83	ACGGTTTTGGTCGAGTGTTCTCGACCCCT	
	FMC.248	ACGGTCTTGGTCGAGTGTTCTCGACCCCC	
Cladophialophora chaetospira	CBS 115468	GTCCTCCGGCGGCCGTGAGGCGGGTCCGCCGAAGCAACA	Hamzehei et al., 2013
Cladophialophora devriesii	CBS147.84	GAATAAATTCACTTAGACAGTAAAATCATGGTTATTCCAGAG	Hamzehei et al., 2013 Heinrichs, De Hoog, Haase 2012
		ACGGCTTGGTAGAGTCCCTACCCCT	
	IFM51369	ACGGCTTGGTAAATTCCCTACCCCT	
Cladophialophora emmonsii	CBS979.96	ACGGCCTGGCGGAGTGCACGACACTCTGCCCCT	Heinrichs, De Hoog, Haase 2012
	CBS640.96	ACGGCCTGGCGGAGTGCACGACACCCTGCCCCT	
Cladophialophora immunda	CBS834.96	GGCGGTCCTCCGGCGGTCCGCTTGAGACGGGTCCGCCGAAGC	Hamzehei et al., 2013; Heinrichs, De Hoog, Haase 2012
		GACACTGGCCAGAGGACGCACGCCACGGTGGGCGGTCCTCC	
		ACGGTTTGGTAGGGCGCCTACCCCT	
	CBS102227	ACGGTTTGGTAGAGTGCCTACCCCT	
Cladophialophora minourae	CBS 987.96	CGGTCCTCCGGCGGTCTTTATGGGACGGGTCCGCCAAAGCAA	Hamzehei et al., 2013
Cladophialophora modesta	CBS985.96	AATCTCAGTTGAGTGATCAACTGGTT	Heinrichs, De Hoog, Haase 2012

Table S-1. Continued.

Cladophialophora mycetomatis	CBS122637	ACGGTTTGGTCGACGACACCCGACCCCT	Heinrichs, De Hoog, Haase 2012
	CBS 454.82	ACGGTTTGGTCGACGACATCCGACCCCT	
Cladophialophora psammophila	CBS110553	CAGAGGACGCCGAGTCGGCAGTCCTCCGGCGGTCAGTTTAA	Hamzehei et al., 2013; Heinrichs, De Hoog, Haase 2012
		ACGGTCTGGCGGAAGTGTCGTGCACGCCGCCCCT	
Cladophialophora samoensis	CBS259.83	AGGGTCCCTGGTCGAGGCTCTACCTCGACCT	Heinrichs, De Hoog, Haase 2012
Cladophialophora saturnica	CBS118724	GAATAGGCTATCGGCGGACAACGGGCCAGAGGACGCCCAGGT	Hamzehei et al., 2013; Heinrichs, De Hoog, Haase 2012
		ACGGCTTGGTAGAGTTCCCTCTACCCT	
Cladophialophora subtilis	CBS122642	ACGGCCTTGGTCGCGCACGCGCCGACCCCT	Heinrichs, De Hoog, Haase 2012
Cladophialophora yegresii	CBS11440	GACGCCTCACGGCGATCCCACGGGCGGTCGTGAGACGGGTCC	Hamzehei et al., 2013; Heinrichs, De Hoog, Haase 2012
		ACGGTTTTGGTCGAGTGTCCCTCGACCCCT	
Cyphellophora europaea	CBS129.96	GGGCCCGGGGTCCTTGCGGACCCCCGGCGGCCTC	Heinrichs, De Hoog, Haase 2012, Feng et al., 2013
		AGCGGCCCCCCCGCTCTT	
Cyphellophora guyanensis	CBS 124764	AACTCAGACGACACGTTTAAGTACAAGAGTTTGTGGTTGGGG	Feng et al., 2013
Cyphellophora laciniata	CBS 190.61	CCCGGCGGCCACCCCTTGCGGGGCGGCGGGCCT	Heinrichs, De Hoog, Haase 2012, Feng et al., 2013
		ACGTCGGCGGCGGCCTTTAGTGGCGTACCGCCCGT	
Cyphellophora oxyspora	CBS 698.73	CCGGGCCCCAAAGGGACACCAGCGGGCCTGCCGAAGC	Feng et al., 2013
Cyphellophora pluriseptata	CBS 286.85	CCCGGTGAGGGGGAGGTTGCGGCGGGCCTGCCGAAG	Heinrichs, De Hoog, Haase 2012, Feng et al., 2013
		ACGCGGCGGCGGCGCACCCGCCCCCGCCCGT	
Cyphellophora suttonii	CBS449.91	ATGTTGGCGGCGGCCTTTAGTGGCGTACCGCCCGT	Heinrichs, De Hoog, Haase 2012
Cyphellophora vermispora	CBS228.86	GGACCCCCGGCGGCTGCTCCTCGCGGGGCGGC	Heinrichs, De Hoog, Haase 2012, Feng et al., 2013
		ACGTCGGCGGCGGCCTTTAGTGGCGTACCGCCCGT	
Exophiala alcalophila	CBS520.82	ACGGCTTGGTTACGCTCCCCGCGTGACCCCT	Heinrichs, De Hoog, Haase 2012
Exophiala angulospora	CBS 119911	GTTGCTTCGGCGGGCCCGTCTTCGTGACCGCCGGAG	Heinrichs, De Hoog, Haase 2012, Najafzadeh et al., 2018
	CBS482.92	ACGGCCTGGTCGAGTCCGCTCGACCCCT	
	CBS122264	ACGGCCTGGTCGAGTCCCCTCGACCCCT	
	CBS109906	ACGGCTTGGTCGAGTCCCCTCGACCCCT	

Table S-1. Continued.

Exophiala aquamarina	CBS 119915	TTGGACGGCTTGGTGGACGCCCCGCACGGGGCGTCCA	Heinrichs, De Hoog, Haase 2012, Najafzadeh et al., 2018
	CBS 119918	ACGGCTTGGTGGACGCCCCGCACGGGGCGTCCACCCCT	
Exophiala asiatica	BMU00015	ACGGCCCGGTCGACGCGTCATTTAGCGCCGACCCCT	Heinrichs, De Hoog, Haase 2012
Exophiala attenuata	CBS110026	ACGGTTTGGTGTCTGGCAACGGTCACCCCT	Heinrichs, De Hoog, Haase 2012
Exophiala bergeri	CBS353.52	ACGGTCTGGTTTAGGCACCGCCTAGACCCCT	Heinrichs, De Hoog, Haase 2012
Exophiala brunnea	CBS587.66	ACGGTTTGGTGGAGGCCCCTCACGGGGCTCCTGCCCCT	Heinrichs, De Hoog, Haase 2012
Exophiala cancerae	CBS120532	ACGGTTTGGTGGAGGGCCTTCGGGCGCCACCCCT	Heinrichs, De Hoog, Haase 2012
Exophiala capensis	CBS128771	ACGGCTCGGTCTAGGTACCGCCTAGACCCCT	Heinrichs, De Hoog, Haase 2012
Exophiala castellanii	CBS 110025	TTGGTGTTGGACGGTTTGGTGTCGGCAACGTCACCCC	Heinrichs, De Hoog, Haase 2012, Najafzadeh et al., 2013
	CBS158.58	ACGGTTTGGTGTCGGCAACGTCACCCCT	
Exophiala dermatitidis	CBS207.35	AGGGGTCGCGCGGAAACGCTCGACCAGACCGTCCAA	Heinrichs, De Hoog, Haase 2012, Najafzadeh et al., 2013
		ACGGTCTGGTCGAGCGTTTCCGCGCGACCCCT	
	CBS100338	ACGGTATGGTCGAGCGTTTCCGCGCGACCT	
	BMU00035	ACCGGTCTGGTCAAGCGTTTCCGCGCGACCCT	
Exophiala equina	CBS 109789	TRGTTAAAGATTTTAATGGTTGGGCTACCGACGAGCG	Heinrichs, De Hoog, Haase 2012, Najafzadeh et al., 2018
	CBS119.23	ACGGTTTGGTGGAGGCCCCCTCGGGGGCTCCTGCCCCT	
	CBS122263	ACGGTTTGGTGGAGGCCCCCTCGGGGGTTCCTGCCCCT	
	CBS122270	ACGGTTTGGTGGAGGTCCCCTCGGGGGCTCCTGCCCCT	
Exophiala exophialae	CBS668.76	ACGGTTTGGTCCCGGGACGCCCCTGGACCCCT	Heinrichs, De Hoog, Haase 2012
Exophiala halophila	CBS121512	ACGGCTTGGTCGCGCCCCCCGGGCACCGACCCCT	Heinrichs, De Hoog, Haase 2012
Exophiala heteromorpha	CBS232.33	ACGGTCTGGTCGAGCCCGCCTCGACCCCT	Heinrichs, De Hoog, Haase 2012
Exophiala jeanselmei	CBS507.90	GTGTACCGGGGGTCGAACAGCCCGCCGTCATTGTCTT	Heinrichs, De Hoog, Haase 2012, Najafzadeh et al., 2013
		ACGGTTTGGTCTCGGGTCCGACCCCCCTTGACCCCT	
	CBS677.76	ACGGTTTGGTCAAAGGGTCCGACCCCCCTAGACCCCT	
Exophiala lacus	CBS117497	ACGGTTTGGCGGAGACCTGTTACAGGCCTCCACCCCT	Heinrichs, De Hoog, Haase 2012
Exophiala lecanii-corni	CBS123.33	ACGGCCTGGCGTCGGCGACGACCCCACCT	Heinrichs, De Hoog, Haase 2012

Table S-1. Continued.

Exophiala mesophila	CBS402.95	ACGGCTTGGTGTCAGCGATGTCACCCCT	Heinrichs, De Hoog, Haase 2012
	CBS121511	ACGGCTTGGTGTCAGCAATGTCACCCCT	
Exophiala moniliae	CBS520.76	ACGGTCTGGTTAGGCGACTGACCCCT	Heinrichs, De Hoog, Haase 2012
Exophiala nishimurae	CBS101538	ACGGCTTGGTCTAGGTCGTCGCCTAGACCCCT	Heinrichs, De Hoog, Haase 2012
Exophiala oligosperma	CBS725.88	TTTGAGGTCCCCGRACCARACCGTCCAACACCAAGCC	Heinrichs, De Hoog, Haase 2012, Najafzadeh et al., 2013
		ACGGTCTGGTCCGGGGACCTCAAACCCCCTGGACCCCT	
	UTHSC95-2041	ACGGTTTGGTTCGGGGACCTCAAACCCCCTGGACCCCT	
	UTHSC91-870	ACGGTTTGGTCCGGGGACCTCAAAGCCCCCTGGACCCCT	
	IFM41701	ACGGTTTGGTCCGGGGACCTCAAAAACCCCCTGGACCCCT	
Exophiala opportunistica	CBS 631.69	CGGACCGCCGGGGGGTCTTYGYACCCCTTGGCCCGC	Heinrichs, De Hoog, Haase 2012, Najafzadeh et al., 2018
	CBS109811	ACGGTTTGGTGGAGACCCCCTTCGCGGGCTTCTACCCCT	
Exophiala pisciphila	CBS119913	GTTGCTTCGGCGAGCCCGTCTGTAATGGACCGCCGGG	Heinrichs, De Hoog, Haase 2012, Najafzadeh et al., 2018
	CBS 537.73	ACGGTTTGGTGGAGGCCCCCTCGCGGGGCCACCGCCCCT	
Exophiala salmonis	CBS 110371	GGGGCAGATGCCCGCAAGGGGCCTCCACCAAACCGTC	Hamzehei et al., 2013; Heinrichs, De Hoog, Haase 2012
	CBS 120274	GGGGCAGATGCCCGCAAGGGGCCTCCACCAAACCGTC	
	CBS157.67	ACGGTTTGGTGGAGGCCCCTTGCGGGCATCTGCCCCT	
Exophiala sideris	CBS121818	ACGGTTTGGTCCAGGTCACCTGGACCCCT	Heinrichs, De Hoog, Haase 2012
Exophiala siphonis	UTHSC88-471	ACGGTCTGGTCGAGCTGCTCGACCCCT	Heinrichs, De Hoog, Haase 2012
Exophiala spinifera	CBS899.68	GAGGGGTCCAGGGGGGTCCCGGGACCAAACCGTC	Heinrichs, De Hoog, Haase 2012, Najafzadeh et al., 2013
		ACGGTTTGGTCCCGGGACCCCCCTGGACCCCT	
Exophiala xenobiotica	CBS118157	CGTGCTCAGTTAAGAAGCTCAGTGTACCGGGGGTTCA	Heinrichs, De Hoog, Haase 2012, Najafzadeh et al., 2013
		ACGGTTTGGTTTAGGTACCCCTAGACCCCT	
	CBS119306	ACGGTTTGGTTTAGGCACCCCTAGACCCCT	
	CBS117665	ACGGTTTGGTTTAGGTACCCCTAGATCCCT	
	CBS117676	AGGTCTTGGTGTAGGGTTCCCCCCTCCACCCCT	
	CBS117641	AGGTCTTGGTGTAGGGTCCCCCCTACACCCCT	

Table S-1. Continued.

Fonsecaea erecta	CBS125763	ACGGTCCGGTGGAGAGTCATCCTTTCCACCCGT	Heinrichs, De Hoog, Haase 2012
	CBS125764	ACGGTCCGGTGGAGAGTCATCCTTTTCACCCGT	
Fonsecaea minima	CBS126865	ACGGTTCGGTGGAGAGTCACACCTCCCACCCGT	Heinrichs, De Hoog, Haase 2012
Fonsecaea monophora	CBS269.37	CAACGCCCGCATTGAGCGGTCCTCCAGC	Heinrichs, De Hoog, Haase 2012, Najafzadeh et al., 2011
		ACGGCTTGGTGGAGTAAGTTCACACTTTCCACCCCT	
	CBS121732	ACGGCTTGGTGGAGCAAGTTCACACTTTCCACCCCT	
	IFM4889	ACGGCTTGGTGGAGTAAGTTCACGCTTTCCACCCCT	
	IFM54446	ACGGCTTGGTGGAGTAGGTTCACGCTTTCCACCCCT	
Fonsecaea multimorphosa	CBS980.96	ACGGCTCGGTGGACTCCTTCCACCCGT	Heinrichs, De Hoog, Haase 2012
Fonsecaea nubica	CBS269.64	CGTCCAACACCAAGCGCAGGGGCTTGAGGGGGTGAT	Heinrichs, De Hoog, Haase 2012, Najafzadeh et al., 2011
		ACGGTTTGGTGGAGCGAGTTCACACTTTCCACCCCT	
	CBS121733	ACGGCTTGGTGGAGCGAGGTCACACTTTCCACCCCT	
	CBS557.76	ACGGCTTGGTGGAGCGAGTTCACACTTTCCACCCCT	
Fonsecaea pedrosoi	CBS271.37	CGATACGTGCTCAATAAAGAAGCTCAGTGTACCGGG	Heinrichs, De Hoog, Haase 2012, Najafzadeh et al., 2011
		ACGGCTTGGTGGAGCGAGTTCACACTTTCCACCCCT	
	CBS122741	ACGGCTTGGTGGAGCGAGTTCACACTTTCCACCCCCT	
Fonsecaea pugnacius	CBS 139214	CGCTGGAGGACCGCCTGTCACGGGTGTTCTC	Schneider et al., 2019
Knufia epidermidis	CBS120353	ACCCAAGTTTTTGGCTATTAAAAACTTGGT	Heinrichs, De Hoog, Haase 2012
Phaeoannelomyces elegans	CBS110172	ACGGTTTGGTGGAGGCCCCCTCGGGGGCTCCTGCCCCT	Heinrichs, De Hoog, Haase 2012
Phialophora americana	CBS840.69	ACGGATTTTGGTCGTGTAACAACGACCCCT	Heinrichs, De Hoog, Haase 2012
Phialophora reptans	CBS113.85	CGCGAAGCTCCCGCCGGGTCCAACAACAAGCCGGGCT	Heinrichs, De Hoog, Haase 2012, Feng et al., 2013
		ACCCGGCGGGAGCTTCGCGCCCCCGCCCGT	
Phialophora verrucosa	CBS273.37	ACGGATCTTGGTCGTGTGATCGCGACCCCT	Heinrichs, De Hoog, Haase 2012
	IMTSP.800	ACGGATCCTGGTCGTGTGATCGCGACCCCT	
	CBS286.47	ACGGATTTTGGTCGTGTGATATCGACCCCT	
	CBS839.68	ACGGATTTTGGTCGTGTGATACCGACCCCT	

Table S-1. Continued.

Rhinocladiella anceps	CBS181.65	AAGGCCTGGTGTCCCCCCGGGGACACCCCT	Heinrichs, De Hoog, Haase 2012
	CBS157.54	AAGGCCTGGTGTCTCCCCCCGGGGATACCCCT	
Rhinocladiella aquaspersa	CBS313.73	ACGGTCGGCCTCTTCACCGAGGCCCCT	Heinrichs, De Hoog, Haase 2012
Rhinocladiella atrovirens	CBS264.49	ACGGCTGGTCGGGCGACGCCCGACCCCT	Heinrichs, De Hoog, Haase 2012
Rhinocladiella basitona	CBS101460	ACGGTTTGGTCTAGGGCCTCCCTAGACCCCT	Heinrichs, De Hoog, Haase 2012
Rhinocladiella mackenziei	CBS650.93	ACGGCCTGGGTTTGGAAGTTTTCTCTCGAGGCCCCT	Heinrichs, De Hoog, Haase 2012
	CBS367.92	ACGGCCTGGGTTTGGAAGTTTTCTCTCGAGCCCCT	
	CBS102590	ACGGCCTGGGTTTGGAAGTTTTCTCTCAAGCCCCT	
Rhinocladiella similis	CBS111763	ACGGTTTGGTCCAGGGCCCCCCCTGGACCCCT	Heinrichs, De Hoog, Haase 2012
	dH13054	ACGGTTTGGTCCAGGGTCCCCCCTGGACCCCT	
Veronaea botryosa	CBS 121506	CGCCGGGGGACCCCTAACAGAGTCTCCTGGCCCGC	Heinrichs, De Hoog, Haase 2012, Najafzadeh et al., 2018
	CBS254.57	ACGGTTTGGTGGAGGCCCCCTCGTCGGGGTCGCTGCCCCT	
	CBS350.65	ACGGTTTGGTGGAGGCCCCCCTCGTCGGGGTCGCTGCCCCT	
Veronaea compacta	CBS268.75	ACGGTTTGGCGGAGGCCCCTCGCGGGGCTCTCGCCCCT	Heinrichs, De Hoog, Haase 2012
Veronaea japonica	CBS776.83	ACGGTTTGGCGGAGGCCCCCTCGGGGGCTCTCGCCCCT	Heinrichs, De Hoog, Haase 2012

Table S-2- Species identified in metagenomic datasets.

Fungi	ASIAN								NORTH AMERICA											
	China		India		Japan		Pakistan		Canada		United States		Mexico		Puerto Rico		Costa Rica		Panama	
	*	**	*	**	*	**	*	**	*	**	*	**	*	**	*	**	*	**	*	**
Cladophialophora arxii CBS	0	595	0	0	0	0	0	0	0	24	0	172	0	0	0	0	0	4	0	1
Cladophialophora bantiana CBS 173.52	74	9	0	0	0	0	0	0	24	2	0	0	0	0	1	0	0	0	0	0
Cladophialophora chaetospira CBS	0	109341	0	0	0	1	0	0	0	9625	0	32562	0	115	0	0	0	5	0	394
Cladophialophora devriesii	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Cladophialophora emmonsii CBS 979.96	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0
Cladophialophora emmonsii CBS 640.96	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0
Cladophialophora immunda	0	2679	0	43	0	0	0	3	0	3	0	12	0	0	0	0	0	0	0	6
Cladophialophora mycetomatis CBS 122637	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	7	0	0	0
Cladophialophora mycetomatis CBS 454.82	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Cladophialophora psammophila CBS 110553	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Cladophialophora saturnica	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Cyphellophora guyanensis CBS	0	819	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	43	0	19
Cyphellophora laciniata CBS 190.61	9629	0	0	0	6	0	1	3	1085	0	8826	49	0	0	0	0	1650	0	2	0
Cyphellophora pluriseptata CBS 286.85	22	32	0	0	0	0	0	0	0	0	32	1	0	0	0	0	0	0	0	0
Cyphellophora suttonii CBS 449.91	2638	0	0	0	0	0	305	0	0	0	0	0	0	0	0	0	0	0	0	0
Cyphellophora vermispora CBS 228.86	0	7417	0	0	0	0	0	0	0	1463	0	2579	0	0	0	0	0	0	0	0
Cyphellophora oxyspora	0	2248	0	74	0	0	0	2962	0	0	0	39	0	0	0	0	0	625	0	0
Cyphellophora europaea	0	144	0	0	0	0	0	0	0	0	0	12	0	0	0	0	0	0	0	0
Cyphellophora reptans CBS 113.85	0	0	0	0	0	0	0	0	1	1	11	10	0	0	0	0	0	0	0	0
Exophiala alcalophila CBS 520.82	313	0	0	0	60	0	0	0	12	0	2276	0	0	0	0	0	0	0	0	0
Exophiala angulospora CBS 482.92	502	384	0	0	3	0	0	0	0	0	0	303	0	0	0	0	51	1	0	0
Exophiala angulospora CBS 122264	9	0	0	0	0	0	0	0	12	0	4	0	0	0	0	0	0	0	0	0
Exophiala angulospora CBS 109906	15	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	10	0	0	0
Exophiala aquamarina CBS 119918	242	240	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0
Exophiala attenuata CBS 110026	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0
Exophiala bergeri CBS 353.52	238	0	0	0	30	0	1	0	9	0	71	0	0	0	0	0	0	0	0	0
Exophiala brunnea CBS 587.66	0	0	0	0	0	0	0	0	0	0	1144	0	0	0	25	0	523	0	112	0
Exophiala cancerae CBS 120532	273	0	0	0	3	0	0	0	1	0	34	0	0	0	4	0	1	0	0	0
Exophiala castellanii CBS 158.58	16	16	0	0	7	7	0	0	0	0	10	10	0	0	3	3	180	171	0	0

*Identification by only padlock probes; **only barcodes; ***padlock probes and barcodes simultaneously

Table S-2- Continued.

Fungi	ASIAN								NORTH AMERICA											
	China		India		Japan		Pakistan		Canada		United States		Mexico		Puerto Rico		Costa Rica		Panama	
	*	**	*	**	*	**	*	**	*	**	*	**	*	**	*	**	*	**	*	**
Exophiala equina CBS 122263	8144	0	0	0	756	0	0	0	27052	0	10097	0	0	0	0	0	0	0	0	0
Exophiala equina CBS 122270	3971	0	0	0	5	0	0	0	41066	0	2497	0	0	0	0	0	0	0	0	0
Exophiala halophila CBS 121512	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Exophiala heteromorpha CBS 232.33	81	0	0	0	0	0	0	0	9	0	245	0	0	0	0	0	0	0	0	0
Exophiala lacus CBS 117497	247	0	0	0	0	0	0	0	1	0	1	0	0	0	0	0	0	0	0	0
Exophiala lecanii-corni CBS 123.33	57	0	0	0	15	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Exophiala jeanselmei CBS 507.90	7	7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Exophiala mesophila CBS 402.95	499	0	0	0	49	0	0	0	0	0	5	0	0	0	5	0	5	0	0	0
Exophiala mesophila CBS 121511	9	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	83	0	1	0
Exophiala pisciphila CBS 537.73	3286	65144	0	0	1	0	0	0	17	22	3309	31095	0	8	6	0	10077	5	0	18
Exophiala oligosperma CBS 725.88	739	0	0	0	0	0	0	0	34	0	15	0	0	0	0	0	0	0	0	0
Exophiala oligosperma UTHSC95-2041	24	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Exophiala oligosperma UTHSC91###870	154	0	8	0	0	0	0	0	0	0	1870	0	0	0	0	0	9	0	7	0
Exophiala opportunistica CBS 109811	4605	0	1	0	0	0	0	0	175	0	356	0	0	0	0	0	0	0	1	0
Exophiala salmonis CBS 157.67	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Exophiala sideris CBS 121818	556	0	0	0	31	0	0	0	36	0	1	0	0	0	0	0	1	0	0	0
Exophiala spinifera CBS 899.68	149	148	0	0	0	0	0	0	0	0	5	5	0	0	0	0	11	11	0	0
Exophiala xenobiotica CBS 118157	1753	2448	0	3	282	445	0	0	3181	3168	506	566	4	4	0	0	0	1	0	0
Exophiala xenobiotica CBS 119306	157	0	0	0	134	0	0	0	30	0	102	0	0	0	0	0	0	0	0	0
Exophiala xenobiotica CBS 117665	112	0	0	0	24	0	0	0	12	0	20	0	0	0	0	0	1	0	0	0
Exophiala xenobiotica CBS 117676	0	0	0	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Fonsecaea minima dH20511	77	0	0	0	0	0	0	0	0	0	15	0	0	0	0	0	0	0	0	0

*Identification by only padlock probes; **only barcodes; ***padlock probes and barcodes simultaneously

Table S-2- Continued.

Fungi	ASIAN								NORTH AMERICA											
	China		India		Japan		Pakistan		Canada		United States		Mexico		Puerto Rico		Costa Rica		Panama	
	*	**	*	**	*	**	*	**	*	**	*	**	*	**	*	**	*	**	*	**
Fonsecaea monophora CBS 269.37	0	775	695	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0
Fonsecaea monophora CBS 121732	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Fonsecaea monophora IFM4889	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Fonsecaea nubica	0	0	0	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Fonsecaea pedrosoi	0	73	0	0	0	2	0	0	0	1	0	33	0	0	0	0	0	181	0	11
Knufia epidermidis CBS 120353	588	0	0	0	23	0	0	0	10	0	20	0	0	0	0	0	1	0	0	0
Phialophora americana CBS 840.69	15	0	0	0	0	0	0	0	0	0	34	0	0	0	0	0	0	0	0	0
Phialophora verrucosa CBS 286.47	4	0	0	0	0	0	1	0	0	0	8	0	0	0	0	0	0	0	1	0
Phialophora verrucosa CBS 839.68	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
Rhinocladiella atrovirens CBS 264.49	99	0	0	0	0	0	0	0	45	0	23	0	0	0	0	0	0	0	0	0
Rhinocladiella anceps CBS 181.65	0	0	0	0	0	0	0	0	4	0	8	0	0	0	0	0	6	0	0	0
Rhinocladiella similis dH13054	284	0	0	0	0	0	0	0	1	0	2	0	0	0	0	0	0	0	0	0
Veronaea botryosa CBS 254.57	0	752	0	0	0	0	0	0	0	0	1	2	0	1	0	0	42	0	8	0
Veronaea botryosa CBS 350.65	8	0	0	0	2	0	0	0	0	0	14	0	0	0	0	0	9	0	14	0
Veronaea compacta CBS 268.75	17	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Veronaea japonica CBS 776.83	251	0	0	0	8	0	0	0	25	0	31	0	0	0	0	0	0	0	0	0
Total	139694	193417	1829	124	2994	460	310	2968	133843	14694	74512	67453	4	128	45	3	12710	1047	147	449

*Identification by only padlock probes; **only barcodes; ***padlock probes and barcodes simultaneously

Figure S-2. Continued.

Fungi	SOUTH AMERICA						AFRICA		EUROPE											
	Chile		Argentina		Colombia		Madagascar		Netherlands		Germany		France		Spain		Portugal		Italy	
	*	**	*	**	*	**	*	**	*	**	*	**	*	**	*	**	*	**	*	**
Cladophialophora arxii CBS	0	44	0	530	0	2	0	0	0	0	0	0	0	4	0	221	0	0	0	12
Cladophialophora bantiana CBS 173.52	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	1	0	0	0	0
Cladophialophora chaetospira CBS	0	316	0	426	0	0	0	0	0	2	0	1258	0	2589	0	10911	0	207	0	3513
Cladophialophora devriesii	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Cladophialophora emmonsii CBS 979.96	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0
Cladophialophora emmonsii CBS 640.96	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	13	0	0	0
Cladophialophora immunda	0	0	0	0	0	0	0	0	0	0	0	0	0	3	0	0	0	0	0	0
Cladophialophora mycetomatis CBS 122637	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Cladophialophora mycetomatis CBS 454.82	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Cladophialophora psammophila CBS 110553	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Cladophialophora saturnica	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Cyphellophora guyanensis CBS	0	7	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0
Cyphellophora laciniata CBS 190.61	0	0	193	0	3	0	628	0	6	0	1421	0	853	0	1443	0	2718	0	102	70
Cyphellophora pluriseptata CBS 286.85	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Cyphellophora suttonii CBS 449.91	0	0	0	0	0	0	0	0	0	0	0	0	0	0	149	0	5	0	0	0
Cyphellophora vermispora CBS 228.86	0	0	193	2	0	0	0	0	0	0	0	0	0	72	0	0	0	0	102	355
Phialophora oxyspora	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Phialophora europaea	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2
Phialophora reptans CBS 113.85	0	0	0	0	0	0	0	0	0	0	340	347	30	15	13	14	0	0	0	0
Exophiala alcalophila CBS 520.82	0	0	0	0	0	0	0	0	0	0	0	0	43	0	0	0	3	0	0	0
Exophiala angulospora CBS 482.92	2	9	0	0	0	0	0	0	0	0	0	5	0	136	1	77	0	0	0	13

*Identification by only padlock probes; **only barcodes; ***padlock probes and barcodes simultaneously

Figure S-2. Continued.

Fungi	SOUTH AMERICA						AFRICA		EUROPE											
	Chile		Argentina		Colombia		Madagascar		Netherlands		Germany		France		Spain		Portugal		Italy	
	*	**	*	**	*	**	*	**	*	**	*	**	*	**	*	**	*	**	*	**
Exophiala angulospora CBS 122264	0	0	0	0	0	0	0	0	0	0	0	0	0	0	38	0	0	0	0	0
Exophiala angulospora CBS 109906	63	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Exophiala aquamarina CBS 119918	0	0	0	0	0	0	0	0	0	0	0	0	0	0	163	154	0	0	0	0
Exophiala attenuata CBS 110026	0	0	0	0	0	0	0	0	0	0	523	0	0	0	0	0	0	0	0	0
Exophiala bergeri CBS 353.52	11	0	0	0	0	0	0	0	0	0	0	0	0	0	5	0	0	0	341	0
Exophiala brunnea CBS 587.66	0	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Exophiala cancerae CBS 120532	0	0	3	0	0	0	0	0	0	0	589	0	1	0	0	0	0	0	0	0
Exophiala castellanii CBS 158.58	1	1	12	12	0	0	0	0	0	0	4	4	4	4	9	8	0	0	0	0
Exophiala dermatitidis CBS 207.35	0	0	4	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Exophiala equina CBS 119.23	2875	0	689	0	10	0	8	0	356	0	79326	0	31354	0	20063	0	10514	0	890	0
Exophiala equina CBS 122263	7	0	8	0	0	0	0	0	43	0	60358	0	14098	0	3651	0	1678	0	303	0
Exophiala equina CBS 122270	3407	0	3203	0	0	0	0	0	1	0	1797	0	683	0	364	0	32	0	433	0
Exophiala halophila CBS 121512	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Exophiala heteromorpha CBS 232.33	0	0	0	0	0	0	0	0	0	0	0	0	0	0	5	0	0	0	0	0
Exophiala lacus CBS 117497	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Exophiala lecanii-corni CBS 123.33	0	0	0	0	0	0	0	0	0	0	0	0	0	0	39	0	0	0	0	0
Exophiala jeanselmei CBS 507.90	0	0	0	0	0	0	8	9	0	0	0	0	2	2	0	0	0	0	0	0
Exophiala mesophila CBS 402.95	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1	0	0	0
Exophiala mesophila CBS 121511	0	0	0	0	4	0	0	0	0	0	0	0	0	0	52	0	2	0	0	0
Exophiala pisciphila CBS 537.73	0	195	20	134	0	0	0	0	0	0	44	1	42	16255	549	377	6421	0	0	110
Exophiala oligosperma CBS 725.88	0	0	0	0	0	0	0	0	2	0	2204	0	0	0	0	0	0	0	0	0
Exophiala oligosperma UTHSC95-2041	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Exophiala oligosperma UTHSC91###870	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Exophiala opportunistica CBS 109811	0	0	0	0	0	0	0	0	2	0	372	0	5423	0	617	0	62	0	8	0
Exophiala salmonis CBS 157.67	0	0	0	0	0	0	0	0	0	0	0	0	0	0	6	0	0	0	0	0

Figure S-2. Continued.

Fungi	SOUTH AMERICA						AFRICA		EUROPE											
	Chile		Argentina		Colombia		Madagascar		Netherlands		Germany		France		Spain		Portugal		Italy	
	*	**	*	**	*	**	*	**	*	**	*	**	*	**	*	**	*	**	*	**
Exophiala sideris CBS 121818	73	0	68	0	0	0	0	0	0	0	602	0	69	0	416	0	7	0	2272	0
Exophiala spinifera CBS 899.68	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Exophiala xenobiotica CBS 118157	1125	2433	22	20	0	0	3	6	0	3	44	60	214	211	59	155	6	9	1256	753
Exophiala xenobiotica CBS 119306	43	0	0	0	0	0	0	0	0	0	0	0	0	0	28	0	0	0	2	0
Exophiala xenobiotica CBS 117665	1433	0	0	0	0	0	0	0	3	0	0	0	0	0	0	0	0	0	0	0
Exophiala xenobiotica CBS 117676	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Fonsecaea minima dH20511	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Fonsecaea monophora CBS 269.37	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Fonsecaea monophora CBS 121732	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Fonsecaea monophora IFM4889	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Fonsecaea nubica	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Fonsecaea pedrosoi	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	10
Knufia epidermidis CBS 120353	0	0	0	0	1	0	0	0	0	0	5	0	0	0	36	0	4	0	0	0
Phialophora americana CBS 840.69	20	0	0	0	0	0	0	0	0	0	0	0	0	0	561	0	159	0	25	0
Phialophora verrucosa CBS 286.47	5	0	0	0	0	0	0	0	0	0	0	0	0	0	101	0	10	0	15	0
Phialophora verrucosa CBS 839.68	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Rhinocladiella atrovirens CBS 264.49	15	0	0	0	0	0	0	0	0	0	0	0	1	0	79	0	0	0	267	0
Rhinocladiella anceps CBS 181.65	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
Rhinocladiella similis dH13054	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	11	0	0	0
Veronaea botryosa CBS 254.57	0	0	0	0	0	0	0	0	0	0	0	0	0	0	5	66	0	0	0	3
Veronaea botryosa CBS 350.65	0	0	0	0	0	0	0	0	0	0	0	0	0	0	5	0	0	0	0	0
Veronaea compacta CBS 268.75	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Veronaea japonica CBS 776.83	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Total	9084	3005	4419	1129	18	2	647	15	413	5	147630	1675	52818	19291	28458	11985	21648	216	6017	4841

Table S-3. Species associated with plants.

Specie	Country	Number of Reads	Substrate
Cladophialhopra immunda	China	145	Wood
Cyphellophora arxii	Chile	42	Endophytic of *Araucaria araucana*
	France	4	Endophytic of grapevines, oaks, and pines
	Italy	2	Leaf of *Castanea sativa*
	USA	2	*Peltigera praetextata, Brachythecium fendleri, Pinus leiophylla var. chihuahuana*
	USA	1	Leaf
	USA	9	*Panicum virgatum* rhizosphere
	China	417	Wood
Cyphellophora chaetospira	Chile	247	Endophytic of *Araucaria araucana*
	Netherlands	2	*Plant of Myriophyllum* sp. (water and sediment)
	France	62	*Arabidopsis thaliana* and grass
	France	11	Endophytic of *Quercus robur*
	France	50	Seeds of *Phelipanche ramosa*
	Spain	1	*Vitis* sp.
	Italy	41	Leaf of *Castanea sativa*
	Italy	1	grass plant
	USA	26	*Peltigera praetextata, Brachythecium fendleri, Pinus leiophylla var. chihuahuana*
	USA	14289	*Panicum virgatum* rhizosphere
	USA	69	*Solidago* sp. rhizosphere
	USA	3	*Pinus rigida* ectomycorrhizae
	China	19	Leaf of *Betula ermanii*
	China	1	*Oryza sativa*
	China	9	Leaf
	China	1	Rice
	China	37191	Wood
	China	2	Endophytic of *Cinnamomum longipanculatum*
	China	1	Leaf *Coix lacryma-jobi*
Cyphellophora vermispora	France	30	*Arabidopsis thaliana* and grass
	France	8	*Zea mays*
	USA	570	*Panicum virgatum* rhizosphere
	USA	1	*Solidago* sp. Rhizosphere
	China	4	Wood
Cyphellophora guyanensis	Costa Rica	37	Banana
	China	3	*Morinda officinali, Amomum villosu*, *Aquilaria sinensi*, *Pogostemon ca/in.*
	China	12	Morus sp.
	China	519	Wood
Cyphellophora laciniata	Italy	3	Leaf of *Castanea sativa*
	Argentina	5	Leaf of *Spartina densiflora*
	Canada	4	Seed of *Hordeum vulgare*
	France	2	*Arabidopsis thaliana* and Grasses

Table S-3. Continued.

Cyphellophora laciniata	France	215	*Zea mays*
	France	3	Seeds of *Hordeum vulgare*
	France	26	Buds of *Juglans regia*
	France	52	Buds of *Juglans regia*
	France	1	Seed of *Acer pseudoplatanus*
	Spain	2	*Pinus nigra*
	Italy	34	Laef of *Vitis* sp.
	USA	2681	Seed of *Triticum aestivum*
	USA	5311	*Zea mays*
	China	88	*Dysphania ambrosioide*s and *Arabis alpina*
	China	3	Not specified
	China	1	leaf litter
Cyphellophora europaea	China	3	*Camellia sinensi*s (Pu-erh tea)
	China	1	*Morinda officinali, Amomum villosu*, *Aquilaria sinensi*, *Pogostemon ca/in.*
	China	58	Leaves of *Cucurbita* sp.
	China	2	Rice phyllosphere
	China	7	Wood
Cyphellophora oxyspora	Costa Rica	501	Banana
	China	6	*Oryza sativa*
	China	1	*Morinda officinali, Amomum villosu*, *Aquilaria sinensi*, *Pogostemon ca/in.*
	China	4	Leaves of *Cucurbita* sp.
	China	2	Rice phyllosphere
	China	1	*Oryza sativa*
Cyphellophora reptans	USA	10	*Sorghastrum nutans*
Cyphellophora pluriseptata	China	32	Wood
Cyphellophora suttonii	China	1	*Zea mays*
Exophiala angulospora	Chile	8	Endophytic of *Araucaria araucana*
	Chile	195	Endophytic of *Araucaria araucana*
	France	136	Wood branche of *Vitis vinifera*
	USA	3	*Peltigera praetextata, Brachythecium fendleri, Pinus leiophylla var. chihuahuana*
	China	204	Wood
Exophiala castellanii	France	1	*Arabidopsis thaliana* and grass
	Spain	1	Not specified
Exophiala dermatitidis	USA	1	*Bothriochloa bladhii*
Exophiala pisciphila	France	1472	*Zea mays*
	France	8	Buds of *Juglans regia*
	France	1	Buds of *Juglans regia*
	France	64	Seeds of *Phelipanche ramosa*
	Spain	300	*Zea mays*
	Italy	6	Leaf of *Castanea sativa*
	USA	19291	*Panicum virgatum*rhizosphere

Table S-3. Continued.

Exophiala pisciphila	USA	75	*Solidago* sp. Rhizosphere
	USA	1	Seed of *Triticum aestivum*
	China	3	*Musa* sp. (banana)
	China	2	*Oryza sativa* phyllosphere
	China	2	*Oryza sativa*
	China	13704	Wood
	China	4	Leaf *Coix lacryma-jobi*
	France	5	*Zea mays*
	USA	21	Zea mays
	China	125	Bambusoideae family (bamboo)
	China	1	*Dysphania ambrosioides* and *Arabis alpina*
	China	1	*Zea mays*
Exophiala spinifera	Costa Rica	11	Banana
	China	20	Not informed
	China	19	Seeds of *Zea mays*
	Costa Rica	11	Banana
	China	20	Aerial material of *Cynodon dactylon*
	China	19	*Zea mays*
Exophiala xenobiotica	Canada	4	Not specified
	Canada	21	Leaf of *Vitis* sp.
	France	5	Seedlings of *Populus tremula alba*
	USA	24	Seed of *Triticum aestivum*
	USA	263	Seed of *Triticum aestivum*
	China	26	Aerial material of *Apocynum venetum*
	China	1	Leaves of *Larix gmelinii* and *Juglans mandshurica*
	China	3	Seeds of *Zea mays*
	Canada	3	Bark, buds, timber, needles and leaves of ornamental woody plant
	Canada	26	Leaf of *Vitis* sp.
	France	5	Seedlings of *Populus tremula alba*
	Spain	71	*Pinus nigra*
	Italy	1	Leaf of *Vitis vinifera*
	USA	1	Seed of *Triticum aestivum*
	China	27	Not specified
	China	1	*Zea mays*
	China	1	*Zea mays*
	USA	17	Seed of *Triticum aestivum*
Exophiala alcalophila	France	7	Zea mays
	USA	44	Seed of *Triticum aestivum*
	USA	890	*Zea mays*
	China	20	Bambusoideae family (bamboo)
Exophiala angulospora	Spain	3	*Pinus nigra*
Exophiala bergeri	Spain	1	*Pinus nigra*
	China	8	*Aquilaria sinensis*

Table S-3. Continued.

Exophiala bergeri	China	9	*Zea mays*
	China	18	*Mussaenda kwangtungensis*
Exophiala cancerae	France	1	Seedlings of *Populus tremula alba*
	China	5	Bambusoideae family (bamboo)
Exophiala castellanii	Spain	1	*Pinus nigra*
Exophiala equina	Colombia	9	Seeds of *Theobroma cacao*
	Canada	1	Bark, buds, timber, needles and leaves of ornamental woody plant
	France	418	*Zea mays*
	France	43	Seeds of *Hordeum vulgare*
	France	5	Buds of *Juglans regia*
	France	1	Buds of *Juglans regia*
	France	2775	Seedlings of *Populus tremula alba*
	Spain	1058	*Pinus nigra*
	USA	1067	Seed of *Triticum aestivum*
	USA	3779	*Zea mays*
	China	939	Bambusoideae family (bamboo)
	China	4	*Oryza sativa*
	China	21	Banana
	China	4	*Oryza sativa*
	China	101	Not specified
	China	2	*Mussaenda kwangtungensis*
	France	2	*Arabidopsis thaliana* e Grasses
	France	41	Zea mays
	France	17	Seeds of *Hordeum vulgare*
	France	5	Buds of *Juglans regia*
	France	7	Seedlings of *Populus tremula alba*
	USA	775	Seed of *Triticum aestivum*
	USA	1078	*Zea mays*
	China	438	Bambusoideae family (bamboo)
	China	2	Not specified
	France	66	Seeds of *Hordeum vulgare*
	France	423	Seedlings of *Populus tremula alba*
	USA	2	*Zea mays*
	China	1	banana
Exophiala halophila	China	2	*Zea mays*
Exophiala heteromorpha	Spain	5	*Pinus nigra*
	China	7	banana
Exophiala lacus	Argentina	1	Leaf of *Spartina densiflora*
	Canada	1	Seed of *Hordeum vulgare*
	China	220	Not specified
Exophiala mesophila	China	1	banana
	China	288	*Aquilaria sinensis*

Table S-3. Continued.

Exophiala mesophila	Colombia	4	Seeds of *Theobroma cacao*
Exophiala opportunistica	France	296	Zea mays
	Spain	462	*Pinus nigra*
Exophiala salmonis	Spain	1	*Pinus nigra*
Exophiala sideris	France	3	Seedlings of *Populus tremula alba*
	Germany	9	Seeds of *Larix gmelinii*
	Spain	385	*Pinus nigra*
	China	4	*Mussaenda kwangtungensis*
Fonsecaea monophora	China	721	Wood
	Costa Rica	1	Banana
Fonsecaea pedrosoi	China	1	Leaf of *Coix lacryma-jobi*
Knufia epidermidis	Colombia	1	Seeds of *Theobroma cacao*
	Spain	4	*Pinus nigra*
	China	22	banana
	China	57	Not specified
Phialophora americana	Spain	58	*Pinus nigra*
Rhinocladiella atrovirens	Canada	6	Bark, buds, timber, needles and leaves of ornamental woody plant
	Spain	41	*Pinus nigra*
	Italy	1	Seeds of *Picea abies*
	USA	4	*Zea mays*
Rhinocladiella similis	China	75	*Zea mays*
Veronaea botryosa	China	484	Wood
Veronaea japonica	China	1	Bambusoideae family (bamboo)
	China	1	Moss crust in desert

Chapter III

Metagenomics reveal abundance of black yeast-like fungi in the skin microbiome

Metagenomics reveals an abundance of black yeast-like fungi in the skin microbiome.

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1. **ABSTRACT**

Background: The skin is the first line of defense against communities of resident viruses, bacteria, and fungi. The composition of the microbiome might change with factors related to the environment and host. The microbiome is dominated by bacteria. Dermatophytes and yeasts are the predominant fungi that are also involved in opportunistic infections of skin, hair, and nails. Among environmental fungi, Chaetothyriales (black yeasts and relatives) are enriched by hydrocarbon pollution in domesticated habitats and comprise numerous species that cause mild-to-severe disease.

Methods: We investigated the presence of black fungi in the skin microbiome by conducting an analysis in the publicly available metagenomic SRA database (NCBI). We focused on the causative agents of chromoblastomycosis and phaeohyphomycosis and used barcodes and padlock probe sequences as diagnostic tools.

Results: A total of 132,159,577 MB was analyzed and yielded 18,360 reads that matched with 24 species of black fungi. *Exophiala* was the most prevalent genus, and *Cyphellophora europaea* was the most abundant species.

Conclusion: This study reveals the abundant presence of Chaetothyriales on the skin without necessarily being associated with infection. Most of the detected causal agents are known from mild skin diseases, while also species were revealed that had been reported from *CARD9*-deficient patients.

Keywords: Black fungi, black yeast, cutaneous colonization, opportunism, microbiome

2. **INTRODUCTION**

The skin is the largest organ of the body and the first line of defense against infections (GALLO, 2017). It forms a physical barrier that protects against environmental factors (BELKAID, TAMOUTOUNOUR, 2016; CALLEWAERT, HELFFER, LEBARON,2020; LEUNG, WILKINS, LEE, 2015). The skin microbiome is the largest ecosystem of the body (GALLO, 2017; FAUST et al 2012; ZHOU et al 2020; GRICE, et al. 2009) and contributes to a healthy cutaneous habitat, preventing colonization by pathogens and modulating innate and adaptive immunity (ZHOU et al 2020; LEUNG et al. 2018). This cutaneous microbial ecosystem contains bacteria, fungi, and viruses (LEUNG, WILKINS, LEE, 2015; FAUST et al 2012; GRICE, et al. 2009) that interact in co-abundance and have co-exclusion relationships that shape the composition of the community (FAUST et al 2012; ZHOU et al 2020; MCCALL et al., 2020).

Species diversity and the relative abundance of the microbiome vary according to individual conditions and the physiology of the site of occurrence (BELKAID, TAMOUTOUNOUR, 2016; BYRD, BELKAID, SEGRE, 2018). Direct interactions with the external environment also affect the microbial composition (CALLEWAERT, HELFFER, LEBARON,2020). *Malassezia* and *Candida* are found throughout the body (OH et al., 2012), particularly on the face and the back (FINDLEY et al., 2013). The dermatophytes are mostly represented by species of *Microsporum*, *Epidermophyton*, and *Trichophyton*, and they are also commonly involved in superficial infections of the skin, hair, and nails (DE HOOG et al. 2020; WEITZMAN, SUMMERBELL, 1995). The composition of the skin microbiota remains stable over time but is influenced by the health status of the host (DORRESTEIN, GALLO, KNIGHT, 2016; LAX et al., 2017; SOHN, 2018) and changes in environmental conditions (CALLEWAERT, HELFFER, LEBARON, 2020).

Black saprobes are ubiquitous in the environment. Among these, the order Chaetothyriales, which includes black yeasts and other related species, is unique. Many of these species are enriched in the hydrocarbon-polluted domesticated environment, while several members in this group are also known for a gamut of human infections (DE HOOG et al. 2020). Thus, their behavior was referred to as having ‘dual ecology’ (PRENAFETA-BOLDÚ, 2006). Clinically relevant species are found in several genera, including *Cladophialophora, Cyphellophora*, *Exophiala*, *Fonsecaea, Phialophora*, and *Rhinocladiella* (DE HOOG et al. 2020; QUEIROZ-TELLES et al., 2009; TORRES-GUERRERO et al., 2012). The order is particularly renowned because it contains agents of serious, mutilating diseases, such as

chromoblastomycosis, phaeohyphomycosis, and primary cerebritis. Chromoblastomycosis is a chronic, granulomatous mycosis of skin and subcutaneous tissue caused by the implantation of contaminated plant spines or wood fragments (QUEIROZ-TELLES et al., 2009; SALGADO et al., 2004; MARQUES et al., 2006; QUEIROZ-TELLES et al., 2017). Epidemiological studies found that mycosis has an environmental origin (QUEIROZ-TELLES et al., 2017; VOIDALESKI et al., 2020; COSTA et al., 2020). Phaeohyphomycosis is a general term used to refer to opportunistic, cutaneous, and systemic infections caused by melanized fungi involving immunocompromised and healthy individuals (REVANKAR et al., 2017). *Cladophialophora bantiana* and *Rhinocladiella mackenziei* are the most virulent species in the Chaetothyriales, causing fatal cerebral infections (HORRÉ, DE HOOG, 1999; KANTARCIOGLU et al., 2017; BADALI et al., 2009).

At the other end of the spectrum, black yeast-like fungi have been described from mild cutaneous infections, e.g., *Cyphellophora europaea* (DE HOOG et al., 2000) or *Knufia epidermidis* (LI et al., 2008). Saunte et al. (2012) discussed whether they had clinical relevance at all, even though known opportunists, such as *Exophiala spinifera*, were reported as cause cutaneous infections (BOHELAY et al., 2016; HARRIS et al., 2009; ESPANHOL et al.,2020).

Several studies have investigated the skin microbiome composition using cultivation-independent DNA sequencing technology, such as metagenomics. Such techniques allow exploration under different environmental conditions and for various host-associated factors (CALLEWAERT, HELFFER, LEBARON,2020; FAUST et al 2012; YING Set al. 2010; LEUNG, CHAN, LEE, 2016). An important driver for changes in the microbiome composition is found in lifestyle-related factors, such as between rural and urban residence ((CALLEWAERT, HELFFER, LEBARON, 2020; MCCALL et al., 2020). As the natural ecology of the potential opportunists in Chaetothyriales is enigmatic, elucidation of their presence and route of infections are required to improve epidemiological understanding (SAUNTE et al., 2012). The aim of the present study is to explore the occurrence of agents chromoblastomycosis, phaeohyphomycosis, and other diseases by members of Chaetothyriales using a metagenomic approach.

3. **MATERIALS AND METHODS**

3.1 DATABASE CONSTRUCTION

We included metagenomics data from the Sequence Read Archive (SRA) (https://www.ncbi.nlm.nih.gov/sra) that focused on the skin microbiome. The database was built with public data collected from metagenomic studies available till June 2022 that reported metagenome sequences of the internal transcribed spacer regions (ITS1, ITS2, or both) and those that were associated with the skin. For searching, we used the term “metagenomic skin”. The data were manually preselected by visual inspection in the Microsoft Excel software. The lines that were not from skin metagenomic studies were removed, and reviews were excluded to prevent redundancy. In total, 1,689 projects were analyzed to identify black yeasts (Table S-1).

3.2 Identification tools

The molecular markers were used as a tool for the identification of species, according to methods described by Costa et al. (2020). For the order Chaetothyriales, was applied 119 molecular markers have been described in the literature (HEINRICHS et al., 2012; NAJAFZADEH et al., 2013; NAJAFZADEH et al., 2011; NAJAFZADEH et al., 2018; FENG et al., 2013; DENG et al., 2014; SCHNEIDER et al., 2019; HAMZEHEI et al., 2013) (Table S-2). In addition, padlock probes available with 41 sequences containing 28–42 bp, and barcode identifiers comprising 105 sequences with 25–41 bp were used. These represented 72 species of chaetothyrialean fungi, including 50 species that were potential causative agents of phaeohyphomycosis and/or chromoblastomycosis (Table S-2). Padlock probes and barcodes were designed in regions with SNPs in one of the rDNA internal transcribed spacers (ITS1, ITS2, or both).

3.3 Identification *in silico*

For fungal identification, the data were clustered into operational taxonomic units (OTUs) at 100% similarity using local BLASTn search (v2.6.0.+). Hits included sequences that satisfied the quality criteria with coverage and identity cutoff of 100% and matches with values below the cutoff were excluded. For identification at the species level using the sequence markers of padlocks and barcodes, slight misalignment and imperfect sequence identity were rejected, as these cannot be used to fully characterize the fungus in the analyses. Metagenome reads from double-strand sequencing were treated as single in the final read count.

4. **RESULTS**

The initial dataset consisted of 132,159,577 MB of data obtained from the public metagenome data available in the SRA (NCBI). Using molecular markers, 18,360 reads were found to be related to chaetothyrialean fungi (Table S-3), of which 68.88% (12,647 reads) were barcode identifiers, 10.10% (1,854 reads) were identified by padlock probe sequences, and 21.02% (3,859 reads) were identified by both molecular marker probe sequences. The species were detected in 496 metagenomic runs (Table S-4) from 19 studies on different anatomical sites of the human body (Table 1). These included upper limbs (hand, forearm, palm, popliteal fossa, and volar forearm), lower limbs (foot and popliteal fossa), head (nose, forehead, and scalp), and trunk (back and umbilicus). According to Table 1, the accessed data refer to healthy patients, and some projects reported microbiomes of individuals with atopic dermatitis, chronic rhinosinusitis, and onychomycosis. No study investigated presented individuals with infections caused by black yeasts.

Table 2. Frequency of Chaetothyriales in the studies.

Project	Total number of reads	Black yeast reads	Frequency of black yeast (%)
PRJEB37496	869,3542	1,226	0.014
PRJEB42399	1,428,212,093	74	0.0000052
PRJNA656308	2,856,440	4,356	0.00030
PRJNA699281	10,134,661,221	15	0.0000011
PRJNA638969	9,832,018	44	0.0000031
PRJNA639280	467,100,965	2	0.00000014
PRJNA704382	26,475,413	269	0.0010
PRJEB19454	4,709,663	46	0.0010
PRJNA478488	703,398,923	89	0.000013
PRJNA630834	15,211,095	4,470	0.029
PRJEB25617	411,811,041	2,474	0.00060
PRJNA421247	22,301,689	2,606	0.012
PRJEB25916	22,197,039	728	0.0033
PRJNA669317	4,630,052	611	0.013
PRJNA438584	36,192,335	2	0.0000055
PRJNA286273	7,894,353	502	0.0064
PRJEB16723	20,194,826	14	0.0001
PRJNA46333	14,509,137,641	1	0.0000000069
PRJNA478488	703,398,923	831	0.00012
Total	**28,538,909,272**	**18,360,000**	**0,1**

Table 1. Summary of the metagenome studies containing sequences of the members of Chaetothyriales.

Project Number	Total Size	Study and/or Description	Age (Years)	Clinical Outbreak	Skin region	Reference
PRJEB19454	4068 MB	Development of modified primer pairs that improved modified 16S rRNA gene and internal transcribed spacer (ITS) Primers for Microbial Community Surveys.	Not Informed	Not Informed	Not Informed	Walters et al., 2015
PRJEB25617	32854 MB	This study addresses the associations between factors associated with westernization including architectural design and the microbial biogeography of households across a gradient of urbanization in South America.	Not Informed	Healthy	Hand and foot	McCall et al., 2020
PRJEB25916	7032 MB	An integrated view of the spatial distribution of microbes in a specific Mediterranean population across a wide age range, in skin, oral and gut in the young, elderly and centenarians in Sardinia.	19 to 107	Healthy	Palm, forehead, umbilicus and oral	Wu et al., 2020
PRJEB37496	3853 MB	To analyze the landscape of fungal diversity in healthy and onychomycotic patients.	26 to 73	Toenail disease and healthy	Nail	Olbrich et al., 2022
PRJEB42399	77430 MB	The impact of skin microbiome sharing in pediatric atopic dermatitis (AD)	Not Informed	Atopic dermatitis and healthy	Volar forearm, antecubital fossae, cheeks and lesions	Chia et al, 2022
PRJNA478488	97939 MB	Chinese human skin metagenome.	25 to 45	Healthy	Face (Cheek) and scalp	Leung et al., 2020
PRJNA614620	1113 MB	Human skin fungal microbiomes in Korean young and elderly women metagenome.	20 to 69	Healthy	Cheek and forehead	Kim et al., 2022
PRJNA630834	4522 MB	Profile of microbiomes of 160 skin samples.	3 to 74	Healthy	Cheek and the abdomen	Li et al., 2020
PRJNA638969	4041 MB	Microbial community in patients with chronic rhinosinusitis; investigation of antimicrobial treatment.	28 to 68	Chronic rhinosinusitis	Nose	Lux et al., 2020
PRJNA639280	44081 MB	Data from Industry Unilever®	Not Informed	Not Informed	Not Informed	NI
PRJNA656308	3566 MB	This is the study to characterize the infant diaper area skin microbiome (the genitals, intertriginous (leg folds), perianal region and buttocks).	5-15 months	Diaper dermatitis	Genital, intertriginous, perianal and buttocks	Teufel et al., 2021
PRJNA669317	1588 MB	Human skin microbiomes in Korean women.	19 to 63	Healthy	Cheek and forehead	Larson et al., 2021
PRJNA699281	1,01TB	Associations of the microbiome with age, frailty, and infectious disease risk factors, of the skin, oral, and gut microbiota in 47 older adults residing in community or skilled nursing facility settings.	65 to 97	Not Informed	Forearm, hand, foot, popliteal fossa, torso, back, nares and face	Larson et al., 2021
PRJNA704382	8904 MB	A prospective, longitudinal study to investigate puberty-associated shifts in skin microbiota from twelve healthy children, evaluated every 6-18 months for up to 6 years of 5 different skin and nares sites.	2 to 40	Healthy	Nares, antecubital fossa, volar forearm and popliteal fossa.	Oh et al., 2012
PRJNA438584	12693 MB	PMS skin microbiome data; healthy human skin microbiome.	3 to 50	Healthy	11 sites of skin (not informed)	Chaudhari et al., 2020
PRJNA421247	6877 MB	Human skin fungal metagenome targeted loci environmental.	Not Informed	Healthy	Forehead, Forearms and palms	Tong et al., 2019
PRJNA46333	2,06TB	This study aims to investigate the skin microbiota of AD patients at specific timepoints (quiescence, disease flares, and post-treatment) to examine how disease state correlates with changes in the skin microflora.	2 to 15	Atopic dermatitis	Not Informed	Kong et al., 2012
PRJNA286273	2384MB	Skin fungal mycobiome of Chinese individuals.	Not Informed	Healthy	Forehead, forearm and palm	Leung, Chan, Lee, 2016;
PRJEB16723	1788 MB	Characterized skin bacterial and fungal microbiotas from healthy and dandruff subjects, comparing scalp and forehead (lesioned and non-lesioned skin sites).	18 to 61	Healthy and Dandruff	Scalp	Soares et al., 2016

Abbreviations: MB, megabytes; TB, terrabytes; NI, Not Informed

Most studies investigated were from the USA (n = 5) and China (n = 4) (Figure 2D). The studies with the largest numbers of reads with a match, i.e., PRJNA630834 (4,470 reads) and PRJNA656308 (4,356 reads), were from these two countries (Figure 2C).

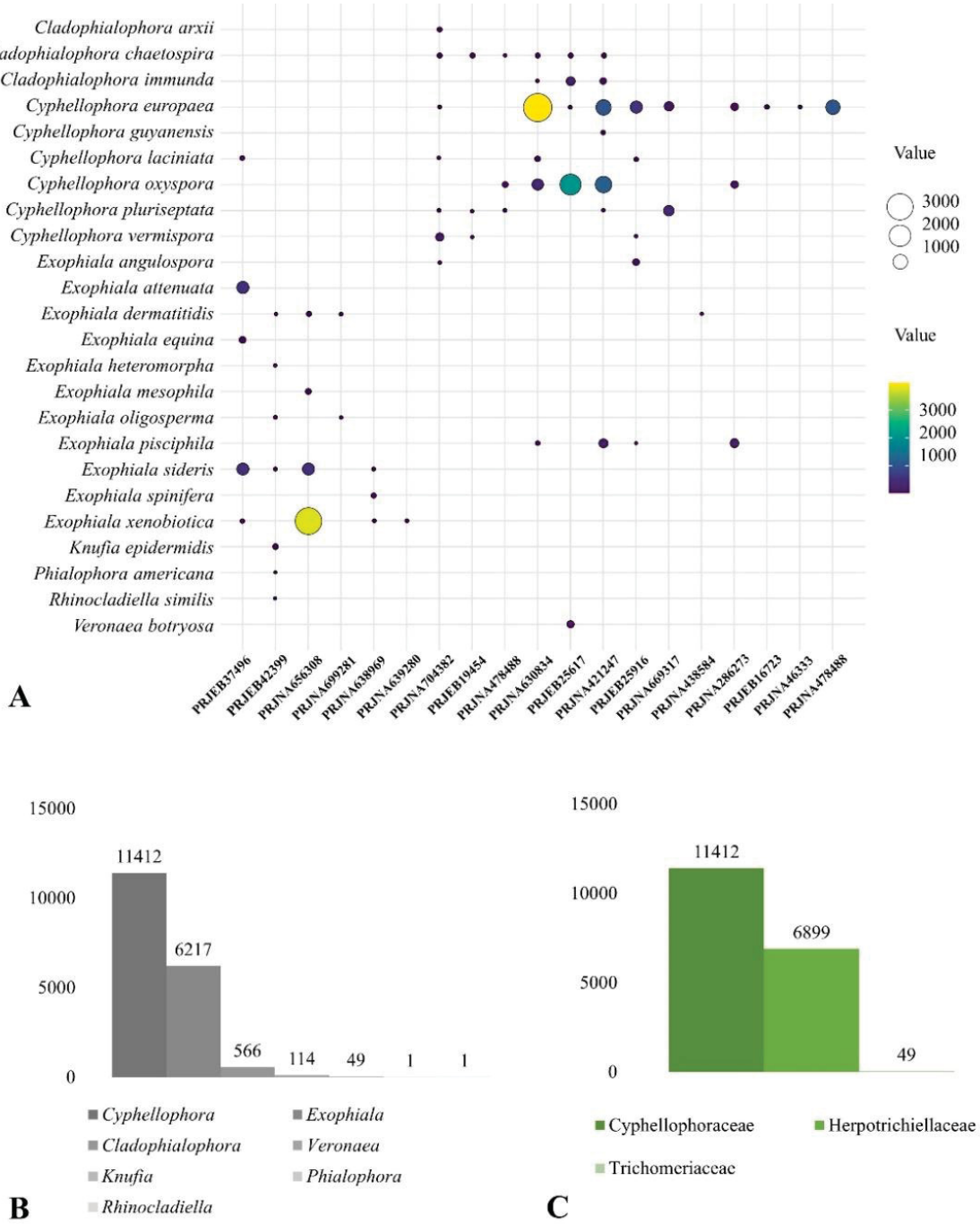

Figure 1. Taxonomic overview of the yeast-like fungi. (A) The bubble chart shows the most abundant species; the size of the bubbles represents the average absolute abundance of each species, and the color gradient represents the most abundant species (yellow) and the least abundant species (purple). (B and C) Number of reads by genus and family, respectively.

Concerning the endemic areas of chromoblastomycosis, PRJEB25617 identified 2,474 reads from a population in South America (Peru and Brazil). In studies from China, 8,409 reads were identified, represented by PRJNA630834 (4,470 reads), PRJNA421247 (2,606 reads), PRJNA478488 (89 reads), and PRJNA286273 (502 reads) (Figure 2D). The metagenome bio-projects analyzed showed that 24 species of Chaetothyriales were present, among which 11 were identified only using probes, nine were identified only using barcodes, and four were identified by both molecular markers. The species belonged to the families Herpotrichiellaceae, Trichomeriaceae, and Cyphellophoraceae (Figure 1D), which accounted for 0.1% of the total number of species analyzed (Table 2 and S-3). *Exophiala* was the most prevalent genus (n = 11 species) (Figure 1C), with *E. xenobiotica* as the most abundant species (3,777 reads) (Figure 1A). The other prevalent genera included *Cyphellophora* (n = 4), *Cladophialophora* (n = 3), *Knufia*, *Rhinocladiella*, *Veronaea*, and *Phialophora* (n = 2), indicating that the frequency of Chaetothyrialean fungi was low (Figure 1C). Out of the 24 species identified, *Cladophialophora chaetospira*, *Exophiala heteromorpha*, and *E. sideris* have never been reported as causative agents of (skin) infections; all other species have been described in clinical case reports or are known as the causative agents of phaeohyphomycosis.

Cyphellophora europaea was the most abundant species with 6,783 reads detected in 10 studies (PRJNA 704382; PRJEB19454; PRJNA478488; PRJNA630834; PRJE B25617; PRJNA421247; PRJEB25916; PRJNA669317; RJNA438584; PRJNA286273; PRJEB 16723; PRJNA 46333; PRJNA 478488), followed by 3,823 reads of *Cyphellophora oxyspora* (3,823 reads), and 3,776 of *Exophiala xenobiotica* (Figure 1A). Opportunistic pathogens, i.e., species that are recurrently involved in human or animal infections, were dominant in our analysis (Figures 2A and B). Only a few strictly environmental species, i.e., *E. sideris* (n = 1,092), *C. chaetospira* (n= 189), *C. guyanensis* (n = 20), and *E. heteromorpha* (n = 2), were found in low frequency in the datasets. Although molecular markers of the major causative agents of chromoblastomycosis, i.e., *Cladophialophora carrionii* and *Fonsecaea* spp. (*F. pedrosoi*, *F. monophora*, and *F. nubica*), were included in the analysis, no match was found in the data. *Rhinocladiella similis* (n = 1) and *Phialophora americana* (n = 1) were the least abundant species (Table S-2). The species reported in this study cause mild skin and nail diseases and phaeohyphomycosis throughout the world, and they are opportunistic pathogens. However, species such as *Fonsecaea* spp. and *Cladophialophora carrionii*, found in endemic areas, were not detected in the data analyzed.

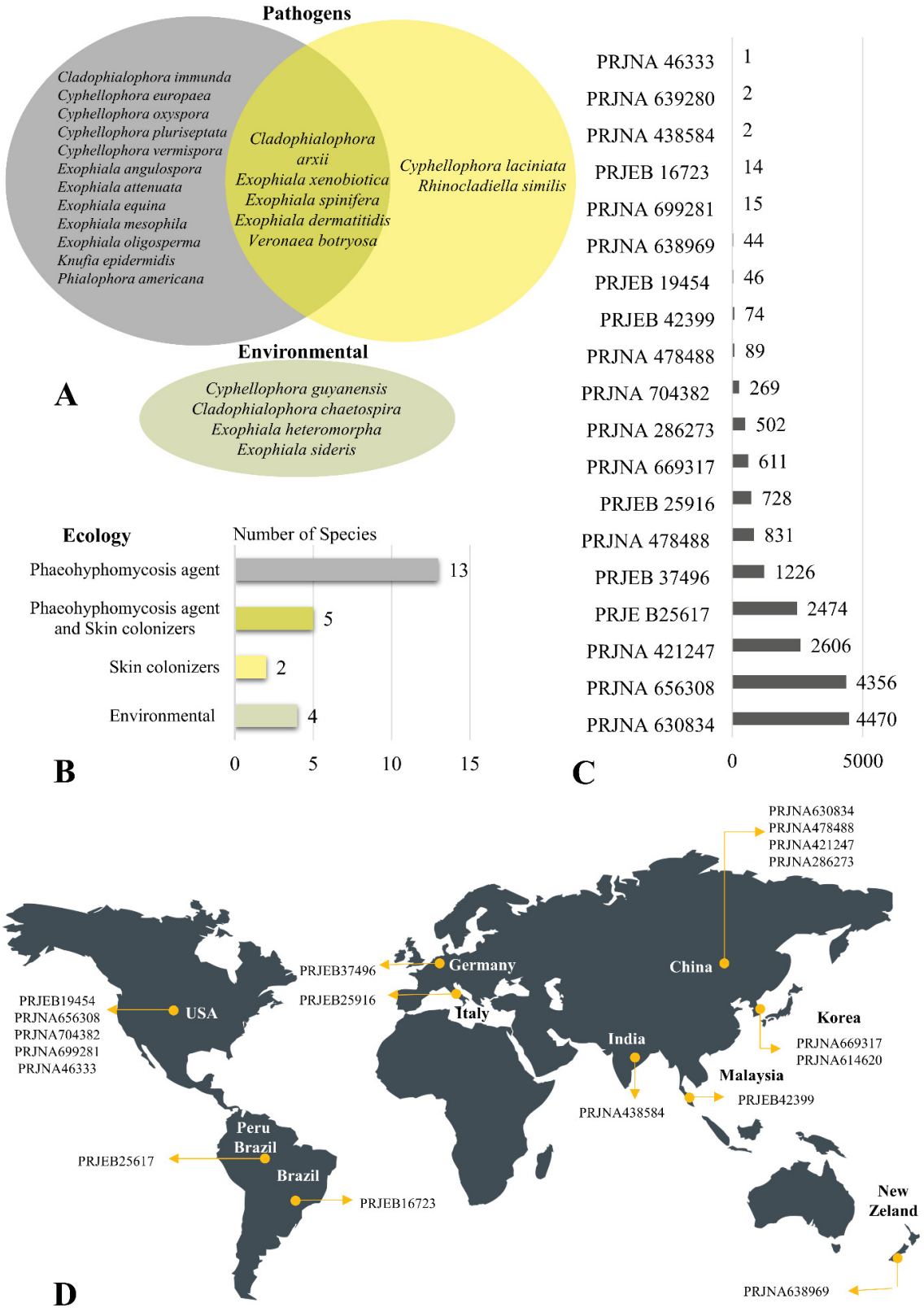

Figure 2. Ecological classification and distribution of the detected species of Chaetothyriales. (A and B) Ecology of the Chaetothyriales species reported in the analysis. C- The number of identified sequences in different studies. (C) Global distribution of the studies analyzed.

5. **DISCUSSION**

The skin microbiome consists of viruses, and bacterial and eukaryotic communities, most of which are natural colonizers while others are ephemeral opportunists or are pathogens potentially involved in cutaneous infections (OLBRICH et al., 2022; DE HOOG et al., 2017). Due to its high exposure to the surroundings, the microbial community of the skin surface constantly loses and acquires microbes (TONG et al., 2019). Among eukaryotes, *Malassezia* is the most frequent colonizer (LEUNG et al., 2020; KIM et al., 2022; TONG et al., 2019; SOARES et al., 2016), while dermatophytes are the most common pathogenic fungi in the skin microbiome (OLBRICH et al., 2022). However, some groups have remained unexplored by conventional culture-based methods. Our study systematically investigated *in silico* the presence of the Chaetothyriales sequences in metagenomic public datasets, using sequences of validated molecular markers following the methodologies proposed by Costa et al., (2020).

Chaetothyriales on the skin that cause only minimal damage were reported by Saunte et al. (2012), who investigated whether these species are coincidental colonizers. In our analysis, we found 24 saprobic and opportunistic species in the genera *Exophiala*, *Cladophialophora*, *Veronaea*, *Phialophora*, and *Rhinocladiella* (family Herpotrichiellaceae), *Cyphellophora* (family Cyphellophoraceae), and *Knufia* (family Trichomeriaceae) as parts of the skin microbiome of healthy human hosts. Species of *Cladophialophora*, *Exophiala*, and *Cyphellophora* were previously reported by Saunte et al., (2012). According to our results, *Exophiala* was the largest genus identified, represented by 10 species, which included *E. dermatitidis*, *E. spinifera*, *E. xenobiotica*, *E. angulospora*, *E. attenuata*, *E. equina*, *E. heteromorpha*, *E. mesophila*, *E. oligosperma*, *E. pisciphila*, and *E. sideris*, although their frequency was moderate. *Exophiala* spp. are found in water, bathing facilities, and indoor environments (MATOS et al., 2003; WANG et al., 2018; SUDHADHAM et al., 2008). Numerous species of this genus are found in occasional opportunistic infections (DE HOOG et al. 2020). *Cyphellophora europaea* (previously classified in *Phialophora*) (REBLOVA et al., 2013) was the species with the largest number of reads in the data analyzed. This finding was similar to that of the study by Saunte et al. (2012), who listed this species as the most prevalent chaetothyrialean species in routine dermatological. *Cyphellophora* species can cause mild skin and nail infections (SAUNTE et al., 2012; FENG et al., 2012) and are rarely reported outside the human host (WANG et al., 2018). Most *Cyphellophora* species are known as yet from just a few isolates (FENG et al., 2012), while in our study they appear relatively common. This

suggests that the skin might be a preferred habitat for these fungi. *Knufia epidermidis* seems to be similar in this respect, being detected regularly from the skin but with very mild or no symptoms. It is a member of Trichomeriaceae, a family where most members are found in low-nutrient surfaces, such as rocks. Although several studies analyzed here were conducted in endemic areas of chromoblastomycosis in China and Brazil, no regular agent of the disease was detected (Table S-3). These fungi might not commonly occur on human skin but might be implanted subcutaneously away from the external environment. In general, members of Cyphellophoraceae were relatively more commonly encountered as skin colonizers. It may be hypothesized that for Cyphellophoraceae, the skin is a permissive habitat.

Despite their irregular occurrence, the presence of Herpotrichiellaceae on the skin might be problematic because of hosts with impaired immunity, such as patients carrying mutations in the caspase recruitment domain 9 (*CARD9*) gene. These individuals are exceptionally susceptible to infections by *Candida*, dermatophytes, and black fungi. Song et al. (2022) showed a remarkable number of *CARD9*-related infections by *Phialophora* species, which do not belong to the common environmental flora. In our study, we detected *Exophiala dermatitidis, E. spinifera, Phialophora americana*, and *Veronaea botryosa*, which were all proven or probable agents of chronic, disseminated, highly mutilating, and eventually fatal *CARD9*-related infections. Patients with immune suppression because of organ transplant are equally vulnerable.

The resident microbiota might have an individual correlation with the characteristics of the host. Species occurring at low frequencies might be associated with a specific condition or external factors. Conversely, black yeast species that are more frequent on human skin, such as *Cyphellophora* and *Exophiala*, may be adapted to this habitat, thus, remaining clinically insignificant.

6. **CONCLUSION**

In summary, we found that members of Cyphellophoraceae are the main group of chaetothyrialean skin colonizers. They might have physiological parameters that favor their growth in the cutaneous habitat. The members in the family Herpotrichiellaceae that cause implantation diseases such as chromoblastomycosis do not normally occur on the skin. However, the significance of occasional presence of other potentially infectious members of

this family should not be underestimated considering that patients with severe clinical syndromes, such as inherited *CARD9* deficiency or immunosuppression, are highly susceptible.

Author Contributions

Morgana Ferreira Voidaleski: writing; original draft preparation; data curation; investigation; formal analysis, review, and editing. Flávia de Fátima Costa: investigation; methodology; data curation; formal analysis. G. Sybren de Hoog: writing, review and editing. Renata Rodrigues Gomes: review and editing; Vania Aparecida Vicente: conceptualization; review and editing.

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SUPPLEMENTARY MATERIAL

Table S-1. Selected projects to database.

BioPorjects													
PRJDB10066	PRJEB22267	PRJEB36022	PRJEB7877	PRJNA268694	PRJNA310288	PRJNA352450	PRJNA395550	PRJNA438728	PRJNA492935	PRJNA531927	PRJNA565423	PRJNA615959	PRJNA656308
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Table S-1. Continued.

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PRJEB12986	PRJEB30063	PRJEB42822	PRJNA237487	PRJNA291994	PRJNA330661	PRJNA384031	PRJNA419407	PRJNA475137	PRJNA515382	PRJNA554499	PRJNA597655	PRJNA635409	PRJNA668348
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PRJEB13722	PRJEB30288	PRJEB44072	PRJNA240174	PRJNA292555	PRJNA335375	PRJNA384210	PRJNA419579	PRJNA476563	PRJNA516879	PRJNA555200	PRJNA598135	PRJNA636582	PRJNA669910
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PRJEB13871	PRJEB30953	PRJEB44542	PRJNA240284	PRJNA293199	PRJNA336369	PRJNA384396	PRJNA421247	PRJNA477528	PRJNA517385	PRJNA555784	PRJNA599608	PRJNA636779	PRJNA670661
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PRJEB14627	PRJEB31281	PRJEB44885	PRJNA242444	PRJNA295605	PRJNA338607	PRJNA385007	PRJNA427374	PRJNA478280	PRJNA518003	PRJNA556256	PRJNA601631	PRJNA639280	PRJNA673077
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Table S-1. Continued.

PRJEB14822	PRJEB31807	PRJEB45604	PRJNA245480	PRJNA297069	PRJNA34067	PRJNA385723	PRJNA429422	PRJNA479402	PRJNA521543	PRJNA556991	PRJNA603431	PRJNA639794	PRJNA673973
PRJEB14891	PRJEB31855	PRJEB45945	PRJNA246019	PRJNA297354	PRJNA34071	PRJNA385862	PRJNA429454	PRJNA479461	PRJNA521554	PRJNA557507	PRJNA603959	PRJNA639958	PRJNA674311
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PRJEB15056	PRJEB32276	PRJEB4630	PRJNA252376	PRJNA298406	PRJNA342418	PRJNA387190	PRJNA430498	PRJNA479997	PRJNA521807	PRJNA558097	PRJNA604820	PRJNA640377	PRJNA674612
PRJEB15173	PRJEB32293	PRJEB4631	PRJNA252600	PRJNA298951	PRJNA342477	PRJNA38727	PRJNA430716	PRJNA480034	PRJNA523146	PRJNA558189	PRJNA604822	PRJNA640465	PRJNA674614
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PRJEB15266	PRJEB32322	PRJEB46654	PRJNA253075	PRJNA299015	PRJNA343146	PRJNA389298	PRJNA431015	PRJNA480529	PRJNA523412	PRJNA559513	PRJNA605329	PRJNA641001	PRJNA675013
PRJEB15287	PRJEB32380	PRJEB46984	PRJNA254018	PRJNA299099	PRJNA343706	PRJNA389321	PRJNA431047	PRJNA480591	PRJNA523711	PRJNA559517	PRJNA605518	PRJNA641451	PRJNA675309
PRJEB15299	PRJEB3250	PRJEB47547	PRJNA254084	PRJNA299237	PRJNA343882	PRJNA389472	PRJNA431642	PRJNA480705	PRJNA523885	PRJNA559522	PRJNA605697	PRJNA641496	PRJNA675470
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PRJEB17744	PRJEB32719	PRJEB51233	PRJNA255445	PRJNA300286	PRJNA343976	PRJNA390139	PRJNA432888	PRJNA482046	PRJNA524711	PRJNA559873	PRJNA606805	PRJNA641999	PRJNA679570
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PRJEB1934	PRJEB33324	PRJEB5758	PRJNA260277	PRJNA304068	PRJNA34637	PRJNA391455	PRJNA434711	PRJNA487648	PRJNA528347	PRJNA561582	PRJNA610303	PRJNA644286	PRJNA683085
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PRJEB20056	PRJEB34022	PRJEB6403	PRJNA263134	PRJNA305211	PRJNA349123	PRJNA393292	PRJNA436005	PRJNA488995	PRJNA529812	PRJNA562087	PRJNA611754	PRJNA647685	PRJNA684689
PRJEB20471	PRJEB34056	PRJEB6499	PRJNA263215	PRJNA305906	PRJNA349189	PRJNA393328	PRJNA436335	PRJNA489249	PRJNA530153	PRJNA562547	PRJNA612419	PRJNA647995	PRJNA685055
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PRJEB20808	PRJEB34686	PRJEB7206	PRJNA266684	PRJNA307339	PRJNA351242	PRJNA393830	PRJNA437700	PRJNA491283	PRJNA531006	PRJNA563272	PRJNA613575	PRJNA649052	PRJNA687260
PRJEB20884	PRJEB34799	PRJEB7243	PRJNA267573	PRJNA308631	PRJNA352101	PRJNA394790	PRJNA437732	PRJNA491518	PRJNA531014	PRJNA564008	PRJNA613666	PRJNA649979	PRJNA687485
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Table S-1- Continued.

PRJEB22016	PRJEB35277	PRJEB7744	PRJNA267733	PRJNA309849	PRJNA352301	PRJNA395375	PRJNA438286	PRJNA492312	PRJNA531247	PRJNA564823	PRJNA614620	PRJNA655516	PRJNA687544
PRJEB22078	PRJEB35345	PRJEB7825	PRJNA268531	PRJNA310265	PRJNA352449	PRJNA395539	PRJNA438584	PRJNA492925	PRJNA531814	PRJNA565155	PRJNA615775	PRJNA655626	PRJNA687766
PRJNA688082	PRJNA732927	PRJNA772107	PRJNA688751	PRJNA734281	PRJNA777885	PRJNA690001	PRJNA736093	PRJNA780377	PRJNA691892	PRJNA739506	PRJNA781752	PRJNA692816	PRJNA740337
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PRJNA704990	PRJNA750940	PRJNA799072	PRJNA706687	PRJNA754815	PRJNA801688	PRJNA713953	PRJNA756230	PRJNA805083	PRJNA714801	PRJNA757748	PRJNA809023	PRJNA766746	PRJNA729106
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PRJNA720147	PRJNA761243	PRJNA813626	PRJNA721448	PRJNA762948	PRJNA823388	PRJNA722964	PRJNA764460	PRJNA828372	PRJNA724031	PRJNA766351	PRJNA835203	PRJNA769088	PRJNA732695
PRJNA836403	PRJNA836734	PRJNA837110	PRJNA838496	PRJNA770514	PRJNA771628	PRJNA771681	PRJNA771717	PRJNA769745					

Table S-2. Molecular markers described in the literature of Chaetothyriales.

Organism	Strain	Source / Host	Number of pb		Sequence (3' 5')	Reference
			Padlock Probe	Barcode		
Cladophialophora arxii	CBS306.94	Human	42	-	TAAACAAAGGGTTGGGAGGTCAGGCCTAGAAGACCCTAACTC	Hamzehei et al., 2013; Heinrichs, De Hoog, Haase, 2012
			-	35	ACGGTTTGGTGGAAGGGTGACACCCTTCCGCCCGT	
	CBS409.96	Human	-	37	ACGGTTCGGTGGGGAAGGGGTACACCCTTCCACCCGT	
Cladophialophora bantiana	CBS173.52	Brain Abcess Human	41	-	ACGCTGGCCAGAGGACGCCGAGTCGGCGGTCCTCCGGCGGT	Hamzehei et al., 2013; Heinrichs, De Hoog, Haase, 2012
			-	34	ACGGTCTGGCGGAAGTGTCGTGCACCCCGCCCCT	
Cladophialophora carrionii	CBS160.54	Human	39	-	AGAGTTTGGGGTTGGCTGTCGGCGGACACGGGCCCAGAG	Hamzehei et al., 2013; Heinrichs, De Hoog, Haase 2012; Deng et al., 2014
			42	-	GTCTTTAGGAGGGGTCGAGAACACTCGACCAAAACCGTCCAA	
			-	29	ACGGTTTTGGTCGAGTGTTCTCGACCCCT	
	CBS163.54	Human	-	29	ACGGTCTTGGTCGAGTGTTCTCGACCCCT	
	CBS260.83	Human	-	29	ACGGTTTTGGTCGAGTGTTCTCGACCCCT	
	FMC.248	Human	-	29	ACGGTCTTGGTCGAGTGTTCTCGACCCCC	
Cladophialophora chaetospira	CBS 115468	Human	39	-	GTCCTCCGGCGGCCGTGAGGCGGGTCCGCCGAAGCAACA	Hamzehei et al., 2013
Cladophialophora devriesii	CBS147.84	Human	42	-	GAATAAATTCACTTAGACAGTAAAATCATGGTTATTCCAGAG	Hamzehei et al., 2013 Heinrichs, De Hoog, Haase 2012
			-	25	ACGGCTTGGTAGAGTCCCTACCCCT	
	IFM51369	Not Reported	-	25	ACGGCTTGGTAAATTCCCTACCCCT	
Cladophialophora emmonsii	CBS979.96	Human subcutaneous lesion of right forearm	0	33	ACGGCCTGGCGGAGTGCACGACACTCTGCCCCT	Heinrichs, De Hoog, Haase 2012
	CBS640.96	Animal subcutaneous lesion	0	33	ACGGCCTGGCGGAGTGCACGACACCCTGCCCCT	

Table S-2. Continued.

Cladophialophora immunda	CBS834.96	Human	42	-	GGCGGTCCTCCGGCGGTCCGCTTGAGACGGGTCCGCCGAAGC	Hamzehei et al., 2013; Heinrichs, De Hoog, Haase 2012
			41	-	GACACTGGCCAGAGGACGCACGCCACGGTGGGCGGTCCTCC	
			-	25	ACGGTTTGGTAGGGCGCCTACCCCT	
	CBS102227	*Syagrus romanzoffiania*, stem	0	25	ACGGTTTGGTAGAGTGCCTACCCCT	
Cladophialophora minourae	CBS 987.96	Rotting wood	42	-	CGGTCCTCCGGCGGTCTTTATGGGACGGGTCCGCCAAAGCAA	Hamzehei et al., 2013
Cladophialophora modesta	CBS985.96	Human	-	26	AATCTCAGTTGAGTGATCAACTGGTT	Heinrichs, De Hoog, Haase 2012
Cladophialophora mycetomatis	CBS122637	Human	-	28	ACGGTTTGGTCGACGACACCCGACCCCT	Heinrichs, De Hoog, Haase 2012
	CBS 454.82	Culture Contaminant	-	28	ACGGTTTGGTCGACGACATCCGACCCCT	
Cladophialophora psammophila	CBS110553	Gasoline-polluted soil	41	-	CAGAGGACGCCGAGTCGGCAGTCCTCCGGCGGTCAGTTTAA	Hamzehei et al., 2013; Heinrichs, De Hoog, Haase 2012
			-	34	ACGGTCTGGCGGAAGTGTCGTGCACGCCGCCCCT	
Cladophialophora samoensis	CBS259.83	Human	-	31	AGGGTCCCTGGTCGAGGCTCTACCTCGACCT	Heinrichs, De Hoog, Haase 2012
Cladophialophora saturnica	CBS118724	Human tinea nigra-like skin lesion	42	-	GAATAGGCTATCGGCGGACAACGGGCCAGAGGACGCCCAGGT	Hamzehei et al., 2013; Heinrichs, De Hoog, Haase 2012
			-	27	ACGGCTTGGTAGAGTTCCCTCTACCCT	
Cladophialophora subtilis	CBS122642	Food Ice tea	-	30	ACGGCCTTGGTCGCGCACGCGCCGACCCCT	Heinrichs, De Hoog, Haase 2012
Cladophialophora yegresii	CBS11440	Plant *Stenocereus griseus*	42	-	GACGCCTCACGGCGATCCCACGGGCGGTCGTGAGACGGGTCC	Hamzehei et al., 2013; Heinrichs, De Hoog, Haase 2012
			-	30	ACGGTTTTGGTCGAGTGTCCCTCGACCCCT	
Cyphellophora europaea	CBS129.96	Human Skin (toe)	34	-	GGGCCCGGGGTCCTTGCGGACCCCCGGCGGCCTC	Heinrichs, De Hoog, Haase 2012, Feng et al., 2013
			-	18	AGCGGCCCCCCCGCTCTT	

Table S-2. Continued.

Cyphellophora guyanensis	CBS 124764	Eucalyptus	42	-	AACTCAGACGACACGTTTAAGTACAAGAGTTTGTGGTTGGGG	Feng et al., 2013
Cyphellophora laciniata	CBS 190.61	Human skin	33	-	CCCGGCGGCCACCCCTTGCGGGGCGGCGGGCCT	Heinrichs, De Hoog, Haase 2012, Feng et al., 2013
			-	35	ACGTCGGCGGCGGCCTTTAGTGGCGTACCGCCCGT	
Cyphellophora oxyspora	CBS 698.73	Decaying leaf Plant	37	-	CCGGGCCCCAAAGGGACACCAGCGGGCCTGCCGAAGC	Feng et al., 2013
Cyphellophora pluriseptata	CBS 286.85	Toenail	36	-	CCCGGTGAGGGGGAGGTTGCGGCGGGCCTGCCGAAG	Heinrichs, De Hoog, Haase 2012, Feng et al., 2013
			-	31	ACGCGGCGGCGGCGCACCCGCCCCCGCCCGT	
Cyphellophora suttonii	CBS449.91	Mamal (Dog ear)	-	35	ATGTTGGCGGCGGCCTTTAGTGGCGTACCGCCCGT	Heinrichs, De Hoog, Haase 2012
Cyphellophora vermispora	CBS228.86	Triticum (Plant)	32	-	GGACCCCCGGCGGCTGCTCCTCGCGGGGCGGC	Heinrichs, De Hoog, Haase 2012, Feng et al., 2013
			-	35	ACGTCGGCGGCGGCCTTTAGTGGCGTACCGCCCGT	
Exophiala alcalophila	CBS520.82	Soil	-	31	ACGGCTTGGTTACGCTCCCCGCGTGACCCCT	Heinrichs, De Hoog, Haase 2012
Exophiala angulospora	CBS 119911	Weedy seadragon	36	-	GTTGCTTCGGCGGGCCCGTCTTCGTGACCGCCGGAG	Heinrichs, De Hoog, Haase 2012, Najafzadeh et al., 2018
	CBS482.92	decorticated wood	-	28	ACGGCCTGGTCGAGTCCGCTCGACCCCT	
	CBS122264	skin and nail	-	28	ACGGCCTGGTCGAGTCCCCTCGACCCCT	
	CBS109906	cod *Gadus morhua*	-	28	ACGGCTTGGTCGAGTCCCCTCGACCCCT	
Exophiala aquamarina	CBS 119915	Little tunnyfish	37	-	TTGGACGGCTTGGTGGACGCCCCGCACGGGGCGTCCA	Heinrichs, De Hoog, Haase 2012, Najafzadeh et al., 2018
	CBS 119918	skin of leafy sea dragon (*Phycodures eques)*	-	38	ACGGCTTGGTGGACGCCCCGCACGGGGCGTCCACCCCT	

Table S-2. Continued.

Exophiala asiatica	BMU00015	tonsil tissue of a human	-	36	ACGGCCCGGTCGACGCGTCATTTAGCGCCGACCCCT	Heinrichs, De Hoog, Haase 2012
Exophiala attenuata	CBS110026	Man, cutaneous lesion of ring finger	-	30	ACGGTTTGGTGTCTGGCAACGGTCACCCCT	Heinrichs, De Hoog, Haase 2012
Exophiala bergeri	CBS353.52	Human	-	31	ACGGTCTGGTTTAGGCACCGCCTAGACCCCT	Heinrichs, De Hoog, Haase 2012
Exophiala brunnea	CBS587.66	*Acacia karro*, litter	-	38	ACGGTTTGGTGGAGGCCCCTCACGGGGCTCCTGCCCCT	Heinrichs, De Hoog, Haase 2012
Exophiala cancerae	CBS120532	Crab	-	34	ACGGTTTGGTGGAGGGCCTTCGGGCGCCACCCCT	Heinrichs, De Hoog, Haase 2012
Exophiala capensis	CBS128771	Leaf bracts *Phaenocoma prolifera*	-	31	ACGGCTCGGTCTAGGTACCGCCTAGACCCCT	Heinrichs, De Hoog, Haase 2012
Exophiala castellanii	CBS 110025	Drinking water	37	-	TTGGTGTTGGACGGTTTGGTGTCGGCAACGTCACCCC	Heinrichs, De Hoog, Haase 2012, Najafzadeh et al., 2013
	CBS158.58	Man, subcutaneous cyst, left wrist	-	28	ACGGTTTGGTGTCGGCAACGTCACCCCT	
Exophiala dermatitidis	CBS207.35	Human	36	-	AGGGGTCGCGCGGAAACGCTCGACCAGACCGTCCAA	Heinrichs, De Hoog, Haase 2012, Najafzadeh et al., 2013
			-	32	ACGGTCTGGTCGAGCGTTTCCGCGCGACCCCT	
	CBS100338	Humidifier	-	32	ACGGTATGGTCGAGCGTTTCCGCGCGACCT	
	BMU00035	Neurotropic infection	-	37	ACCGGTCTGGTCAAGCGTTTCCGCGCGACCCT	
Exophiala equina	CBS 109789	Human, dialysis	37	-	TRGTTAAAGATTTTAATGGTTGGGCTACCGACGAGCG	Heinrichs, De Hoog, Haase 2012, Najafzadeh et al., 2018
	CBS119.23	Horse skin	-	38	ACGGTTTGGTGGAGGCCCCCTCGGGGGCTCCTGCCCCT	
	CBS122263	Unknown	-	38	ACGGTTTGGTGGAGGCCCCCTCGGGGGTTCCTGCCCCT	
	CBS122270	Human skin	-	38	ACGGTTTGGTGGAGGTCCCCTCGGGGGCTCCTGCCCCT	
Exophiala exophialae	CBS668.76	Straw in burrow of *Dasypus septemcinctus*	-	32	ACGGTTTGGTCCCGGGACGCCCCTGGACCCCT	Heinrichs, De Hoog, Haase 2012

Table S-2. Continued.

Exophiala halophila	CBS121512	Man, axillary	-	34	ACGGCTTGGTCGCGCCCCCCGGGCACCGACCCCT	Heinrichs, De Hoog, Haase 2012
Exophiala heteromorpha	CBS232.33	Wood pulp	-	29	ACGGTCTGGTCGAGCCCGCCTCGACCCCT	Heinrichs, De Hoog, Haase 2012
Exophiala jeanselmei	CBS507.90	Human Man	37	-	GTGTACCGGGGGTCGAACAGCCCGCCGTCATTGTCTT	Heinrichs, De Hoog, Haase 2012, Najafzadeh et al., 2013
			-	36	ACGGTTTGGTCTCGGGTCCGACCCCCCTTGACCCCT	
	CBS677.76	Skin, abscess of foot	-	37	ACGGTTTGGTCAAAGGGTCCGACCCCCCTAGACCCCT	
Exophiala lacus	CBS117497	Water	-	37	ACGGTTTGGCGGAGACCTGTTACAGGCCTCCACCCCT	Heinrichs, De Hoog, Haase 2012
Exophiala lecanii-corni	CBS123.33	Human	-	29	ACGGCCTGGCGTCGGCGACGACCCCACCT	Heinrichs, De Hoog, Haase 2012
Exophiala mesophila	CBS402.95	Silicone seal, in shower room of hospital	-	28	ACGGCTTGGTGTCAGCGATGTCACCCCT	Heinrichs, De Hoog, Haase 2012
	CBS121511	Nasal tissue	-	28	ACGGCTTGGTGTCAGCAATGTCACCCCT	
Exophiala moniliae	CBS520.76	Quercus, twig	-	26	ACGGTCTGGTTAGGCGACTGACCCCT	Heinrichs, De Hoog, Haase 2012
Exophiala nishimurae	CBS101538	Bark Plant	-	32	ACGGCTTGGTCTAGGTCGTCGCCTAGACCCCT	Heinrichs, De Hoog, Haase 2012
Exophiala oligosperma	CBS725.88	Human	37	-	TTTGAGGTCCCCGRACCARACCGTCCAACACCAAGCC	Heinrichs, De Hoog, Haase 2012, Najafzadeh et al., 2013
			-	38	ACGGTCTGGTCCGGGGACCTCAAACCCCCTGGACCCCT	
	UTHSC95-2041	Human	-	38	ACGGTTTGGTTCGGGGACCTCAAACCCCCTGGACCCCT	
	UTHSC91-870	Human	-	39	ACGGTTTGGTCCGGGGACCTCAAAGCCCCCTGGACCCCT	
	IFM41701	Soil	-	40	ACGGTTTGGTCCGGGGACCTCAAAAACCCCCTGGACCCCT	
Exophiala opportunistica	CBS 631.69	Unknown	36	-	CGGACCGCCGGGGGGTCTTYGYACCCCTTGGCCCGC	Heinrichs, De Hoog, Haase 2012, Najafzadeh et al., 2018
	CBS109811	Water	-	39	ACGGTTTGGTGGAGACCCCCTTCGCGGGCTTCTACCCCT	

Table S-2. Continued.

Exophiala pisciphila	CBS119913	Potbelly seahorse	37	-	GTTGCTTCGGCGAGCCCGTCTGTAATGGACCGCCGGG	Heinrichs, De Hoog, Haase 2012, Najafzadeh et al., 2018
	CBS 537.73	Fish *Ictalurus punctatus*	-	39	ACGGTTTGGTGGAGGCCCCCTCGCGGGGCCACCGCCCCT	
Exophiala salmonis	CBS 110371	Frog	37	-	GGGGCAGATGCCCGCAAGGGGCCTCCACCAAACCGTC	Hamzehei et al., 2013; Heinrichs, De Hoog, Haase 2012
	CBS 120274	Drinking water tap	37	-	GGGGCAGATGCCCGCAAGGGGCCTCCACCAAACCGTC	
	CBS157.67	Fish *Salmo clarkii*	-	37	ACGGTTTGGTGGAGGCCCCTTGCGGGCATCTGCCCCT	
Exophiala sideris	CBS121818	Berry, *Sorbus aucuparia*	-	29	ACGGTTTGGTCCAGGTCACCTGGACCCCT	Heinrichs, De Hoog, Haase 2012
Exophiala siphonis	UTHSC88-471	Human	-	27	ACGGTCTGGTCGAGCTGCTCGACCCCT	Heinrichs, De Hoog, Haase 2012
Exophiala spinifera	CBS899.68	Human Man, nasal granuloma	34	-	GAGGGGTCCAGGGGGGTCCCGGGACCAAACCGTC	Heinrichs, De Hoog, Haase 2012, Najafzadeh et al., 2013
			-	32	ACGGTTTGGTCCCGGGACCCCCCTGGACCCCT	
Exophiala xenobiotica	CBS118157	Oil sludge	37	-	CGTGCTCAGTTAAGAAGCTCAGTGTACCGGGGGTTCA	Heinrichs, De Hoog, Haase 2012, Najafzadeh et al., 2013
			-	30	ACGGTTTGGTTTAGGTACCCCTAGACCCCT	
	CBS119306	Human	-	30	ACGGTTTGGTTTAGGCACCCCTAGACCCCT	
	CBS117665	Human	-	30	ACGGTTTGGTTTAGGTACCCCTAGATCCCT	
	CBS117676	Human	-	33	AGGTCTTGGTGTAGGGTTCCCCCCTCCACCCCT	
	CBS117641	Human	-	32	AGGTCTTGGTGTAGGGTCCCCCCTACACCCCT	
Fonsecaea erecta	CBS125763	Living Japecanga plant	-	33	ACGGTCCGGTGGAGAGTCATCCTTTCCACCCGT	Heinrichs, De Hoog, Haase 2012
	CBS125764	Rotting wood	-	33	ACGGTCCGGTGGAGAGTCATCCTTTTCACCCGT	
Fonsecaea minima	CBS126865	Tucum palm tree	-	33	ACGGTTCGGTGGAGAGTCACACCTCCCACCCGT	Heinrichs, De Hoog, Haase 2012

Table S-2. Continued.

Fonsecaea monophora	CBS269.37	Human Skin	28	-	CAACGCCCGCATTGAGCGGTCCTCCAGC	Heinrichs, De Hoog, Haase 2012, Najafzadeh et al., 2011
			-	36	ACGGCTTGGTGGAGTAAGTTCACACTTTCCACCCCT	
	CBS121732	Human Skin	-	36	ACGGCTTGGTGGAGCAAGTTCACACTTTCCACCCCT	
	IFM4889	Human Skin	-	36	ACGGCTTGGTGGAGTAAGTTCACGCTTTCCACCCCT	
	IFM54446	Cervical lymph nodes	-	36	ACGGCTTGGTGGAGTAGGTTCACGCTTTCCACCCCT	
Fonsecaea multimorphosa	CBS980.96	Cat Brain Abcess	-	27	ACGGCTCGGTGGACTCCTTCCACCCGT	Heinrichs, De Hoog, Haase 2012
Fonsecaea nubica	CBS269.64	Human Skin	36	-	CGTCCAACACCAAGCGCAGGGGCTTGAGGGGGTGAT	Heinrichs, De Hoog, Haase 2012, Najafzadeh et al., 2011
			-	36	ACGGTTTGGTGGAGCGAGTTCACACTTTCCACCCCT	
	CBS121733	Human Skin	-	36	ACGGCTTGGTGGAGCGAGGTCACACTTTCCACCCCT	
	CBS557.76	Unknown	-	36	ACGGCTTGGTGGAGCGAGTTCACACTTTCCACCCCT	
Fonsecaea pedrosoi	CBS271.37	Human Skin	36	-	CGATACGTGCTCAATAAAGAAGCTCAGTGTACCGGG	Heinrichs, De Hoog, Haase 2012, Najafzadeh et al., 2011
			-	36	ACGGCTTGGTGGAGCGAGTTCACACTTTCCACCCCT	
	CBS122741	Human Skin	-	37	ACGGCTTGGTGGAGCGAGTTCACACTTTCCACCCCCT	
Fonsecaea pugnacius	CBS 139214	Human Brain Cerebral Absess	31	-	CGCTGGAGGACCGCCTGTCACGGGTGTTCTC	Schneider et al., 2019
Knufia epidermidis	CBS120353	Human	-	30	ACCCAAGTTTTTGGCTATTAAAAACTTGGT	Heinrichs, De Hoog, Haase 2012
Phaeoannelomyces elegans	CBS110172	Railway tie	-	38	ACGGTTTGGTGGAGGCCCCCTCGGGGGCTCCTGCCCCT	Heinrichs, De Hoog, Haase 2012
Phialophora americana	CBS840.69	Decaying timber	-	30	ACGGATTTTGGTCGTGTAACAACGACCCCT	Heinrichs, De Hoog, Haase 2012
Phialophora reptans	CBS113.85	Environment Food	37	-	CGCGAAGCTCCCGCCGGGTCCAACAACAAGCCGGGCT	Heinrichs, De Hoog, Haase 2012, Feng et al., 2013
			-	30	ACCCGGCGGGAGCTTCGCGCCCCCGCCCGT	

Table S-2. Continued.

Phialophora verrucosa	CBS273.37	Human	-	30	ACGGATCTTGGTCGTGTGATCGCGACCCCT	Heinrichs, De Hoog, Haase 2012
	IMTSP.800	Not Reported	-	30	ACGGATCCTGGTCGTGTGATCGCGACCCCT	
	CBS286.47	Fruit of *Musa sapientum*	-	30	ACGGATTTTGGTCGTGTGATATCGACCCCT	
	CBS839.68	Pathogen *Actinidia deliciosa*	-	30	ACGGATTTTGGTCGTGTGATACCGACCCCT	
Rhinocladiella anceps	CBS181.65	Soil under *Thuja plicata*	-	30	AAGGCCTGGTGTCCCCCCGGGGACACCCCT	Heinrichs, De Hoog, Haase 2012
	CBS157.54	*Fagus sylvatica*, stem	-	32	AAGGCCTGGTGTCTCCCCCCGGGGATACCCCT	
Rhinocladiella aquaspersa	CBS313.73	Human	-	27	ACGGTCGGCCTCTTCACCGAGGCCCCT	Heinrichs, De Hoog, Haase 2012
Rhinocladiella atrovirens	CBS264.49	Honey	-	28	ACGGCTGGTCGGGCGACGCCCGACCCCT	Heinrichs, De Hoog, Haase 2012
Rhinocladiella basitona	CBS101460	Human subcutaneous lesion	-	31	ACGGTTTGGTCTAGGGCCTCCCTAGACCCCT	Heinrichs, De Hoog, Haase 2012
Rhinocladiella mackenziei	CBS650.93	Human Brain infection	-	37	ACGGCCTGGGTTTGGAAGTTTTCTCTCGAGGCCCCCT	Heinrichs, De Hoog, Haase 2012
	CBS367.92	Human Brain infection	-	35	ACGGCCTGGGTTTGGAAGTTTTCTCTCGAGCCCCT	
	CBS102590	Human Brain infection	-	35	ACGGCCTGGGTTTGGAAGTTTTCTCTCAAGCCCCT	
Rhinocladiella similis	CBS111763	Human chronic cutaneous ulcer	-	32	ACGGTTTGGTCCAGGGCCCCCCCTGGACCCCT	Heinrichs, De Hoog, Haase 2012
	dH13054	Water	-	32	ACGGTTTGGTCCAGGGTCCCCCCTGGACCCCT	

Table S-2. Continued.

Veronaea botryosa	CBS 121506	Human, wrist skin	35	-	CGCCGGGGGACCCCTAACAGAGTCTCCTGGCCCGC	Heinrichs, De Hoog, Haase 2012, Najafzadeh et al., 2018
	CBS254.57	Plant Sansa, olive slag	-	40	ACGGTTTGGTGGAGGCCCCCTCGTCGGGGTCGCTGCCCCT	
	CBS350.65	Dung of goat	-	41	ACGGTTTGGTGGAGGCCCCCCTCGTCGGGGTCGCTGCCCCT	
Veronaea compacta	CBS268.75	Unknown	-	38	ACGGTTTGGCGGAGGCCCCTCGCGGGGCTCTCGCCCCT	Heinrichs, De Hoog, Haase 2012
Veronaea japonica	CBS776.83	Dead bamboo culm	-	38	ACGGTTTGGCGGAGGCCCCCTCGGGGGCTCTCGCCCCT	Heinrichs, De Hoog, Haase 2012

Table S-3. Species identified in skin metagenomic datasets.

Specie	PRJEB 37496		PRJEB 42399		PRJNA 656308		PRJNA 699281		PRJNA 638969		PRJNA 639280		PRJNA 704382		PRJEB 19454		PRJNA 478488		PRJNA 630834	
	N.Reads	%	N.Reads	%	N.Reads	%	N.Reads	%	N.Reads	%	N.Reads	%	N.Reads	%	N.Reads	%	N.Reads	%	N.Reads	%
C.arxii	0	0	0	0	0	0	0	0	0	0	0	0	8**	0,00003	0	0	0	0	0	0
C. chaetospira	0	0	0	0	0	0	0	0	0	0	0	0	40**	0,0002	42**	0,0009	2**	0,0000003	35**	0,0002
C. europaea	0	0	0	0	0	0	0	0	0	0	0	0	17**	0,0001	0	0	0	0	3970**	0,026099
C. guyanensis	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
C. immunda	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2**	0,00001
C. oxyspora	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	77**	0,00001	367**	0,002
C. pluriseptata	0	0	0	0	0	0	0	0	0	0	0	0	5**	0,00002	3**	0,00006	10**	0,000001	0	0
C. vermispora	0	0	0	0	0	0	0	0	0	0	0	0	193**	0,0007	1**	0,00002	0	0	0	0
C.laciniata	40*	0,0005	0	0	0	0	0	0	0	0	0	0	2**	0,00001	0	0	0	0	78**	0,0005
E. angulospora	0	0	0	0	0	0	0	0	0	0	0	0	4**	0,00002	0	0	0	0	0	0
E. attenuata	523*	0,006	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
E. dermatitidis	0	0	4***	0,0000003	40***	0,000003	12***	0,0000008	0	0	0	0	0	0	0	0	0	0	0	0
E. equina	88*	0,001	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
E. heteromorpha	0	0	2*	0,0000001	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
E. mesophila	0	0	0	0	46*	0,000003	0	0	0	0	0	0	0	0	0	0	0	0	0	0
E. oligosperma	0	0	6*	0,0000004	0	0	3*	0,0000002	0	0	0	0	0	0	0	0	0	0	0	0
E. pisciphila	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	18**	0,0001
E. sideris	570*	0,0066	11*	0,0000008	504*	0,00004	0	0	7*	0,0000005	0	0	0	0	0	0	0	0	0	0
E. spinifera	0	0	0	0	0	0	0	0	34***	0,000002	0	0	0	0	0	0	0	0	0	0
E. xenobiotica	5**	0,00006	0	0	3766***	0,0003	0	0	3***	0,0000002	2*	0,0000001	0	0	0	0	0	0	0	0
K.epidermidis	0	0	49*	0,000003	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
P. americana	0	0	1*	0,00000007	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
R. similis	0	0	1*	0,00000007	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
V. botryosa	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Total	**1226**	**0**	**74**	**0**	**4356**	**0**	**15**	**0**	**44**	**0**	**2**	**0**	**269**	**0**	**46**	**0**	**89**	**0,00001**	**4470**	**0**

*Identification by only padlock probes; **only barcodes; ***padlock probes and barcodes simultaneously.

Table S-3. Continued.

Specie	PRJE B25617		PRJNA 421247		PRJEB 25916		PRJNA 669317		PRJNA 438584		PRJNA 286273		PRJEB 16723		PRJNA 46333		PRJNA 478488	
	N.Reads	%	N.Reads	%	N.Reads	%	N.Reads	%	N.Reads	%	N.Reads	%	N.Reads	%	N.Reads	%	N.Reads	%
C.arxii	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
C. chaetospira	35**	0,000008	35**	0,0002	0	0	0	0	0	0	0	0	0	0	0	0	0	0
C. europaea	8**	0,000002	1045**	0,005	566**	0,003	206**	0,004	0	0	125**	0,002	14**	0,00007	1*	0,00000001	831**	0,0001
C. guyanensis	0	0	20**	0,00009	0	0	0	0	0	0	0	0	0	0	0	0	0	0
C. immunda	255**	0,00006	112**	0,0005	0	0	0	0	0	0	0	0	0	0	0	0	0	0
C. oxyspora	2062**	0,0005	1179**	0,005	0	0	0	0	0	0	138**	0,002	0	0	0	0	0	0
C. pluriseptata	0	0	4**	0,00002	0	0	405**	0,009	0	0	0	0	0	0	0	0	0	0
C. vermispora	0	0	0	0	2**	0,00001	0	0	0	0	0	0	0	0	0	0	0	0
C.laciniata	0	0	0	0	43**	0,0002	0	0	0	0	0	0	0	0	0	0	0	0
E. angulospora	0	0	0	0	116**	0,001	0	0	0	0	0	0	0	0	0	0	0	0
E. attenuata	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
E. dermatitidis	0	0	0	0	0	0	0	0	2**	0,00001	0	0	0	0	0	0	0	0
E. equina	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
E. heteromorpha	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
E. mesophila	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
E. oligosperma	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
E. pisciphila	0	0	211**	0,001	1**	0,000005	0	0	0	0	239**	0,003	0	0	0	0	0	0
E. sideris	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
E. spinifera	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
E. xenobiotica	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
K.epidermidis	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
P. americana	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
R. similis	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
V. botryosa	114**	0,00003	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Total	**2474**	**0**	**2606**	**0**	**728**	**0**	**611**	**0**	**2**	**0**	**502**	**0**	**14**	**0**	**1**	**0**	**831**	**0**

Table S-4. Summary of identifiers datasets contain sequences of black yeast-like fungi in this study.

BioProject	**Number of Accession Samples***
PRJEB19454	ERR1839522; ERR1845406; ERR1839524; ERR1839522
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PRJNA614620	SRR11426392; SRR11426398; SRR11426356; SRR11426383; SRR11426387; SRR11426390; SRR11426401

Table S-4. Continued.

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PRJNA638969	SRR12000113; SRR12000124; SRR12000146; SRR12000173; SRR12000174; SRR12000176; SRR12000102; SRR12000113; SRR12000124; SRR12000146; SRR12000168; SRR12000173 SRR12000175; SRR12000179; SRR12000113; SRR12000174; SRR12000179; SRR12000102; SRR12000113; SRR12000124; SRR12000146; SRR12000168; SRR12000173; SRR12000175; SRR12000179; SRR12000113; SRR12000174; SRR12000179
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PRJNA656308	SRR12425756; SRR12425762; SRR12425743; SRR12425810; SRR12425659; SRR12425756; SRR12425780; SRR12425813; SRR12425628; SRR12425629; SRR12425630; SRR12425641; SRR12425642; SRR12425651; SRR12425652; SRR12425657; SRR12425665; SRR12425673; SRR12425674; SRR12425688; SRR12425692; SRR12425707; SRR12425729; SRR12425734; SRR12425739; SRR12425740; SRR12425749; SRR12425750; SRR12425753; SRR12425763; SRR12425781; SRR12425792; SRR12425793; SRR12425797; SRR12425800; SRR12425629; SRR12425642; SRR12425688; SRR12425708; SRR12425729; SRR12425797; SRR12425688; SRR12425756; SRR12425762; SRR12425628; SRR12425629; SRR12425630; SRR12425641; SRR12425642; SRR12425651; SRR12425652; SRR12425657; SRR12425665; SRR12425665; SRR12425671; SRR12425673; SRR12425688; SRR12425692; SRR12425707; SRR12425708; SRR12425729; SRR12425734; SRR12425739; SRR12425740; SRR12425749; SRR12425750; SRR12425753; SRR12425763; SRR12425781; SRR12425788; SRR12425793; SRR12425797; SRR12425800
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Table S-4.Continued.

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PRJNA438584	SRR6847010
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PRJNA46333	SRR997246
PRJNA286273	SRR2061508; SRR2061543; SRR2061550; SRR2061582; SRR2061589; SRR2061594; SRR2061598; SRR2061646; SRR2061651; SRR2061653; SRR2061664; SRR2061666; SRR2061667; SRR2061668
PRJEB16723	ERR1701224; ERR1701229; ERR1701238; ERR1701241; ERR1701245; ERR1701247; ERR1701250

*The accession number of samples provide a complete information about the specific run that contain sequences of fungi identified by molecular markers in this study, accessing https://www.ncbi.nlm.nih.gov/sra/.

CHAPTER IV

Exploring the environmental occurrence of the black yeast like fungi diversity in Maranhão State, Brazil, with description of Chaetothyriales specie

Exploring the environmental occurrence of the black yeast like fungi diversity in Maranhão State, Brazil, with description of Chaetothyriales specie

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1. **ABSTRACT**

Black yeasts like fungi are a ubiquitous class that mediate many of biotic and abiotic processes in the ecosystems. In hot spots of diversity like Amazon rainforest this class could be poor understanding due to extremely slow growth rate and lack of distinguishing morphological characteristics. In this study, we surveyed the presence of melanized fungi in environmental samples from four cities in Maranhão State, Amazon region, Brazil by selective methods. Among 175 isolates, after preliminary delimitation using the internal transcribed spacer region (ITS) sequences, it was identified fungi as members of the genera *Cladophialophora*, *Cyphellophora*, *Exophiala*, among other taxa. In this work, we reported endophytic, saprobes and pathogenic black fungi present in live plants, plant debris and soil. The genes ITS and the nuclear ribosomal large subunit (LSU) suggest one specie not described in the Chaetothyriales order.

Keywords: Black Yeast; Chaetothyriales; Environmental isolation

2 . INTRODUCTION

Fungi are inconspicuous organism and an important part of ecosystems. In tropical biomes, forests provide the environmental services through high above- and below-ground biodiversity (WARDLE et al., 2004) Brazil shelter Amazonia Forest, the world's most diverse rainforest (ANTONELLIA et al., 2018, VLEMINCKX et al., 2019). Due the heterogeneous diversity, many of biotic and abiotic processes in this ecosystem are mediated by fungi (RITTER et al., 2020). Black fungi are ubiquitous organisms, which can be ecological classified as saprobes, opportunist and pathogens and climate conditions in rainforest allied to high variability of organic matter facilitate the development of these group (REVANKAR; SUTTON, 2010).

Maranhão state are part of Amazon region, Cerrado biome and the transition of both biomes an area and a rich area to explore the diversity of melanized fungi, although is described as an important endemic area of chromoblastomycosis in Latin America. The disease is characterized as an implantation mycosis caused mainly by black fungi from the Herpotrichiellaceae family (QUEIROZ-TELLES et al., 2017; AZEVEDO et al., 2015). Previous isolation studies realized in Maranhão (MARQUES et al, 2006; VICENTE et al., 2008; 2013) demonstrated environmental niches of the pathogens including the description of new species associated to living plants.

Environmental black fungi isolation studies performed in Amazonian region (NASCIMENTO et al., 2017; VICENTE et al., 2014) demonstrated that these niches still need to be explored. In this context, molecular markers have shown the presence of pathogenic species in several environmental niches (VOIDALESKI et al., 2020). Molecular techniques such as metagenomic and new generation sequencing have been used to elucidate biodiversity and classified the taxonomic groups of pathogens fungi (SOUSA et al., 2016; COSTA et al., 2022) in the environmental niches. However, the isolation techniques, are essential to access these fungi and better exploration the potential of these organisms. In this context, the previous molecular study developed in this endemic region by Voidaleski et al., 2020 using padlock probes indicated that this represents an efficient and sensitive molecular tool for the environmental screening of chromoblastomycosis agents.in environmental samples.

Thus, based on it the aim of this study was characterized the black yeast like fungi in environmental samples from four cities in Maranhão State, in order to elucidate niches joining metagenomic techniques and isolation methods with a description of new taxa.

3. **MATERIALS AND METHODS**

3.1 SAMPLING SITES AND SAMPLING

3.1.2 **Sampling areas**

A preselection of samples was done based on the previously study by molecular methods by Voidaleski et al., 2020. Samples were collected from ecosystems in Maranhão State, north of Brazil (Figure 1). These ecosystems are part of Amazon Region, Cerrado biome and the transition of both biomes an area of tropical and humid climate with an extensive rainforest with an important diversity of plants, including native palm trees, as Babassu coconut (*Orbignya phalerata* Mart., Arecaceae) has great economic importance for local development, explored for application in food, soap and skin products (SOUZA et al., 2011).

Pinheiro and Bacabeira City A and B respectively are part of Amazon Region in Maranhão State, Nina Rodrigues (C) belongs to biome transition zone and São Benedito do Rio Preto (D) represents the Cerrado Biome (Figure 1). The environmental samples collected was soil, decomposing plant material, and living plants as *Solanum paniculatum*, *Astrocaryum vulgare*, *Platonia insignis*, *Scoparia dulcis*, *Murraya paniculata* and *Urtica* spp. were collected in four cities of Maranhão state. Samples were sealed in sterilized paper bags and transported to the laboratory at ambient temperatures.

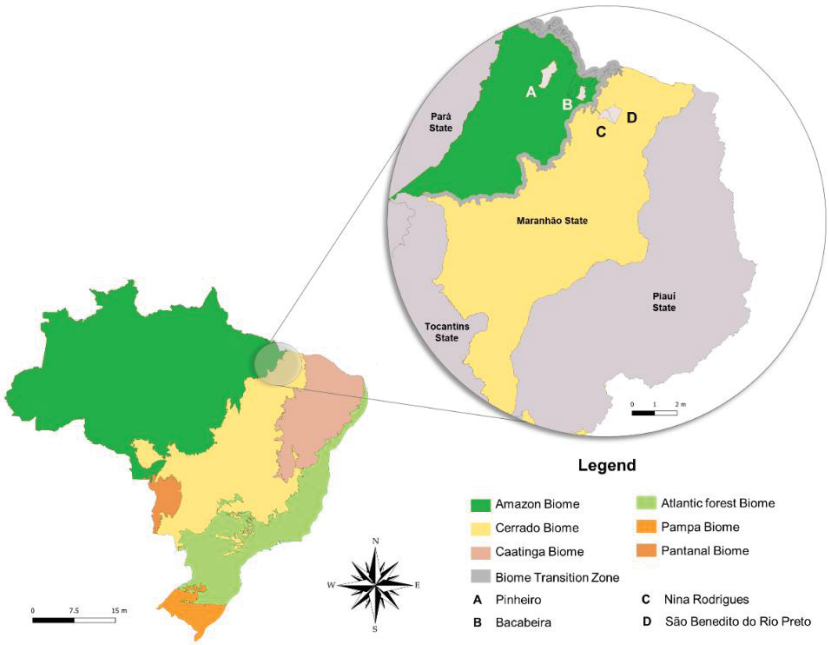

Figure 1. Study area of Maranhão state. The area of study was in the North and East of Maranhão state. Pinheiro (A) and Bacabeira (B) are part of Amazon biome; Nina Rodrigues (C) as a part of Biome Transition Zone and São Benedito do Rio Preto are in Cerrado Biomes.

3.2 METAGENOMIC ANALYSIS

3.2.1 **DNA extraction of environmental samples**

About 250 mg of each environmental sample was transferred to a 2 mL microtube containing 300 µL CTAB (cetyltrimethylammonium bromide) and about 80 mg of a silica mixture. Cells were grinded manually with a sterile pestle for approximately 5 min. Subsequently, 700 µL CTAB buffer was added; the mixture was vortexed for 5 min and incubated for 60 min at 65 °C; 600µL 24:1 chloroform: isoamylalcohol was added, mixed carefully, and centrifuged for 10 min at 12,000 g force. The supernatant was transferred to a new tube and 800 µL ice-cold 100 % isopropyl alcohol was added. DNA was allowed to precipitate for 45 min at -20 °C and then centrifuged again for 15 min at 12,000 g. The pellet was washed twice with 500 µL cold 70 % ethanol and once with 500 µL of cold 100 % ethanol. After drying at room temperature, samples were resuspended in 100 µL of ultrapure water. Purity and integrity of the DNA were evaluated by spectrophotometry (NanoDrop®, Thermo Scientific) and on agarose gel 1 % [16, 28].

3.2.2 **Target libraries ITS and metabarcoding analysis**

A pool of samples was submitted to a metagenomic analysis, in order to characterize the; Chaetothyriales species, and the taxonomic diversity of the samples. MiSeq for ITS2 region Metabarcoding Sequencing was performed by a commercial company (Zymo Research South America, Botucatu, Brazil,). PCR of the ITS2 region for fungi rRNA was performed using primers ITS3 (5'-TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGGCATCGATGAAGAACGCAGC-3') and ITS4 (5'-GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGTCCTCCGCTTATTGATATGC-3') (White et al., 1990), according to Illumina's amplicon-based metagenomics sequencing protocol. Fragments of 250 bp reads from forward and reverse primers were analyzed using Qiime2 (BOLYEN et al., 2019), following the Casava 1.8 paired end demultiplexed FASTQ to import. The elimination of primers and filtering of sequences was according to their quality and DADA2 package (CALLAHAN et al. 2016) was used to infer amplicon sequence variants (ASVs). Following primer removal, paired reads were loaded into the DADA2 pipeline and trimmed (forward and reverse reads 3′ truncated at 230 nt), filtered (max. 2 errors per read, minimum length after trimming = 220 nt), and finally merged with a minimum required overlap of 10 nt. Chimeras were removed from merged pairs (59.04%). Following filtration and chimera

removal results in a total of 68,655 reads. Taxonomic classification of curated ASVs was performed using the feature-classifier 'classify-sklearn' (PEDREGOSA et al., 2011), with VSEARCH global alignment, and the databanks UNITE for fungi (v8.3.2021, threshold 99%) (KÕLJALG et al., 2020).

3.3 ISOLATION OF ENVIRONMENTAL SAMPLES

Fungi were isolated using selective isolation by oil flotation (IWATSU et al. 1981, VICENTE et al., 2008). Plants materials were fragmented into leaves, stems, and thorns (when present). A total of 435 replicates were analyzed, with 5 replicates of eighty-seven samples (20 of soil, 20 plant debris, 20 leaves, 20 stems, 07 thorns).

Approximately 20 g from each environmental sample were transferred to an Erlenmeyer with100 mL of a sterilized saline solution. Antibiotics solutions as penicillin (200 U), streptomycin (200 µg/L) and chloramphenicol (200 µg/L) was added to control bacterial grow and antimicrobial cycloheximide solution (500 µg/L) was included. The solution was incubated at room temperature (20ºC+/- 2ºC) for 30 min. sterilized mineral oil (20mL) was added to the solution and vigorously shaken manually for 5 min. The oil-water was left to settle for 40 min and then aliquot of the interphase oil-water (100mL) was inoculated onto Mycosel Agar (Difco) and incubated for 4 weeks at 28 ºC. Darkly pigmented colonies were transferred to Sabouraud Dextrose Agar (SAB; peptone 1%, glucose 4%, and agar 1.5%) for purification and identification by monosporic method. The isolates specimens were deposited in Microbiological Collections of Paraná Network – Taxonline (CMRP/Taxonline).

3.3.1 **Morphology**

Preliminary identification was carried out based on macro and microscopic features of the monosporic colonies after slide culturing on 2% malt extract agar (MEA; malt extract 1%, peptone 1%, glucose 1%, and agar 1.5%) after twenty-one days of incubation at 28ºC (de Hoog et al.2000a). The slide culture method was applied to observe the conidiogenese.

Digital images of samples were acquired using a Zeiss AXIO microscope with a Nikon digital sight DS-Fi1 camera.

3.3.2 **Physiology**

Cardinal growth temperatures and optimal pH growth were determined on 2% malt extract agar (MEA; malt extract 1%, peptone 1%, glucose 1%, and agar 1.5%). The temperature range available was 18–36 °C at intervals of 3 °C incubated in the dark for 3 weeks. In addition to determine the potential human infection, the growth was also recorded at 37 °C and at 40 °C. The pH values were available of 4,0 to 7,5 and the plates was incubated in the optimal temperature determined previously. All tests were performed in triplicate and the diameters of the colonies were recorded weekly. The results were plotted using temperature (°C) versus colony diameter (mm) as parameters.

3.4 MOLECULAR IDENTIFICATION

3.4.1 **DNA Extraction**

The total DNA was extracted from the monosporic colony on SAB plates according described by Vicente et al., 2008. Fungi mycelium with 15 d-old cultures was transferred to a 2 mL microtube with of a silica mixture (80 mg) (silica gel H, Merck / BIOTEC Celite 545, 2:1, w/w) and CTAB buffer (300mL) [CTAB 2 % (w/v), NaCl 1.4 M, Tris-HCl 100 mM, pH 8.0; EDTA 20 mM]. Cell disruption occurred manually with a sterile pestle for approximately 5 min and incubation for 10 min at 65 °C. CIA solution (24:1 chloroform: isoamylalcohol) (500uL) was added, and the DNA samples was mixed and centrifuged for 5 min at 20,500 g. DNA was precipitated ice-cold 96 % ethanol (800uL) for 2h at -20 °C. The pellet was obtained by centrifugation for 5 min at 20,500 g, washed with cold 70 % ethanol (500uL), drying at room temperature, and then resuspended in ultrapure water (100 µL). The integrity of DNA was available on agaroses gel 1% and the quantification was performed in a spectrophotometer spectrum (NanoDrop®, Thermo Scientific, Waltham, MA, EUA).

3.4.2 **PCR and Amplification**

The ITS rDNA region was chosen to initial characterization of isolates. Additional identification of the new specie phylogeny was performed by PCR-amplification with corresponding primer of LSU (the nuclear ribosomal large subunit) (Table 1).

PCR amplifications were made from 12.5 µL reaction mixtures, comprising 1× PCR buffer, 2.0 mM $MgCl_2$, 25 µM of deoxynucleoside triphosphates (dNTPs), 0.5 µM of each forward and reverse primer, 1 U of Taq DNA polymerase (Ludwig Biotec, Bela Vista, Brazil), and 10 ng of genomic DNA. PCR parameters for amplification were 95 °C for 4 min, followed by 35 cycles consisting of 95 °C for 45 s, 52 °C for 30 s, and 72 °C for 2 min, and a delay at 72 °C for 7 min, performed in an ABI Prism 2720 thermocycler (Applied Biosystems, Foster City, USA). The annealing temperatures was changing according to the gene are described in Table 1.

Purification of amplicons were performed with exonuclease I (Applied Biosystems, Foster City, CA, USA) and shrimp alkaline phosphatase (SAP) (Applied Biosystems, Foster City, CA, USA) according to the manufacturer's instructions. Amplicons were subjected to sequencing with BigDye™ Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, USA) according to the manufacturer's instructions and the amplification condition as follow: 95 °C for 1 min, followed by 30 cycles consisting of 95 °C for 10 s, 50 °C for 5 s and 60 °C in an ABI Prism 2720 thermocycler (Applied Biosystems, Foster City, USA). The primers used for each region was the same of the PCR amplification.

Table 1- Primers for Identification

Locus	Target region	Primer	Oligonucleotides (5'–3')	Annealing Temperature	Reference
Internal transcribed spacer (ITS)	ITS1-5.8S-ITS2 rDNA	ITS1	TCCGTAGGTGAACCTGCGG	52ºC	White et al., 1990
		ITS4	TCCTCCGCTTATTGATATGC		
Large subunit ribosomal (LSU)	26S rDNA	NL1	GCATATCAATAAGCGGAGGAAAAG	52ºC	O'Donnell, 1992; Vilgalys et al., 1990
		LR5	TCC TGA GGG AAA CTT CG		

3.4.3 **Phylogenetic Analysis**

Consensus sequences were visually inspected using BIOEDIT v7.2 (HALL, 1999) and compared with those in the GenBank database via BLAST and CBS databank (www.cbs.knaw.nl) to find the most similar taxonomic identification. To assess the phylogenetic position of isolates the phylogenetic analysis was conducted according to the preliminary identification. The alignments were made by MAFFT v7 (http://mafft.cbrc.jp/) and optimized manually using MEGA v7.2 (KUMAR, 2012).

Maximum likelihood (ML) was employed on phylogenetic analyses implemented in Mega v.7 software, and the best evolutionary model corresponding to the data set was used. In order

to determinate the position of new Chaetothyriales specie, phylogenetic analyses of the LSU (Large subunit ribosomal) and ITS (internal transcribed spacer) loci were performed with sequences of species described belong to order (QUAN et al., 2020). *Capnodium coffeae*, CBS 147.52 and *Capnodium salicinum*, CBS 131.34 were taken as outgroups of the tree (QUAN et al., 2020)

Sequence alignments were made by MAFFT v7 (http://mafft.cbrc.jp/) and optimized manually using MEGA v7.2 (KUMAR, 2012). For species delimitation, the region of ITS (internal transcribed spacer) was analyzed separately by algorithms maximum likelihood (ML) implemented in MEGA v7.0.26 with the best model corresponding to the data set was used. All analysis was conducted with statistical bootstrapping involving 1000 replicates, and values equal to or greater than 80% were considered statistically significant.

4 **RESULTS**

From the metagenomic analysis, 116,287 reads for each forward and reverse ITS primers were generated, with fragments having a minimum length of 280nt and maximum length of 482nt. After denoising, merging and chimeric filtering, 68,655 reads valid sequences (ASVs) were obtained for ITS. In the ITS library, ASVs arranged according to length from minimal for maximal of 220–423 bp were mapped against the UNITE database. The composition of fungal data revealed members of three phyla (Ascomycota, Basidiomycota, and Chytridiomycota) (Figure 1A). Most identifications concerned members of Ascomycota and Basidiomycota, while over 70,139% did not have association with any phylum, i.e., only with the Kingdom Fungi (Figure 2A). The order Capnodiales represents the major number of reads (8.371%) while Chaetothyriales order represents 0.556% of the identified reads (Figure 2C).

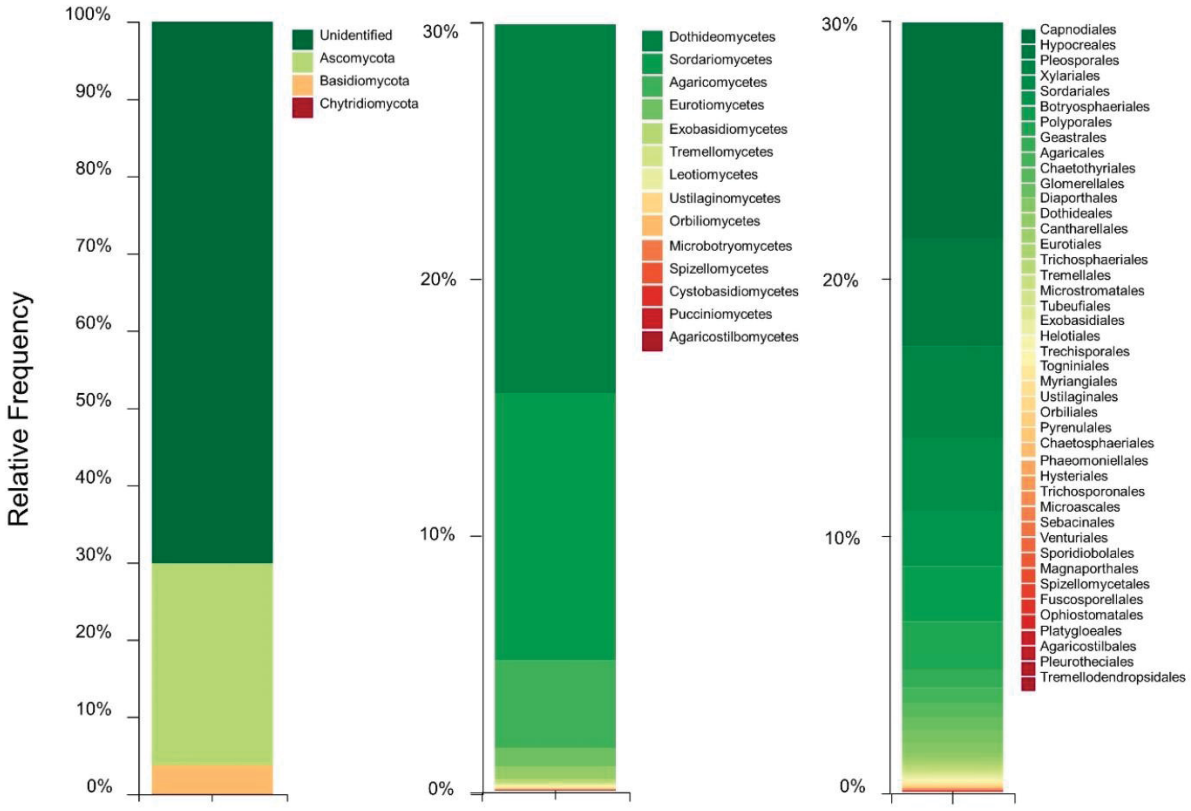

Figure 2. Microbial composition of environmental samples from Maranhão state, Brazil. A-Relative frequency (%) for each Phylum of fungi legend in color showing three Phyla for the ITS library, Ascomycota (26.154%), Basidiomycota (3.7%), and Chytridiomycota (0.007%). B. Dothideomycetes | class represents 14.369% of the reads identified and Eurotiomycetes 0.721%. C. Capnodiales is the major order identified (8.371%), Chaetothyriales was identified as law frequency (0.556%).

A total of 175 black yeast-like fungi were isolated from environmental samples analyzed. All substrates were able to isolate black-fungi and most isolates (n=80) was obtained in living plants (leave n=34, steam n=31, torn n=15) followed by plant debris (n=64) and soil (n=31). Micromorphology reveals a diversity of morphology isolates and rDNA ITS sequences are able to classify the taxonomic positions. Reference sequences from each group was taken to phylogenetic analysis. In the Chaetothyriales order, comparison of sequence data led to assignment of genera, i.e., *Fonsecaea*, *Exophiala* and *Cyphellophora*.

Exophiala spinifera an agent of cutaneous infection was recovered of soil from Bacabeira city. *F. brasiliensis* and *F. multimorphosa* both species involved in animal infections was present in plant debris from Pinheiro and Nina Rodrigues city. The *Cyphellophora* genus are present in samples from Bacabeira city, represents by *C. oxyspora* in torn, steam and leave of *A. vulgare* plant and soil. Moreover, the region is considered endemic, no chromoblastomycosis agents was isolate. *Arthrocladium* sp. e (n=1) was identified in low frequency.

Table 2. Isolates from environmental samples investigated in this study.

ID Molecular	CMRP	Source, Geography	Reference
Exophiala spinifera	CMRP2603	Soil, Bacabeira, Brazil	This study
	CMRP2623	Soil, Bacabeira, Brazil	
	CMRP2607	Soil, Bacabeira, Brazil	
Exophiala alcalophila	CMRP3075	Plant Debris, Brazil	
Fonsecaea brasiliensis	CMRP2988	Plant Debris, Nina Rodrigues, Brazil	
	CMRP2991	Plant Debris, Nina Rodrigues, Brazil	
	CMRP3066	Plant Debris, Nina Rodrigues, Brazil	
Fonsecaea multimorphosa	CMRP3063	Plant Debris, Nina Rodrigues, Brazil	
Rhinocladiella similis	CMRP2858	Soil, Bacabeira, Brazil	
	CMRP2861	Plant Debris, Bacabeira, Brazil	
Arthrocladium sp.	CMRP2610	Soil, Brazil	Voidaleski et al., 2020
Cypelhophora oxyspora	CMRP2608	Stem *S. paniculatum*, Bacabeira, , Brazil	
Exophiala spartinae	CMRP2611	Soil, Bacabeira, Brazil	This study
	CMRP3115	Soil, Bacabeira, Brazil	
	CMRP3096	Decomposing Material, Pinheiro, Brazil	
	CMRP3071	Decomposing Material, Nina Rodrigues, Brazil	
Chaetothyriales sp.	CMRP3087	Decomposing Material, Pinheiro, Brazil	
	CMRP3088	Decomposing Material, Bacabeira, Brazil	
	CMRP2855	Decomposing Material, Pinheiro, Brazil	
	CMRP3086	Steam *A. vulgare* Bacabeira, Brazil	
	CMRP2856	Decomposing Material, Pinheiro, Brazil	
	CMRP2865	Decomposing Material, Pinheiro, Brazil	
	CMRP2869	Decomposing Material, Pinheiro, Brazil	Voidaleski et al., 2020
	CMRP2874	Decomposing Material, Pinheiro, Brazil	
	CMRP3074	Stalk, *S. dulcis*, Pinheiro, Brazil	
	CMRP2875	Decomposing Material, Pinheiro, Brazil	
	CMRP2562	Stalk, *S. paniculatum*, Bacabeira, Brazil	
	CMRP3002	Decomposing Material, Pinheiro, Brazil	

CMRP- Microbiological Collections of Paraná Network – Taxonline (CMRP/Taxonline).

In order to assess the phylogenetic position of specie that did not match with any described taxon in Chaetothyriales order, a combined LSU and ITS tree (Figure 3) was provided by ML (Figure 3). The LSU analyses showed that isolates Chaetothyriales sp. CMRP 2869; CMRP2856; CMRP2855; CMRP3074 was clustered in a separate cluster in Incertae sedis clade close to *Capronia villosa* (Figure 3).

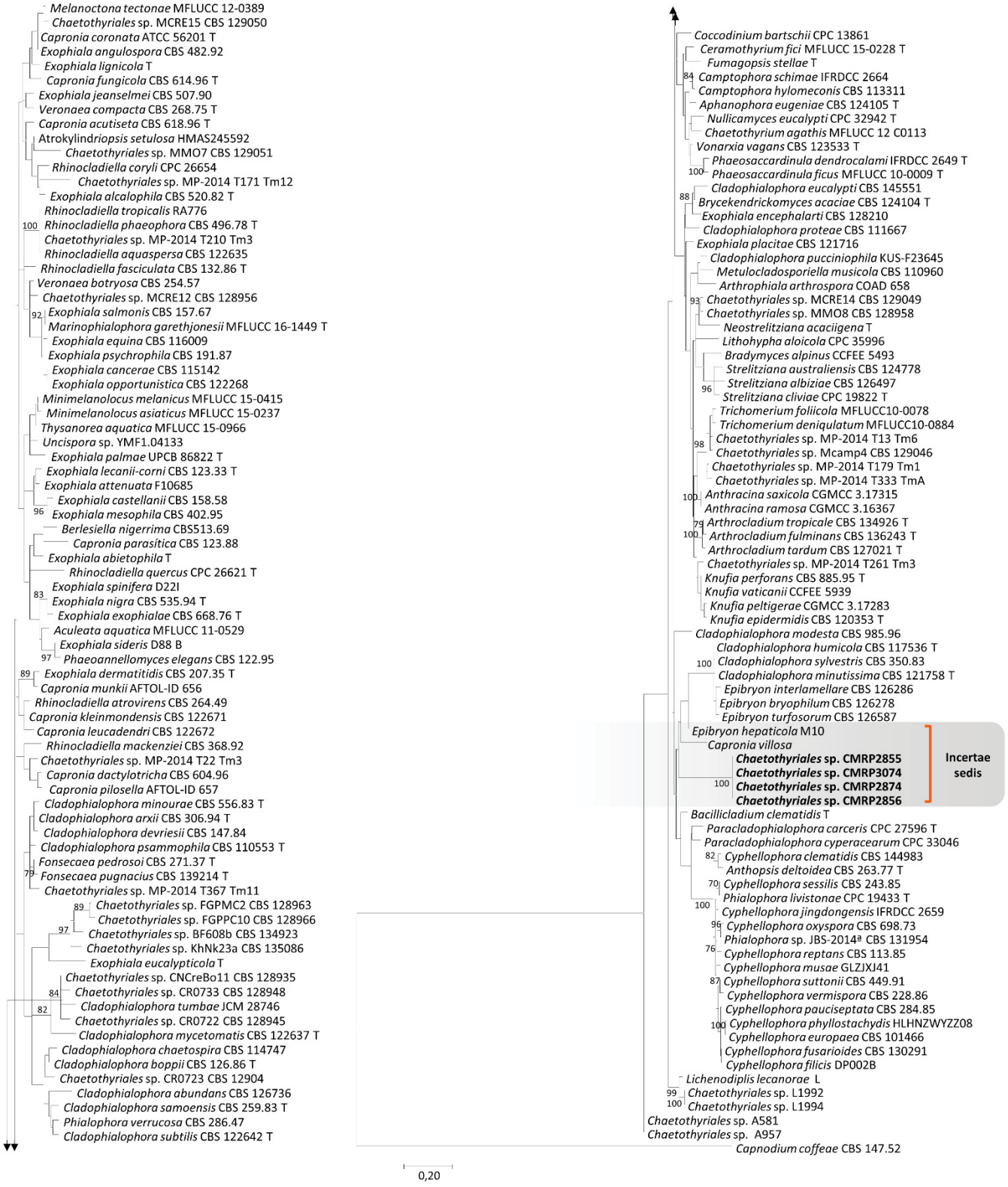

Figure 3- Phylogenetic tree of Chaetothyriales order based on LSU and ITS sequences, constructed with Maximum likelihood implemented in MEGA 7.0. Bootstrap support was calculated from 1000 replicates; values >80 % are considered significant. Capnodium coffeae CBS 147.52 was taken as outgroup. New species are indicated in bold belong to Incertae sedis. The species in the tree was recognized by de Quan et al. (2020). (T) = type strain of the species.

Chaetothyriales sp. nov. CMRP 3074 (Voidaleski, Vicente & de Hoog, sp. nov.)

Cultural characteristics: Colonies moderately expanding, with black color black in malt extract agar with olivaceous black reverse. Septate hyphae, pale brown to brown. No exudates or soluble pigments observed (Figure 6).

Type: Brazil, Pinheiro, Maranhão state, plant debris in rainforest; dried holotype will bewas deposited at Department of Botany Herbarium at Federal University of Paraná (UPCB) number (In process). Type strain culture *Chaetothyriales* sp. CMRP3074. Description after 2 weeks incubation on MEA, 28 ºC.

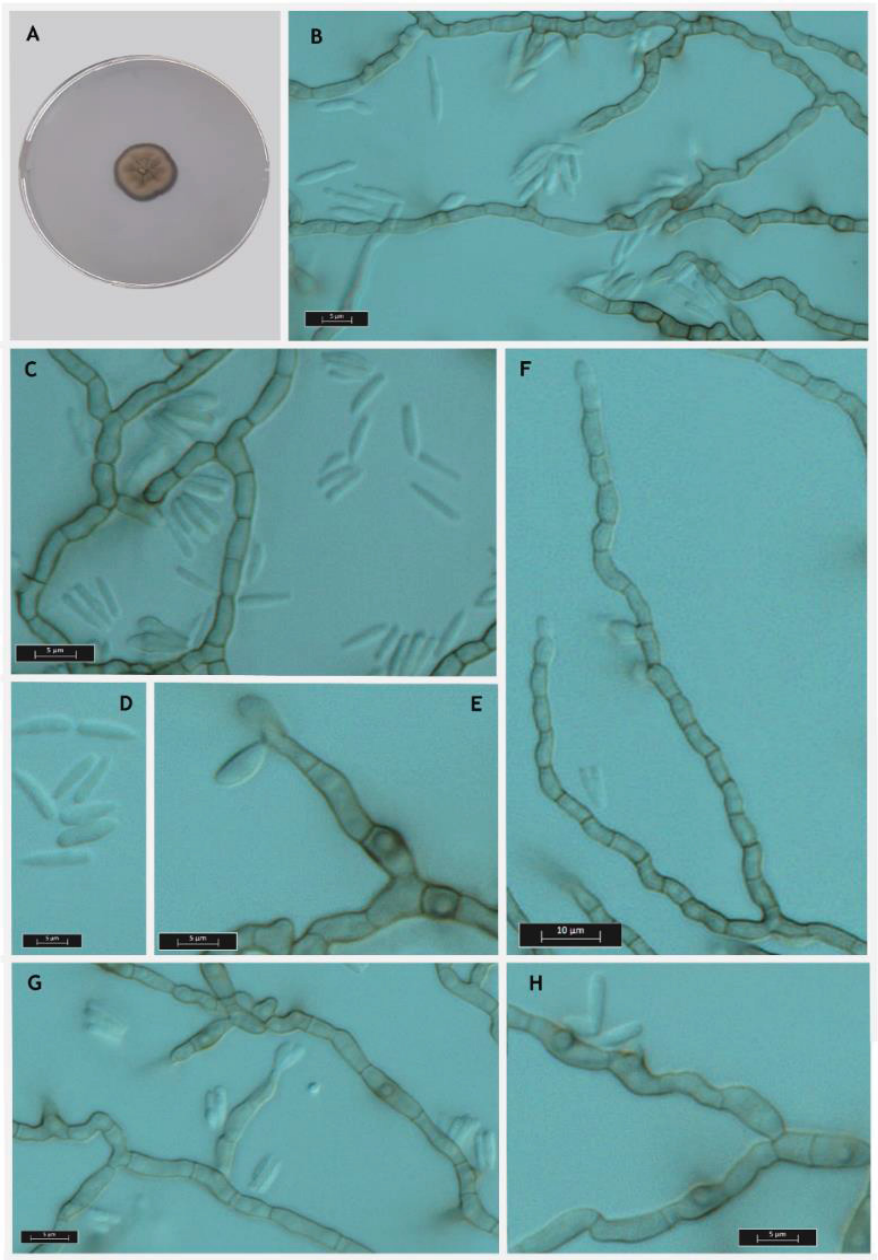

Figure 4. *Chaetothyriales* sp. nov. CMRP3074 microscopic morphology. (A) colony on MEA; (B-D) conidia; (E-F) Conidiophore. Scale bars 5-10 µm

4.1 PHYSIOLOGY

The evaluation of cardinal growth of *Chaetothyriales* sp. showed that the optimal temperature is 27°C +/- 2ºC (Figure 7A), related to environmental origin as a saprobe. The optimal value of pH was performed in 27°C, 31°C and 33°C, however the optimal temperature available above (27°C) (Figure 7A), demonstrate the best values to analyze the range of pH tolerate by the isolate. *Chaetothyriales* sp. CMRP3074 growth in acid and neutral values, 4,5 to 7,0, respectively, with the best value is 4,5, an acid Ph (Figure 7B).

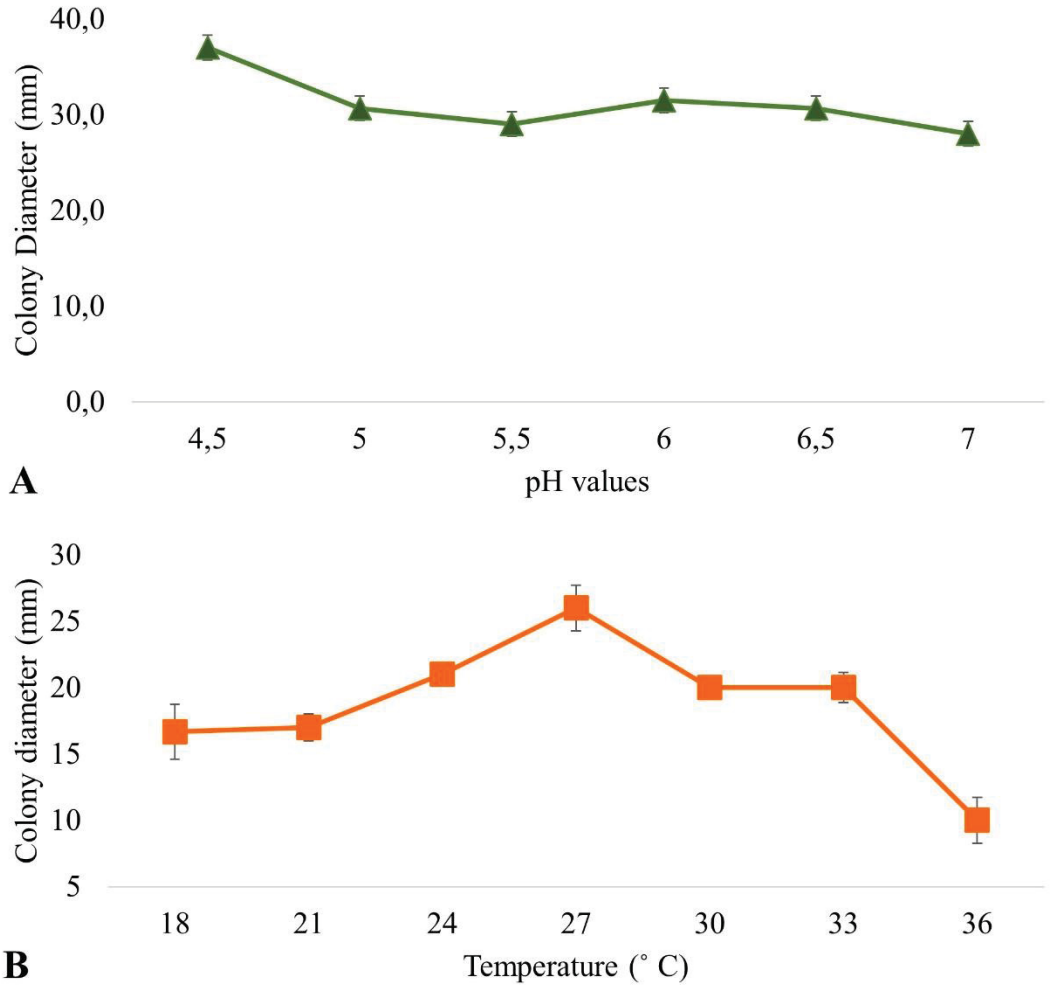

Figure 5. A. Colony diameters of novel species at different pH values ranging from 4,5 to 7,0 at 27ºC, measured after twenty-one days on 2 % MEA. B. Colony diameters of novel species at different temperatures ranging from 18 to 36 °C, measured after twenty-one days on 2 % MEA.

5 **DISCUSSION**

The diversity of black fungi has increase in the recent years. Several studies of eco epidemiological data of mycosis agents (VICENTE et al., 2012,2014; COSTA et al., 2020; VOIDALESKI et al., 2020) including environmental source before not related (LIMA et al, 2020) are reported. In addition, the diversity in this group has been explored to biotechnological application (IDE-PÉREZ et al., 2020; ISOLA et al, 2021). The hypothesis is that in the environment the black fungi species occurs in specific micro-habitats (SUN et al., 2020), and in conventional methods isolation the species are often low available due to ecological competitive with other organisms (VICENTE et al., 2014; IDE-PÉREZ et al., 2020). The oligotrophic characteristic is important to recover these fungi by selective methods based on aromatic hydrocarbons as mineral oil (VICENTE et al.,2001, 2008; SATOW et al., 2008; IDE-PÉREZ et al., 2020; ISOLA et al, 2021).

The Maranhão state are considered an endemic area in Brazil to chromoblastomycosis QUEIROZ-TELLES et al., 2017; SANTOS et al., 2020) The diversity of black fungi was explored with focus on agents of disease (MARQUES et al., 2006; VICENTE et al., 2014; COSTA et al., 2020; VOIDALESKI et al., 2020; LIMA et al., 2020). However, in this study we reported the black fungi diversity in this Amazon and Cerrado region, a hot spot of ecological diversity in Brazil. The selective oil flotation isolation technique (IWATSU et al. 1981, VICENTE et al., 2008) showed efficient in order to obtaining black yeasts (n=175 isolates) as previously established in other studies (IWATSU et al. 1981, VICENTE et al., 2001, 2008, 2012; MARQUES et al., 2006; GUERRA et al., 2013).

In this study, we investigate the black fungi in living plants as *S. paniculatum*, *A. vulgare*, *P. insignis*, *S. dulcis*, *M. paniculata* and *Urtica* spp. Black fungi can be found as endophytic and epiphytic (FORNARI et al., 2018 In Cyphellophoraceae family, *C. oxyspora* are found in stalk and thorns of *S. paniculatum* and thorn of *A. vulgare*, this species was reported from decaying leaves (GAO et al., 2015).

In natural environment soil is a part of a rich and diverse ecosystem by presence of microorganisms. Our data showed the presence of isolates belong to *Cypelhophora oxyspora* (n=3), *Fonsecaea brasiliensis* (n=3), *Fonsecaea multimorphosa* (n=1) and *Exophiala spinifera* (n=6). *E. spinifera* is a cosmopolitan dematiaceous fungus which can cause phaeohyphomycosis and chromoblastomycosis, with potential of systemic and disseminate infections (DE HOOG et al., 2011, HARRIS et al., 2009). Cases of disease in human host of are reported in country as India (SRINIVAS et al., 2016), Argentina (PEREZ et al., 2021) and

Brazil (DABOIT et al., 2012). Cutaneous phaeohyphomycosis in feline animal host are also reported (DALYA et al., 2020).

Environmental sources of plant debris are characterized by vegetal material as woody debris as leaf, little thorns and stalk that compound organic matter, this material provides energy, nutrient, and habitat for microbes during decomposition process cycling of forest ecosystems (ZHU et al., 2017). This source demonstrated was the most richness of isolates in this study with 17 isolated belong 6 taxons (*E. alcalophila*, *F. brasiliensis*, *F. multimorphosa*, *R. simlis*, *Chaetothyriales* sp. and *Exophiala spartinae*). Interesting that some isolates was identified in living plants sources from the same area, suggesting that these organisms can survive in the plant material after decaying of this material in soil and acts as saprobe in the ecosystem. *E. alcalophila* are reported indoor environment (BARON et al., 2021) and the biotechnological potential was observed by ability to grow under alkaline conditions and on a wide range of carbon sources (GOTO et al., 1981). The *Fonsecaea* genus are known by *F. pedrosoi* a major agent of chromoblastomycosis in the world and the region studied the cases of disease are essentially by this agent (QUEIROZ-TELLES et al., 2017; SANTOS et al., 2020). In our report, this genus was represented by *F. brasiliensis* and *F. multimorphosa*, both related in environmental sources by Vicente et al., (2008; 2013) and with potential infection phaeohyphomycosis in animal host (VICENTE et al., 2012; NAJAFZADEH et al., 2011).

Microorganisms of the genus *Exophiala* are commonly found in the environment in plant materials, wood, and soil (NASCIMENTO et al., 2017; SUN et al., 2020). The *Exophiala spartinae* CMRP 3096 was isolate in plant debris source from Pinheiro city, a recent proposed specie described in USA, in association with flooded environments. The new *Chaetothyriales* isolate CMRP 3074 was recovered in stalk of *S. dulcis* and plant debris sources from Pinheiro city, classified in the incertae sedis, a group proposed by Quan et al., 2020 that are composed by species *Epibryon hepaticola*, *Capronia villosa*, *Cladophialophora modesta*, *Cladophialophora hostae*, *Cladophialophora scillae*, could not be affiliated to any of the known families and are therefore regarded as incertae sedis.

The physiology of new specie presents the optimal temperatures at 27 °C, while that growth was observed over the entire range between 19 - 33°C, demonstrating that these fungi are saprobes, since the isolates do now grow at 36ºC. Both isolates have attributes as ability to thrive under extreme pH and temperature, for this reason the tolerance to high metal concentrations are evaluated to remediation applicability as a promising alternative.

Besides an endemic area to chromoblastomycosis in Brazil, our study recovered few chromoblastomycosis agents, belong to *Exophiala* genus. Phaeohyphomycosis agents are

represents by some species like *F. multimorphosa* and *F. brasiliensis*, classified as opportunistic in infections. However, it may be stated that the present study contributes to bioprospecting the diversity of black yeast-like-fungi in the biomes analyzed in Maranhão state Brazil. New species was explored with an alternative biotechnological potential of environmental species to tolerate heavy metals, including a bioremediation application. Authors should discuss the results and how they can be interpreted from the perspective of previous studies and of the working hypotheses. The findings and their implications should be discussed in the broadest context possible. Future research directions may also be highlighted.

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SUPPLEMENTARY MATERIAL

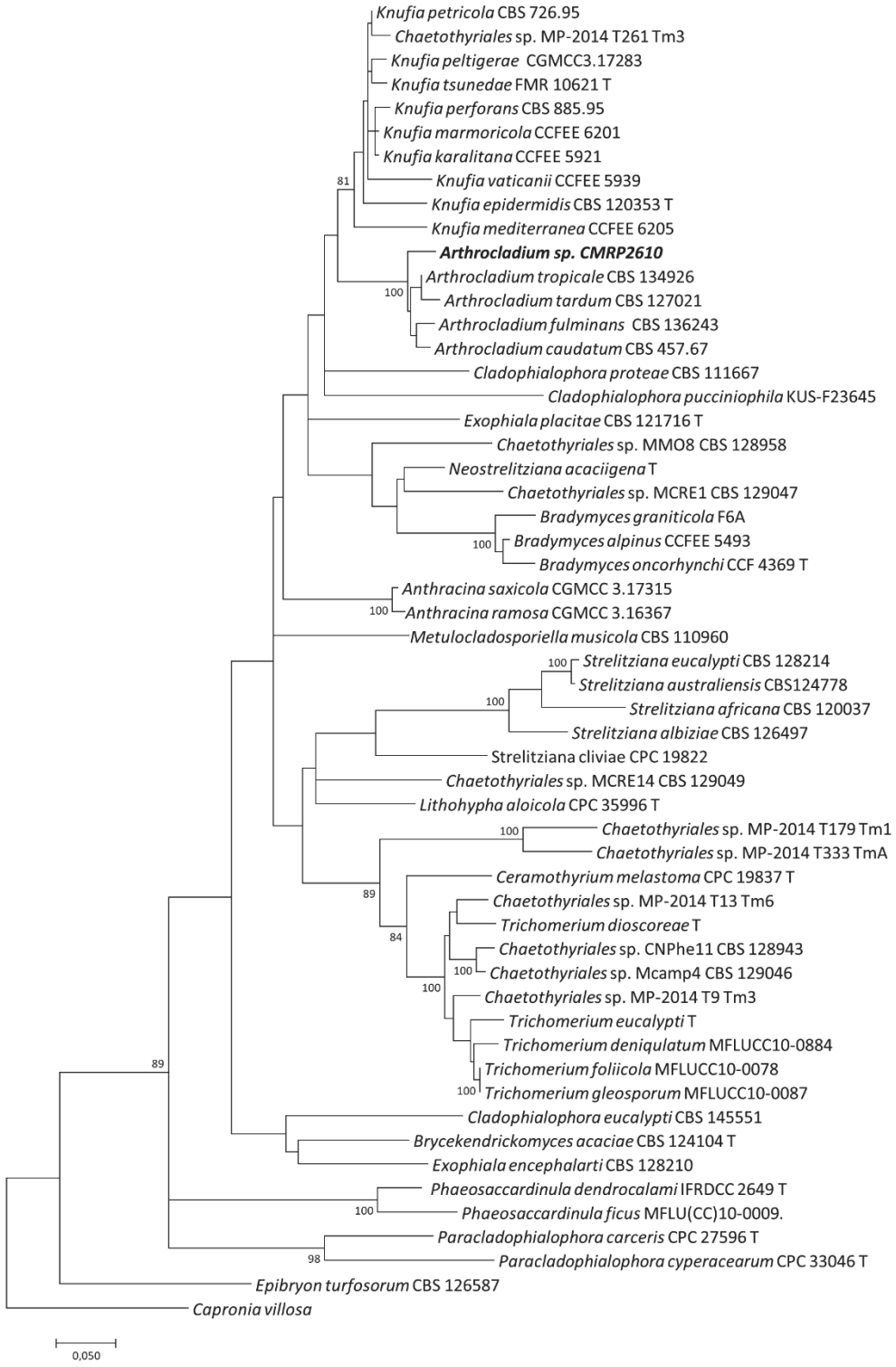

Figure S1- Phylogenetic tree of Trichomeriaceae family based on ITS sequences, constructed with Maximum likelihood implemented in MEGA 7.0. Bootstrap support was calculated from 1000 replicates; values >80 % are considered significant. *Capronia villosa* ATCC 56206 was taken as outgroup. The species in the tree was recognized by de Quan et al. (2020). (T) = type strain of the species.

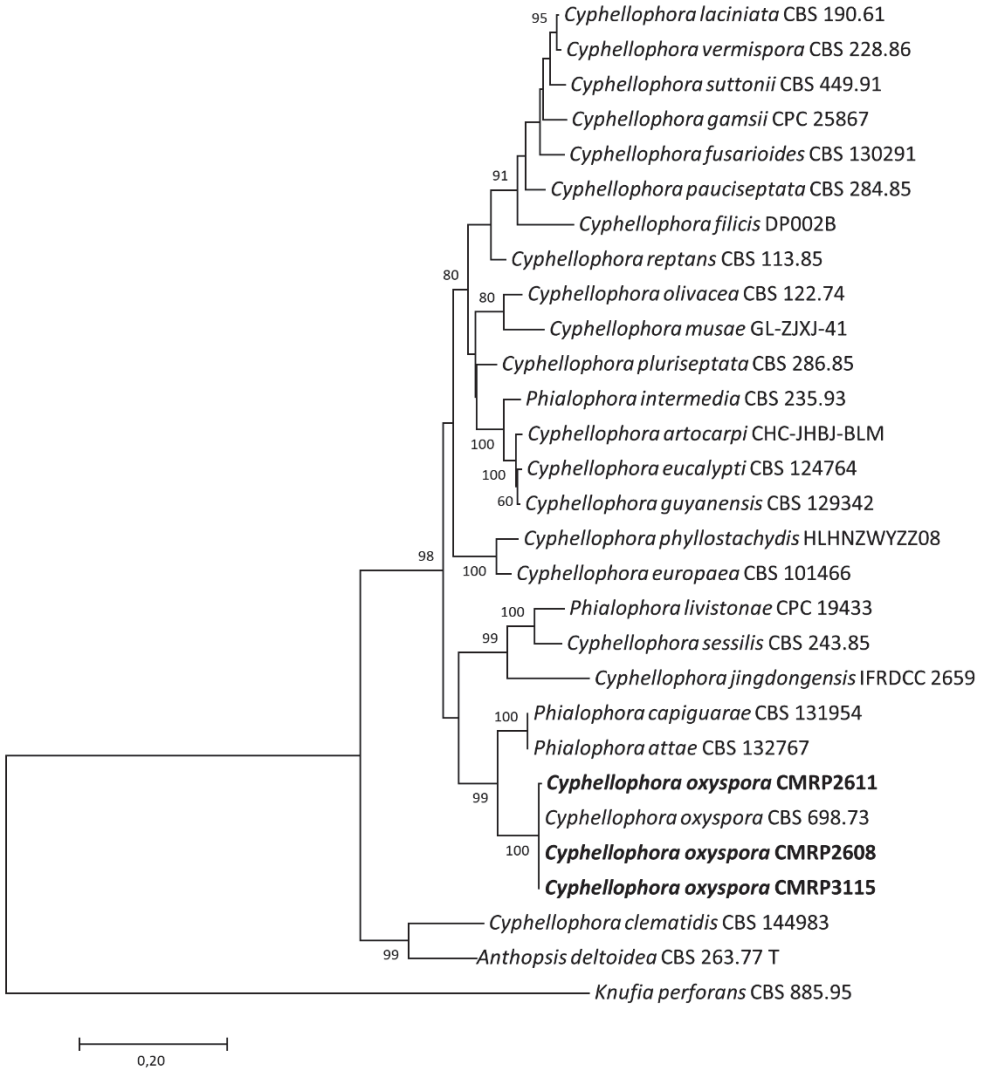

Figure S2- Phylogenetic tree of Cyphellophoraceae family based on ITS sequences, constructed with Maximum likelihood implemented in MEGA 7.0. Bootstrap support was calculated from 1000 replicates; values >80 % are considered significant. *Knufia perforans* CBS 885.95 was taken as outgroup. The species in the tree was recognized by de Quan et al. (2020). (T) = type strain of the species.

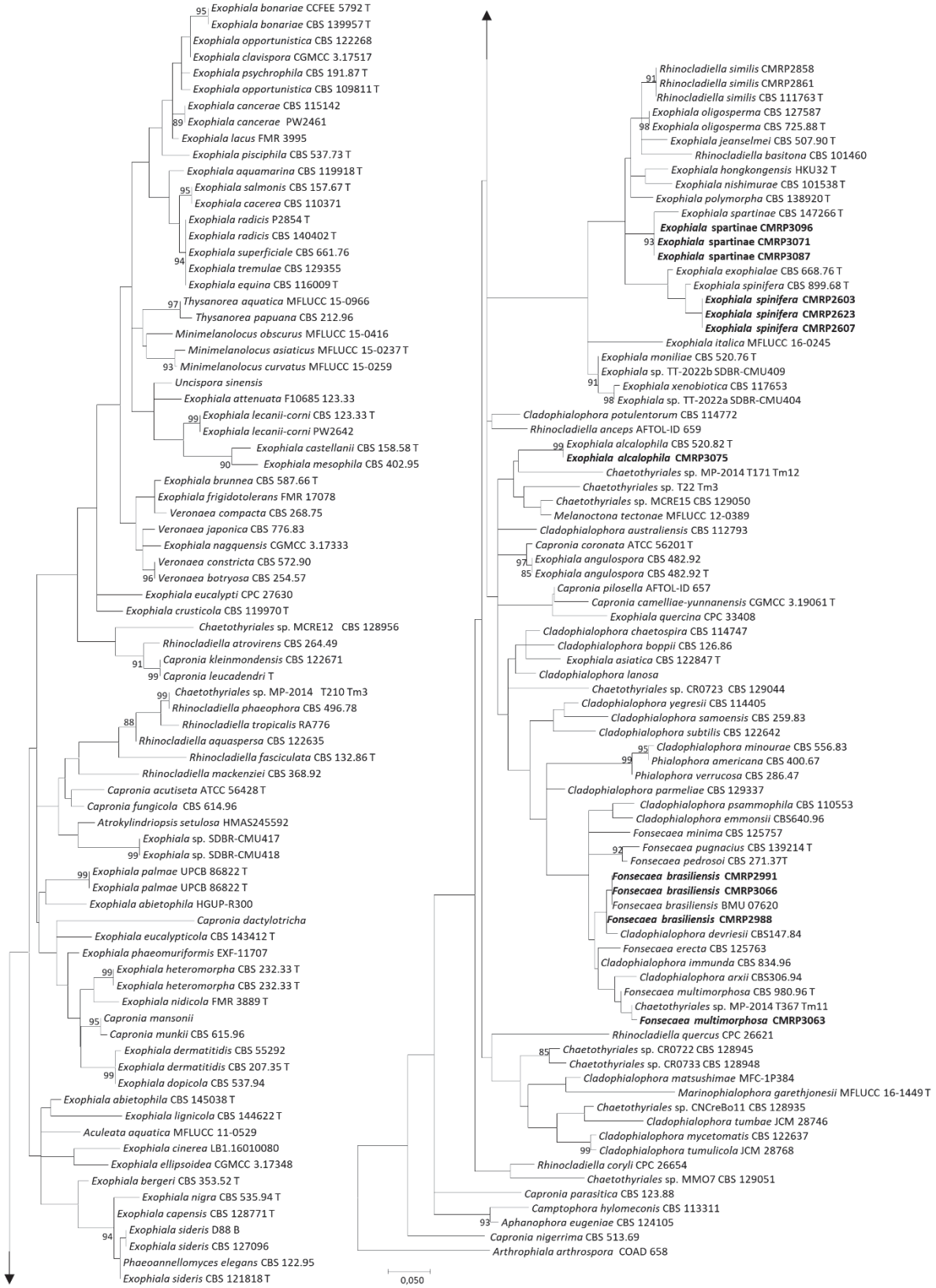

Figure S3- Phylogenetic tree of Herpotrichiellaceae family based on ITS sequences, constructed with Maximum likelihood implemented in MEGA 7.0. Bootstrap support was calculated from 1000 replicates; values >80 % are considered significant. *Arthrophiala arthrospora* COAD 658 was taken as outgroup. The species in the tree was recognized by de Quan et al. (2020). (T) = type strain of the species.

CHAPTER V

Conclusions and final considerations

CONCLUSIONS AND FINAL CONSIDERATIONS

As general conclusion of the thesis:

- The Chaetothyriales are worldwide distributed, and we described new substrates associated to this order, including animal and plant association.
- The environmental occurrence of some restricts pathogens as *Fonsecaea pugnacius* and *Rhinocladiella mackenziei* remains not elucidate.
- Metagenomics is an effective methodology to understand the epidemiology and distribution of Chaetothyriales order.
- Black yeast like fungi of Chaetothyriales are present on the skin without necessarily being associated with infection. *Cyphellophora* and *Exophiala* genus seems be adapted to skin, thus, the clinical aspects remaining unclear.
- Environmental substrates from endemic areas to mycosis associated to black yeast like fungi are a rich environment to explore the diversity of this fungi.
- We proposed the included new species in the Chaetothyriales order.

As final consideration of the thesis:

This thesis focused on infer insights on eco-epidemiology and the diversity of black yeast-like-fungi in Chaetothyriales, concentrating effort in clarify the different ecological trends among this group, based on metagenomic and isolation methodology.

In the Chapter II and III, through the analysis in silico with metagenomics data was possible infer insights on eco-epidemiology of the black yeast fungi belongs to Chaetothyriales in environmental samples of 19 countries, endemic and non-Endemic area. *Exophiala* show the most abundant species, with several niches associated to this group, including the skin microbiome. Despite *Fonsecaea pedrosoi* is considered the major agent of chromoblastomycosis and was frequent associated to environmental inoculation, the group was a low frequency in the data set analyzed. The consistent finds in the present thesis (chapters II and III) validate that the of black yeasts can be inside of plants and in your compartments, as root, rhizosphere, leaf and stalk, in addition to insects, animals and ice environments.

As observed in the chapter IV, Maranhão is an endemic area to chromoblastomycosis in Brazil, however the diversity of black yeast like fungi goes beyond pathogens associated to mycosis. The isolation methods were recovered several species belong to *Fonsecaea, Exophiala*, *Cyphellophora and Arthrocladium* genus in the Chaetothyriales order. The oil flotation method showed high success in bioprospecting black yeast, which is attributed to the

hydrophobicity of fungi melanized cells, and a new species *Chaetothyriales* sp. was obtained through this method. The alternative biotechnological potential of saprobic species in order to explore the potential use of enzymes from the fungi, without any biohazard problems**.**

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