UNIVERSIDADE FEDERAL DO ACRE – UFAC
PROGRAMA DE PÓS-GRADUAÇÃO EM BIODIVERSIDADE
E BIOTECNOLOGIA - REDE BIONORTE

OCORRÊNCIA DE FUNGOS DO GÊNERO *Paracoccidioides* (Onygenales: Ajellomycetaceae) NA AMAZÔNIA OCIDENTAL

IASMINY RANIELLY SILVA FERREIRA

Rio Branco – AC
2024

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Tese de doutorado apresentada ao Curso de Doutorado do Programa de Pós-Graduação em Biodiversidade e Biotecnologia - Rede BIONORTE, na Universidade Federal do Acre – UFAC, como requisito para obtenção do Título de Doutor em Biodiversidade e Biotecnologia.

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Rio Branco - AC
ABRIL/ 2024

Ficha catalográfica elaborada pela Biblioteca Central da UFAC

F383o Ferreira, Iasminy Ranielly Silva, 1994 -
Ocorrência de fungos do gênero *Paracoccidioides* (Onygenales: Ajellomycetaceae) na Amazônia ocidental / Iasminy Ranielly Silva Ferreira; orientadora: Profa. Dra. Clarice Maia Carvalho. – 2024.
68 f.

Tese (Doutorado) – Universidade Federal do Acre, Programa de Pós-graduação em Biodiversidade e Biotecnologia da Rede BIONORTE. Rio Branco, 2024.
Inclui referências bibliográficas.

1. Solos. 2. Tatu. 3. Fungos - Doenças. I. Carvalho, Clarice Maia, 1978 – (orientadora). II. Título.

CDD: 338.1

Bibliotecária: Alanna Santos Figueiredo – CRB 11º/1003.

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Dedico aos meus pais, João Lopes Ferreira (*in memoriam)* e Ozair Jardim da Silva, que com amor e apoio constante, me ensinaram a importância do conhecimento e me guiaram rumo à realização dos meus sonhos, e ao meu esposo, Atilon Vasconcelos de Araújo, pelo apoio incondicional.

AGRADECIMENTOS

A Deus, por me conceder proteção, saúde, paciência e discernimento para chegar ao dia de hoje.

À minha família, em especial a minha mãe Ozair Jardim da Silva e a minha irmã Jordana Jardim Ferreira, obrigado por todo amor e apoio.

Ao meu esposo, Atilon Vasconcelos de Araújo, obrigado por ser meu alicerce e companheiro constante.

À professora Dra. Clarice Maia Carvalho, agradeço pela sua paciência, carinho, profissionalismo e competência.

À professora Dra. Leila Priscila Peters, Gostaria de expressar minha profunda gratidão por seus ensinamentos, paciência incansável e amizade.

Ao curso de Pós-Graduação em Biodiversidade e Biotecnologia da Rede BIONORTE, em especial ao coordenador regional do Acre, prof. Dr. Luís Eduardo Maggi.

“Esforçai-vos, e animai-vos; não temais, nem vos espanteis diante deles; porque o senhor teu Deus é o que vai contigo não te deixará nem te desamparará”
(Deuteronômio: 31:6)

FERREIRA, Iasminy Ranielly Silva. **Ocorrência de fungos do gênero *Paracoccidioides* (Onygenales: Ajellomycetaceae) na Amazônia Ocidental.** 2024. Tese (Doutorado em Biodiversidade e Biotecnologia) – Universidade Federal do Acre, Rio Branco, 2024.

RESUMO

A paracoccidioidomicose (PCM) é uma micose granulomatosa sistêmica considerada endêmica na América Latina, com registros de casos principalmente no Brasil. Esta doença é causada por fungos dimórficos do gênero *Paracoccidioides*. Este patógeno acomete humanos, assim como animais domésticos e silvestres por meio da inalação das estruturas infectantes conhecidas como conídios, presentes no ambiente, que podem colonizar tecido pulmonar com conversão para a forma leveduriforme, ocasionando lesões nos sistema respiratório e pele. Estudo prévios registram a ocorrência destes microrganismos principalmente em solos, a principal fonte natural para disseminação da doença. Neste sentido, este estudo tem como objetivo avaliar a ocorrência de *Paracoccidioides* spp. em amostras de solo da Amazônia Sul-Ocidental. Inicialmente, foi realizada a análise sistemática de artigos publicados durante o período de 1963 a 2021, com a utilização dos termos *Paracaccidioides*, América do Sul, solos, áreas ambientais associadas com cada país desta região. Este estudo resultou na identificação de 65 publicações que detectaram a ocorrência deste agente predominantemente no Brasil (86.16%). Com relação as amostras utilizadas, os cães domésticos e bovinos, foram os mais investigados em (36,69%) e (24,94%) dos artigos, respectivamente, sendo utilizadas as técnicas de ELISA (66,40%). Este estudo também teve como objetivo detectar a ocorrência destes fungos na Amazônia Sul-ocidental, por meio da coleta de 60 amostras de solos de tocas de tatus em propriedades rurais localizadas nos municípios de Rio Branco, Sena Madureira, Acrelândia, Plácido de Castro, Bujari e Senador Guiomard, no Estado do Acre. Foi realizada a extração de DNA do fungo por meio da utilização do kit DNEASY® PowerSoil – Quiagen, seguida pela técnica de PCR e Nested PCR. Foram detectados em seis amostras (10%), sendo sequenciados e identificados como *P. brasiliensis*. Esses achados indicam que os solos do estado do Acre podem ser considerados uma fonte potencial para *Paracoccidioides* spp., sugerindo que infecções locais são prováveis.

Palavra-chave: PCM; solos; tatu; PCR; América Latina; Acre.

FERREIRA, Iasminy Ranielly Silva. **Ocorrência de fungos do gênero *Paracoccidioides* (Onygenales: Ajellomycetaceae) na Amazônia Ocidental.** 2024. Tese (Doutorado em Biodiversidade e Biotecnologia) – Universidade Federal do Acre, Rio Branco, 2024.

ABSTRACT

Paracoccidioidomycosis (PCM) is a systemic granulomatous mycosis considered endemic in Latin America, with cases predominantly reported in Brazil. This disease is caused by dimorphic fungi of the genus *Paracoccidioides*. This pathogen affects humans, as well as domestic and wild animals, through the inhalation of infective structures known as conidia, present in the environment. These conidia can colonize lung tissue with a conversion to the yeast form, causing lesions in the respiratory and skin systems. Previous studies have documented the occurrence of these microorganisms mainly in soils, the primary natural source for the dissemination of the disease. In this context, this study aims to evaluate the occurrence of *Paracoccidioides* spp. in soil samples from the Southwestern Amazon. Initially, a systematic analysis of articles published from 1963 to 2021 was conducted, using terms such as *Paracoccidioides*, South America, soils, and environmental areas associated with each country in this region. This study identified 65 publications that predominantly detected the occurrence of this agent in Brazil (86.16%). Regarding the samples used, domestic dogs and cattle were the most investigated in 36.69% and 24.94% of the articles, respectively, with ELISA techniques being used in 66.40% of cases. This study also aimed to detect the occurrence of these fungi in the Southwestern Amazon by collecting 60 soil samples from armadillo burrows on rural properties located in the municipalities of Rio Branco, Sena Madureira, Acrelândia, Plácido de Castro, Bujari, and Senador Guiomard, in the state of Acre. Fungal DNA was extracted using the DNEASY® PowerSoil kit - Qiagen, followed by PCR and Nested PCR techniques. *Paracoccidioides* spp. were detected in six samples (10%), sequenced, and identified as *P. brasiliensis*. These findings indicate that the soils of the state of Acre could be considered a potential source for *Paracoccidioides* spp., suggesting that local infections are likely.

Keywords: PCM; soils; armadillo; PCR; Latin America; Acre.

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1. INTRODUÇÃO

As alterações ambientais relacionadas com o desmatamento, as mudanças climáticas, o uso incorreto dos solos, a migração humana e a consequente perda da biodiversidade, são fatores que tem contribuído para a disseminação de patógenos fúngicos em diferentes regiões do mundo (FISHER et al., 2020). Neste cenário, os estudos que descrevem a ocorrência e distribuição destes fungos são fundamentais para as estratégias de saúde pública.

A paracoccidioidomicose (PCM) destaca-se neste contexto como a micose sistêmica com maior prevalência na América Latina, principalmente no Brasil (MARTINEZ, 2017). Esta infecção tem como agente etiológico o fungo *Paracoccidioides* spp. descrito no Brasil em 1908 por Adolf Lutz (LUTZ, 1908). A espécie *P. brasiliensis* foi proposta após comparações morfológicas com o fungo *Coccidioides immitis* (ALMEIDA, 1930), sendo classificado como um Ascomycota da família Ajellomycetaceae, ordem Onygenales (MARTINEZ, 2017).

Estudos filogenéticos indicam que este fungo constitui um complexo de espécies. Assim, podem ser classificados sete grupos distintos identificados como *P. brasiliensis* (S1a e S1b), *P. americana* (PS2), *P. restrepiensis* (PS3), *P. venezuelensis* (PS4), *P. lutzii*, *P. cetii* e *P. lobogeorgii* (MATUTE, 2006; TEXEIRA et al., 2014; MUNÕZ et al., 2016; TURISSINI et al., 2017). Estas análises têm possibilitado a detecção de *Paracoccidioides* spp. por meio de técnicas moleculares (COSTA et al., 2021).

Estes fungos também são classificados como termodimórficos, com apresentação na forma micelial (saprofítica) à temperatura ambiente de 28ºC e leveduriforme (parasitária) à 37ºC (PINHEIRO et al., 2020). A forma micelial produz conídios, clamidoconídios e artroconídios que podem se dispersar no ambiente em aerossóis antes de infectar hospedeiros susceptíveis (BOCCA et al., 2013). Após a inalação dos propágulos, o fungo se diferencia em leveduras com multibrotamentos, levando a progressão da doença (BOCCA et al., 2013).

As principais formas clínicas da PCM são classificadas em aguda, subaguda ou crônica. A forma aguda ou subaguda, 5% a 25% dos casos, acomete principalmete crianças e adolescentes, ocasionando linfonodomegalia e hepatoesplenomegalia, além de lesões na mucosa oral e intestinal (SHIKANAI-YASUDA et al., 2017). A forma crônica é relatada em 74% a 96% dos casos, acometendo indivíduos do sexo masculino com faixa etária entre 30 a 50 anos, gerando comprometimento pulmonar em 90% dos pacientes, podendo disseminar-se para o sistema respiratório superior e pele (SHIKANAI-YASUDA et al., 2017).

A subnotificação de casos, associado a escassez de ferramentas diagnósticas precisas e rápidas, dificultam a determinação da incidência e prevalência da PCM. Além disso, nos últimos anos foram observadas mudanças na distribuição geográfica da doença decorrente da

expansão das fronteiras agrícolas e um extenso processo de migração populacional limitando a detecção da distribuição fúngica, exigindo mais estudos em espécimes biológicas e ambientais (HRYCYK et al., 2018; TEXEIRA et al., 2022).

Aproximadamente 80% dos casos de PCM registrados na América Latina são descritos no Brasil (MARTINEZ, 2017). Estudos prévios indicam que a doença foi responsável por 3.181 óbitos no país entre 1980 e 1995, representando uma média anual de 198,81 óbitos e uma taxa de mortalidade de 1,45 por milhão de habitantes, apresentando a maior taxa de mortalidade entre as micoses sistêmicas e representando a oitava causa de óbitos entre as doenças crônicas infecciosas e parasitarias (COUTINHO et al., 2002). Estes achados indicam a necessidade de estratégias em saúde pública para reduzir os impactos socioeconômicos desta infecção.

Neste sentido, a identificação de áreas de riscos associadas às diferentes espécies pode contribuir para o melhor entendimento da biogeografia do gênero *Paracoccidioides*, assim como auxiliar no diagnóstico e tratamento precoce da doença (ARANTES et al., 2016). Portanto, este estudo tem como objetivo determinar a ocorrência de fungos do gênero *Paracoccidioides* na Amazônica Sul-Ocidental.

1.1 OBJETIVOS

1.1.1 Geral

- Descrever a ocorrência de *Paracoccidioides* spp. em amostras de solo da Amazônia Sul-Ocidental.

1.1.2 Específicos

- Analisar o panorama do fungo *Paracoccidioides* em áreas ambientais da América do Sul, com base em dados da literatura;
- Realizar a detecção molecular de *Paracoccidioides* spp. em amostras ambientais do Sudoeste da Amazônia.

REFERÊNCIAS

ALMEIDA, F. P. **Estudos comparativos do granuloma coccidióico nos Estados Unidos e no Brasil. Novo gênero para o parasito brasileiro**. Faculdade de Medicina da Universidade de São Paulo. v. 5. p. 125-41. 1930.

ARANTES, T. D.; THEODORO, R. C.; DE MELO TEIXEIRA, M.; BOSCO, S. d. M. G.; BAGAGLI, E. Environmental mapping of *Paracoccidioides* spp. in Brazil reveals new clues into genetic diversity, biogeography and wild host association. **PLoS Neglected Tropical Diseases**, v. 10, n. 4, p. 1-18, 2016.

BOCCA, A. L.; AMARAL, A. C.; TEIXEIRA, M. M.; SATO, P. K.; SHIKANAI-YASUDA, M. A.; SOARES FELIPE, M. S. Paracoccidioidomycosis: eco-epidemiology, taxonomy and clinical and therapeutic issues. **Future Microbiology**, v. 8, n. 9, p. 1177-1191, 2013.

COUTINHO, Z. F.; SILVA, D. S..; LAZÉRA, M. PETRI, V.; OLIVEIRA, R. M.; SABROZA, P. C.; WANKE, B. Mortalidade por paracoccidioidomicose no Brasil (1980-1995). **Cadernos de Saúde Pública**, v. 18, p. 1-14, 2002.

FISHER, M. C.; GURR, S. J.; CUOMO, C. A.; BLEHERT, D. S.; JIN, H.; STUKENBROCK, E. H.; STAJICH, J. E.; KAHMANN, R.; BOONE, C.; DENNING, D. W.; GROW, N. A.R.; KLEIN, B. S.; KRONSTAD, J. W.; SHEPPARD, D. C.; TAYLOR, J. W.; WRIGHT, G. D.; HEITMAN, J.; CASADEVALL, A.; COWEN, L. E.; CHOWDHARY, A. Threats posed by the fungal kingdom to humans, wildlife, and agriculture. **American Society for Microbiology**, v. 11, n. 3, p. 1-17, 2020.

HRYCYK, M. F.; GARCIA GARCES, H.; BOSCO, S. d. M. G.; DE OLIVEIRA, S. L.; MARQUES, S. A.; BAGAGLI, E. Ecology of *Paracoccidioides brasiliensis*, *P. lutzii* and related species: infection in armadillos, soil occurrence and mycological aspects. **Medical mycology**, v.1, n.1, p. 1-13, 2018.

LUTZ, A. Uma mycose pseudococcidica localisada na bocca e observada no Brasil. Contribuição ao conhecimento das hyphoblastomycoses americanas. **O Brazil-Medico**, **Revista Semanal de Medicina e Cirurgia**, n. 22, p. 121–141, 1908.

MARTINEZ, R. New trends in paracoccidioidomycosis epidemiology. **Journal of Fungi**, v. 3, n. 1, p. 1-13, 2017.

MATUTE, D. R.; MCEWEN, J. G.; PUCCIA, R.; MONTES, B. A.; SAN-BLAS, G.; BAGAGLI, E.; RAUSCHER, J. T.; RESTREPO, A.; MORAIS, F.; NIÑO-VEGA, G. Cryptic speciation and recombination in the fungus *Paracoccidioides brasiliensis* as revealed by gene genealogies. **Molecular biology and evolution**, v. 23, n. 1, p. 65-73, 2006.

MUÑOZ, J. F.; FARRER, R. A.; DESJARDINS, C. A.; GALLO, J. E.; SYKES, S.; SAKTHIKUMAR, S.; MISAS, E.; WHISTON, E. A.; BAGAGLI, E.; SOARES, C. M. Genome diversity, recombination, and virulence across the major lineages of *Paracoccidioides.* **American Society for Microbiology**, v. 1, n. 5, p. 1-18, 2016.

PINHEIRO, B. G.; HAHN, R. C.; CAMARGO, Z. P.; RODRIGUES, A. M. Molecular tools for detection and identification of *Paracoccidioides* species: Current status and future perspectives. **Journal of fungi**, v. 6, n. 4, p. 1-24, 2020.

SHIKANAI-YASUDA, M. A.; MENDES, R. P.; COLOMBO, A. L.; QUEIROZ-TELLES, F. d.; KONO, A. S. G.; PANIAGO, A. M.; NATHAN, A.; VALLE, A. C. F.; BAGAGLI, E.; BENARD, G. Brazilian guidelines for the clinical management of paracoccidioidomycosis. **Revista da Sociedade Brasileira de Medicina Tropical**, v. 50, n. 5, p. 715-740, 2017.

TEIXEIRA, A. G. K.; YAMAUCHI, D. H.; ROSSI, A.; SOUSA, H. R.; GARCES, H. G.; JÚNIOR, L. J.; JÚNIOR, A. O. S.; FELIPE, M. S. S.; BAGAGLI, E.; ANDRADE, H. F.; TEXEIRA, M. M. Clinical and Eco-Epidemiological Aspects of a Novel Hyperendemic Area of Paracoccidioidomycosis in the Tocantins-Araguaia Basin (Northern Brazil), Caused by Paracoccidioides sp. **Journal of Fungi**, v. 8, n. 5, p. 1-11, 2022.

TEIXEIRA, M. M.; THEODORO, R. C.; NINO-VEGA, G.; BAGAGLI, E.; FELIPE, M. S. *Paracoccidioides* species complex: ecology, phylogeny, sexual reproduction, and virulence. **PLoS Pathogens**, v. 10, n. 10, p. 1-4, 2014.

TURISSINI, D. A.; GOMEZ, O. M.; TEIXEIRA, M. M.; MCEWEN, J. G.; MATUTE, D. R. Species boundaries in the human pathogen *Paracoccidioides*. **Fungal Genetics and Biology**, v. 106, n.1, p. 9-25, 2017.

2. CAPÍTULO I - Overview of the genus *Paracoccidioides* in environmental areas of South America

EUROPEAN ACADEMIC RESEARCH
ISSN 2286-4822
www.euacademic.org

EUROPEAN ACADEMIC RESEARCH
Vol. XI, Issue 5/ August 2023

Impact Factor: 3.4546 (UIF)
DRJI Value: 5.9 (B+)

Overview of the genus *Paracoccidioides* in environmental areas of South America

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Resumo

Os fungos do gênero Paracoccidioides são agentes causadores de micoses sistêmicas consideradas endêmicas na América Latina, principalmente no Brasil. Estudos científicos têm investigado a distribuição deste fungo na natureza e sua associação com outras espécies, como o Tatu (Dasypus sp.) com a finalidade de fornecer estratégias para vigilância desta infecção em áreas de risco. Neste contexto, foi realizada a análise do panorama do fungo Paracoccidioides spp. em áreas ambientais da América do Sul por meio da análise sistemática de artigos publicados durante o período de 1963 a 2022, com a utilização dos termos Paracoccidioides, América do Sul, solos, áreas ambientais associadas com cada país desta região. Foram selecionados artigos em inglês, português e espanhol. Esta análise permitiu a identificação de 65 publicações que detectaram estes fungos em seis países da América Latina, predominantemente no Brasil (86,16%). Com relação as amostras utilizadas, os cães domésticos e bovinos, foram os mais investigados em 36,69% e 24,94% dos artigos, respectivamente. Além disso, a ocorrência também foi relatada em equinos, ovinos e tatu. As técnicas mais utilizadas nestes estudos foram ELISA (66,40%), Testes intradérmicos (25,95%), PCR e Nested PCR (4,14%) e Cultura (1,21%). Estas análises fornecem, portanto, informações fundamentais para as estratégias de vigilância da doença, indicando áreas ambientais com risco potencial para a disseminação do fungo Paracoccidioides ssp.

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Overview of the genus *Paracoccidioides* in environmental areas of South America

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Abstract

The fungi of the *Paracoccidioides* genus are causative agents of systemic mycoses considered endemic in Latin America, primarily in Brazil. Scientific studies have investigated the distribution of this fungus in nature and its association with other species, such as the Armadillo (*Dasypus* sp.), with the aim of providing strategies for monitoring this infection in at-risk areas. In this context, an analysis of the panorama of *Paracoccidioides* spp. fungus in environmental areas of South America was conducted through a systematic analysis of articles published from 1963 to 2022, using the terms *Paracoccidioides*, South America, soils, and environmental areas associated with each country in this region. articles in english, portuguese, and spanish were selected. This analysis led to the identification of 65 publications that detected these fungi in six Latin American countries, predominantly in Brazil (86.16%). Regarding the samples used, domestic dogs and bovines were the most investigated in 36. 69% and 24.94% of the articles, respectively. Furthermore, occurrences were also reported in horses, sheep, and armadillos. The most commonly used techniques in these studies were ELISA (64,60%), Intradermal Tests (25.22%), PCR and Nested PCR (4.13%), and Culture (1.58%). These analyses therefore provide crucial information for disease surveillance strategies, indicating environmental areas with potential risk for the dissemination of *Paracoccidioides* spp. Fungus.

Keywords: Latin America; Systemic mycosis; Soil; Armadillo.

1. Introduction

In recent years, there has been an observed rise in the incidence and dissemination of emerging fungal infections (Fisher et al., 2020). It is estimated that over 200 fungal species have been identified as agents responsible for severe diseases in humans (Fisher et al., 2020). Among these fungi, the order Onygenales stands out due to its extensive diversity of pathogenic

species, and it is responsible for triggering approximately 650,000 infections per year (Dyke; Teixeira; Barker, 2019).

Among these fungi, the genus *Paracoccidioides* stands out, which is the etiological agent of paracoccidioidomycosis (PCM), a prevalent systemic granulomatous mycosis in South American countries (Shikanai-Yasuda et al., 2017). Despite not being a compulsorily notifiable disease, it is known that PCM affects individuals engaged in soil-related activities (Martinez et al., 2017). Furthermore, it is estimated that in endemic regions, there are 1 to 3 new cases per thousand inhabitants per year, resulting in a mortality rate of 1.45 per million inhabitants. This classification ranks it as the eighth leading cause of mortality among infectious and parasitic diseases (Shikanai-Yasuda et al., 2017).

The genus *Paracoccidioides* is composed of two main species, *P. brasiliensis* and *P. lutzii* (Shikanai-Yasuda et al., 2017). Genotypic studies have revealed variations within *P. brasiliensis*, which have been attributed to cryptic species: *P. brasiliensis* (S1a and S1b), *P. americana* (PS2), *P. restrepiensis* (PS3), and *P. venezuelensis* (PS4) (Teixeira et al., 2020). The species S1a, S1b, PS2, and *P. lutzii* are widely distributed across the South American continent, whereas the species PS3 and PS4 are restricted to Colombia and Venezuela, respectively (Martinez et al., 2017).

Recently, the cryptic species *P. ceti* and *P. lobogeorgii* have been included in the phylogenetic complex of *P. brasiliensis*. *P. ceti* has been described in Brazil, Cuba, the United States, and Japan (Ueda et al., 2013; Esperon et al., 2012; Vilela et al., 2016; Minakawa et al., 2016; Sacristan et al., 2017), while *P. lobogeorgii* has predominantly been identified in the state of Acre and in São Paulo, Brazil (Taborda et al., 1999; Vilela et al., 2023).

While there is a consensus regarding the widespread distribution of this fungus in the soil of the american continent, few studies have been able to demonstrate the isolation of this microhabitat, thus limiting our understanding of the ecoepidemiology of the genus *Paracoccidioides* (Arantes et al., 2016). Previous studies, utilizing molecular analyses, have indicated the presence of this fungus in soil, armadillo specimens, domestic animals, as well as in aerosols in different regions of Brazil (Arantes et al., 2016; Hrycyk et al., 2018).

The presented evidence indicates a broad dispersion of this fungus in the soil, along with its capacity for adaptation not only in humans but also in domestic and wild animals (Arantes et al., 2016; Martinez et al., 2017). Organizing this information, through the identification of risk areas and the description of cases involving accidental hosts, can provide an understanding of the dynamics of *Paracoccidioides* spp. fungus in the environment. Therefore, this study aimed to describe the landscape of the Paracoccidioides genus in environmental areas of South America.

2. Methods

This is a quantitative and retrospective systematic review, conducted following an adaptation of the guidelines proposed in the "Preferred Reporting Items for Systematic Reviews and Meta-Analyses" guide (PRISMA) (Moher et al., 2015). The objective was to provide an overview of the genus *Paracoccidioides* spp. in environmental areas of South America.

Over a one-year period, two reviewers identified articles indexed in PubMed, Scientific Electronic Library Online (Scielo), Web of Science, and Google Scholar. The search was conducted using the descriptors "*Paracoccidioides*," "soils," "domestic and wild animals," "aerosol," "Argentina," "Bolivia," "Brazil," "Chile," "Colombia," "Ecuador," "Guyana," "French Guiana," "Paraguay," "Peru," "Suriname," "Uruguay," and "Venezuela." Only articles published in English, Portuguese, and Spanish between 1963 and 2022 were included in the review.

Inclusion criteria for this review encompassed studies investigating the detection of the genus *Paracoccidioides* in environmental areas within the South American countries. Conversely, exclusion criteria were established to eliminate studies conducted outside the specified geographic area and data related to human disease that did not align with the proposed objective of this review. Additionally, duplicate articles in the search platform and those outside the established timeframe were excluded.

Information from the selected articles was meticulously compiled and systematized in an Excel® spreadsheet to organize and facilitate data analysis. Additionally, using Prism-GraphPad® software, figures were created representing the frequencies of different variables, such as types of collected samples, regions of study origin, and techniques used for fungus detection. This approach provided a clear and concise visualization of patterns and trends related to the Paracoccidioides spp. genus in environmental areas and domestic and wild animals in South America.

3. Results

A total of 3.007 records were identified, comprising 1.112 from Pubmed, 73 from Scielo, 142 from Web of Science, and 1.957 from Medline. Among these, 1.278 records were duplicated across the databases, resulting in the identification of 1.878 unique documents. Following the application of selection criteria involving a thorough review of titles and abstracts, 500 documents were chosen for further assessment. Within this final selection, only 53 articles demonstrated alignment with the objectives of this study.

It is important to emphasize that the exploration of the reference listings of these additional 53 articles led to the identification of an additional twelve documents that met the same pre-established selection criteria. Consequently, a cumulative total of 65 articles were selected for comprehensive review and subsequent systematization (Figure 1).

IDENTIFICATION

Identified Studies: Pubmed n=1.112; Scielo n=73; Web of Science n=142; Medline n=1.957. → Descriptors: "Paracoccidioides", "soils", "domestic and wild animals", "aerosol", "Argentina", "Bolivia", "Brazil", "Chile", "Colombia", "Ecuador", "Guyana", "French Guiana", "Paraguay", "Peru", "Suriname", "Uruguay", and "Venezuela".

↓

Total n=3.156 → Excluded studies due to duplication. n=1.278

↓

SELECTION

Selected studies n=1.878

↓

Studies selected after reading abstracts and titles n=500

↓

ELIGIBILITY

Studies that conducted the detection of the genus *Paracoccidioides* in environmental areas within the countries comprising South America.

↓

INCLUDED

Studies included in the review n=65

Figure 1 - Flowchart of identification and selection of literature review articles.

The systematic analysis of scientific articles detected the presence of *Paracoccidioides* spp. in six Latin American countries, namely Brazil, Colombia, French Guiana, Venezuela, Argentina, Uruguay, and Bolivia. A predominance of investigations was observed in Brazil, with 56 (86.16%) studies (Table 1).

Table 1 - Detection of genus *Paracoccidioides* fungi in Latin American countries, considering the types of samples used, techniques employed, total analyzed samples, quantity of positive identifications, and publication authorship.

Local de coleta	Tipo de amostra	Técnica para detecção	Total de amostras	Positivas (%)	Autor
Argentina	Domestic dog	Western blot	89	01 (1,12)	(Canteros et al., 2010)
	Soil	Culture	12	10 (83,33)	(Negroni, 1966)
Bolvia	Titi monkey	Direct exam	1	01 (100)	(Jhonson; lang, 1977)
Brazil	Soil	Culture	1	01 (100)	(Shome et al., 1993)
			887	05 (0,56)	(Montenegro et al., 1996)
			760	01 (0,13)	(Vergara et al., 1997)
		PCR and Nested PCR	4	3 (75)	(Theodoro et al., 2005)
			9	4(44,44)	(Terçarioli et al., 2007)
			27	07 (25,92)	(Arantes et al., 2013)
			44	23 (52,27)	(Arantes et al., 2016)
			11	05 (45,45)	(Hrycyk et al., 2018)
			30	08 (26,66)	(Mendes et al., 2019)
			18	03 (16,66)	(Macedo et al., 2020)
			16	07 (43,75)	(Mendes et al., 2020)
			15	09 (60)	(Teixeira et al., 2022)
	Armadillo[1,2,3,4]	Direct exam	21	01 (4,76)	(Vergara; martinez et al.,1999)
			16	02 (12,5)	(Vergara et al., 2000)
		Culture	20	04 (20)	(Naiff et al., 1986)
			4	02 (50)	(Bagagli et al., 1998)
			16	03 (18,75)	(Vergara et al., 2000)
			15	10 (66,66)	(Bagagli et al., 2003)
			7	06 (85,71)	(Hrycyk et al., 2018)
		Elisa	47	28(59,60)	(Fernandes et al., 2004)

		1	01(100)	(Albano et al., 2014)
		1	01(100)	(Albano et al., 2014)
	Double immunodiffusion	4	03(75)	(Bagagli et al., 1998)
		16	04(25)	(Vergara et al., 2000)
	Immunoblotting	4	4(100)	(Bagagli et al., 1998)
	PCR	4	03(75)	(Bagagli et al., 1998)
		16	03(18,75)	(Vergara et al., 2000)
	PCR and Nested PCR	9	1 (11,11)	(Bosco et al., 2005)
		1	01(100)	(Pereira et al., 2008)
		2	02(100)	(Pereira et al., 2008)
		7	06 (85,71)	(Hrycyk et al., 2018)
		18	4 (22,22)	(Costa et al., 2021)
		7	6 (85,71)	(Bagagli et al., 2021)
Aerosol	PCR and Nested PCR	06	05 (83,33)	(Arantes et al., 2013)
		16	13 (81,25)	(Arantes et al., 2016)
Primates[5,6,7,8]	Intradermal test	52	11 (21,15)	(Costa et al., 1995[a])
		33	9 (27,30)	(Costa et al., 1995[b])
	Elisa	39	25(64,10)	(Corte et al., 2005)
		93	45 (48,38)	(Corte et al., 2007)
Equines	Intradermal test	109	84 (77,06)	(Costa; Netto,1978)
		234	147 (62,82)	(Costa et al., 1995[a])
	Elisa	100	30 (30)	(Corte et l., 2009)
		200	24 (12)	(Albano et al., 2015)

		200	26 (13)	(Mendes et al., 2017)
Ring-tailed coati[9]	Intradermal test	37	35 (94,60)	(Costa et al., 1995[a])
Wild felids[10]	Intradermal test	11	05 (45,45)	(Costa et al., 1995[a])
Fish[11]	Elisa	214	25 (11,68)	(Suguiura et al., 2020)
	PCR and Nested PCR	100	03 (3)	(Suguiura et al., 2020)
Nutria[12]	Elisa	4	1 (25)	(Albano et al., 2014)
Rodent[13,14,,15,16,17,18]	Elisa	3	1 (33,3)	(Sbeghen et al., 2015)
		7	1 (14,28)	(Sbeghen et al., 2015)
		8	1 (12,50)	(Sbeghen et al., 2015)
		12	6(50)	(Sbeghen et al., 2015)
	PCR and Nested PCR	4	1 (25)	(Losnak et al., 2018)
		12	1 (8,33)	(Losnak et al., 2018)
Wild mammals	Elisa	85	27 (31,76)	(Mendes et al., 2017)
Boar[19]	Elisa	106	40 (37,73%)	(Belitardo et al., 2014)
White-eared opossum[20]	Elisa	51	8 (15,68)	(Albano et al., 2014)
Crab-eating raccoon[21]	Elisa	2	2 (100)	(Albano et al., 2014)
Capybara[22]	Elisa	1	1(100)	(Albano et al., 2014)
Brocket deer[23]	Elisa	8	3 (37,50)	(Albano et al., 2014)
Lesser grison[24]	Elisa	2	1 (50)	(Albano et al., 2014)

Crab-eating fox[25]	Elisa	3	1 (33,33)	(Albano et al., 2014)
Pampas fox[26]	Elisa	9	1 (11,11)	(Albano et al., 2014)
Margay[27]	Elisa	3	1(33,33)	(Albano et al., 2014)
Geoffroy's cat[28]	Elisa	9	1 (11,11)	(Albano et al., 2014)
Guinea pig[29]	PCR and Nested PCR	1	1 (100)	(Pereira et al., 2008)
Grison[30]	PCR and Nested PCR	2	1 (50)	(Pereira et al., 2008)
Raccoon[31]	PCR and Nested PCR	2	1 (50)	(Pereira et al., 2008)
Porcupine[32]	PCR and Nested PCR	2	1 (50)	(Pereira et al., 2008)
Velvety-free-tailed-bat[33]	PCR and Nested PCR	95	2 (2,10)	(Paz et al., 2017)
Dolphin	PCR and Nested PCR	1	1 (100)	(Sacristán et al., 2017)
Crab-eating fox	PCR and Nested PCR	18	02 (11,11)	(Costa et al., 2021)
Paca[34]	PCR and Nested PCR	18	01 (5,55)	(Costa et al., 2021)
Ovine	Intradermal test	98	42 (42,8)	(Costa; Netto et al.,1978)
		98	40 (40,80)	(Costa et al., 1995[a])
	Elisa	262	97 (37,02)	(Oliveira et al., 2012)
Bovine	Intradermal test	254	114 (44,88)	(Costa; Netto et al.,1978)
		632	254 (40,18)	(Costa et al., 1995[a])
	Elisa	400	350 (87,50)	(Silveira et al., 2005)
		400	70 (17,50)	(Silveira et al., 2008)

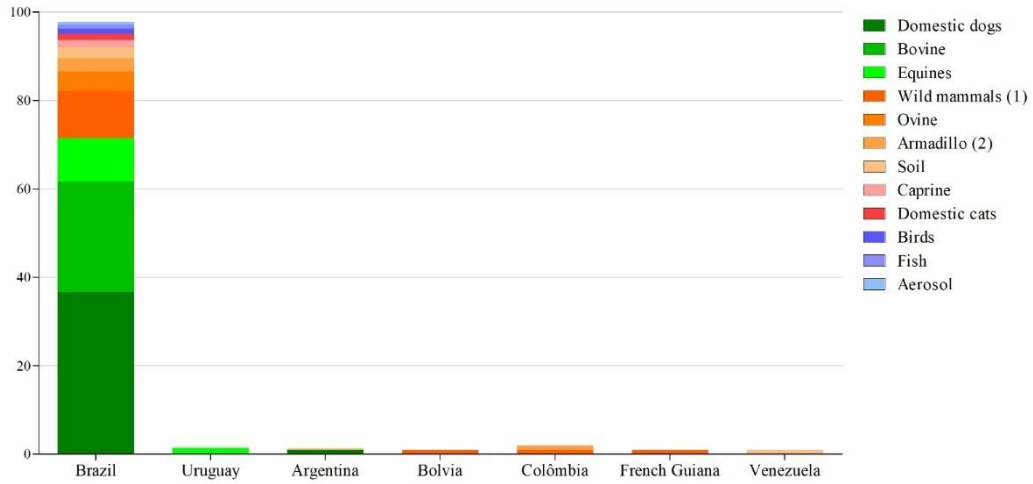
	Domestic dogs	Direct exam	01	01 (100)	(Farias et al., 2011)
		Culture	01	01 (100)	(Bosco et al., 2005)
			01	01 (100)	(Headley et al., 2017)
		Intradermal test	149	9 (6,04)	(Fontana et al., 2010)
		Complement fixation	145	109 (75,17)	(Môs; Netto, 1974)
		Elisa	305	85 (27,86)	(Ono et al., 2001)
			836	567 (67,82)	(Silveira et al., 2006)
			275	182 (66,18)	(Fontana et al., 2010)
			126	69 (54,76)	(Corte et al., 2012)
			196	58 (29,60)	(Teles et al., 2015)
			196	52 (26,53)	(Mendes et al., 2017)
			300	22 (7,33)	(Petroni et al., 2017)
		PCR and Nested PCR	1	01 (100)	(Ricci et al., 2004)
		PCR	1	01 (100)	(Bosco et al., 2005)
			1	01 (100)	(Headley et al., 2017)
	Birds	Elisa	140	38 (27,14)	(Oliveira et al., 2011)
	Goats	Elisa	202	53 (26,23)	(Ferreira et al., 2013)
	Rabbit	Elisa	170	46 (27,05)	Belitardo et al., 2014
	Domestic cats	Elisa	136	43 (31,62)	(Oliveira et al., 2013)
Colômbia	Bat guano[35]	Culture	243	03 (1,23)	(Grose, Tamsitt, 1965)
	Armadillo[36,37]		2	01 (50)	(Corredor et al., 1999)
			1	01 (100)	(Corredor et al., 2005)

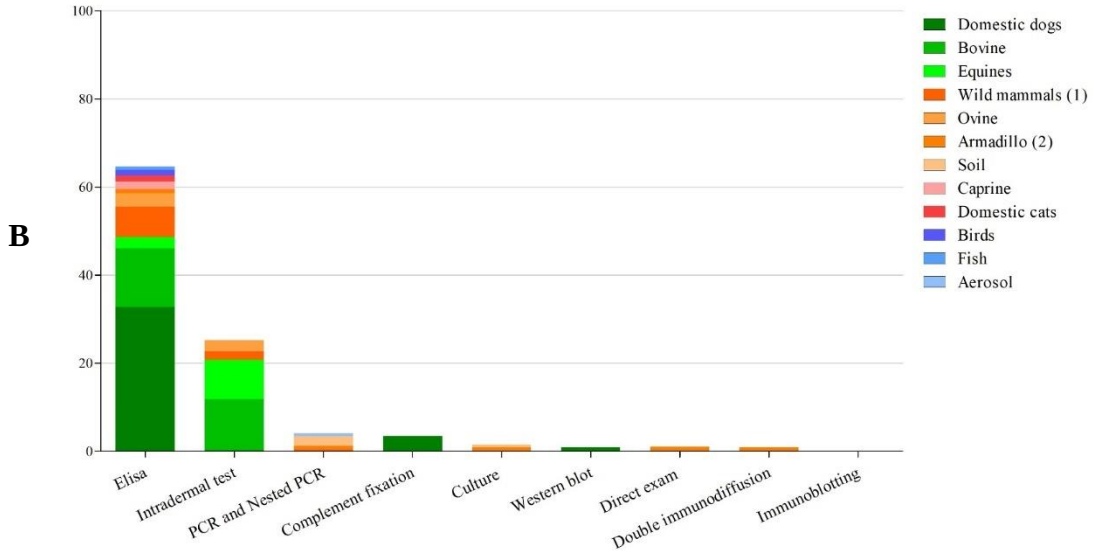
French Guiana	Two-toed sloth[38]	Direct exam	1	01 (100)	(Chavez et al., 2011)
Uruguay	Equine	Intradermal test	195	45 (23,07)	(Díaz et al., 1972)
Venezuela	Soil	Culture	84	03(3,44)	(Albornoz, 1971)

[1,4] Armadillo (*Dasypus novemcinctus*); Armadillo[2] (*Dasypus hibridus*); Armadillo[3] (*Euphractus sexcinctus*); Primates[5] (*Callithrix jacchus*, *Cebus apella*); Primates[6,7] (*Cebus apella*); Primates [8] (*Cebus sp*; *Alouatta caraya*); Ring-tailed coati[9] (*Nasua nasua*); Wild felids[10] (*Panthera onca*, *Felis pardalis*, *Felis tigtina*, *Felis wiedii*, *Felis geoffroyi*); Fish[11] (*Oreochromis niloticus*); Nutria[12] (*Myocastor coypus*); Rodent[13] (*Oligoryzomys nigripes*), Rodent[14](*Euryoryzomys russatus*) , Rodent[15] (*Thaptomys nigrita*), Rodent[16] (*Akodon sp*), Rodent[17] (*Euryoryzomys russatus*), Rodent[18] (*Oxymycterus sp*); Boar [19] (*Sus scrof*); White-eared opossum[20] (*Didelphis albiventris*); Crab-eating raccoon[21] (*Procyon cancrivorus*); Capybara[22] (*Hydrochoerus hydrochaeris*); Brocket deer[23] (*Mazama guazoubira*); Lesser grison[24] (*Galictis cuja*); Crab-eating fox[25] (*Cerdocyon thous*); Pampas fox[26] (*Lycalopex gymnocercus*); Margay[27] (*Leopardus wiedii*); Geoffroy's cat[28] (*Leopardus geoffroyi*); Guinea pig[29] (*Cavia aperea*); Grison[30] (*Gallictis vittata*); Raccoon[31] (*Procyon cancrivorus*); Porcupine[32] (*Sphiggurus spinosus*); Velvety-free-tailed-bat[33] (*Molossus molossus*); Paca[34] (*Cuniculus paca*); Bat guano[35] (*Artibeus lituratus*); Armadillo[36] (*Dasypus novemcinctus*); Armadillo[37] (*Cabassous centralis*); Two-toed sloth[38] (*Choloepus didactylus*).

Additionally, variations were observed in the types of samples and techniques employed for the detection of *Paracoccidioides* spp. (Figure 2). Noteworthy among the utilized samples are domestic dogs (36.69%) and cattle (24.94%). The identification of this fungus was also reported in other domestic and wild animals, such as horses (11.27%), sheep (4.40%), and armadillos (3.07%). Furthermore, the presence of the fungus was identified in soil samples (2.82%), which is considered to be the natural reservoir of this fungus (Figure 2).

A





[1]Armadillo (*Dasypus novemcinctus*; *Dasypus hibridus*; *Euphractus sexcinctus; Cabassous centralis*); Wild mammals[1] (Primates - *Callithrix jacchus*, *Cebus apella*, *Cebus apella*, *Cebus sp*; *Alouatta caraya*); (Ring-tailed coati - *Nasua nasua*); (Wild felids *Panthera onca*, *Felis pardalis*, *Felis tigtina*, *Felis wiedii*, *Felis geoffroyi*); (Fish - *Oreochromis niloticus*); (Nutria - *Myocastor coypus*); Rodent - *Oligoryzomys nigripes*, (*Euryoryzomys russatus*, *Thaptomys nigrita*, *Akodon sp*, *Euryoryzomys russatus*, *Oxymycterus sp*); (Boar -*Sus scrof*); (White-eared opossum - *Didelphis albiventris*); (Crab-eating raccoon - *Procyon cancrivorus*); (Capybara - *Hydrochoerus hydrochaeris*); (Brocket deer - *Mazama guazoubira*); (Lesser grison - *Galictis cuja*); (Crab-eating fox - *Cerdocyon thous*); (Pampas fox - *Lycalopex gymnocercus*); (Margay - *Leopardus wiedii*); (Geoffroy's cat - *Leopardus geoffroyi*); (Guinea pig - *Cavia aperea*); (Grison - *Gallictis vittata*); (Raccoon - *Procyon cancrivorus*); (Porcupine - *Sphiggurus spinosus*); (Velvety-free-tailed-bat - *Molossus molossus*); (Paca - *Cuniculus paca*); (Bat guano - *Artibeus lituratus*); (Two-toed sloth - *Choloepus didactylus*).

Figure 2 - Frequency of *Paracoccidioides* spp. detection in the analyzed studies. These records were correlated with the country of origin (A) and the technique used (B).

The detection of the *Paracoccidioides* fungus was predominantly in Brazil (97.91%) compared to other regions of Latin America (2.09%) (Figure 1A). These findings were established through the application of diverse methodologies, with the highest utilization of ELISA techniques (64.60%), followed by intradermal tests (25.22%), PCR and Nested PCR (4.13%), and culture (1.58%) (Figure 1B).

4. Discussion

The results underscored the detection of *Paracoccidioides* particularly in samples collected in Brazil, aligning with previous studies describing the region as endemic to the disease, accounting for approximately 80% of the reported cases only in Latin America (Martinez, 2017). Climatic factors specific to this region, such as average temperatures and rainfall indices, also contribute to these findings by maintaining soil moisture conducive to the fungus's persistence (Mendes et al., 2020; Macedo et al., 2020).

Furthermore, the biodiversity present in Brazil offers a wide array of species susceptible to the disease, contributing to the fungus's dispersal within the environment. Among these species, armadillos stand out as significant indicators of *Paracoccidioides* occurrence in nature, given their close association with soils – the natural habitat for the fungus. Studies frequently document the infection of these animals, as indicated by the preceding results (Figure 2).

In this context, previous studies have also investigated the fungus's occurrence in other wildlife species to enhance our understanding of its natural dispersion. Consequently, additional cases have been detected in primates, such as the Common Marmoset (*Callithrix jacchus*) and the Capuchin Monkey (*Cebus apella*) (Costa et al., 1995[a]). Furthermore, newly identified potential hosts from the region, like the Crab-eating Fox (*Cerdocyon thous*) and the Paca (*Cuniculus paca*), have also shown evidence of infection (Costa et al., 2021).

Other studies have documented the presence of *Paracoccidioides* in domestic animals, further underscoring the potential of these species as indicative of the fungus's occurrence in the environment. Cases have been recorded in dogs (Petroni et al., 2017; Farias et al., 2011; Ricci et al., 2004) and felines (Oliveira et al., 2013), as well as in production animals like cattle (Silveira et al., 2008) and pigs (Belitardo et al., 2014). Despite these analyses, the significance of these findings in relation to the occurrence of the disease in humans should be explored in subsequent studies.

The variation in techniques employed across the articles was also observed, serving as a pivotal factor for the accurate detection of cases within the environment. In this context, experiments predominantly utilized ELISA, especially for the analysis of both domestic and wild animals. Conversely, the detection in armadillos and soil samples was primarily conducted through Nested PCR and culture techniques (Figure 2B).

Despite some studies identifying the occurrence of *Paracoccidioides* through culture isolation (Figure 2B), this technique can be influenced by various factors, particularly in soil samples. Factors such as the presence of saprophytic fungi and the use of pesticides in these regions can potentially hinder the fungal mycelial growth (Oliveira et al., 2011).

This challenge encountered in isolation has spurred the development of more sensitive methods for the detection of *Paracoccidioides* spp. in the environment, including the Nested PCR technique. This methodology was employed in 33.33% of articles with positive samples, standing out as an efficient approach for fungus detection and identification. It enables the classification of new risk areas for the disease, thereby facilitating a more accurate assessment of its geographical spread.

5. Conclusion

These studies contribute to the understanding of the occurrence and distribution of *Paracoccidioides* spp. in Latin American environments, providing essential insights for disease control strategies within these areas. Moreover, recent findings, especially in new animal species, suggest the possibility of describing this fungus in previously unexplored regions.

Reference

Albano, A. P. N.; Klafke, G. B.; Brandolt, T. M.; Hora, V. P.; Minello, L. F.; Jorge, S.; Santos, E. O.; et al. 2014. Wild animals as sentinels of *Paracoccidioides brasiliensis* in the state of Rio Grande do Sul, Brazil. *Mycopathologia*, 177 (3): 207-215.

Albano, A. P. N.; Klafke, G. B.; Brandolt, T. M.; Hora, V. P.; Nogueira, C. E. W.; Xavier, M. O.; Meireles, M. C. A. 2015. Seroepidemiology of *Paracoccidioides brasiliensis* infection in horses from Rio Grande do Sul, Brazil. *Brazilian Journal of Microbiology*, 46 (2): 513-517.

Albornoz, M. B. 1971. Isolation of *Paracoccidioides brasiliensis* from rural soil in Venezuela. *Medical Mycology*, 9 (3): 248-253.

Arantes, T. D.; Theodoro, R. C.; Teixeira, M. M; Bosco, S. D. M. G.; Bagagli, E. 2016. Environmental mapping of *Paracoccidioides* spp. in Brazil reveals new clues into genetic diversity, biogeography and wild host association. *PLoS Neglected Tropical Diseases*, 10 (4): 1-18.

Arantes, T. D.; Theodoro, R. C.; Macoris, S. A. G.; Bagagli, E. 2013. Detection of *Paracoccidioides* spp. in environmental aerosol samples. *Medical Mycology*, 51 (1): 83-92.

Bagagli, E.; Coelho, K. I.; Alquati, S.; Miyaji, M.; Camargo, Z. P.; Gomes, G. M.; Franco, M.; et al. 1998. Isolation of *Paracoccidioides brasiliensis* from armadillos (*Dasypus noveminctus*) captured in an endemic area of paracoccidioidomycosis. *The American journal of tropical medicine and hygiene*, 58 (4): 505-512.

Bagagli, E.; Franco, M.; Bosco, S. M. G.; Barbosa, F. H.; Trinca, L. A.; Montenegro, M. R. 2003. High frequency of *Paracoccidioides brasiliensis* infection in armadillos (*Dasypus novemcinctus*): an ecological study. *Medical mycology*, 41 (3): 217-223.

Bagagli, E.; Matute, D. R.; Garces, H. G.; Tenório, B. G.; Garces, A. G.; Alves, L. G. B.; Yamauchi, D. H.; et al. 2021. *Paracoccidioides brasiliensis* isolated from nine-banded armadillos (*Dasypus novemcinctus*) reveal population structure and admixture in the Amazon Basin. *Journal of fungi*, 7 (1): 1-11.

Belitardo, D. R.; Calefi, A. S.; Borges, I. K.; Oliveira, G. G.; Sbeghen, M. R.; Itano, E. N.; Camargo, Z. P.; Ono, M. A. 2014. Detection of antibodies against *Paracoccidioides brasiliensis* in free-range domestic pigs (*Sus scrofa*). Mycopathologia, 177 (2): 91-95.

Bosco, S. M. G.; Theodoro, R. C.; Macoris, S. A.G.; Farias, M. R.; Muro, M.; Ribeiro, M. G.; Bagagli, E. 2005. Morphological and molecular characterization of the first isolate of *Paracoccidioides brasiliensis* from dog (*Canis familiaris*). *Revista do Instituto de Medicina Tropical de São Paulo*, 47(1): 62-63.

Canteros, C. E.; Madariaga, M. J.; Lee, W.; Rivas, M. C.; Davel, G.; Lachini, R. 2010. Agentes de micosis endémicas en un área rural de Argentina: estudio seroepidemiológico en perros. *Revista Iberoamericana de Micología*, 27 (1): 14-19.

Chávez, A. T.; Romero, R. R.; Rodríguez, J. A.; Garza, A. M. N. Tovar, L. E. R. 2011. Disseminated paracoccidioidomycosis in a Southern two-toed sloth (*Choloepus didactylus*). *Journal of Comparative Pathology*, 144 (2): 231-234.

Corredor, G. G. Peralta, L. A.; Castaño, J. H.; Zuluaga, J. S.; Henao, B.; Arango, M.; Tabares, A. M.; et al. 2005. The naked-tailed armadillo Cabassous centralis (Miller 1899): a new host to Paracoccidioides brasiliensis. Molecular identification of the isolate. *Medical Mycology*, 43 (3): 275-280.

Corredor, G. G.; Castaño, J. H.; Peralta, L. A.; Díez, S.; Arango, M.; Mcewen, J.; Restrepo, A. 1999. Isolation of *Paracoccidioides brasiliensis* from the nine-banded armadillo *Dasypus novemcinctus*, in an endemic area for paracoccidioidomycosis in Columbia. *Revista Iberoamericana de Micologia*, 16 (4): 216-220.

Corte, A. C.; Itano, E. N.; Freire, R. L.; Camargo, Z. P.; Ono, M. A. 2009. Detection of antibodies to *Paracoccidioides brasiliensis* in horses from northern Region of Paraná State. Semina: *Ciências Agrárias*, 30 (2): 441-446.

Corte, A. C.; Itano, E. N.; Ono, M. A.; Malanski, L. S.; Navarro, I. T.; Shiozawa, M. M.; Svoboda, W. K.; et al. 2005. Paracoccidioidomycosis-infection in free-living monkeys of northwest Paraná State, Brazil. *Revista do Instituto de Medicina Tropical de São Paulo*, 47(1): 62-63.

Corte, A. C.; Svoboda, W. K.; Navarro, I. T.; Freire, R. L.; Malanski, L. S.; Shiozawa, M. M.; Ludwig, G.; et al. 2007. Paracoccidioidomycosis in wild monkeys from Paraná State, Brazil. *Mycopathologia,* 164 (5): 225-228.

Corte, C. A.; Gennari, S. M; Labruna, M. B.; Camargo, L. M. A.; Itano, E. N.; Freire, R. L.; Camargo, Z. P.; et al. 2012. *Paracoccidioides brasiliensis* infection in dogs from Western Brazilian Amazon. *Pesquisa Veterinária Brasileira*, 32 (7): 649-652.

Costa, B. S. S.; Silva, F. A.; Wanke, B. Weksler, M.; Moratelli, R.; Valle, A. C. F.; Oliveira, R. M. Z.; et al. 2021. Molecular eco-epidemiology of *Paracoccidioides brasiliensis* in road-killed mammals reveals *Cerdocyon thous* and *Cuniculus paca* as new hosts harboring this fungal pathogen. *Plos one,* 16 (8): 1-11.

Costa, E. O.; Diniz, L. S. M.; Fava Netto, C. 1995[a]. The prevalence of positive intradermal reactions to paracoccidioidin in domestic and wild animals in São Paulo, Brazil. *Veterinary research communications*, 19 (1): 127-130.

Costa, E. O.; Diniz, L. S. M.; Fava Netto, C; Arruda, C.; Dagli, M. L. Z. 1995[b]. Delayed hypersensitivity test with paracoccidioidin in captive Latin American wild mammals. *Journal of medical and veterinary mycology*, 33 (1): 39-42.

Costa, E. O.; Netto, C. F. 1978. Contribution to the epidemiology of paracoccidioidomycosis and histoplasmosis in the state of São Paulo, Brazil: Paracoccidioidin and histoplasmin intradermic tests in domestic animals. *Sabouraudia*, 16 (2): 93-101.

Díaz, I. A. C.; Alvarez, B. J.; Gezuele, E.; Marini, H. G.; Duarte, J.; Falcón, J. 1972. Skin-test survey with paracoccidioidin and histoplasmin in horses. *Revista do Instituto de Medicina Tropical de São Paulo*, 14 (6): 372-376.

Dyke, M. C. C. V.; Teixeira, M. M.; Barker, B. M. 2019. Fantastic yeasts and where to find them: the hidden diversity of dimorphic fungal pathogens. *Current Opinion in Microbiology*, 1 (52): 55-63.

Esperón, F.; García, D. P.; Bellière, E. N.; Sánchez, J. M. V. 2012. Molecular diagnosis of lobomycosis-like disease in a bottlenose dolphin in captivity. Medical Mycology, 50(1):106–109.

Farias, M. R.; Condas, L. A. Z.; Ribeiro, M. G.; Bosco, S. M. G.; Muro, M. D.; Werner, J.; Theodoro, R. C.; Bagagli, E. 2011. Marques, S. A.; Franco, M. Paracoccidioidomycosis in a dog: case report of generalized lymphadenomegaly. *Mycopathologia*, 172 (2): 147-152.

Fernandes, G. F.; Deps, P.; Yamashita, J. T.; Camargo, Z. P. 2004. Resposta de anticorpos IgM e IgG para *Paracoccidioides brasiliensis* em tatus selvagens naturalmente infectados (*Dasypus novemcinctus*). *Micologia Médica*, 42 (4): 363-368.

Ferreira, J. B.; Navarro, I. T.; Freire, R. L.; Oliveira, G. G.; Omori, A. M.; Belitardo, D. R.; Itano, E. N.; et al. 2013. Evaluation of *Paracoccidioides brasiliensis* infection in dairy goats. *Mycopathologia*, 176 (1): 95-99.

Fischer, M. C.; Gurr, S. J; Cuomo, C. A.; Blehert, D. S.; Jin, H.; Stukenbrock, E. H.; Stajich, J. E.; et al. 2020. Threats Posed by the Fungal Kingdom to Humans, Wildlife, and Agriculture. *American Society for Microbiology*, 11 (3): 1-17.

Fontana, F. F.; Santos, C. T. B.; Esteves, F. M.; Rocha, A.; Fernandes, G. F.; Amaral, C. C.; Domingues, M. A.; et al. 2010. Seroepidemiological survey of paracoccidioidomycosis infection among urban and rural dogs from Uberaba, Minas Gerais, Brazil. *Mycopathologia*, 169 (1): 159-165.

Grose, E.; Tamsitt, J. R. 1965. *Paracoccidioides brasiliensis* recovered from the intestinal tract of three bats (*Artibeus lituratus*) in Colombia, SA. *Medical Mycology*, 4 (2): 124-125.

Headley, S. A.; Giordano, L. G. P.; Santis, G. W.; Gomes, L. A.; Macagnan, R.; Nóbrega, D. F.; Leite, K. M.; et al. 2017. *Paracoccidioides brasiliensis*-associated dermatitis and lymphadenitis in a dog. *Mycopathologia*, 182 (1): 425-434.

Hrycyk, M. F.; Garces, H. G.; Bosco, S. M. G.; Oliveira, S. L.; Marques, S. A.; Bagagli, E. 2018. Ecology of *Paracoccidioides brasiliensis*, *P. lutzii* and related species: infection in armadillos, soil occurrence and mycological aspects. *Medical Mycology*, 56 (8): 950-962.

Johnson, W. D.; Lang, C. M. 1977. Paracoccidioidomycosis (South American blastomycosis) in a squirrel monkey (*Saimiri sciureus*). *Veterinary pathology*, 14 (4): 368-371.

Losnak, D. O.; Rocha, F. R.; Almeida, B. S.; Batista, K. Z. S.; Althoff, S. L.; Haupt, J.; Ruiz, L. S.; et al. 2018. Molecular detection of fungi of public health importance in wild animals from Southern Brazil. *Mycoses*, 61 (7): 455-463.

Macedo, P. M.; Costa, B. S. S.; Paes, R. A.; Trilles, L.; Oliveira, R. M. Z.; Valle, A. C. F.; Wanke, B. 2020. *Paracoccidioides brasiliensis* habitat: far beyond armadillo burrows?. *Memórias do Instituto Oswaldo Cruz*, 115 (1): 1-3.

Martinez, R. New trends in paracoccidioidomycosis epidemiology. 2017. *Journal of Fungi*, 3 (1): 1-13.

Mendes, J. F.; Groll, A. V.; Poester, V. R.; Brasil, C. L.; Brandolt, T. M.; Klafker, G.B.; Nogueira, C. E. W.; et al. 2019. O. *Paracoccidioides* spp. in soil from the Pampa Biome in Southern Brazil. *Current microbiology*, 76 (1): 258-262.

Mendes, J. F.; Klafke, G. B.; Albano, A. P. N.; Cabana, A. L.; Teles, A. J.; Camargo, Z. P.; Xavier, M. O.; Meireles, M. C. 2017. Paracoccidioidomycosis infection in domestic and wild mammals by *Paracoccidioides lutzii*. *Mycoses*, 60 (6): 402-406.

Mendes, J. F.; Poester, V. R.; Groll, A. V.; Meireles, M. C. A.; Xavier, M. O. 2020. Molecular detection of *Paracoccidioides* in soil from an urban area of southern Brazil. *Revista da Sociedade Brasileira de Medicina Tropical*, v. 53 (1): 1-4.

Minakawa, T.; Ueda, K.; Tanaka, M.; Tanaka, N.; Kuwamura, M.; Izawa, T.; et al. 2016. Detection of multiple budding yeast cells and a partial sequence of 43-kDa glycoprotein coding gene of *Paracoccidioides brasiliensis* from a case of lacaziosis in a female Pacific white-sided dolphin (*Lagenorhynchus obliquidens*). *Mycopathologia*, 181 (1):523–529.

Moher, D.; Shamseer, L.; Clarke, M.; Ghersi, D.; Liberati, A.; Petticrew, M.; Shekelle, P., et al. 2015. A. Preferred reporting items for systematic review and meta-analysis protocols (PRISMA-P) 2015 statement. *Systematic reviews*, 4 (1): 1-9.

Montenegro, M. R.; Miyaji, M.; Franco, M.; Nishimura, K.; Coelho, K. I.; Horie, Y.; Mendes, R. P.; et al. 1996. Isolation of fungi from nature in the region of Botucatu, state of São Paulo, Brazil, an endemic area of paracoccidioidomycosis. *Memórias do Instituto Oswaldo Cruz*, 91 (6): 665-670.

Mós, E. N.; Netto, C. F. Contribuição ao estudo da paracoccidioidomicose I – possível papel dos cães. Estudo sorológico e anatomo-patológico. *Revista do Instituto Medicina Tropical de São Paulo*, 16(3): 154-159.

Naiff, R. D.; Ferreira, L. C. L.; Barrett, T. V.; Naiff, M. F.; Arias, J. R. 1986. Paracoccidioidomicose enzoótica em tatus (*Dasypus novemcinctus*) no estado do Pará. *Revista do Instituto de Medicina Tropical de São Paulo*, 28 (1): 19-27.

Negroni, P. 1966. El *Paracoccidioides brasiliensis* vive saprofiticamente en el suelo argentino. Prensa Médica Argentina, 53 (1): 2381–2382.

Oliveira, G. G.; Belitardo, D. R.; Balarin, M. R. S.; Freire, R. L.; Camargo, Z. P.; Ono, M. A. 2013. Serological survey of paracoccidioidomycosis in cats. *Mycopathologia*, 176 (3): 299-302.

Oliveira, G. G.; Navarro, I. T.; Freire, R. L.; Belitardo, D. R.; Silveiram L. H.; Camargo, Z. P.; Itano, E. N.; Ono, M. A. 2012. Serological survey of Paracoccidioidomycosis in sheep. *Mycopathologia*, 173 (1): 63-68.

Oliveira, G. G.; Silveira, L. H.; Itano, E. N.; Soares, R. M.; Freire, R. L.; Watanabe, M. A. E.; Camargo, Z. P.; Ono, M. A. 2011. Serological evidence of *Paracoccidioides brasiliensis* infection in chickens from Paraná and Mato Grosso do Sul States, Brazil. *Mycopathologia*, 171 (3):197-202.

Ono, M. A.; Bracarense, A. P. F. R.; Morais, H. S. A.; Trapp, S. M.; Belitardo, D. R. 2001. Canine paracoccidioidomycosis: a seroepidemiologic study. *Medical Mycology*, 39 (3): 277-282.

Paz, G. S.; Adorno, B. M.; Pereira, V. B. R.; Bosco, S. M. G.; Langoni, H. 2018. Infection by *Histoplasma capsulatum*, *Cryptococcus* spp. and *Paracoccidioides brasiliensis* in bats collected in urban areas. *Transboundary and emerging diseases*, 65 (6): 1797-1805.

Pereira, V. B. R.; Bosco, S. M. G.; Griese, J.; Theodoro, R. C.; Macoris, S. A.G.; Silva, R. J.; Barrozo, L.; et al. 2008. Molecular detection of *Paracoccidioides brasiliensis* in road-killed wild animals. *Medical Mycology*, 46 (1): 35-40.

Petroni, T. F.; Bonfietti, L. X.; Zaninelli, T. H.; Itano, E. N.; Ono, M. A. 2017. Serological evidence of infection by *Paracoccidioides brasiliensis* in dogs with leishmaniasis. *Mycopathologia*, 182 (9): 947-952.

Ricci, G.; Mota, F. T.; Wakamatsu, A.; Serafim, R. C.; Borra, R. C.; Franco, M. 2004. Canine paracoccidioidomycosis. Medical Mycology, 42(4):379–83.

Sacristán, C.; Esperón, F.; Ewbank, A. C.; Kolesnikovas, C. K. M.; Dias, J. L.C. 2018. Paracoccidioidomycosis ceti in an Atlantic bottlenose dolphin (*Tursiops truncatus*), Brazil. *Transboundary and Emerging Diseases*, 65 (2): 585-587.

Sbeghen, M. R.; Zanata, T. B.; Macagnan, R.; Abreu, K. C.; Cunha, W. L.; Watanabe, M. A. E.; Camargo, Z. P.; et al. 2015. *Paracoccidioides brasiliensis* infection in small wild mammals. *Mycopathologia*, 180 (5): 435-440.

Shikanai-Yasuda, M. A.; Mendes, R. P.; Colombo, A. L.; Queiroz-Telles, F. D.; Kono, A. S. G.; Paniago, A. M.; Nathan, A.; Valle, A. C. F.; Bagagli, E.; Benard, G. 2017. Brazilian guidelines for the clinical management of paracoccidioidomycosis. *Revista da Sociedade Brasileira de Medicina Tropical*, 50 (5): 715-740.

Shome, S. K.; Batista, A. C. 1963. Occurrence of *Paracoccidioides brasiliensis* in the soil of Recife, Brazil. *Revista de Medicina da Universidade Federal do Ceará*, 3 (1): 90-94.

Silveira, L. H.; Domingos, I. H.; Kouchi, K.; Itano, E. N.; Silva, E. A.; Landgraf, V. O.; Werneck, S. M.; et al. 2006. Serological detection of antibodies against *Paracoccidioides brasiliensis* in dogs with leishmaniasis. *Mycopathologia*, 162 (1): 325-329.

Silveira, L. H.; Paes, R. C. S.; Medeiros, E. V.; Itano, E. N.; Camargo, Z. P.; Ono, M. A. 2008. Occurrence of antibodies to *Paracoccidioides brasiliensis* in dairy cattle from Mato Grosso do Sul, Brazil. *Mycopathologia*, 165 (1): 367-371.

Silveira, L.H.; Camargo, Z.P.; Ono, M.A. Domingos, I. H.; Kouchi, K.; Silva, E A.; Landgraf, V. O.; et al. 2005. Paracoccidioidomycosis infection in dogs seropositive and seronegative to leishmaniosis. *Revista do Instituto Medicina Tropical de São Paulo*, 47(14): 32.

Suguiura, I. M. S.; Macagnan, R.; Omori, A. M.; Buck, E. L.; Scarpassa, J. A.; Giordano, L. G. P.; Boas, L. A. V.; et al. 2020. First report of *Paracoccidioides brasiliensis* infection in fish. *Medical Mycology*, 58 (6): 737-743.

Taborda, P. R.; Taborda, V. A.; Mcginnis, M. R. 1999. Lacazia loboi gen. nov., comb. nov., the etiologic agent of lobomycosis. *Journal of Clinical Microbiology*, 37 (6): 2031-2033.

Teixeira, A. G. K..; Yamauchi, D. H.; Rossi, A.; Sousa, H. R.; Garces, H. G.; Júnior, J. L.; Júnior, A. O. S.; et al. 2022. Clinical and Eco-Epidemiological Aspects of a Novel Hyperendemic Area of Paracoccidioidomycosis in the Tocantins-Araguaia Basin (Northern Brazil), Caused by *Paracoccidioides* sp. *Journal of Fungi*, 8 (5): 1-11.

Teixeira, M. M.; Cattana, M. E.; Matute, D. R.; Muñoz, J. F.; Arechavala, A.; Isbell, K.; Schipper, R.; et al. 2020. Genomic diversity of the human pathogen *Paracoccidioides* across the South American continent. *Fungal genetics and biology*, 140 (1): 1-28.

Teles, A. J.; Klafke, G. B.; Cabana, A. L.; Albano, A. P. N.; Xavier, M. O.; Meireles, M. C. A. 2016. Serological investigation into *Paracoccidioides brasiliensis* infection in dogs from Southern Rio Grande do Sul, Brazil. *Mycopathologia*, 181 (1): 323-328.

Terçarioli, G. R.; Bagagli, E.; Reis, G. M.; Theodoro, R. C.; Bosco, S. M. G.; Macoris, S. A. G.; Pereira, V. B. R. 2007. Ecological study of *Paracoccidioides brasiliensis* in soil: growth ability, conidia production and molecular detection. *BMC microbiology*, 7 (92): 1-8.

Theodoro, R. C.; Candeias, J. M. G.; Araújo Jr, J. P.; Bosco, S. D. M. G.; Macoris, S. A. D. G.; Junior, L. O. P.; Franco, M.; et al. 2005. Molecular detection of *Paracoccidioides brasiliensis* in soil. *Medical Mycology*, 43 (8): 725-729.

Ueda K, Sano A, Yamate, J.; Nakagawa, E. I.; Kuwamura, M.; Izawa, T.; Tanaka, M.; et al. 2013. Two cases of lacaziosis in bottlenose dolphins (Tursiops truncates) in Japan. *Case Reports in Veterinary Medicines*, 1 (1): 1-9.

Vergara, M. L. S.; Martines, R.; Camargo, Z. P.; Malta, M. H. B.; Maffei, C. M. L.; Chadu, J. B. et al. 2000. Isolation of *Paracoccidioides brasiliensis* from armadillos (*Dasypus novemcinctus*) in an area where the fungus was recently isolated from soil. *Medical Mycology*, 38 (3): 193-199.

Vergara, M. L. S.; Martinez, R. 1998. Role of the armadillo *Dasypus novemcinctus* in the epidemiology of paracoccidioidomycosis. *Mycopathologia*, 144 (3):131-133.

Vergara, M. L.; Martines, R.; Chadu, A.; Madeira, M.; Silva, G. F.; Maffei, C. M, L. 1997. Isolation of a *Paracoccidioides brasiliensis* strain from the soil of a coffee plantation in Ibiá, State of Minas Gerais, Brazil. *Medical mycology*, 36 (1): 37-42.

Vilela, R.; Hoog, S.; Bensch, K.; Bagagli, E.; Mendoza, L. 2023. A taxonomic review of the genus *Paracoccidioides*, with focus on the uncultivable species. *PLOS Neglected Tropical Diseases*, 17 (4): 1-25.

Vilela, R; Bossart, G. D.; Leger, J. A. S.; Dalton, L. M.; Reif, J. S.; Schaefer, A. M.; Mccarthy, P. J.; et al. 2016. Cutaneous granulomas in dolphins caused by novel uncultivated *Paracoccidioides brasiliensis*. *Emerging Infectious Diseases*, 22 (12): 1-7.

3. CAPÍTULO II - Molecular detection of *Paracoccidioides* spp. in environmental samples from the Southwestern Amazon

Brazilian Journal of Microbiology
https://doi.org/10.1007/s42770-024-01256-7

SOIL AND AGRICULTURAL MICROBIOLOGY - RESEARCH PAPER

Molecular detection of *Paracoccidioides* spp. in environmental samples from the Southwestern Amazon

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Received: 25 September 2023 / Accepted: 9 January 2024
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Abstract
Paracoccidioidomycosis is an infection with the potential for environmental dissemination, especially in regions of hot and humid climate, where human cases have been recorded in the Southwestern Amazon of Brazil, specifically in the state of Acre. Despite studies providing information about the presence of these fungi in soil and animal samples, such as armadillos, further investigations are still needed to determine the epidemiological distribution of the genus *Paracoccidioides*. The aim of this study was to detect the occurrence of *Paracoccidioides* fungi in the Southwestern Amazon. To achieve this, 60 soil samples were collected from armadillo burrows on rural properties in the in the municipalities of Acrelândia, Bujari, Plácido de Castro, Rio Branco, Sena Madureira, and Senador Guiomard, located in the state of Acre, Brazil. Fungal DNA was extracted from these samples using the DNEASY® PowerSoil kit—Quiagen, followed by Nested PCR technique with ITS4 and ITS5 as external primers, and PBITS-E and PBITS-R as internal primers. DNA amplification products of about 380 bp compatible with *Paracoccidioides* spp. were detected in six samples (10%), being sequenced and identified as *P. brasiliensis*. These findings indicate that the soils of the Acre state could be considered a potential source for *Paracoccidioides* spp., suggesting that local infections are likely.

Keywords Brazil · Acre · Armadillo · Paracoccidioidomycosis · *P.brasiliensis*

Introduction

Paracoccidioidomycosis (PCM) is a systemic mycosis caused by the genus *Paracoccidioides*, which currently comprises seven distinct species: *P. brasiliensis* (S1a and S1b), *P. americana* (PS2), *P. restrepiensis* (PS3), *P. venezuelensis* (PS4), *P. lutzii*, *P. lobogeorgii*, and *P. cetii* [1, 2]. This fungus is widely distributed in Latin America, with a higher incidence in Brazil, Colombia, and Venezuela [1, 2].

In Brazil, approximately 80% of cases occur in the South, Southeast, and Midwest regions, where the mortality rate is 1.45 cases per million inhabitants [3]. In recent years, PCM has undergone notable changes in its epidemiology and demographics of the affected population [4]. These transformations have been driven by the expansion of agricultural activities as well as the intense migration process, becoming relevant factors for the spread of the disease in the northern region of Brazil [4].

In the Amazon region, the states of Rondônia and Tocantins have reported the presence of the genus *Paracoccidioides* in environmental areas, suggesting favorable conditions for the growth and survival of this fungus [5, 6]. Furthermore, this same region has been identified as a new hyperendemic area, with cases of the disease reported in Rondônia and along the eastern border of the Amazon region, encompassing the states of Pará and Tocantins [4].

In the state of Acre, PCM still remains an underestimated disease. However, the identification of its presence in the

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Introduction

Paracoccidioidomycosis (PCM) is a systemic mycosis caused by the genus *Paracoccidioides*, which currently comprises seven distinct species: *P. brasiliensis* (S1a and S1b), *P. americana* (PS2), *P. restrepiensis* (PS3), *P. venezuelensis* (PS4), *P. lutzii*, *P. lobogeorgii*, and *P. cetii* [1,2]. This fungus is widely distributed in Latin America, with a higher incidence in Brazil, Colombia, and Venezuela [1,2].

In Brazil, approximately 80% of cases occur in the South, Southeast, and Midwest regions, where the mortality rate is 1.45 cases per million inhabitants [3]. In recent years, PCM has undergone notable changes in its epidemiology and demographics of the affected population [4]. These transformations have been driven by the expansion of agricultural activities as well as the intense migration process, becoming relevant factors for the spread of the disease in the northern region of Brazil [4].

In the Amazon region, the states of Rondônia and Tocantins have reported the presence of the genus *Paracoccidioides* in environmental areas, suggesting favorable conditions for the growth and survival of this fungus [5,6]. Furthermore, this same region has been identified as a new hyperendemic area, with cases of the disease reported in Rondônia and along the eastern border of the Amazon region, encompassing the states of Pará and Tocantins [4].

In the state of Acre, PCM still remains an underestimated disease. However, the identification of its presence in the region has gained greater prominence based on recent studies. An epidemiological survey identified 114 cases of the disease [7]. Additionally, molecular investigations conducted on 35 clinical samples from patients initially diagnosed with lobomycosis (*Lacazia loboi*) revealed notable similarities with the genus *Paracoccidioides*, leading to the classification of a new cryptic species: *P. lobogeorgii* [2,8].

These findings indicate the epidemiological significance of the disease in the state of Acre. However, the presence of this fungus in environmental areas is still undetermined. Consequently, the identification of risk areas will contribute to a better understanding of the biogeography of the genus *Paracoccidioides* [5]. Thus, this study aimed to perform the molecular detection of *Paracoccidioides* spp. in environmental samples from the South Western Amazon.

Materials and Methods

Selection of the study areas and environmental samples collection

Soil samples from armadillo burrows were collected in the municipalities of Acrelândia, Bujari, Plácido de Castro, Rio Branco, Sena Madureira, and Senador Guiomard, located in the state of Acre, Brazil. In each of the six study municipalities, ten soil samples were collected from different rural properties, totaling sixty samples (Fig 1 and Table SI1). The collection of

approximately 50g of soil was performed using an iron-shafted shovel to scrape the inner part of the burrows. During this process, the shovel was decontaminated with 70% v/v ethanol solutions before each sampling to prevent cross-contamination. Subsequently, the soils were placed in sterile universal containers, labeled, and stored in isothermal boxes at room temperature.

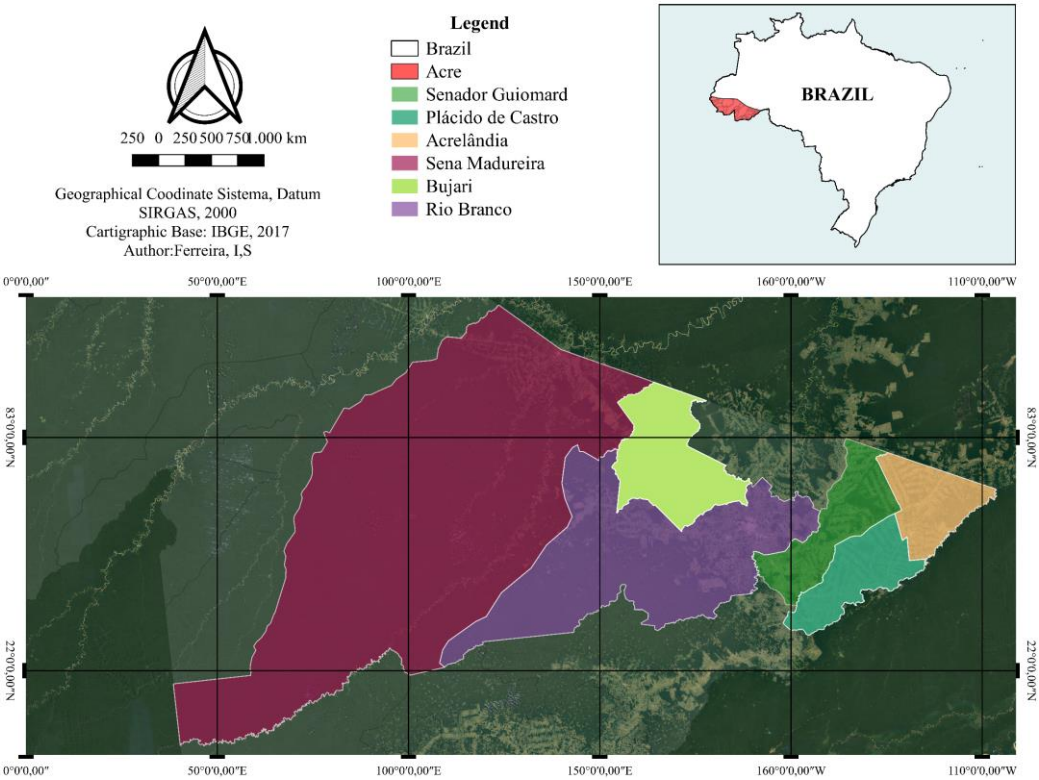

Fig 1 Collection areas of soil samples in the Southwestern Amazon. Geographic location of the collection site. Graphics program: QGis 2.18

DNA extraction

The samples underwent total deoxyribonucleic acid (DNA) extraction using the DNeasy PowerSoil kit (QIAGEN, Germany). The total DNA was suspended in nuclease-free water and quantified using a NanoVue spectrophotometer (GE Healthcare®) [5]. Polymerase chain reactions (PCR) were conducted targeting the universal fungal region of the rRNA ITS1-5.8S-ITS2 (Internal Transcribed Spacer) using the external primers ITS-4 and ITS-5 (Table 1) [9].

Table 1 Primer sequences and PCR conditions used to detect *Paracoccidioides* spp. DNA in soil samples Southwestern Amazon [9-10]

PCR procedure	Primer	Sequence	PCR conditions
Nested PCR	ITS4	TCCTCCGCTTATTGATATGC	Initial cycle: 95°C/5 min. + 35 cycles: 95°C/50 seg. + 55°C/50 min. + 72°C/ 50 min. + final cycles: 72°C/5 min.
	ITS5	GGAAGTAAAAGTCGTAACA AGG-	
Nested PCR	PbITS-E	GAGCTTTGACGTCTGAGACC	Initial cycle: 95°C/5 min. + 35 cycles: 95°C/ 45seg. + 55°C/ 50 min + 72°C/ 50 min. + final cycles: 72°C/5 min.
	PbITS-R	AAGGGTGTCGATCGAGAGA G	

Subsequently, the Nested-PCR technique was performed using the product from the first reaction, employing the internal primers PbITS-E (sense) and PbITS-R (antisense), specific to *P. brasiliensis* (Table 1) [10]. Genomic DNA from *P. brasiliensis* (T19F33) [11], isolated from a *Dasypus novemcinctus* and provided by the Department of Microbiology and Immunology, Institute of Biosciences, Botucatu, São Paulo State University (UNESP), served as the positive control, and nuclease-free water was used as the negative control. The samples were considered positive upon identification of the PCR product via agarose gel electrophoresis (1.5%).

Phylogenetic analysis

To confirm the specificity of the amplicons, the PCR products were sequenced using the Sanger sequencing method (ACTGene Molecular Analyses Ltda, Porto Alegre, RS, Brasil), using the automatic sequencer ABI-Prism 3500 Genetic Analyzer (Applied BiosystemsTM, Foster City, CA, EUA). The obtained sequences were identified as *P. brasiliensis* and stored in the GenBank database (accession number: OQ456013, OQ456014, OQ456015, OQ456016, OQ456017, OQ456018).

The sequences were analyzed and aligned using a Multiple sequence alignment program (MAFFT®) software version 7.0 [12]. The similarity between the strains was determined using the Basic Local Aligment Search Tool (BLAST®) on the website of National Center for Biotechnology Information (NCBI). The ModelFinder [13] in the IQ TREE 2 software [14] was used to find the best-fit model for each gene according to the Bayesian Information Criterion (BIC).

The sequences generated in this study were subjected to analysis along with other sequences obtained using the BLAST tool (Table SI2). In addition, the organism *L. loboi* was included as an outgroup for comparative purposes. Subsequently, a phylogenetic tree was

constructed using maximum likelihood methods. To assess the reliability of the branches in the phylogenetic tree, branch support values were utilized, measured through ultrafast bootstrap (UFBoot) and approximate likelihood ratio test (SH-aLRT).

Results

An amplicon (approximately 380 bp) consistent with *Paracoccidioides* spp. was observed in six (10%) of the analyzed samples (Table SI1). In the municipality of Plácido de Castro, four samples (66.60%) were recorded, and one (16.60%) each in the municipalities of Acrelândia and Senador Guiomard. The six environmental amplicons obtained were subjected to sequencing and then compared with previously deposited sequences in GenBank. The results revealed 100% identity with *P. brasiliensis*. Furthermore, phylogenetic analysis of the amplicons associated with *P. brasiliensis* exhibited significant genetic variability (Fig 2).

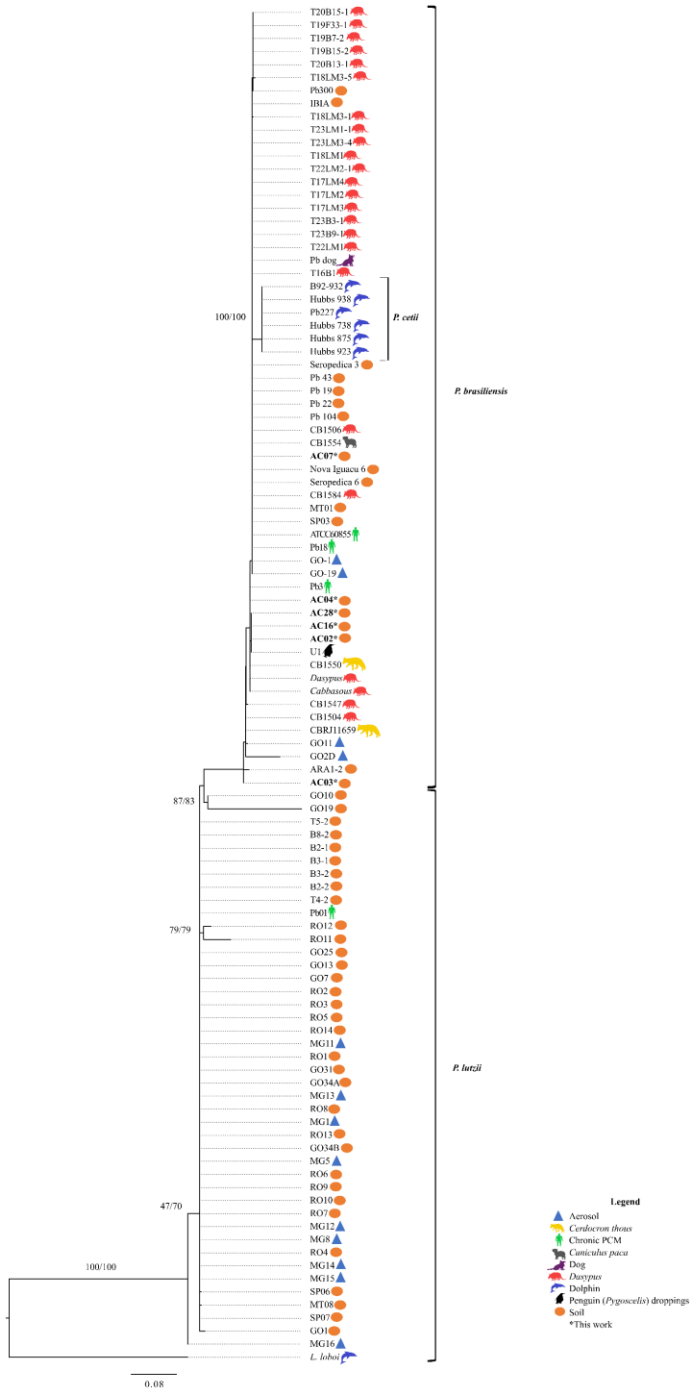

Fig 2 Phylogenetic tree of *Paracoccidioides* based on ITS sequences obtained by maximum likelihood using the Bayesian Information Criterion. **Graphics program**: ModelFinder in the IQ-TREE 2 software

Discussion

This study represented the first detection of *P. brasiliensis* in soil samples from rural areas of the state of Acre. These samples tested positive in three different municipalities, indicating a widespread distribution of the fungus in the investigated region. This suggests that the local population is significantly exposed to this fungus and, consequently, at risk of

infection. These findings are in agreement with previous studies that documented *Paracoccidioides* spp. infection and cases of the disease in patients treated at healthcare facilities in the state [2,8].

Molecular detection in soil samples was positive in the municipalities of Acrelândia, Plácido de Castro, and Senador Guiomard, but negative in Bujari, Rio Branco and Sena Madureira. The highest detection rate of *Paracoccidioides* spp. was identified in samples from Plácido de Castro (Table SI1). However, it is important to note that these samples were collected during a period of lower rainfall, which may suggest that the fungus exhibits greater resistance to adverse environmental conditions in this region [5].

The genus *Paracoccidioides* has the soil as its natural reservoir but can infect accidental hosts such as humans, domestic, and wild animals. Among these animals, the armadillo stands out for having a less active immune system and lower body temperature, factors that make them more susceptible to infection by this fungus [15]. Furthermore, these animals have a habit of digging for food and living underground, which increases their exposure to the fungus present in the environment [15].

Paracoccidioides spp. was detected in soil samples from armadillo burrows. Previous studies have also reported similar findings in Rondônia, Goiás, and Tocantins [5,6]. These investigations reinforce the hypothesis that abiotic factors within the armadillo burrow, such as temperature, humidity, and protection from solar radiation, favor the development of the fungus. Consequently, these factors are considered suitable for mapping the geographical distribution of the fungus in the environment [16].

The samples were collected from soil in rural properties engaged in cattle farming and family agriculture. In the municipalities where the detection of *Paracoccidioides* was positive, approximately 50% of their territories are deforested, with cattle farming being the main economic activity [17]. These results reinforce the hypothesis that agricultural expansion with deforestation of preserved areas exposes the soil and alters its physicochemical characteristics, favoring the emergence of new endemic areas of the disease in the Amazon region [5].

The isolation of *Paracoccidioides* spp. from soil is a significant challenge, as the diversity of saprophytic fungi in these samples, along with the use of pesticides, can inhibit the growth and identification of these microorganisms under laboratory conditions [18]. With this perspective, molecular techniques have shown promise in documenting the occurrence of this fungus in the environmentt.

In this study, molecular methods were employed, resulting in the detection of six (10%) samples identified as *P. brasiliensis*. Similar results have also been found in other states, such as São Paulo (10%) and Tocantins (6.66%) [6,11]. The PCR and Nested-PCR techniques exhibit

high sensitivity and specificity, allowing for the identification of the fungus in environmental samples with low DNA concentrations. This contributes to the detection of this genus in various regions [5, 19].

Although the ITS region is not useful for distinguishing different species of the *Paracoccidioides* genus, the observation of *P. lutzii* clustered in a distinct clade suggests that the six amplicons in this study belong to the *P. brasiliensis* species. Previous studies suggest that *P. lutzii* is composed of a single monophyletic and recombined population, identified in Ecuador as well as in the Central-West and Northern regions of Brazil [19].

On the other hand, *P. brasiliensis* encompasses a complex of different cryptic species, such as *P. brasiliensis* (S1a and S1b), *P. americana*, *P. restrepiensis*, *P. venezuelensis*, *P. lobogeorgii*, and *P. ceti* [1, 2]. It is relevant to highlight that the species *P. lobogeorgii* has been predominantly identified in the state of Acre, as well as in São Paulo, Brazi [2]. Given this diversity of species, it is suggested that future studies utilize the ADP-ribosylation factor (ARF) and the 43kDa immunodominant glycoprotein (GP43) genes to analyze samples from this region, to determine the predominant cryptic species in the Southwestern Amazon [20].

Conclusion

The estimate of PCM cases in the state of Acre may be underestimated. However, the detection of *Paracoccidioides* in 10% of the samples reinforces the hypothesis of a gradual expansion of this fungus over the years in the northern region of the country, possibly driven by the growth of agricultural activities. Furthermore, these findings also indicate favorable climatic conditions for the dispersion of the fungus in the Southwestern Amazon, suggesting that the local population is at risk of contracting the disease.

Acknowledgments

We thank the Coordination for the Improvement of Higher Education Personnel (CAPES) and the Graduate Support Program (PROAP) for their support.

Declarations

Funding: Partial financial support was received from the Coordination for the Improvement of Higher Education Personnel (CAPES) and the Graduate Support Program (PROAP).

Conflict of interest: All authors declare that they have no conflicts of interest pertaining to this work.

Informed consente: Informed consent was obtained from all individual participants reported in the study.

Availability of data and material: The data presented in this study are openly available in NCBI, accession number OQ456013, OQ456014, OQ456015, OQ456016, OQ456017, OQ456018.

Author Contributions

All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by [Iasminy R. Silva Ferreira], [Marcus de M. Teixeira], [Atilon V. de Araújo], [Eduardo Bagagli], [Hans G. Garces], [Leila P. Peters] and [Clarice M. Carvalho]. The first draft of the manuscript was written by [Iasminy R. Silva Ferreira] and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Ethics approval: Not applicable.

Consent to participate: Not applicable.

Consent for publication: Not applicable.

Code availability: Not applicable.

Reference

1. Teixeira MM, Cattana ME, Matute DR, Muñoz JF, Arechavala A, Isbell K, Schipper R, Santioso G, Tracogna F, Sosa MA, Cech N, Alvarado P, Barreto L, Chacón Y, Ortellado J, Lima CM, Chango R, Vega GN, Shikanai-Yasuda MA, Felipe MSS, Giusiano G (2020) Genomic diversity of the human pathogen *Paracoccidioides* across the South American continent. Fungal Genet. Biol 140 (1): 1-28. https://doi.org/10.1016/j.fgb.2020.103395

2. Vilela R, Hoog S, Bensch K, Bagagli E, Mendoza L (2023) A taxonomic review of the genus *Paracoccidioides*, with focus on the uncultivable species. PLoS Negl Trop Dis 17(4): 1-25. https://doi.org/10.1371/journal.pntd.0011220

3. Shikanai-Yasuda MA, Mendes RP, Colombo AL, Queiroz-Telles FD, Kono AS G, Paniago AM, Nathan A, Valle ACF, Bagagli E, Benard G (2017) Brazilian guidelines for the clinical management of paracoccidioidomycosis. Rev Soc Bras Med Trop 50 (5): 715-740. https://doi.org/10.1590/0037-8682-0230-2017

4. Martinez R (2017) New trends in paracoccidioidomycosis epidemiology. J. Fungi 3 (1): 1-13. https://doi.org/10.3390/jof3010001

5. Arantes TD, Theodoro RC, Teixeira MM, Bosco SDMG, Bagagli E (2016) Environmental mapping of *Paracoccidioides* spp. in Brazil reveals new clues into genetic diversity, biogeography and wild host association. PLoS Negl Trop Dis 10 (4): 1-18. https://doi.org/10.1371/journal.pntd.0004606

6. Teixeira AGK, Yamauchi DH, Rossi A, Sousa HR, Garces HG, Júnior JL, Júnior AOS, Felipe MSS, Bagagli E, Junior HFA, Teixeira MM (2022) Clinical and Eco-Epidemiological Aspects of a Novel Hyperendemic Area of Paracoccidioidomycosis in the Tocantins-Araguaia Basin (Northern Brazil), Caused by *Paracoccidioides* sp. J. Fungi 8 (5): 1-11. https://doi.org/10.3390/jof8050502

7. Trompier RGE, Costa AR, Silva RSU (2020) Paracoccidioidomicose no estado do Acre, Brasil: aspectos epidemiológicos e clínicos. In: Silva RSU, Meneguetti DUO (org.) Ciências Médicas na Amazônia, 1º ed. Stricto Sensu, Rio Branco, pp 81-94. https://doi.org/10.35170/ss.ed.9786586283235.06

8. Taborda PR, Taborda VA, Mcginnis MR (1999) *Lacazia loboi* gen. nov., comb. nov., the etiologic agent of lobomycosis. J. Clin. Microbiol 37 (6): 2031-2033. https://doi.org/10.1128/jcm.37.6.2031-2033.1999

9. White TJ, Bruns T, Lee S, Taylor JIMA (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Sninsky JJ, White TJ, editors. PCR protocols: a guide to methods and applications. San Diego: Academic, pp 315–322

10. Theodoro R, Candeias JM, Araújo JP Jr, Bosco SDMG, Macoris SADG, Junior LOP, Franco M, Bagagli E (2005) Molecular detection of *Paracoccidioides brasiliensis* in soil. Med Mycol 43(8):725–729. https://doi.org/10.1080/13693780500129418

11. Hrycyk MF, Garces HG, Bosco SMG. Oliveira SL, Marques AS, Bagagli E (2018) Ecology of *Paracoccidioides brasiliensis*, *P. lutzii* and related species: infection in armadillos, soil occurrence and mycological aspects. Med. Mycol. J 56 (8): 950-962. https://doi.org/10.1093/mmy/myx142

12. Steenwyk JL, Buida T J, Shen XX, Rokas A (2020) ClipKIT: a multiple sequence alignment trimming software for accurate phylogenomic inference. PLoS Biol 18 (12): p. 1-25. https://doi.org/10.1371/journal.pbio.3001007

13. Kalyaanamoorthy S, Minh BQ, Wong TK, Haeseler AV, Jermiin LS (2017) ModelFinder: fast model selection for accurate phylogenetic estimates. Nat. Methods 14 (6): 587-589. https://doi.org/10.1038/nmeth.4285

14. Nguyen LT, Schmidt HÁ, Haeseler AV, Minh BQ (2015) IQ-TREE: a fast and effective stochastic algorithm for estimating maximum-likelihood phylogenies. Mol. Biol. Evol 32 (1): 268-274. https://doi.org/10.1093/molbev/msu300

15. Bagagli E, Bosco SDMG, Theodoro RC, Franco M (2006) Phylogenetic and evolutionary aspects of *Paracoccidioides brasiliensis* reveal a long coexistence with animal hosts that explain several biological features of the pathogen. Infect Genet Evol 6 (5): 344-351. https://doi.org/10.1016/j.meegid.2005.12.002

16. Bagagli E, Sano A, Coelho KI, Alquati S, Miyaji M, Camargo ZP, Gomes GM, Franco M, Montenegro MR (1998) Isolation of *Paracoccidioides brasiliensis* from armadillos (*Dasypus noveminctus*) captured in an endemic area of paracoccidioidomycosis. Am. J. Trop. Med. Hyg 58 (4): 505-512

17. Programa Estadual de Zoneamento Ecológico Econômico do Estado do Acre (ZEE). http://semapi.acre.gov.br/wp-content/uploads/sites/20/2021/12/COMPLETO-Resumo-Executivo-do-ZEE-Acre-Fase-III_V16_WEB.pdf. Accessed Jun 2023

18. Macedo PM, Costa BSS, Paes RA, Trilles, L.; Oliveira RMZ, Valle ACF, Wanke B (2020) *Paracoccidioides brasiliensis* habitat: far beyond armadillo burrows?. Mem. Inst. Oswaldo Cruz 115 (1): 1-3. https://doi.org/10.1590/0074-02760200208

19. Teixeira MM,Theodoro RC, Nino-Veja G, Bagagli E, Felipe MS (2014) *Paracoccidioides* species complex: ecology, phylogeny, sexual reproduction, and virulence. PLoS Pathog 10 (10):1-4. https://doi.org/10.1371/journal.ppat.1004397

20. Theodoro RC, Teixeira MM, Felipe MSS, Paduan KS, Ribolla PM, Blas GS, Bagagli E (2012) Genus *Paracoccidioides*: species recognition and biogeographic aspects. PloS one, 7 (5): 1-15. https://doi.org/10.1371/journal.pone.0037694

4. Conclusão

- O Brasil é uma região endêmica para o fungo *Paracoccidioides*, com o tatu se destacando como um indicador significativo da presença desse fungo na natureza, devido à sua estreita associação com solos, que são o habitat natural do fungo. Além disso, a detecção molecular é reconhecida como o método mais sensível para detectar o fungo no ambiente.
- *Paracoccidioides* sp. foi detectado em 10% das amostras de solo analisadas, indicando que a região Sudoeste da Amazônia possui condições climáticas favoráveis para o desenvolvimento e dispersão do fungo no ambiente. Portanto, sugerindo que a população dessa região está em risco de contrair a paracoccidioidomicose.

APÊNDICE

Supplementary Material - Molecular detection of *Paracoccidioides* spp. in environmental samples from the Southwestern Amazon

Table SI1 – Results of molecular detection of *Paracoccidioides brasiliensis* by Nested-PCR in soil samples from rural areas of the South Western Amazon

Collection Point	Sample ID*	Geographic region (area)	Soil sample	Molecular detection	Species
P01		Plácido de Castro/AC	Burrow	Negative	Negative
P02	AC02	Plácido de Castro/AC	Burrow	Positivo	*P. brasiliensis*
P03	AC03	Plácido de Castro/AC	Burrow	Positivo	*P. brasiliensis*
P04	AC04	Plácido de Castro/AC	Burrow	Positivo	*P. brasiliensis*
P05		Plácido de Castro/AC	Burrow	Negative	Negative
P06		Plácido de Castro/AC	Burrow	Negative	Negative
P07	AC07	Plácido de Castro/AC	Burrow	Positivo	*P. brasiliensis*
P08		Plácido de Castro/AC	Burrow	Negative	Negative
P09		Plácido de Castro/AC	Burrow	Negative	Negative
P10		Plácido de Castro/AC	Burrow	Negative	Negative
P11		Acrelândia/AC	Burrow	Negative	Negative
P12		Acrelândia/AC	Burrow	Negative	Negative
P13		Acrelândia/AC	Burrow	Negative	Negative
P14		Acrelândia/AC	Burrow	Negative	Negative
P15		Acrelândia/AC	Burrow	Negative	Negative
P16	AC16	Acrelândia/AC	Burrow	Negative	*P. brasiliensis*
P17		Acrelândia/AC	Burrow	Negative	Negative

Collection Point	Sample ID*	Geographic region (area)	Soil sample	Molecular detection	Species
P18		Acrelândia/AC	Burrow	Negative	Negative
P19		Acrelândia/AC	Burrow	Negative	Negative
P20		Acrelândia/AC	Burrow	Negative	Negative
P21		Senador Guiomard/AC	Burrow	Negative	Negative
P22		Senador Guiomard/AC	Burrow	Negative	Negative
P23		Senador Guiomard/AC	Burrow	Negative	Negative
P24		Senador Guiomard/AC	Burrow	Negative	Negative
P25		Senador Guiomard/AC	Burrow	Negative	Negative
P26		Senador Guiomard/AC	Burrow	Negative	Negative
P27		Senador Guiomard/AC	Burrow	Negative	Negative
P28	AC28	Senador Guiomard/AC	Burrow	Positivo	*P. brasiliensis*
P29		Senador Guiomard/AC	Burrow	Negative	Negative
P30		Senador Guiomard/AC	Burrow	Negative	Negative
P31		Rio Branco/AC	Burrow	Negative	Negative
P32		Rio Branco/AC	Burrow	Negative	Negative
P33		Rio Branco/AC	Burrow	Negative	Negative
P34		Rio Branco/AC	Burrow	Negative	Negative
P35		Rio Branco/AC	Burrow	Negative	Negative
P36		Rio Branco/AC	Burrow	Negative	Negative
P37		Rio Branco/AC	Burrow	Negative	Negative
P38		Rio Branco/AC	Burrow	Negative	Negative

Collection Point	Sample ID*	Geographic region (area)	Soil sample	Molecular detection	Species
P39		Rio Branco/AC	Burrow	Negative	Negative
P40		Rio Branco/AC	Burrow	Negative	Negative
P41		Bujari/AC	Burrow	Negative	Negative
P42		Bujari/AC	Burrow	Negative	Negative
P43		Bujari/AC	Burrow	Negative	Negative
P44		Bujari/AC	Burrow	Negative	Negative
P45		Bujari/AC	Burrow	Negative	Negative
P46		Bujari/AC	Burrow	Negative	Negative
P47		Bujari/AC	Burrow	Negative	Negative
P48		Bujari/AC	Burrow	Negative	Negative
P49		Bujari/AC	Burrow	Negative	Negative
P50		Bujari/AC	Burrow	Negative	Negative
P51		Sena Madureira/AC	Burrow	Negative	Negative
P52		Sena Madureira/AC	Burrow	Negative	Negative
P53		Sena Madureira/AC	Burrow	Negative	Negative
P54		Sena Madureira/AC	Burrow	Negative	Negative
P55		Sena Madureira/AC	Burrow	Negative	Negative
P56		Sena Madureira/AC	Burrow	Negative	Negative
P57		Sena Madureira/AC	Burrow	Negative	Negative
P58		Sena Madureira/AC	Burrow	Negative	Negative
P59		Sena Madureira/AC	Burrow	Negative	Negative

Collection Point	Sample ID*	Geographic region (area)	Soil sample	Molecular detection	Species
P60		Sena Madureira/AC	Burrow	Negative	Negative

Table SI2 - Sequences obtained from amplicons of *Paracoccidioides* spp. with the universal primers ITS1; 5.8S; ITS2 deposited on the GenBank platform of the NCBI database

Clone/Strain	Country	State	Species	Source	Reference	Accession Number
AC02	Brazil	Acre	*P. brasiliensis* complex	Burrow	This work	OQ456013.1
Seropedica 3	Brazil	Rio de Janeiro	*P. brasiliensis* complex	Soil	[1]	MT726207.1
AC03	Brazil	Acre	*P. brasiliensis* complex	Burrow	This work	OQ456015.1
GO-1	Brazil	Goiás	*P. brasiliensis* complex	Aerosol	[2]	KP636448.1
GO19	Brazil	Goiás	*P. brasiliensis* complex	Aerosol	[2]	KP636451.1
GO11	Brazil	Goiás	*P. brasiliensis* complex	Aerosol	[2]	KP636450.1
Pb18	Brazil	São Paulo	*P. brasiliensis* complex	Chronic PCM	[3]	KT155977.1
ATCC60855	Colômbia	Antioquia	*P. brasiliensis* complex	Chronic PCM	[3]	KJ540971.1
Pb3	Brazil	São Paulo	*P. brasiliensis* complex	Chronic PCM	[3]	EU870315.1
Pb300	Venezuela	Miranda	*P. brasiliensis* complex	Soil	[3]	EU118553.1
IBIA	Brazil	Minas Gerais	*P. brasiliensis* complex	Soil	[3]	EU118545.1
T23LM3-4	Brazil	São Paulo	*P. brasiliensis* complex	Dasypus	[4]	KX774409.1
T23LM1-1	Brazil	São Paulo	*P. brasiliensis* complex	Dasypus	[4]	KX774408.1
T23B9-1	Brazil	São Paulo	*P. brasiliensis* complex	Dasypus	[4]	KX774407.1
T23B3-1	Brazil	São Paulo	*P. brasiliensis* complex	Dasypus	[4]	KX774406.1
T22LM2-1	Brazil	São Paulo	*P. brasiliensis* complex	Dasypus	[4]	KX774405.1
T22LM1	Brazil	São Paulo	*P. brasiliensis* complex	Dasypus	[4]	KX774404.1
T18LM1	Brazil	São Paulo	*P. brasiliensis* complex	Dasypus	[4]	KX774396.1
T17LM4	Brazil	São Paulo	*P. brasiliensis* complex	Dasypus	[4]	KX774395.1
T17LM3	Brazil	São Paulo	*P. brasiliensis* complex	Dasypus	[4]	KX774394.1

Clone/Strain	Country	State	Species	Source	Reference	Accession Number
T17LM2	Brazil	São Paulo	*P. brasiliensis* complex	Dasypus	[4]	KX774393.1
T20B15-1	Brazil	Mato Grosso	*P. brasiliensis* complex	Dasypus	[4]	KX774403.1
T20B13-1	Brazil	Mato Grosso	*P. brasiliensis* complex	Dasypus	[4]	KX774402.1
T19F33-1	Brazil	Mato Grosso	*P. brasiliensis* complex	Dasypus	[4]	KX774401.1
T19B15-2	Brazil	Mato Grosso	*P. brasiliensis* complex	Dasypus	[4]	KX774400.1
T19B7-2	Brazil	Mato Grosso	*P. brasiliensis* complex	Dasypus	[4]	KX774399.1
T18LM3-1	Brazil	São Paulo	*P. brasiliensis* complex	Dasypus	[4]	KX774397.1
T18LM3-5	Brazil	São Paulo	*P. brasiliensis* complex	Dasypus	[4]	KX774398.1
Dasypus	Colômbia	Caldas	*P. brasiliensis* complex	Dasypus	[3]	AY631234.1
U1	Uruguai	-	*P. brasiliensis* complex	Penguin (Pygoscelis) droppings	[3]	AY631235.1
Cabbasous	Colômbia	Caldas	*P. brasiliensis* complex	Dasypus	[3]	AY618999.1
T16B1	Brazil	São Paulo	*P. brasiliensis* complex	Dasypus	[5]	JQ675762.1
AC28	Brazil	Acre	*P. brasiliensis* complex	Burrow	This work	OQ456014.1
AC16	Brazil	Acre	*P. brasiliensis* complex	Burrow	This work	OQ456018.1
AC07	Brazil	Acre	*P. brasiliensis* complex	Burrow	This work	OQ456016.1
CB1506	Brazil	Rio de Janeiro	*P. brasiliensis* complex	Dasypus	[6]	MZ233474.1
Nova Iguaçu 6	Brazil	Rio de Janeiro	*P. brasiliensis* complex	Soil	[1]	MT726209.1
Seropedica 6	Brazil	Rio de Janeiro	*P. brasiliensis* complex	Soil	[1]	MT726208.1
SP03	Brazil	São Paulo	*P. brasiliensis* complex	Burrow	[4]	MF078066.1
MT01	Brazil	Mato Grosso	*P. brasiliensis* complex	Soil	[4]	MF078064.1
CB1584	Brazil	Rio de Janeiro	*P. brasiliensis* complex	Dasypus	[6]	MZ233472.1

Clone/Strain	Country	State	Species	Source	Reference	Accession Number
CB1554	Brazil	Minas Gerais	*P. brasiliensis* complex	*Cuniculus paca*	[6]	MZ233471.1
CB1550	Brazil	Minas Gerais	*P. brasiliensis* complex	*Cerdocyon thous*	[6]	MZ233470.1
CB1504	Brazil	Minas Gerais	*P. brasiliensis* complex	Dasypus	[6]	MZ233473.1
CBRJ11659	Brazil	Rio de Janeiro	*P. brasiliensis* complex	*Cerdocyon thous*	[6]	MZ233476.1
CB1547	Brazil	Rio de Janeiro	*P. brasiliensis* complex	Dasypus	[6]	MZ233475.1
ARA1-2	Brazil	Tocantins	*P. brasiliensis* complex	Burrow	[7]	MZ424096.1
AC04	Brazil	Acre	*P. brasiliensis* complex	Burrow	This work	OQ456017.1
T5 -2	Brazil	São Paulo	*P. brasiliensis* complex	Burrow	[5]	JQ669673.1
T4-2	Brazil	São Paulo	*P. brasiliensis* complex	Burrow	[5]	JQ669672.1
B8-2	Brazil	São Paulo	*P. brasiliensis* complex	Burrow	[5]	JQ669671.1
B3-2	Brazil	São Paulo	*P. brasiliensis* complex	Burrow	[5]	JQ669670.1
B3-1	Brazil	São Paulo	*P. brasiliensis* complex	Burrow	[5]	JQ669669.1
B2-2	Brazil	São Paulo	*P. brasiliensis* complex	Burrow	[5]	JQ669668.1
B2-1	Brazil	São Paulo	*P. brasiliensis* complex	Burrow	[5]	JQ669667.1
GO7	Brazil	Goiás	*P. lutzii*	Burrow	[2]	KP636440.1
GO13	Brazil	Goiás	*P. lutzii*	Burrow	[2]	KP636442.1
GO31	Brazil	Goiás	*P. lutzii*	Burrow	[2]	KP636445.1
GO34A	Brazil	Goiás	*P. lutzii*	Burrow	[2]	KP636446.1
GO34B	Brazil	Goiás	*P. lutzii*	Burrow	[2]	KP636447.1
MG1	Brazil	Minas Gerais	*P. lutzii*	Aerosol	[2]	KP636452.1
MG5	Brazil	Minas Gerais	*P. lutzii*	Aerosol	[2]	KP636453.1
MG8	Brazil	Minas Gerais	*P. lutzii*	Aerosol	[2]	KP636454.1

Clone/Strain	Country	State	Species	Source	Reference	Accession Number
MG11	Brazil	Minas Gerais	*P. lutzii*	Aerosol	[2]	KP636455.1
MG12	Brazil	Minas Gerais	*P. lutzii*	Aerosol	[2]	KP636456.1
MG13	Brazil	Minas Gerais	*P. lutzii*	Aerosol	[2]	KP636457.1
MG14	Brazil	Minas Gerais	*P. lutzii*	Aerosol	[2]	KP636458.1
MG15	Brazil	Minas Gerais	*P. lutzii*	Aerosol	[2]	KP636459.1
RO1	Brazil	Rondônia	*P. lutzii*	Burrow	[2]	KP636461.1
RO2	Brazil	Rondônia	*P. lutzii*	Burrow	[2]	KP636462.1
RO3	Brazil	Rondônia	*P. lutzii*	Burrow	[2]	KP636463.1
RO4	Brazil	Rondônia	*P. lutzii*	Burrow	[2]	KP636464.1
RO5	Brazil	Rondônia	*P. lutzii*	Burrow	[2]	KP636465.1
RO6	Brazil	Rondônia	*P. lutzii*	Burrow	[2]	KP636466.1
RO7	Brazil	Rondônia	*P. lutzii*	Burrow	[2]	KP636467.1
RO8	Brazil	Rondônia	*P. lutzii*	Burrow	[2]	KP636468.1
RO9	Brazil	Rondônia	*P. lutzii*	Burrow	[2]	KP636469.1
RO10	Brazil	Rondônia	*P. lutzii*	Burrow	[2]	KP636470.1
RO13	Brazil	Rondônia	*P. lutzii*	Burrow	[2]	KP636473.1
RO14	Brazil	Rondônia	*P. lutzii*	Burrow	[2]	KP636474.1
GO25	Brazil	Goiás	*P. lutzii*	Burrow	[2]	KP636444.1
MG16	Brazil	Minas Gerais	*P. lutzii*	Aerosol	[2]	KP636460.1
Pb01	Brazil	Goiás	*P. lutzii*	Chronic PCM	[8]	XR_001551846.1
GO10	Brazil	Goiás	*P. brasiliensis* complex	Burrow	[2]	KP636441.1
SP07	Brazil	São Paulo	*P. lutzii*	Burrow	[4]	MF078069.1

Clone/Strain	Country	State	Species	Source	Reference	Accession Number
SP06	Brazil	São Paulo	*P. lutzii*	Soil	[4]	MF078073.1
MT08	Brazil	Mato Grosso	*P. lutzii*	Burrow	[4]	MF078070.1
RO12	Brazil	Rondônia	*P. lutzii*	Burrow	[2]	KP636472.1
Pb43	Brazil	Rio Grande do Sul	*P. brasiliensis* complex	Soil	[9]	MN271902.2
Pb22	Brazil	Rio Grande do Sul	*P. brasiliensis* complex	Soil	[9]	MN271901.1
Pb19	Brazil	Rio Grande do Sul	*P. brasiliensis* complex	Soil	[9]	MN271900.1
Pb104	Brazil	Rio Grande do Sul	*P. brasiliensis* complex	Soil	[9]	MN271899.2
B92-932	EUA	Florida	*P. brasiliensis* complex	Dolphin	[10]	NR_177553.1
Hubbs 923	EUA	Florida	*P. brasiliensis* complex	Dolphin	[10]	MW566084.1
Hubbs 738	EUA	Florida	*P. brasiliensis* complex	Dolphin	[10]	MW566083.1
Hubbs 938	EUA	Florida	*P. brasiliensis* complex	Dolphin	[10]	MW566082.1
Hubbs 875	EUA	Florida	*P. brasiliensis* complex	Dolphin	[10]	MW566081.1
Pb227	Espanha	Valência	*P. brasiliensis* complex	Dolphin	[11]	HQ413323.1
Pb dog	Brazil	Paraná	*P. brasiliensis* complex	Dog	[12]	JF289270.1
GO1	Brazil	Goiás	*P. lutzii*	Burrow	[2]	KP636439.1
GO2D	Brazil	Goiás	*P. brasiliensis* complex	Aerosol	[2]	KP636449.1
RO11	Brazil	Rondônia	*P. lutzii*	Burrow	[2]	KP636471.1
GO19	Brazil	Goiás	*P. brasiliensis* complex	Burrow	[2]	KP636443.1

Clone/Strain	Country	State	Species	Source	Reference	Accession Number
L. loboi	EUA	Texas	*Lacazia loboi*	Dolphin	[13]	AF035675.1

Reference

1. Macedo PM, Costa BSS, Paes RA, Trilles, L.; Oliveira RMZ, Valle ACF, Wanke B (2020) *Paracoccidioides brasiliensis* habitat: far beyond armadillo burrows?. Mem. Inst. Oswaldo Cruz 115 (1): 1-3. https://doi.org/10.1590/0074-02760200208

2. Arantes TD, Theodoro RC, Teixeira MM, Bosco SDMG, Bagagli E (2016) Environmental mapping of *Paracoccidioides* spp. in Brazil reveals new clues into genetic diversity, biogeography and wild host association. PLoS Negl Trop Dis 10 (4): 1-18. https://doi.org/10.1371/journal.pntd.0004606

3. Matute DR, Mcewen JG, Puccia R, Montes BA, Blas GS, Bagagli E, Rauscher JT, Restrepo A, Morais F, Vega GN, Taylor JW (2006) Cryptic speciation and recombination in the fungus *Paracoccidioides brasiliensis* as revealed by gene genealogies. Mol. Biol. Evol 23 (1): 65-73. https://doi.org/10.1093/molbev/msj008

4. Hrycyk MF, Garces HG, Bosco SMG. Oliveira SL, Marques AS, Bagagli E (2018) Ecology of *Paracoccidioides brasiliensis*, *P. lutzii* and related species: infection in armadillos, soil occurrence and mycological aspects. Med. Mycol. J 56 (8): 950-962. https://doi.org/10.1093/mmy/myx142

5. Arantes TD, Theodoro RC, Macoris SAG, Bagagli E (2013) Detection of *Paracoccidioides* spp. in environmental aerosol samples. Med. Mycol. J 51 (1): 83-92. https://doi.org/10.3109/13693786.2012.698444

6. Costa BSS, Silva FA, Wanke B, Weksler M, Moratelli R, Valle ACF, Oliveira RMZ, Paes RA, Bueno C, Macedo PM (2021) Molecular eco-epidemiology of *Paracoccidioides brasiliensis* in road-killed mammals reveals Cerdocyon thous and Cuniculus paca as new hosts harboring this fungal pathogen. Plos one 16 (8): 1-11. https://doi.org/10.1371/journal.pone.0256668

7. Teixeira MM, Cattana ME, Matute DR, Muñoz JF, Arechavala A, Isbell K, Schipper R, Santioso G, Tracogna F, Sosa MA, Cech N, Alvarado P, Barreto L, Chacón Y, Ortellado J, Lima CM, Chango R, Vega GN, Shikanai-Yasuda MA, Felipe MSS, Giusiano G (2020) Genomic diversity of the human pathogen *Paracoccidioides* across the South American continent. Fungal Genet. Biol 140 (1): 1-28. https://doi.org/10.1016/j.fgb.2020.103395

8. Teixeira MM, Theodoro RC, Carvalho MJ, Fernandes L, Paes HC, Hahn RC, Mendoza L., Bagagli E, Blas GS, Felipe MSS (2009) Phylogenetic analysis reveals a high level of speciation in the *Paracoccidioides* genus. Mol. Phylogenet. Evol 52 (2): 273-283. https://doi.org/10.1016/j.ympev.2009.04.005

9. Mendes JF, Poester VR, Groll AV, Meireles MCA, Xavier MO (2020) Molecular detection of *Paracoccidioides* in soil from an urban area of southern Brazil. Rev. Soc. Bras. Med. Trop 53 (1): 1-4. https://doi.org/10.1590/0037-8682-0172-2019

10. Vilela R, Bossart GD, Leger JAS, Dalton LM, Reif JS, Schaefer AM Mccarthy PJ, Fair PA, Mendoza L (2016) Cutaneous granulomas in dolphins caused by novel uncultivated *Paracoccidioides brasiliensis*. Emerg Infect Dis 22 (12): 1-7.

https://doi.org/10.3201/eid2212.160860

11. Esperón F, Parraga DG, Bellieri EN, Vizcaino JMS (2012) Molecular diagnosis of lobomycosis-like disease in a bottlenose dolphin in captivity. Med. Mycol. J 50 (1): 106-109. https://doi.org/10.3109/13693786.2011.594100

12. Farias MR, Condas LAZ, Ribeiro MG, Bosco SMG, Muro MD, Wener J (2011) Theodoro, R. C.; Bagagli, E.; Marques, S. A.; Franco, M. Paracoccidioidomycosis in a dog: case report of generalized lymphadenomegaly. Mycopathologia 172(1):147-152. https://doi.org/10.1007/s11046-011-9412-z

13. Haubold EM, Aronson JF, Cowan DF, Mcginnis MR, Cooper CR (1998) Isolation of fungal rDNA from bottlenose dolphin skin infected with *Loboa loboi*. Med. Mycol. J 36 (5): 263-267. https://doi.org/10.1080/02681219880000421