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CASE REPORT

An atypical case of chronic paracoccidioidomycosis in a dog caused by a fungus from the *Paracoccidioides brasiliensis* complex

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Abstract

Paracoccidioidomycosis (PCM) is a systemic mycosis endemic in Latin American countries and one of the most important fungal diseases regarding incidence and mortality in humans. PCM has also been described in some animal species such as dogs. In this study we describe a new case of PCM disease in a dog that differed from previous records in the literature which includes a progressive evolution of fungal dermatitis causing a deforming lesion in the nose, like those found in human patients, and humoral response against gp70 instead of gp43, the major diagnostic antigen for human PCM. The clinical isolate through the ITS and partial gp43 gene phylogenetic analysis was grouped in the *Paracoccidioides brasiliensis* complex. This case describes several features which may contribute to improving diagnosis and understanding of canine paracoccidioidomycosis.

Keywords Veterinary · Diagnosis · Mycosis · LAMP · Serology · Animals

Background

Paracoccidioidomycosis (PCM) is a systemic mycosis caused by species of the *Paracoccidioides brasiliensis* complex and *Paracoccidioides lutzii*. The elevation of different cryptic groups of this complex to species level has been proposed, but still under debate. Endemic in Latin American countries, PCM is associated with different clinical signs in humans including lymphadenitis, dermatitis, and hepatosplenomegaly. However, most infected individuals will not develop the disease. Serological evidence of PCM infection in domestic and wild animals has been reported by our group in several species including mammals, birds, and fish (de Souza Suguiura et al. 2020; Ferreira et al. 2013; Oliveira et al. 2011; Petroni et al. 2017).

The occurrence of PCM disease in animals is much less frequent than in humans with few reports in the literature, including dogs, a cat, a monkey, and a sloth (de Farias et al. 2011; Gonzalez et al. 2010; Headley et al. 2017; Johnson and Lang 1977; Ricci et al. 2004; Trejo-Chávez et al. 2011). The clinical signs in dogs include emaciation, lymphadenomegaly, and dermatopathy (de Farias et al. 2011; Headley et al. 2017; Ricci et al. 2004). In the present study we describe a new case of canine PCM, the first showing progressive

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deforming lesions in the nose diagnosed through molecular methods, histology and serology.

Case presentation

In October of 2021 a mixed-breed, intact, 4-year-old male dog was presented to a private veterinary service with emaciation and a chronic wound in the nose with purulent secretion with approximately one year of evolution (Fig. 1a). The dog was brought from a farm at 8-months-old and since then had been living in a periurban area in Northern Paraná, Brazil. Neither the owners nor other pets, another dog and two cats, in the household had any apparent sign of disease.

At the appointment, the dog showed generalized lymphadenomegaly, ulcerative lesions in both nostrils, crusted skin lesions on the muzzle, and enlargement of the right carpal joint. The dog resented the palpation of the lymph nodes and the affected joint. An empirical treatment was prescribed composed of cephalexin 30 mg/kg/BID for 7 days and itraconazole 10 mg/kg/SID for 30 days.

In February 2022, due to the non-remission of the nose wound but with the regression of the submandibular lymphadenomegaly the dog returned for biopsy and laboratorial examination. Hematological findings showed hyperproteinemia, and mild, regenerative, and microcytic hypochromic anemia. Nose skin samples for cytology were collected but the result was inconclusive showing only neutrophils and sparse macrophages, then an upper nostril fragment was collected for histopathology in early March 2022. The differential diagnosis included carcinoma and leishmaniasis. The skin samples were processed according to traditional histology techniques, and 5 µm thick sections were stained with hematoxylin and eosin (HE), and periodic acid-schiff (PAS).

The histological evaluation of the biopsy samples of glabrous skin of the nose with HE demonstrated a severe, multifocal to coalescing, pyogranulomatous dermatitis within the superficial and deep dermis disorganizing dermal collagen (Fig. 2b and c). Special staining with PAS revealed numerous budding, intracytoplasmic (within macrophages) and extracellular yeast cells. These organisms were round to oval in shape with pronounced variation in size (between 2 and 15 µm in diameter). Budding organisms consisted of two or more yeast cells arranged in chains. Some presented the classical ‘‘mariner steering-wheel’’ morphology, formed by a mother cell surrounded by several peripheral daughter yeasts, which were suggestive of *Paracoccidioides* spp. organisms (Fig. 2d).

In late March 2022, serum, nasal swabs, and aspirates of the submandibular and popliteal lymph nodes were collected for immunological, molecular and mycological analyses.

The sample from the fine needle aspirate obtained from the enlarged popliteal lymph node, and nasal swab was plated in Sabouraud Dextrose Agar and BHI agar with chloramphenicol, and then incubated at 35° C for 30 days. Plates were observed weekly for colony growth. DNA was extracted from nasal swabs and lymph nodes aspirate using traditional phenol-chloroform extraction protocol and used as template for molecular analysis such as LAMP (Kanegae et al. 2021), Nested-PCR for ITS1-5.8 S-ITS2 amplification (Theodoro et al. 2005), and PCR for partial gp43 gene amplification, using the same set of primers F3 and B3 as described by Endo et al. (2004). Amplicons from ITS and gp43 reactions were purified by ammonium acetate and sequenced by Sanger method. The sequencing analysis was performed using the BioEdit program, and the sequence of nucleotides obtained was compared with the National Center database for Biotechnology Information (www.ncbi.nlm.nih.gov) searched through BLAST (http://blast.ncbi.nlm.nih.gov/). Phylogenetic analyses were performed on MEGA-X (ver. 10.1) using the sequences for the ITS1-5.8 S-ITS2 region of the rRNA, and partial gp43, of previously reported sequences obtained on GenBank (Supplemental Table S1) using the parameters as described by de Souza Suguiura et al. (2020). Serology investigation was performed double immunodiffusion (DID), ELISA, and Western blot with cell-free antigens obtained from the Pb18 isolate, and recombinant gp43 (g43△NT) (Camargo et al. 1991; Petroni et al. 2017).

No fungal colony was obtained with morphological characteristics of *Paracoccidioides* spp. from the nasal swabs or popliteal lymph node aspirate.

The dog serum was negative in DID and showed reactivity only against CFA from *P. brasiliensis* (Pb18) in the ELISA. In the immunoblotting analysis it reacted solely against the gp70 and it did not react to gp43 or any other antigen from Pb18 CFA or g43△NT.

A ladder-like electrophoretic pattern in the LAMP amplification product was observed from extracted samples of the popliteal lymph node aspirate and nasal swab (Fig. 2b). The same samples were positive in Nested-PCR being amplified by the ITS E-R primers. The amplicon obtained from the popliteal lymph was submitted to the Genbank under the ON746176 access number with 100% similarity to other sequences of *Paracoccidioides* spp. (KJ540971, KX774406, MN519724, KT155978). The same samples positive in LAMP and Nested-PCR were also positive in traditional gp43 PCR. The sequence obtained from this study was grouped in the *P. brasiliensis* complex and differed from *P. lutzii* isolates based on the analysis of the ITS 1–2 region (Fig. 2a). In the partial gp43 analysis the obtained sequence was grouped in the same cluster of *P. brasiliensis sensu stricto* and *P. restrepiensis* and it was submitted to the Genbank under the PP475427 access number (Fig. 2c).

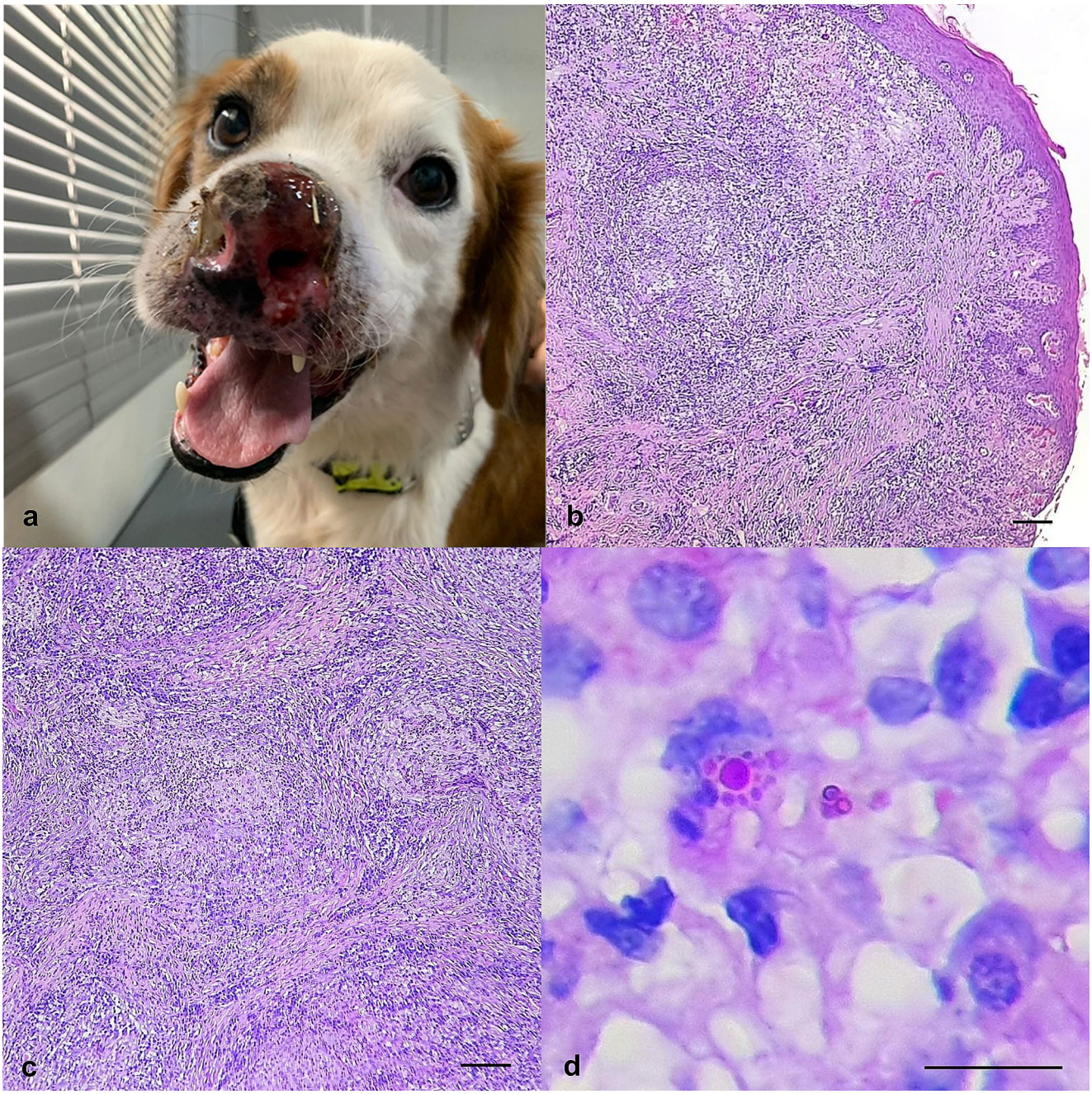

Fig. 1 Gross and histologic aspect of the nose. **a** Aspect of the dog lesions when presented in October 2021, mild nasal discharge of serosanguinous/purulent exudate. **b** Pyogranulomatous inflammation within the superficial and deep dermis accompanied by ulceration, mild epidermal hyperplasia and parakeratotic hyperkeratosis, scale bar corresponds to 100 µm. **c** Suppurative granulomas disorganizing dermal collagen, scale bar corresponds to 75 µm. **d** Periodic Acid-Schiff special stain demonstrating evidence of the classical "mariner steering-wheel" morphology of *Paracoccidioides* spp, scale bar corresponds to 10 µm

Discussion and conclusions

To the best of our knowledge, there are just three reports in the literature of natural PCM disease in dogs, until now all of them have been observed in large size females (Table 1) and this study is the first reported case of PCM in a male dog. In contrast to the other reported cases of PCM in this species, the present case showed a chronic evolution of cutaneous granulomatous lesions which resembles the chronic form observed in human patients.

The geographical area of this dog represents the third ranked in cumulative incidence of human cases of PCM in Paraná, and the same State as the second and third reported

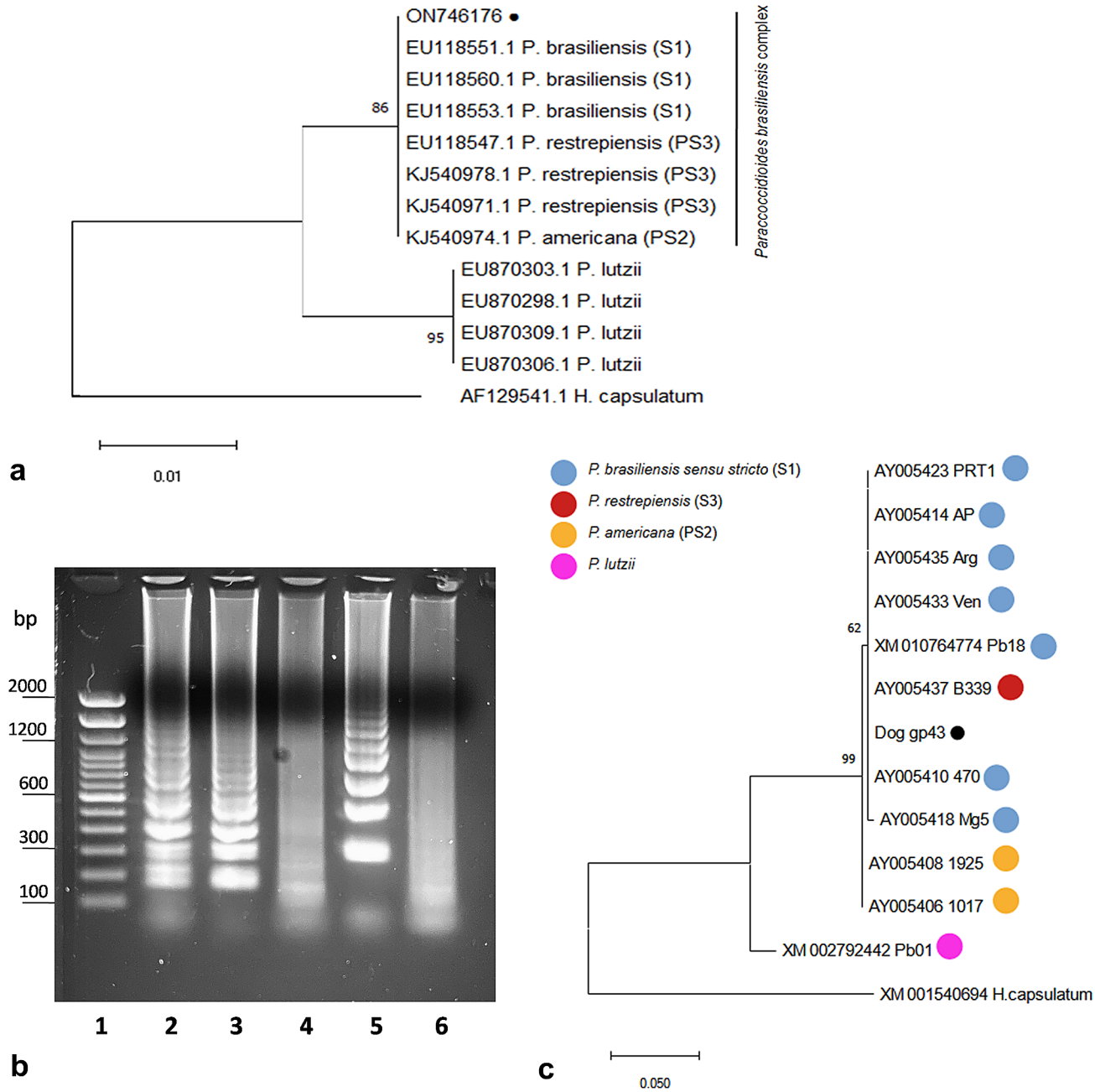

Fig. 2 Molecular investigation of the clinical specimens obtained from the dog. **a** Phylogenetic tree of ITS regions of several sequences obtained from GenBank (accession numbers are given) and the sequence amplified by Nested-PCR from the popliteal lymph node from this study (black dot), the ITS sequences derived from this study were grouped within the species of the *Paracoccidioides brasiliensis* complex. **b** LAMP using the set of primers F3, B3, FIP and BIP for partial gp43 region amplification in 2% agarose gel. Lanes: (1) DNA ladder 100 bp, (2) nasal swab, (3) popliteal lymph node aspirate, (4) submandibular lymph node aspirate, (5) positive control (Pb18), and (6) negative control. **c** Phylogenetic tree of partial gp43 amplification, the dog sequence (black dot) was grouped in the same cluster of *P.brasiliensis sensu stricto* and *P. restrepiensis*. The trees were generated by the Jukes Cantor method and analyzed by the maximum likelihood method. The bootstrap values from 1000 resampling are indicated along the branches. Scale bar indicates nucleotide substitutions per site

Table 1 Peer reviewed cases of canine paracoccidioidomycosis in the literature regarding, origin, patient characterization, diagnosis and treatment

State	Sex	Breed	Clinical signs	Diagnosis method	Antifungal therapy	Resolution	Reference
São Paulo	Female	Dobermann pinscher	Apathy, lymphadenomegaly	Histopathology, Immunohistochemistry, PCR	Ketoconazole	Euthanisia after 18 months by clinical recurrence	Ricci et al. 2004
Paraná	Female	Dobermann pinscher	Emaciation, lymphadenomegaly, and hepatosplenomegaly	Cytology, immunohistochemistry, isolation	Itraconazole (10 mg/kg/SID/PO)	Remission of signs and no clinical recurrence after 24 months	de Farias 2011
Paraná	Female	Labrador retriever	Apathy, hyperthermia, lymphadenomegaly	Cytology, isolation, PCR	Itraconazole (10 mg/kg/BID/PO)	Remission of signs and no clinical recurrence after 18 months	Headley et al. 2017
Paraná	Male	Mixed-breed	Nasal discharge, dermatitis, lymphadenomegaly	Histopathology, ELISA, immunodifusion, PCR, LAMP	Itraconazole (10 mg/kg/BID/PO)	Remission of signs and no clinical recurrence after 24 months, nasal obstruction	Present study

canine cases in the literature (de Farias et al. 2011; Headley et al. 2017; Suguiura and Ono 2022). PCM may be easily diagnosed by cytology, histology and its inclusion as a differential diagnosis in dogs showing emaciation, apathy, lymphadenomegaly, and non-healing wounds must be considered by veterinary clinicians in PCM endemic areas.

Regarding serology, the dog sample was negative in DID. Immunodiffusion has been used in PCM serosurveys in dogs followed by other immunoassays such as ELISA and immunoblotting (Eisele et al. 2004; Ono et al. 2001; Petroni et al. 2017). Due to the period from the onset of clinical signs until the serum collection, a positive DID was expected because it is known that dogs show positivity in this test from the 20th day post infection, together with rise in IgG levels against *Paracoccidioides* spp. (Eisele et al. 2004). Moreover, negative results in immunodiffusion are expected to occur at a rate of approximately 10% as described in studies with human patients microbiologically proved for PCM (Do Valle et al. 2001). The reactivity against gp70 instead of gp43 as observed in western blot is a possible explanation for the negative DID result because crude *Paracoccidioides* spp. antigen preparations, such as CFA, contain higher concentrations of gp43 than gp70.

The dog was treated for almost 30 days with itraconazole, three months before the collection of samples for mycological isolation, a possible reason for the failure in *Paracoccidioides* sp. Isolation by culture. Moreover, the brief treatment cycle did not impair the diagnosis by molecular methods.

LAMP has been proven to be an interesting technique in PCM diagnosis in humans and animals due to its speed, sensitivity and specificity (Endo et al. 2004; Kanegae et al. 2021). A disadvantage of the method is the lack of downstream applications such as sequencing to identify the *Paracoccidioides* species.

The clinical specimen from this study was grouped in the *Paracoccidioides* complex cluster differentiating it from *P. lutzii*. In previous reports of PCM in dogs the identification of *Paracoccidioides* spp. as proposed by Turissini et al. (2017) was carried out unevenly in which only the second recorded case had their isolate identified as *P. americana* (former cryptic species PS2) (Turissini et al. 2017). In our study using the partial gp43 gene amplification it was not possible to differentiate the species of the fungi because the sample was grouped in the same cluster as *P. brasiliensis sensu stricto* and *P. restrepiensis*. Despite the new proposed classification for *Paracoccidioides* spp. little is known about its practical implication in the diagnosis, treatment and prognosis of PCM.

In brief, we described a new case of PCM disease in dogs in an endemic area for human PCM. This new case showed some unique features not observed in the previously reported cases such as the chronicity of the disease, serological response of the dog, the employment of LAMP in the diagnosis. Despite being traditionally employed for definitive PCM diagnosis, the isolation of the fungi may lack in sensitivity as observed in this case after the treatment with antifungal azoles, but other assays such as LAMP offer a fast and reliable result even in the absence of viable cells. The early detection of the disease is very important to avoid further sequelae therefore PCM should be considered as a differential diagnosis of disease with mononuclear phagocytic system involvement and chronic wounds in dogs from endemic regions for the mycosis.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s11259-024-10413-0.

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Author contributions I.M.S.S. wrote the manuscript and contributed to mycological and molecular biological studies. F.N. and A.P.F.L.B. contributed to the histopathological analysis and diagnosis. G.G.C.I., R.M. and A.S. contributed to molecular biological studies. B.A.S. and M.L.S.S. contributed to clinical management and patient follow-up. M.A.O. and E.N.I. contributed to manuscript revision and data curation.

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Data availability Sequence data that support the findings of this study have been deposited in the GenBank database with the accession codes ON746176 and PP475427.

Declarations

Competing interests The authors declare no competing interests.

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