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CLINICAL MICROBIOLOGY - RESEARCH PAPER

Molecular characterization of clinical and environmental isolates from the *Cryptococcus neoformans/C. Gattii* species complexes of Maceió, Alagoas, Brazil

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Abstract

Cryptococcosis is one of the major life-threatening opportunistic/systemic fungal diseases of worldwide occurrence, which can be asymptomatic or establish pneumonia and meningoencephalitis mainly in immunosuppressed patients, caused by the *Cryptococcus neoformans* and *C. gattii* species complexes. Acquisition is by inhaling fungal propagules from avian droppings, tree hollows and decaying wood, and the association of the molecular types with geographic origin, virulence and antifungal resistance have epidemiological importance. Since data on cryptococcosis in Alagoas are limited, we sought to determine the molecular types of etiological agents collected from clinical and environmental sources. We evaluated 21 isolates previously collected from cerebrospinal fluid and from environment sources (pigeon droppings and tree hollows) in Maceió-Alagoas (Brazil). Restriction fragment length polymorphism of *URA5* gene was performed to characterize among the eight standard molecular types (VNI-VNIV and VGI-VGIV). Among isolates, 66.67% (14) were assigned to *C. neoformans* VNI – 12 of them (12/14) recovered from liquor and 2 from a tree hollow (2/14). One isolate from pigeon droppings (4.76%) corresponded to *C. neoformans* VNIV, while five strains from tree hollows and one from pigeon droppings (6, 28.57%) to *C. gattii* VGII. VNI-type was present in clinical and environmental samples and most *C. neoformans* infections were observed in HIV-positive patients, while types VNIV and VGII were prevalent in environmental sources in Alagoas. This is the first molecular characterization of *Cryptococcus* spp. in Alagoas, our study provides additional information on the ecoepidemiology of *Cryptococcus* spp. in Brazil, contributing to a closer view of the endemic species.

Keywords *Cryptococcus neoformans* · *Cryptococcus gattii* · Cryptococcosis · Molecular typing · RFLP

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Introduction

Cryptococcus neoformans and *C. gattii* form two species complexes that are etiological agents of cryptococcosis, a systemic disease acquired through the inhalation of the fungal cells – desiccated blastoconidia or basidiospores – dispersed in the environment [1]. After inhalation, the infection develops into an initial dormant state and may further progress into the most common symptom, meningoencephalitis, with other manifestations such as pneumonia and lesions in different tissues due to its systemic nature [2–4].

The infection with *C. neoformans* has major significance for causing cryptococcosis in immunosuppressed individuals, with estimates of 152,000 cases of cryptococcal meningitis and 112,000 worldwide annual deaths in 2020, responsible for 19% of death in patients with HIV/AIDS

[5], while infections caused by *C. gattii* affect immunocompetent patients [6], primarily children and the elderly, showing a primary infection trait and pathogenic aptitude [1, 7]. Recently, the World Health Organization (WHO) (2022) included *C. neoformans* in the critical group and *C. gattii* in the medium group of the Fungal Priority Pathogens List [8], to direct research and raise awareness of this fungal disease, strengthening new actions to control these pathogens.

The two species complexes are subdivided into eight major molecular types, proposed in 2009 by the International Society for Human and Animal Mycology (ISHAM) working group [9], recognizable by different molecular techniques, such as PCR-fingerprinting, restriction fragment length polymorphism (RFLP) of the *URA5* gene, amplified fragment length polymorphism (AFLP), multi-locus sequence typing (MLST) and whole genome sequencing (WGS), subdividing *C. neoformans* in VNI, VNII, VNIII and VNIV and *C. gattii* in VGI, VGII, VGIII and VGIV [9–11]. One of the most applied techniques is the PCR-RFLP of *URA5*, a low-cost methodology which benefits from the nucleotide sequence of *URA5* in *C. neoformans* and in *C. gattii* differing in only about 8%, showing identical size and introns position, and having the product (orotidine monophosphate pyrophosphorylase) structure with homology in 98% of the amino acids, demonstrating a recent phylogenetic relation between these species [12, 13].

Recently, 3 other molecular types have been identified (VNB, VGV and VGVI) [14–18], however these demonstrate low distribution and a smaller frequency compared to the 8 stablished genotypes [11]. Additionally, a nomenclature change has been proposed by Hagen et al. (2015) [17], moving the molecular types into species level: *C. neoformans* (VNI, VNII), *C. neoformans* X *C. deneoformans* hybrid (VNIII), *C. deneoformans* (VNIV), *C. gattii* (VGI), *C. deuterogattii* (VGII), *C. bacillisporus* (VGIII), *C. tetragattii* (VGIV) and *C. decagattii* (VGIV/VGIIIc). In face of this proposal, Kwon-Chung et al. (2017) [19] suggested the use of "species complexes", reasoned by the new molecular types being discovered and the lack of biological differences among the clades, and later Hagen et al. (2017) [20] defended their perspective, however there is still no consensus among researchers about this matter, thus we decided to use "species complexes" in this study following recent literature [11, 21].

C. neoformans sensu lato occurs in all continents and is the most common pathogen of cryptococcosis in all regions of Brazil [22, 23]. It is usually found on avian droppings (mainly pigeon) and decaying organic material [1, 24]. On the other hand, *C. gattii sensu lato* occurs mainly in tropical and subtropical regions of the world [1], it is endemic in the northern and northeastern regions of Brazil [22], usually colonizing decaying wood material and tree hollows from different species [4]. Limited data on cryptococcosis is still a reality in Latin America [11, 24] and the ecoepidemiological data in Brazil are restricted to a few regions, lacking information on the correlation of the environmental and clinical isolates, with some states without any molecular characterization to date [22, 24–26]. Our purpose was to determine for the first time the molecular types of clinical and environmental isolates collected in Maceió (Alagoas, Brazil) by *URA5*-RFLP, aiming to update the ecoepidemiological distribution of *C. neoformans/C. gattii* species complexes in Brazil.

Materials and methods

Fungal isolates

This study evaluated 21 isolates collected from 2013 to 2016, previously identified by the Clinical Microbiology Laboratory (LMC), Federal University of Alagoas (UFAL), as *Cryptococcus* sp. through phenotypic methods (india ink staining followed by urease and phenoloxidase tests), these isolates were stocked at the mycological collection of the LMC (SisGen access nº. A180447/A329989). Fourteen clinical isolates were collected from the cerebrospinal fluid (CSF) of patients with suspected cryptococcal meningoencephalitis assisted at Hospital Escola Doutor Hélvio Auto (HEHA), besides seven environmental isolates collected from decaying material in a tree hollow and pigeon droppings located in public squares from Maceió (Alagoas, Brazil).

Standard strains of each molecular type provided by Instituto Nacional de Infectologia Evandro Chagas – Fundação Oswaldo Cruz (INI-Fiocruz) were utilized: *C. neoformans* WM 148 (VNI), WM 626 (VNII), WM 628 (VNIII), WM 629 (VNIV) and *C. gattii* WM 179 (VGI), WM 178 (VGII), WM 175 (VGIII), WM 779 (VGIV).

Isolate preparation

Collection of the isolates were maintained stocked at -20ºC after saturated cultures were grown in Sabouraud dextrose broth, with new stock cultures made every 6 months. For the use, isolates were inoculated in Sabouraud dextrose agar (SDA) for the period of 48 h at 30ºC, then approximately 10 µL aliquot of cells were harvested by an inoculation loop and inserted in 1.5 mL sterilized microtubes, this material was incubated overnight at -20ºC for mechanical breaking of the yeast capsule. Alternatively, the isolates were inoculated in 1 mL of YEPD broth (yeast 1%, peptone 2% and dextrose 1%) with 0.5 M of NaCl, to avoid capsule formation, in 1.5 mL sterilized microtubes and incubated for 48 h

at 30ºC in a shaker at 150 rpm until cultures were saturated. Then, the samples were pelleted by centrifuging in 17,000 x *g* for 2 min to collect the cells.

DNA extraction

Genomic DNA was extracted and purified using Wizard® Genomic DNA Purification Kit (Cat. A1120; Promega, Madison, USA) or following the methodology described by Ferrer et al. (2001) [27] and Rede de Criptococose Brasileira (RCB) [28], with some modifications: 10 µL aliquot of cells was suspended in 500 µL of lysis buffer (0.5% sodium dodecyl sulfate, 1.4% NaCl, 0.73% dihydrate EDTA and Tris-HCl 0.2 M, pH = 8.0) and 5 µL of 2-mercapethanol, followed by incubation at 65ºC for 1 h. Then, 500 µL of phenol: chloroform: isoamyl alcohol (25:24:1) was added into the tubes and mixed thoroughly for 2 min, followed by centrifugation for 15 min at 32,000 x *g*. The supernatant was transferred to new tubes and mixed with an equal volume of isopropanol for incubation at -20ºC overnight, aiming the precipitation of nucleic material. Following, the tubes were centrifuged at 32,000 x *g* (15 min) for pellet formation and removal of the supernatant. The pellet was suspended in 200 µL of 70% ethanol for cleaning and centrifuged at 32,000 x g (15 min), removing the supernatant. The DNA pellet was air dried, resuspended in 100 µL of sterile ultrapure water for treatment with RNase A (Cat. A7973; Promega, Madison, USA) (1 h at 37ºC) and the material was stocked at 4ºC.

Molecular typing by *URA5*-RFLP

URA5 PCR was performed individually as described by Meyer et al. 2003 [29], in a final volume of 50 µL with an aliquot of genomic DNA, 1X PCR buffer (5X Colorless GoTaq® Flexi Buffer, Promega; Cat. M890A), 0.2 mmol of each dNTP (Cat. DNTP100; Sigma-Aldrich, Burlington, USA), 3 mmol of magnesium chloride (Cat. A351B; Promega, Madison, USA), 1.5 U of GoTaq® Flexi DNA polymerase (Cat. M829A; Promega, Madison, USA), 50ng of primers *URA5* (5'-ATGTCCTCCCAAGCCCTCGACTCC G-3') and *SJ01* (5'-TTAAGACCTCTGAACACCGTACT C-3'). The thermocycler (Bio-Rad®, Hercules, USA) was configured to the following cycles: 2 min at 94 °C for initial denaturation, followed by 35 cycles of 45 s at 94 °C for denaturation, 1 min at 61 °C for annealing, 2 min at 72 °C for extension and a final cycle at 72 °C for 10 min for final extension.

The amplified product was stocked at 4 °C for further procedures. Products were visualized on a 1.4% agarose gel with 1X TBE and Nancy-520 (Cat. 01494; Sigma-Aldrich, Burlington, USA) after electrophoresis with a 100 bp DNA ladder (Cat. 15,628,019; Invitrogen, Waltham, USA).

According to Meyer et al. (2003) [29], the *URA5* amplified products were double digested by restriction enzymes *Sau96I* (5 U/µL; Cat. R0165S; New England Biolabs, Massachusetts, USA) and *HhaI* (10 U/µL; Cat. R0139S; New England Biolabs, Massachusetts, USA) in a final volume of 30 µL with 1X NEBuffer (Cat. B7202; New England Biolabs, Massachusetts, USA) for incubation at 37ºC for 3 h. For evaluation, electrophoresis with an agarose gel (3%) stained with Nancy-520 was performed and the genotypes were assigned by comparison with their respective reference strain.

Brazilian ecoepidemiological map

Articles reporting different molecular types of *Cryptococcus neoformans*/*C. gattii* species complexes in Brazil were selected from PubMed and Scielo databases, using the primary descriptors "molecular type" OR "genotype" AND "cryptococcus" AND "Brazil" to select articles published from 2008 to 2024 with information about place, description of sample and molecular type. After data compilation, we constructed a Brazilian map using the QGIS v.3.34.3 software and the Instituto Brasileiro de Geografia e Estatística (IBGE) database, separating *Cryptococcus* molecular types by states and including the types described in Alagoas.

Results

The clinical isolates (n = 12) were collected from cerebrospinal fluid of 9 patients that underwent CSF recollection to monitor the effectiveness of the treatment and remission of the yeast. Out of the 9 patients with cryptococcal meningoencephalitis, 4 were HIV-positive (44.44%), 2 HIV-negative (22.22%) and 3 had no information about seropositivity in their medical records (33.34%). The mortality rate was 75% (3/4) among the HIV positive individuals and 50% (1/2) in the HIV negative. One HIV-negative patient was released from medical assistance and 4 patients had no clinical evolution information registered. The environmental strains (n = 9) were isolated from a tree hollow and from pigeon droppings (Table 1).

DNA extraction was performed by two methodologies, the method described by Ferrer et al. (2001) [27] was effective and allowed the recovery of large amounts of fungal genomic material. However, the usage of the Wizard Kit allowed faster and more purified acquisition of the DNA, being used in most of the samples. *URA5* PCR-RFLP of the 21 *Cryptococcus* spp. clinical and environmental isolates compared with the 8 molecular reference strains revealed 71.43% (15/21) as *C. neoformans sensu lato*, whereas 28.57% (6/21) were *C. gattii sensu lato*. The molecular

Table 1 List of clinical and environmental isolates and their complementary information

Clinical isolates								Environmental isolates				
Isolate	Molecular type	Patient	Gender	Age	HIV	Outcome	Sample source	Local	Isolate	Molecular type	Sample source	Local
1	VNI	1	M	16	-	Death	CSF	HEHA – Maceió, AL.	13	VGII	Tree hollow	Praça São Vicente – Centro, Maceió-AL.
2	VNI								14	VGII		
3	VNI								15	VNI		
4	VNI	2	M	23	+	Death			16	VGII		
5	VNI								17	VGII		
6	VNI	3	M	47	-	Released			18	VNI		
7	VNI	4	F	29	+	Death			19	VGII		
8	VNI	5	F	14	+	Death			20	VNIV	Pigeon droppings	Praça Santa Rita de Cássia– Farol, Maceió-AL.
9	VNI	6	M	44	NA	NA						
10	VNI	7	F	47	+	NA			21	VGII	Pigeon droppings	Praça São Vicente – Centro, Maceió-AL.
11	VNI	8	M	23	NA	NA						
12	VNI	9	M	NA	NA	NA						

M: male; F: female; NA: data not available on their medical records; CSF: cerebrospinal fluid; HEHA: Hospital Escola Dr. Helvio Auto

types characterized in Maceió (Alagoas, Brazil) were VNI (66.67% – 14/21), VGII (28.57% – 6/21) and VNIV (4.76% – 1/21) (Fig. 1). All clinical isolates (12) corresponded to genotype VNI, in both HIV-positive and HIV-negative patients, while the most common molecular type in the 9 environmental isolates were VGII (6/9), followed by VNI (2/9) and VNIV (1/9). It was possible to identify 2 molecular types (VNI and VGII) collected from the same tree hollow, whereas in the pigeon droppings the genotypes identified were VNIV and VGII.

After compiling the public molecular epidemiological data of *Cryptococcus* spp. in Brazil, it was possible to construct an ecoepidemiological map to assess the distribution of the molecular types in different regions (Fig. 2; Table S1). The genotypes VNI and VGII are the most commomly identified among the studies, distributed in all regions of Brazil including the present record in the state of Alagoas, while VNIV is rarely identified.

Discussion

Cryptococci eradication requires a rapid identification and effective treatment for the host survival, but in several countries these measures are not available for the population, making cryptococcal infection one of the most important life-threatening opportunistic diseases for AIDS patients [5]. The molecular types of *Cryptococcus neoformans and C. gattii* species complexes differ in their ecology, geographical distribution, pathogenicity and antifungal susceptibility, even with the phylogenetic proximity and morphological similarities among the groups [20].

In this study, the isolates had origin in clinical and environmental samples from Maceió, Alagoas (northeastern Brazil), indicating the presence of molecular types VNI, VNIV and VGII. Studies about the ecoepidemiology of *Cryptococcus* spp. should take into account both clinical and environmental samples for a better understanding of its geographical distribution, as the infection is disseminated through borne fungal propagules from the environment and is also considered as a zoonosis, indicating that molecular types collected from clinical samples were disseminated in certain regions [26, 30].

The mortality rate observed in our study was 44.44% (4/9). However, the rate was higher among HIV-positive patients (75%; 3/4). Several factors are involved in the disease mortality, such as delay in diagnosis, the lack of effective treatment, virulence of the strains and level of immunosuppression of the patient. In the world, the mortality rate in individuals with cryptococcosis is high, even in places with first-line treatment availability, reaching 40% in developed countries and up to 70% in low-income countries

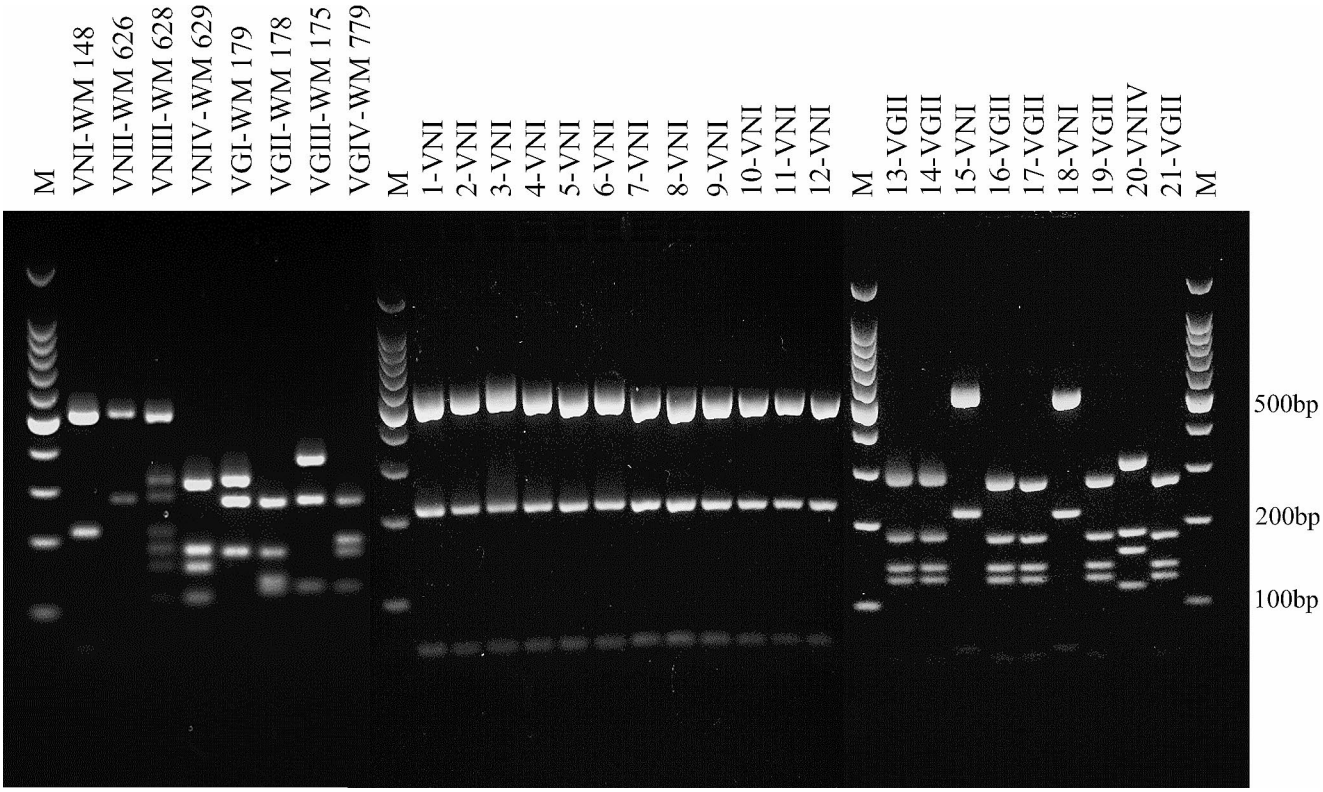

Fig. 1 Characterization of clinical and environmental isolates of *Cryptococcus* sp. by *URA5* PCR-RFLP. Restriction fragment length polymorphism (RFLP) profile of *URA5* gene double-digested with restriction enzymes *Sau96I* and *HhaI*. M: 100 bp DNA ladder (molecular weight); lanes VNI-VGIV: reference strains; lanes 1–12: clinical isolates; lanes 13–21: environmental isolates; bp: base pairs

[31]. In Brazil, mortality rate may vary from 26 to 70%, depending on the evaluated place, treatment availability and patient immunosuppression [32, 33]. Current data show a decrease of 28% of deaths related to AIDS in the world from 2014 to 2020 [5], in accordance with the Brazilian Ministry of Health [34], which has suggested a decrease in individuals living with HIV in Brazil, although an increase occurred in the northern and northeastern regions, where the cryptococcal surveillance is at most scarcity.

Even though clinical isolates were from HIV-infected and HIV-uninfected patients, all of them were characterized as VNI in our study. This molecular type is characterized as the primary infecting agent of cryptococcal meningoencephalitis in individuals with advanced immunosuppression stage due to AIDS [1]. This is observed by Tsujisaki et al. (2013) [35], Favalessa et al. (2014) [36], Aguiar et al. (2017) [32] and da Silva et al. (2020) [33] that also identified VNI as the predominant molecular type in infections of HIV-positive individuals in Mato Grosso do Sul, Mato Grosso, Minas Gerais and São Paulo (Brazil), respectively.

In immunocompetent patients, the infections are usually associated with VGII. Despite that, there are evidences of VNI also infecting these individuals as observed in China, where VNI is frequently associated [37]. These distinctions possibly occur due to virulence and pathogeny differences among strains of *C. neoformans*, expressing genes that guarantee the ability to cause infections in immunocompetent individuals [1]. Recently, there are also reports of VNI causing infections in HIV-uninfected patients in Brazil [25, 36, 38–40], this evidence demonstrates the ability of the molecular type VNI in causing infections to HIV-negative individuals, as well as in 2 patients from Alagoas.

Considering the environmental samples, the predominance of each type may vary according to the niche with which the sampling is made, due to better adaptation that each genotype has to specific habitats [3, 41]. Tree hollows are considered to be predominantly colonized by *C. gattii* [42], but likewise, this niche is supportive for *C. neoformans* to grow [3]. According to Trilles et al. (2003) [43], different molecular types of *Cryptococci* can inhabit a common tree, as observed in a study carried out in Teresina (Piauí, Brazil) and also found in Italy by Cogliati et al. (2020) [4], when they observed the types VNI and VNIV sharing the same tree, but with different populational density in different parts of it. Barbosa et al. (2013) [44] identified VGI and VNI isolates in tree hollows in Rio de Janeiro, while Costa et al. (2009) [45] reported VGII and VNI associated with decaying material from a tree hollow in Belém (Pará, Brazil). This

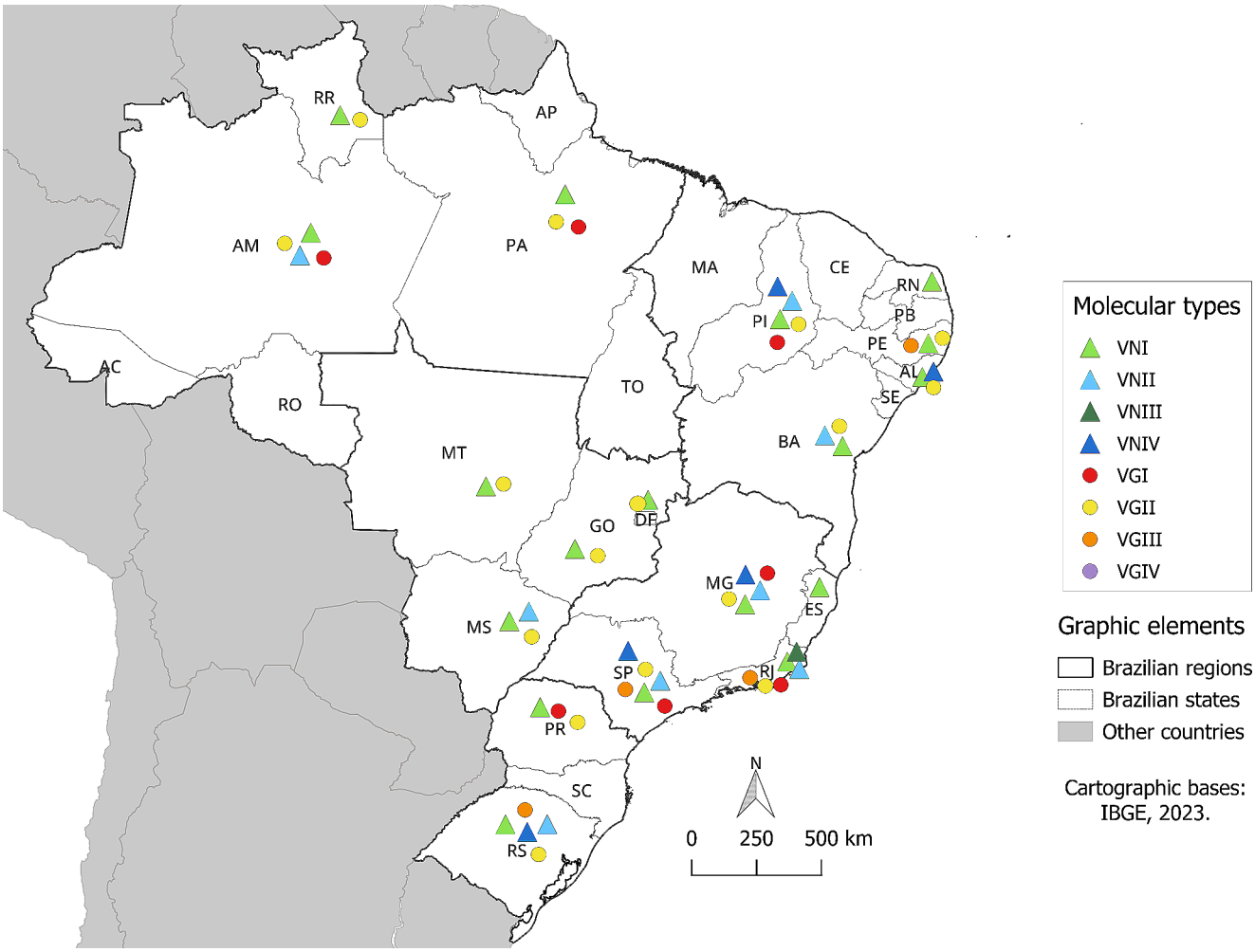

Fig. 2 Map showing the molecular types distribution of *Cryptococcus neoformans* and *C. gattii* species complexes in Brazil. Data described in the literature between 2008 and 2024, with the inclusion of molecular types described for the first time in Alagoas. Molecular types were indicated by triangle and circle shapes with different colors for each type. The QGIS v.3.34.3 software and the Instituto Brasileiro de Geografia e Estatística (IBGE) database were used to construct the map. Abbreviations correspond to each Brazilian state. Reference list: [15, 22, 25, 32, 33, 35, 36, 38–40, 44, 45, 50, 56, 57, 59, 69, 71–116]

pattern was also observed in our study, with five VGII isolates and two VNI identified inhabiting a single tree hollow. An interesting observation is that different molecular types of *C. gattii* have not been detected in association in the same location, such as a tree, which may suggest a stronger competition among VG types.

C. neoformans tends to be the dominant species in pigeon droppings, once it utilizes urea and creatinine from the substrate as nitrogen source [41, 46]. The molecular type VNI is the most common type in pigeon droppings in Brazil [22, 23]. However, in the present study we did not recover any strain from this type in pigeon droppings, only 1 VNIV and 1 VGII isolates. *C. gattii* is unusual in this environment and can grow without adequate adaptation to inhabit this niche for long periods [41]. Despite that, Teodoro et al. (2013) [47] have reported about the recovery of *C. gattii* in 5.2% of the identified species from bird droppings in São Paulo.

There is a possibility that the pigeon droppings that were positive for VGII in our study were contaminated with the strains inhabiting a neighboring tree, approximately 3 m away – from which we also identified the same molecular type. This may be considered due to the fact that *Cryptococcus* spp. has already demonstrated great achievement to disperse to and colonize new habitats [48]. As an example, there is the report of type VGII described as the infecting agent in a cryptococcosis outbreak in Vancouver, Canada [49], demonstrating its ability to adapt to new locations and be dispersed through different means, such as wood export, air and water currents, as well as biological sources (as birds and insects), allowing colonization in areas far from its endemic region [4, 22, 25, 50].

The molecular type VNI occurs worldwide and is the most prevalent in clinical and environmental samples [51], with less frequency only in samples collected from

tree hollows [41], and predominant in cryptococcal infections in Brazil [22–24, 44]. Considering the environmental samples from our study, the VNI prevalence was lower than expected, once this type was not identified on its preferential niche – pigeon droppings.

The molecular type VGII is the most identified group in *C. gattii* infections, distributed primarily in the Americas [23, 52] and considered endemic from the northern and northeastern regions of Brazil [24, 53]. It is collected mainly from decaying organic matter and is associated with primary infections [3]. In our study, VGII was the predominant type among the environmental isolates, identified in a tree hollow and in pigeon droppings, but not on clinical samples. As this type is endemic to the northern and northeastern regions, its prevalence is usually high, as shown by Trilles et al. (2008) [22]. VGII was the most prevalent type (63.3%) in clinical and environmental isolates (n = 107) collected from the states of Roraima, Amazonas, Piauí, Pernambuco and Bahia [22].

Molecular type VNIV exhibited low prevalence in our study, as only one isolate was identified from pigeon droppings, similar to what is described in Brazil [24]. It is distributed primarily in the European countries, probably due to its better adaptation to temperate weather [4], and identified from the environment commonly from pigeon droppings or associated with different tree species [52]. It shows scarce clinical association, being cutaneous cryptococcosis one of the few clinical manifestations related [54]. There are reports of this type in tree hollows from Rio Grande do Sul [55], São Paulo [56], and in a patient from Minas Gerais [57]. Firacative et al. (2021) [23] reported type VNIV in 6.32% of isolates from Latin America and Trilles et al. (2008) [22] in 4.7% of the isolates from the northern and northeastern regions of Brazil.

The most prevalent genotypes demonstrated in this study are the main agents of opportunistic infections in immunosuppressed individuals (VNI) and primary infections in healthy people (VGII) [1]. It has been shown that VGII is more resistant to azoles, as fluconazole and itraconazole, regardless of the original geographic area of the isolate [58, 59]. According to Trilles et al. (2012) [58], VGII was more resistant than VNI to fluconazole, albaconazole, voriconazole, itraconazole, ravuconazole and 5-flucytosine, being amphotericin B the only antifungal that did not show response variation from different molecular types.

In Brazil, cryptococcosis is still a disease that does not require compulsory notification [60], even though there are reports of mortality rate reaching 10.96/million inhabitants in Mato Grosso, in direct cause of death, or 70.41/million inhabitants in Santa Catarina, in associated cause of death [61]. Only a few Brazilian states have measures forcing the inclusion of cryptococcosis among the diseases with epidemiological control. However, the brand-new fungal priority pathogens list released by WHO (2022) [8] demonstrates an increased concern about this infection, thus a gradual expansion in epidemiological information related to infections by *Cryptococcus* spp. is expected, with better surveillance around the world.

Ecological and clinical studies can provide information on the epidemiology of these pathogens and characterization of niches favorable for the colonization of these microorganisms, which represent a risk to the population when exposed to propagules [30]. Prevention of infection is hampered by the invasive and disseminative capacity of these organisms [62], but the chances of exposure can be minimized through the use of personal protective equipment (PPE) for respiratory protection in places with high concentration of avian excreta or wood cutting. In cases of cleaning of contaminated areas, the decontamination and spraying with water or oil of the material should be previously made to avoid aerobic propagules [63].

Here we report VNI, VNIV and VGII as prevalent molecular types in Maceió (Alagoas, Brazil), with VNI identified in clinical and environmental samples, which highlights dissemination points of this genotype in our area. Although VNIV and VGII have not been identified in clinical samples, their presence in the environment is already a determinant for the possibility of infection, since the dispersion of any genotype occurs from environmental sources and dissemination from human to human has not yet been reported [4, 64]. Despite the fact that transmission by organ donation [65] and direct contamination of wounds [66] is rarely reported in humans, there are reports of maternal-fetal transmission in cetaceans [67], demonstrating the great disseminative capacity of these microorganisms, in addition to the fact that *Cryptococcus* sp. manages to infect a wide range of wild and domestic animals, from terrestrial to marine environments [68], making constant exposure to contaminated excreta of avian pets an important mean of zoonotic transmission [69, 70]. Such information leads us to take more cautious measures regarding the possibility of contamination in these situations.

The importance of determining molecular types of the *C. neoformans* and *C. gattii* species complexes goes behind ecoepidemiology, since there are clinically important differences between each evaluated types, and until our research there was no information available about the molecular types of *Cryptococcus* spp. prevalent in the state of Alagoas.

Conclusions

C. neoformans was the prevalent species in both HIV-infected and HIV-uninfected patients with cryptococcal meningoencephalitis from Alagoas, being VNI-type the most frequent, while among the environmental samples *C. gattii* VGII prevailed. The VNI, VNIV and VGII genotypes are adapted to survive in organic matter associated with tree hollows and in pigeon excreta in different urban areas of the capital, evidencing that VNI and VGII genotypes may coexist in the same ecological niche and following the same pattern of prevalence observed in Brazil and other north-eastern states. Evidence of VNI strains in both clinical and environmental samples indicate a potential risk of human infection, mainly to immunosuppressed individuals.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s42770-024-01313-1.

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Authors contributions (CRediT) Douglas Lyra (Investigation; data curation; writing – original draft preparation; writing – review and editing), Denise Wanderlei (Supervision; conceptualization; funding acquisition; methodology; project administration; data curation; writing – review and editing) and Fernanda Maranhão (Supervision; conceptualization; funding acquisition; methodology; project administration; data curation; writing – review and editing).

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Data availability All data generated or analyzed during this study are included in this published article and its supplementary files.

Declarations

Ethics approval and consent to participate This study was approved by the Research Ethics Committee (number 19035713.8.0000.5013) in accordance with the resolution 466/2012 of the National Council of Health from Brazil. Informed consent was obtained from all individuals participants in this study.

Conflict of interest The authors declare that they have no conflict of interests to this work.

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