First isolation of *Cryptococcus gattii* molecular type VGII and *Cryptococcus neoformans* molecular type VNI from environmental sources in the city of Belém, Pará, Brazil

Solange do PSE Costa1/+, Márcia dos S Lazéra2, Wallace RA Santos2, Bernardina P Morales2, Cláudia CF Bezerra2, Marília M Nishikawa4, Gláucia G Barbosa2, Luciana Trilles2, José LM do Nascimento1, Bodo Wanke2

1Instituto de Ciências Biológicas 2Instituto de Ciências da Saúde, Universidade Federal do Pará, Rua Augusto Corrêa 1, 66075-110 Belém, PA, Brasil 3Laboratório de Micologia, Instituto de Pesquisa Clínica Evandro Chagas-Fiocruz 4Instituto Nacional de Controle de Qualidade em Saúde-Fiocruz, Rio de Janeiro, RJ, Brasil

Cryptococcosis is a life-threatening systemic mycosis affecting healthy and immunocompromised hosts and is globally endemic. It is caused by two species of *Cryptococcus: Cryptococcus neoformans* (serotypes A, D, and hybrid AD) and *Cryptococcus gattii* (serotypes B and C), which differ genotypically, phenotypically, in epidemiology, as well as in their geographic distribution and ecologies (Perfect & Casadevall 2002, Kwon Chung & Varma 2006). Mating type in this species is determined by a single genomic locus, with two idiomorphs termed MAT α and MAT a, making this species bipolar. MAT α strains are 30-40 times more prevalent than MAT α strains in most clinical and environmental sampling studies (Kwon-Chung & Bennett 1992). Eight major molecular types have been identified within these two pathogenic species: *C. neoformans* is grouped into the molecular types VNI/AFLP1 (serotype A), VNII/AFLP1A (serotype A), VNIII/AFLP2 (serotype AD) and VNI/AFLP3 (serotype B); *C. gattii* is grouped into molecular types VGI/AFLP4, VGII/AFLP6, VGIII/AFLP5 and VGIV/AFLP7 (serotypes B and C) (Meyer et al. 1999, Boekhout et al. 2001). These molecular classifications have been used for global epidemiological studies on cryptococcosis.

Cryptococcal infections are most often acquired through the inhalation of viable propagules from the environment. Although the lungs are the primary sites for the infection, dissemination leads to meningoencephalitis, which is the most frequently diagnosed clinical manifestation in humans. *C. neoformans* has a worldwide distribution, can be found in avian guano and tree hollows, and affects mainly immunocompromised hosts. *C. gattii* distribution prevails in tropical and subtropical regions and the organism is often associated with decomposing wood in the hollows of tropical trees. Several tree species have been found to be colonized by *C. gattii*, including *Eucalyptus camaldulensis*, in Australia and Mexico (Ellis & Pfeiffer 1990, Licea et al. 1996), *Terminalia catappa*, in Colombia (Callegas et al. 1998), and *Syzygium jambolana*, *Cassia grandis*, *Senna multijuga*, *Ficus microcarpa*, *Moquilea tomentosa* and *Guettarda acreana*, in Brazil (Lazera et al. 1996, 2000, 2005, Fortes et al. 2001).

*C. gattii* has the potential to cause life-threatening disease in immunocompetent hosts and is recognized as the main agent of endemic primary cryptococcosis in the Northeast Region of Brazil (Nishikawa et al. 2003). In addition, *C. gattii* has been the agent of one outbreak in captive psittacine birds in São Paulo, Brazil (Raso et al. 2004). *C. gattii* has attracted particular attention as a primary emerging pathogen on Vancouver Island, Canada, where an ongoing large-scale cryptococcosis outbreak in both humans and animals has been caused almost exclusively by *C. gattii* molecular type VGII (Kidd et al. 2004).

In 1999, Corrêa et al. reported a total of 19 cases of cryptococcosis in children in PA and nine of these cases...
were caused by C. gattii infections. A recent study in
the same region showed that C. gattii is an endemic pri-
mary mycosis affecting HIV-negative hosts, including an
unexpectedly high number of children, with most cases
caused by molecular type VGII (Santos et al. 2008).
Considering that some of these patients were from the
Metropolitan area of Belém, the capital of PA, potential
environmental sources for cryptococcal infection in this
area were investigated in this current study.

The city of Belém (01°27'20"S 48°30'15"W) has
approximately 1,280,614 inhabitants. Located at the
mouth of the Amazon River, the city has a tropical and
humid climate, with a consistently high relative humid-
ity of about 85% (Prefeitura Municipal de Belém 2006).

For a preliminary analysis, sampling was performed
in the quarters São Braz and Umarizal, both close to
the Tropical Medicine Centre of the Federal University
of PA, near the downtown region of the city. Four
samples of caged psittacine dried bird excreta in three
avian stores were collected. In addition, seven samples
of decaying wood material were collected from each tree
trunk hollow from trees found along the sidewalk. The
trees included four Senna sp., one Senna siamea tree,
one living tree that was not identified, and one trunk of a
dead tree. Processing was conducted according to previ-
ously published protocols from Lazera et al. (1996).

Positive phenoloxidase colonies were isolated and
tested for both thermotolerance at 37°C and cyclohex-
imide sensitivity. The canavanine-glycine-bromothymol
blue medium was used to determine if isolates were C.
gattii (positive reaction and growth) or C. neoformans
(negative reaction and no growth). Carbon and nitrogen
compounds assimilation was performed by the use of
the Vitek 32-BioMerieux System (Vitek ICB, bioMeriux,
Durham, USA) and the Crypto check kit (Iatron Labora-
tories, Tokyo, Japan) was used for serotyping of the
isolates. After identification, the isolates were stored in
Skim Milk medium (DIFCO) at -20°C.

Genomic DNA was extracted from isolates accord-
ing to protocols from Ferrer et al. (2001). The mating
type was determined using specific PCR primer pairs
for mating type α and α, according to Chaturvedi et
al. (2000). The α-mating-type-specific primers were
5'-CTTCACTGCATCTTCAAC-3’ and 5’-GACA-
CAAAGGGTCATGCCA-3’, While the a-mating-type-
specific primers were 5’-CGCCCTTACGTGCTACCT-
TCT-3’ and 5’-AAGCAGAGTAAAGTCGGGC-3’. Two
mating types, ATCC 28957 (Mat α) and ATCC 28958
(Mat a), were used as positive controls.

Restriction Fragment Length Polymorphism (RFLP)
analysis using the URA5 gene was performed as de-
scribed by Meyer et al. (2003) using the primers URA5
(5’ATGTCTCCACAAGCCTCGACTCCG-3’) and SJ01
(5’TTAGACCTCCTGAACACCGTGACTC 3’). RFLP
patterns were assigned visually by comparing them with
the patterns obtained from the standard type strains
(VNI-VNIV and VGI-VGIV).

One aviary store and one tree were positive for Cryp-
tococcus. Five darkbrown colonies were obtained from
a sample of bird droppings and all were identified as C.
neoformans serotype A, MAT α, molecular type VNI.
Two dark brown colonies were obtained from decaying
wood inside a hollow of a S. siamea tree (kassod tree);
one colony was identified as C. neoformans serotype A,
MAT a, molecular type VNI and the other colony was
identified as C. gattii serotype B, molecular type VGII.
Mating type analysis is shown in Fig. 1 and the RFLP
profiles are illustrated in Fig. 2.

Few studies on the eco-epidemiology of C. neo-
formans and C. gattii have been performed in Northern
Brazil. Fortes et al. (2001) demonstrated the occurrence
of C. gattii in G. acreana in a wild area of the Amazon
rainforest. In the present study, we report for the first
time the isolation of C. neoformans and C. gattii from
environmental sources in the city of Belém, PA. Trilles
et al. (2008) analyzed the geographic distribution of C.
neoformans and C. gattii molecular types from isolates
within Brazil, and described VNI as the most common
molecular type and VGII as the prevailing molecular type
in immunocompetent hosts in the North and Northeast
Regions of Brazil. Although a large number of isolates
were analyzed (n = 443), none were from PA. Recently,
Santos et al. (2008) analyzed the agents of 43 cases of
cryptococcosis and found that VNI was the most com-
mon type in immunocompromised patients, while mo-
lecular type VGII was the major cause of endemic pri-

![Fig. 1: PCR amplification of environmental isolates with primers MAT α1, MAT α2 and MAT α1, MAT α2. Lanes: M: molecular weight marker: 100 bp DNA ladder; 1: LMM 1082; 2: LMM 1083; 3: LMM 1084; 4: LMM 1088; 5: LMM 1089; 6: LMM 1090; 7: ATCC 28957; 8: ATCC 28958; 9: negative control.](image1)

![Fig. 2: molecular typing profiles generated via Restriction fragment length polymorphism analysis of URA5 from environmental isolates. Lanes: M: molecular weight marker: 100 bp DNA ladder; 1: LMM 1082; 2: LMM 1083; 3: LMM 1084; 4: LMM 1088; 5: LMM 1089; 6: LMM 1090; 7: LMM 794; 9: LMM 795; 10: LMM 796; 11: LMM 797; 12: LMM 798; 13: LMM 799; 14: LMM 800; 15: LMM 801; 16: negative control. All samples are molecular types VNI, except Lane 7, which is VGII. Lanes 8-15 are molecular type standard strains (VNI-VNIV and VGI-VGIV).](image2)
ary mycosis in HIV-negative individuals, including an unexpectedly high number of children in PA.

Kidd et al. (2004) reported C. gattii molecular type VGII as the causative agent of the cryptococcosis outbreak on Vancouver Island (British Columbia, Canada). In addition, molecular studies of clinical and environmental isolates reported by Escandón et al. (2006) demonstrated the predominance of molecular type VGII within C. gattii isolates from Colombia. Moreover, the detection of molecular type VGII in a hollow of a tree in the city of Belém reinforces that this molecular type deserves increased attention in other parts of the Brazilian Amazonia as well as in other South American countries.

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