Molecular Typing of IberoAmerican *Cryptococcus neoformans* Isolates

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A network was established to acquire basic knowledge of *Cryptococcus neoformans* in IberoAmerican countries. To this effect, 340 clinical, veterinary, and environmental isolates from Argentina, Brazil, Chile, Colombia, Mexico, Peru, Venezuela, Guatemala, and Spain were typed by using M13 polymerase chain reaction-fingerprinting and orotidine monophosphate pyrophosphorylase (*URA5*) gene restriction fragment length polymorphsm analysis with *Hha*I and *Sau*96I in a double digest. Both techniques grouped all isolates into eight previously established molecular types. The majority of the isolates, 68.2% (n=232), were VNI (var. *grubii*, serotype A), which accords with the fact that this variety causes most human cryptococcal infections worldwide. A smaller proportion, 5.6% (n=19), were VNII (var. *grubii*, serotype A); 4.1% (n=14), VNIII (AD hybrid), with 9 isolates having a polymorphism in the *URA5* gene; 1.8% (n=6), VNIV (var. *neoformans*, serotype D); 3.5% (n=12), VGI; 6.2% (n=21), VGII; 9.1% (n=31), VGIII, and 1.5% (n=5) VGIV, with all four VG types containing var. *gattii* serotypes B and C isolates.

Cryptococcosis is among the most prevalent life-threatening mycoses and has a worldwide distribution. The etiologic agent is the basidiomycetous yeast *Cryptococcus neoformans* (1,2); three varieties are recognized: *C. neoformans* var. *grubii*, serotype A (3), *C. neoformans* var. *neoformans*, serotype D, *C. neoformans* var. *gattii*, serotypes B and C, and the hybrid serotype AD (4).

Humans are infected by inhaling infectious propagules from the environment, which primarily colonize the lung and subsequently invade the central nervous system (4). *C. neoformans* var. *grubii*/*neoformans* has been isolated worldwide from soil enriched with avian excreta (4,5). More recently, decaying wood from certain species of trees has been proposed as environmental habitat for this variety (6). In contrast, the distribution in nature for *C. neoformans* var. *gattii* is geographically restricted to mainly tropical and subtropical regions (7,8). To date, specific host trees are represented by *Eucalyptus* species, *Moquilea tomentosa, Cassia grandis, Ficus microcapra*, and *Terminalia catappa* (7–11).

Worldwide, in immunocompromised hosts, most infections are caused by *C. neoformans* var. *grubii* (4,5). In contrast, *C. neoformans* var. *gattii* virtually always affects immunocompetent hosts (8).

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In the last decade, a number of DNA typing techniques have been used to study the epidemiology of *C. neoformans*. These techniques include karyotyping, random amplification of polymorphic DNA, restriction fragment length polymorphism (RFLP), DNA hybridization studies, amplified fragment length polymorphism (AFLP), and polymerase chain reaction (PCR) fingerprinting (12–17).

PCR fingerprinting has been used as the major typing technique in the ongoing global molecular epidemiologic survey of *C. neoformans* (14,18), dividing >400 clinical and environmental isolates into eight major molecular types: VNI (var. *grubii,* serotype A), VNII (var. *grubii,* serotype A), VNIII (serotype AD), VNIV (var. *neoformans*, serotype D), VGI, VGII, VGIII, and VGIV (var. *gattii*, serotypes B and C). No correlation between serotype and molecular type has been found for *C. neoformans* var. *gattii*. The molecular types were recently confirmed by RFLP analysis of the orotidine monophosphate pyrophosphorylase (*URA5*) gene and the phospholipase (*PLB1*) gene (19).

Globally, most of the isolates recovered from AIDS patients belong to the genotypes VNI and VNIV, whereas the genotypes VNI and VGI are predominant throughout the world for *C. neoformans* var. *grubii* and *C. neoformans* var. *gattii,* respectively. The larger number of genotype VNI isolates agrees with the fact that *C. neoformans* var. *grubii* causes most human cryptococcal infections worldwide (18,19).

The aims of this study were the following: 1) to extend the molecular epidemiologic survey to other parts of the world, 2) to establish a regional network of participating reference laboratories, and 3) to apply PCR fingerprinting and *URA5* RFLP typing to investigate the genetic structure and possible epidemiologic relationships between clinical and environmental isolates obtained in Latin America and Spain. The results of this study permitted us to determine the major molecular types and their distribution within each participating country.

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Materials and Methods

Study Design

During the 12th International Society for Human and Animal Mycoses meeting in Buenos Aires, Argentina, in March 2000, it was decided to establish an IberoAmerican Cryptococcal Study Group under the coordination of E. Castañeda and W. Meyer. Each of the participating laboratories was asked to submit 10–30 isolates. For the clinical isolates, the following data were requested: isolation date, demographic data (age and gender of patient), collection location, risk factors, source and variety, and serotype. For the environmental and veterinary isolates, the data collected included isolation date, source, collection location, variety, and serotype.

Fungal Isolates

An online appendix of cryptococcal isolates studied in this study is available at: URL: http://www.cdc.gov/ncidod/EID/vol9no2/02-0246_app.htm. The isolates were obtained by the participating laboratories of the IberoAmerican Cryptococcal Study Group and maintained on Sabouraud dextrose agar slants at 4°C and as water cultures at room temperature. Isolates were identified as *C. neoformans* by using standard methods (20). The variety was determined by the color reaction test on L-canavanine-glycine-bromothymol blue medium (21), and the serotype was determined, in selected isolates, by the use of the Crypto Check Kit (Iatron Laboratories Inc., Tokyo, Japan).

The isolates were sent for molecular typing to the Molecular Mycology Laboratory at the University of Sydney at Westmead Hospital, Sydney, Australia, either as water cultures or on Sabouraud dextrose agar slants. For long-term storage, the isolates were maintained as glycerol stocks at –70°C.

Reference Strains

A set of laboratory standard *C. neoformans* reference strains representing each molecular type were used in PCR fingerprinting and *URA5* RFLP as follows: WM 148 (serotype A, VNI), WM 626 (serotype A, VNII), WM 628 (serotype AD, VNIII), WM 629 (serotype D, VNIV), WM 179 (serotype B, VGI), WM 178 (serotype B, VGII), WM 161 (serotype B, VGIII), and WM 779 (serotype C, VGIV) (14).

DNA Extraction

High-molecular-weight DNA was isolated as described previously (14). Briefly, *C. neoformans* isolates were grown on Sabouraud's dextrose agar at 37°C for 48 h, a loopful of cells from the culture was mixed with sterile deionized water and centrifuged. The supernatant was discarded, and the tube containing the yeast cell pellet was frozen in liquid nitrogen. The pellet was ground with a miniature pestle. The cell lysis solution (100 mg triisopropylnapthalene sulfonic acid, 600 mg para-aminosalicylic acid, 10 mL sterile deionized water, 2.5 mL extraction buffer (1 M Tris-HCl, 1.25 M NaCl, 0.25 M EDTA, pH 8.0) and 7.5 mL phenol saturated with Tris-EDTA was preheated to 55°C, and 700 µL of this mixture was added to the frozen, ground cells. The tubes were incubated for 2 min at 55°C, shaken occasionally, and then 500 µL chloroform was added, and the mixture was incubated for a 2 min at 55°C and shaken occasionally. The tubes were centrifuged for 10 min at 14,000 rpm, and the aqueous phase was transferred to a new tube. Then, 500 µL of phenol-chloroform-isoamyl alcohol (25:24:1) was added, shaken for 2 min at room temperature, and centrifuged as above. The aqueous phase was transferred to a new tube, 500 µL of chloroform was added, shaken, and centrifuged as above. To precipitate the genomic DNA, the aqueous phase was again transferred to a new tube, and 0.03 volumes 3.0 M sodium acetate (pH 5.2) and 2.5 volumes cold 96% ethanol were added, and the mixture was gently shaken and incubated at –20°C for at least 1 h or overnight. The solution was centrifuged for 30 min at 14,000 rpm to pellet the DNA. The DNA pellet was washed with 70% ethanol and centrifuged for 10 min at 14,000 rpm and air-dried. The DNA was resuspended in 200 µL sterile deionized water at 4°C overnight and stored at –20°C.

PCR Fingerprinting

The minisatellite-specific core sequence of the wild-type phage M13 (5′ GAGGGTGGCGGTTCT 3′) (22) was used as single primer in the PCR. The amplification reactions were performed in a volume of 50 µL containing 25 ng high-molecular-weight genomic DNA, 10 mM Tris-HCl, pH 8.3, 50 mM KCl, 1.5 mM MgCl, 0.2 mM each of the dATP, dCTP, dGTP and dTTP (Roche Diagnostics GmbH, Mannheim, Mannheim, Germany), 3 mM magnesium acetate, 30 ng primer, and 2.5 U Amplitaq DNA polymerase (Applied Biosystems, Foster City, CA). PCR was performed for 35 cycles in a Perkin-Elmer thermal cycler (model 480) with 20 s of denaturation at 94°C, 1 min annealing at 50°C, and 20 s extension at 72°C, followed by a final extension cycle for 6 min at 72°C. Amplification products were removed, concentrated to approximately 20 µL and separated by electrophoresis on 1.4% agarose gels (stained with ethidium bromide, 10 mg/mL stock) in 1X Tris-borate-EDTA (TBE) buffer at 60 V for 14 cm, and visualized under UV light (14). Molecular types (VNI–VNIV and VGI–VGIV) were assigned, according to the major bands in the patterns. All visible bands were included in the analysis, independent of their intensity (14,18).

URA5 Gene RFLP

PCR of the *URA5* gene was conducted in a final volume of 50 µL. Each reaction contained 50 ng of DNA, 1X PCR buffer (10 mM Tris-HCl, pH 8.3, 50 mM KCl, 1.5 mM $MgCl_2$; Applied Biosystems, Foster City, CA), 0.2 mM each of dATP, dCTP, dGTP, and dTTP (Roche Diagnostics GmbH), 3 mM magnesium acetate, 1.5 U AmpliTaq DNA polymerase (Applied Biosystems), and 50 ng of each primer URA5 (5′ATGTCCTCCCAAGCCCTCGACTCCG 3′) and SJ01 (5′ TTAAGACCTCTGAACACCGTACTC 3′). PCR was performed for 35 cycles in a Perkin-Elmer thermal cycler (model 480) at 94°C for 2-min initial denaturation, 45 s of denaturation at 94°C, 1 min

annealing at 61°C, and 2-min extension at 72°C, followed by a final extension cycle for 10 min at 72°C. Amplification products were mixed with one fifth volume of loading buffer (15% Ficoll 400, 0.25% orange G, MilliQ water), 15 µL of PCR products were double digested with *Sau*96I (10 U/µL) and *Hha*I (20 U/µl) for 3 h or overnight and separated by 3% agarose gel electrophoresis at 100 V for 5 h. RFLP patterns were assigned visually by comparing them with the patterns obtained from the standard strains (VNI-VNIV and VGI-VGIV) (Jackson et al. unpub. data).

Statistical Analysis

Initially, individual PCR fingerprints were visually compared to those of the standard strains, amplified in parallel, to determine the major molecular type for each isolate. The computer program GelCompar*II*, version 1.01 (Applied Maths, Kortrijk, Belgium) was used to determine the genetic relationship of the strains. DNA bands of each fingerprint pattern were defined manually with a band-position tolerance of 0.9%, being the optimal settings needed to define the molecular size marker bands as 100% identical. Similarity coefficients were calculated by using the Dice algorithm, and cluster analyses were performed by the neighbor-joining algorithms by using the "Fuzzy Logic" and "Area Sensitive" option of the GelcomparII program.

Results

During the course of this investigation, a network was established with 15 laboratories from nine countries participating in this study. The participant countries were: Argentina, Brazil, Chile, Colombia, Guatemala, Mexico, Peru, Spain, and Venezuela.

A total of 340 *C. neoformans* isolates, comprising 266 clinical, 7 veterinary, and 67 environmental isolates were submitted for molecular typing. Of these, 57 were from Argentina (53 clinical and 4 environmental), 66 from Brazil (56 clinical, 9 environmental, and 1 veterinary), 19 from Chile (15 clinical and 4 environmental), 62 from Colombia (39 clinical and 23 environmental), 15 from Guatemala (all clinical), 69 from Mexico (46 clinical and 23 environmental), 13 from Peru (all clinical), 19 from Spain (9 clinical, 6 veterinary, and 4 environmental), and 20 from Venezuela (all clinical). From the total isolates investigated, 271 (79.6%) were *C. neoformans* var. *grubii/neoformans;* 251 (92.6%) of them were *C. neoformans* var. *grubii*, 6 (1.8%) were *C. neoformans* var. *neoformans,* and 13 (4.8%) were AD hybrid isolates. The remaining 69 (20.4%) isolates were *C. neoformans* var. *gattii*.

All 340 isolates were typed by PCR fingerprinting by using the minisatellite-specific oligonucleotide M13 as a single primer and RFLP analysis of the *URA5* gene with the restriction enzymes *Sau*96I and *Hha*I in a double digest. The molecular types were determined for each isolate by comparing the obtained PCR fingerprint profiles and *URA5* RFLP patterns with the respective standard patterns for each molecular type.

The serotyping results (Iatron) correlated with the molecular subtyping results in all serotype B (n=31) and C (n=13) isolates. Regarding serotype A, 99 from a total of 102 (97%) isolates correlated; the remaining 3 were serotype A by the Iatron and serotype AD by the molecular typing method. Regarding serotype D, one of four reported was confirmed by molecular typing; the other three were serotype AD. For serotype AD, two isolates were found when typed with the Iatron kit and eight when typed with molecular typing techniques. All the changes were found in the isolates from Spain. This finding is not surprising, taking into account that problems with the serotyping concerning potential serotype AD hybrids are known (4). A list of the characteristics of the studied isolates by participating country, laboratory, laboratory code, clinical, veterinary or environmental origin, isolate characteristics (isolation year, isolation location, source, gender, age and risk factor), variety, serotype and the molecular type identified during this study is available in an online appendix (http://www.cdc.gov/ncidod/EID/vol9no2/02-0246_app.htm).

Both molecular typing techniques grouped the isolates in the eight previously established major genotypes (Figures 1 and 2). From the isolates investigated, 232 (68.2%) were molecular type VNI (serotype A, var. *grubii*), 19 (5.6%) were molecular type VNII (serotype A, var. *grubii*), 14 (4.1%) were molecular type VNIII (serotype A/D, hybrid between the serotypes A and D), with 5 having a RFLP pattern of the *URA5* gene with seven bands, indicated by VNIII in the online Appendix and Figure 3B and 4B, corresponding to a hybrid between VNI, VNII, and VNIV and 9 isolates having an RFLP pattern of the *URA5* gene with six bands, indicated by VNIII* in the online Appendix and Figure 4B and 5B, corresponding to a hybrid between VNII and VNIV, 6 (1.8%) were molecular type VNIV (serotype D, var. *neoformans*), 12 (3.5%) were molecular type VGI (serotypes B and C, var. *gattii*), 21 (6.2%) were molecular type VGII (serotypes B and C, var. *gattii*), 31 (9.1%) were molecular type VGIII (serotypes B and C, var. *gattii*), and 5 (1.5%) were molecular type VGIV (serotypes B and C, var. *gattii*).

Figures 3A and 4A show examples of PCR fingerprints obtained from Mexican and Spanish isolates; Figures 3B and 4B show the corresponding *URA5* RFLP patterns for the same isolates. The Mexican isolates were selected because they were representative of the patterns observed from all the isolates submitted by the Latin American participating laboratories. The Spanish isolates were selected because they represented a distribution of molecular types corresponding to those observed in previous studies on European isolates (14).

From the 340 isolates studied 277, marked with "#" in the online Appendix, have been included in the GelCompar*II* analysis. Sixty-three isolates were excluded from the analysis since their PCR fingerprinting patterns were not sharp or had been run under slightly different electrophoresis conditions, making the band positions impossible to compare. Cluster analysis of the PCR-fingerprinting profiles by using the GelCompar*II* program grouped all isolates into three major clusters according to variety and into eight major groups according to the molecular type. The overall homology observed was 50.4% among isolates of *C. neoformans* var.

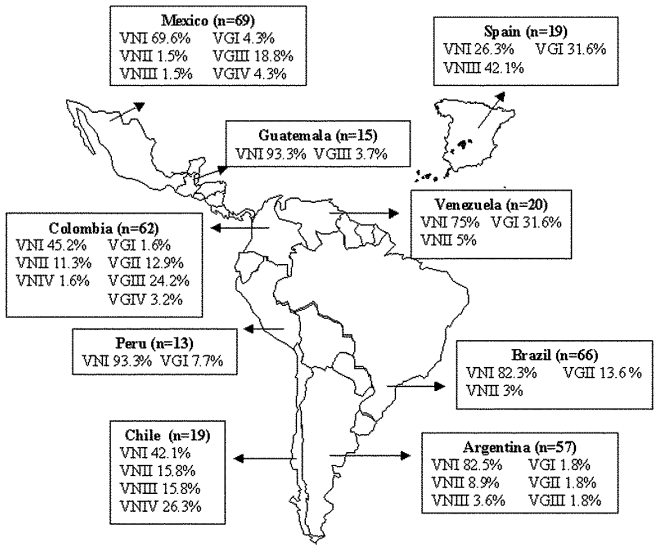

Figure 1. Geographic distribution of the molecular types obtained from IberoAmerican *Cryptococcus neoformans* isolates by polymerase chain reaction fingerprinting and *URA5* gene restriction fragment length polymorphis analysis (total numbers studied per country given in parentheses).

grubii, 50.9% for *C. neoformans* var. *neoformans,* and 51.2% for *C. neoformans* var. *gattii* (Figure 2). The homology within a given molecular type was as follows: 54.8% VNI, 57.3% VNII, 51.9% VNIII, 50.9% VNIV, 56.4% VGI, 56.4% VGII, 54.4% VGIII, and 68.3% VGIV.

Besides grouping all isolates into the eight major molecular patterns, the molecular type VNI could be subdivided into eight main subclusters, with most of these subclusters' grouping isolates obtained from specific countries. The similarity between isolates obtained from any individual country varied from 65% to 82%. Most of the main subclusters within molecular type VNI also contained isolates from different countries, indicating gene flow and strain dispersal between South American countries, Spain, or both. However, all isolates could be separated by their unique PCR-fingerprinting pattern, with the highest homology being 84% between two unrelated environmental isolates from Mexico City (LA 22, budgerigar [parakeet] droppings, and LA 25, pigeon droppings).

Of the 266 clinical isolates, 177 (66.5%) were obtained from HIV-positive patients, with 139 (78.5%) being VNI, 14 (7.9%) VNII, 13 (7.4%) VNIII, 6 (3.4%) VNIV, 3 (1.7%) VGII, and 2 (1.1%) VGIII. Most (86.4%) isolates from HIV-positive patients belonged to the molecular types VNI and VNII, representing serotype A, *C. neoformans* var. *grubii*. Of these, 266 clinical isolates, 51 (19.2%) were recovered from patients with no reported risk factors. From those, 23 (45.1%) were var. *grubii* with the molecular type VNI (n=21) or VNII (n=2), and 28 (54.9%) were var. *gattii* with the molecular types VGI (n=3), VGII (n=10), VGIII (n=14), and VGIV (n=1). For 26 of the clinical isolates, no data concerning risk factors were available. Six veterinary isolates were molecular type VGI, and one was VGII. Most of the environmental isolates belonged to *C. neoformans* var. *grubii* with 73.1% being VNI (n=49) and 1.5% VNIII (n=1) AD hybrids. The remaining 17 (25.3%) isolates were *C. neoformans* var. *gattii* with the molecular type VGI (n=1), VGII (n=3), VGIII (n=12), and VGIV (n=1).

Cryptococcal isolates included in the IberoAmerican study were more frequently obtained from men than from women. The male-to-female ratio was 2l1 to 41, i.e., cryptococcosis was 5.1 times more common in men than in women. In the HIV-positive population alone, the incidence of cryptococcosis was 5.5 times more frequent in men than in women, based on the data obtained from the isolates investigated in this study. The age of the patients with clinically manifested cryptococcosis ranged from 4 to 73; 175 (65.9%) were between 21 and 40 years old.

The clinical isolates submitted to this study were collected over a period of 41 years, 1961–2001; most (92.5%) of the isolates were collected in the mid-1990s. The veterinary isolates were recovered from goats in Spain in 1995 and from a parrot in Brazil in 2000. The environmental isolates submitted were collected over a period of 7 years, 1993–2000.

Discussion

This retrospective study of cryptococcosis in IberoAmerica was set up in an effort to establish a network of medical

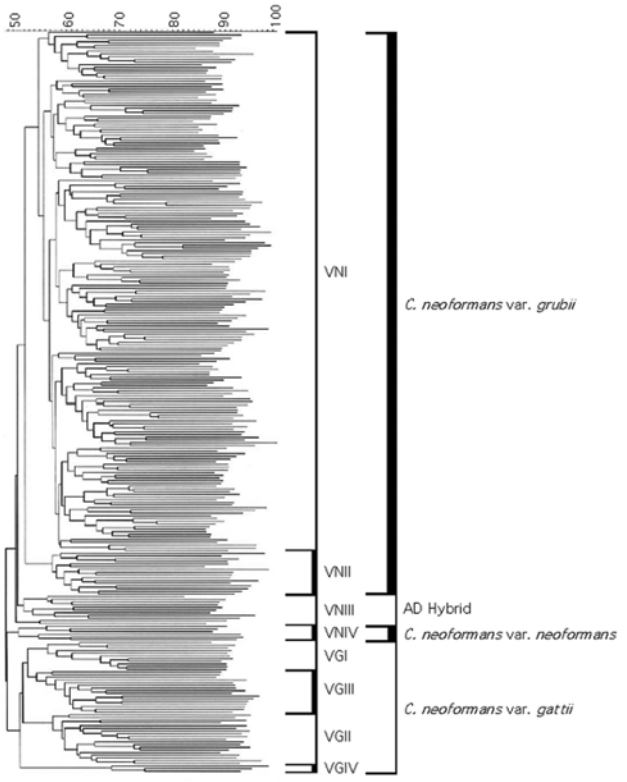

Figure 2. Dendrogram of the polymerase chain reaction-fingerprinting patterns obtained with the primer M13 from a selection of the IberoAmerican isolates studied. All the isolates fall into eight major molecular types, which fall into three major groups corresponding to *Cryptococcus neoformans* var. *grubii*, serotype A, with two molecular types VNI and VNII; *C. neoformans* var. *neoformans*, serotype D, with the molecular type VNIV; and *C. neoformans* var. *gattii* serotypes B and C, with the molecular types VGI, VGII, VGIII and VGIV. In addition to the three major clusters we can see the intermediate molecular type VNIII, representing the AD hybrids.

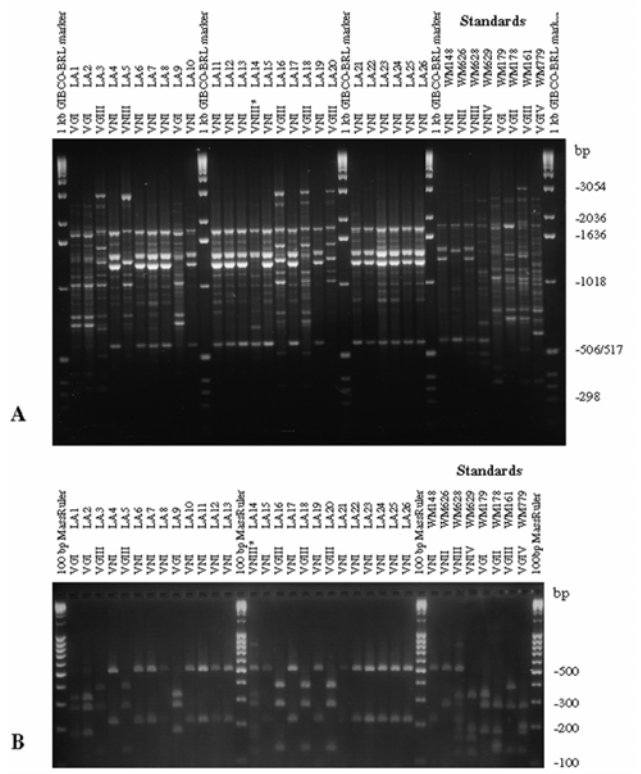

Figure 3. Polymerase chain reaction (PCR) fingerprints generated with the primer M13 (3A), and *URA5* gene restriction fragment length polymorphism (RFLP) profiles identified by double digest of the gene with *Sau*96I and *Hha*I (3B) obtained from a selection of Mexican *Cryptococcus neoformans* isolates, given as a representative example for the patterns obtained from clinical and environmental isolates from Latin America. Standard patterns obtained from the reference strains of the major molecular types by PCR-fingerprinting patterns with the microsatellite specific primer M13 as a single primer in the PCR (right-hand side of 3A) and *URA5* gene RFLP profiles generated after double digest with *Sau*96I and *Hha*I (right-hand side of 3B) (VNIII correspond to the seven-band *URA5* RFLP pattern and VNIII* correspond to the six-band *URA5* RFLP pattern).

mycology laboratories to study the distribution of cryptococcal isolates, including the varieties and molecular types within the participating countries. The network was aimed at generating PCR-fingerprint and *URA5* RFLP patterns under standardized conditions in the Molecular Mycology Laboratory of the University of Sydney at Westmead Hospital, for a subset of clinical and environmental *C. neoformans* isolates from each participating country. These reference profiles are now available to each participating laboratory so they can set up the molecular typing techniques in their own laboratories, and to serve as internal controls in future extended studies of cryptococcal isolates in each country.

The data were obtained from a random selection of cryptococcal isolates from each participating country and laboratory, which do not necessarily reflect the true situation in IberoAmerica. Nonetheless, the data offer a general overview of molecular types and variety distribution of *C. neoformans* in IberoAmerica. For the first time, two different molecular typing techniques, PCR-fingerprinting with the minisatellite specific primer M13 and *URA5* gene RFLP analysis, were applied simultaneously to the same set of cryptococcal isolates, demonstrating identical groupings to the eight major molecular types previously described (14,18).

Previous pilot studies that used PCR-fingerprinting at the University of Sydney at Westmead Hospital distributed more than 400 clinical and environmental isolates obtained from Argentina, Australia, Belgium, Brazil, Germany, Italy, New Zealand, Papua New Guinea, South Africa, Thailand, Uganda, and the United States in eight major molecular types, VNI and VNII (serotype A), VNIII (serotype AD), VNIV (serotype D), VGI, VGII, VGIII and VGIV (serotypes B and C) (14,18). At the time of this original work, the molecular types VNI and VGI were found to be the most common genotypes worldwide. The present study, which includes more isolates from Latin America, showed the same results as regards variety *grubii,* with VNI being the predominant molecular type, accounting for 68.2% of all isolates. However, the situation changed drastically for variety *gattii*; as in this study, the predominant molecular type was VGIII, accounting for 9.1% of all isolates, in contrast to previous studies which showed that the molecular type VGIII was geographically restricted to India and the United States (18,19). In the present study, VGIII was also found in Argentina, Colombia, Guatemala, Mexico

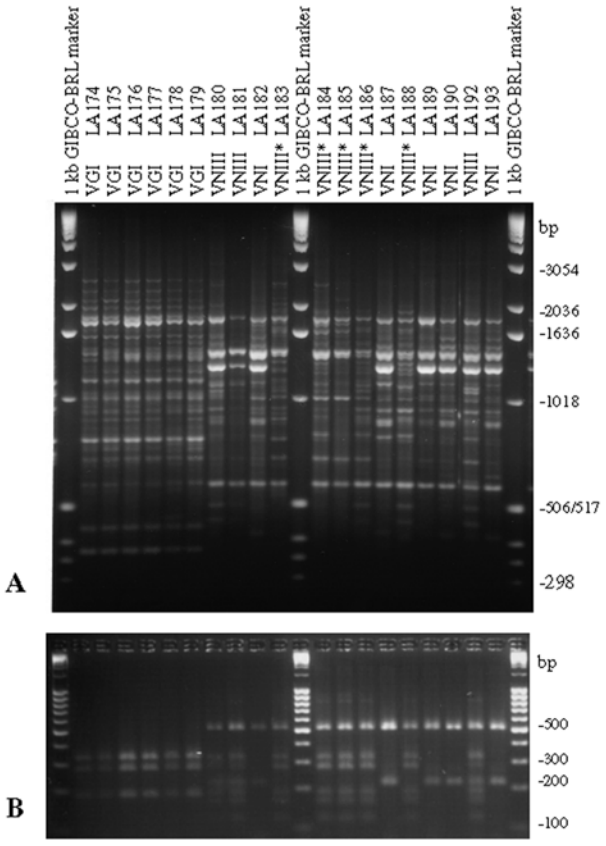

Figure 4. Polymerase chain reaction fingerprints generated with the primer M13 (4A) and *URA5* gene restriction fragment length polymorphism (RFLP) profiles identified via double digest of the gene with *Sau*96I and *Hha*I (4B) obtained from the Spanish clinical, veterinary, and environmental *Cryptococcus neoformans* isolates (VNIII correspond to the seven-band *URA5* RFLP pattern and VNIII* correspond to the six-band *URA5* RFLP pattern). 100bp MassRuler used as molecular marker in 4B.

and Venezuela, suggesting that it is not as limited as previously suggested. The same was true for the molecular type VGIV, previously assigned only to India and South Africa (18,19); its presence in Colombia and Mexico, although in very low numbers, indicates a wider geographic distribution.

In general, the most common variety was *C. neoformans* var. *grubii,* 73.8% (n=251), followed by variety *gattii,* 20.3% (n=69). Much less common were the AD hybrids, 4.1% (n=14) and variety *neoformans,* 1.8% (n=6), which reflects the global distribution previously established (14,18,23).

The overall grouping of the isolates into eight major molecular types by PCR-fingerprinting with the minisatellite specific primer M13, obtained in this study and the previous pilot study by Meyer et al. 1999 (14) and Ellis et al. 2000 (18), agrees with the findings by Boekhout et al. 2001 (23) and by Cogliati et al. in 2000 (24). Boekhout et al. (23) used AFLP analysis to study 206 global isolates of *C. neoformans*, and grouped them into six major AFLP groups, whereas Cogliati et al. (24), using a slightly modified PCR-fingerprinting technique with the microsatellite specific primer $(GACA)_4$, grouped Italian isolates of *C. neoformans* var. *grubii* and var. *neoformans* into four major molecular types. Comparable molecular/genotypes, which where identified in the four cited independent studies, are VNI = AFLP1 = Cogliati VN6 (serotype A, var. *grubii*); VNII = AFLP1A (serotype A, var. *grubii*); VNIII = AFLP3 = Cogliati VN3 and VN4 (serotype AD, hybrid between var. *grubii* and var. *neoformans*); VNIV = AFLP2 = Cogliati VN1 (serotype D, var. *neoformans*); VGI = AFLP4, VGII = AFLP6, VGIII = AFLP5 and VGIV (all corresponding to serotypes B and C, var. *gattii*) (14,18,23,24).

The overall results show some clonality between isolates obtained from a certain country or even between different countries, suggesting partial clonal spread of the pathogenic yeast within South America. However, the approximately 50%, overall similarity between *C. grubii* isolates, with the highest being 82%, suggests that these South American isolates are more varied than those obtained in a previous study by Franzot et al. (25), in which they examined a limited number of isolates from Brazil by using less discriminatory molecular techniques (CNRE-1 RFLP analysis and *URA5* sequencing). In Franzot's study, the highest similarity was >94% between the Brazilian isolates, suggesting a higher clonality than observed in the isolates obtained from New York City studied in the same paper (25).

Interestingly, Chile and Spain share similar molecular types. Both countries have a large number of molecular type VNIII isolates (AD hybrids), 15.8% and 42.1%, respectively, although VNIV serotype D isolates were present only in Chile (26.3%). These groups are usually common in a number of European countries, such as France and Italy (26–28). However, only these two countries show two different *URA5* RFLP patterns, one consisting of seven bands, indicating a hybrid between VNI, VNII, and VNIV, and a second new hybrid *URA5* RFLP pattern, consisting of six bands, indicating a hybrid between VNII and VNIV. As a result of the IberoAmerican study, these hybrid patterns had recently been reported as part of Jackson's honor's thesis work (Jackson and Meyer unpub. data, 2000). The seven-band *URA5* RFLP pattern was exclusively found in Spain (n=3) and Chile (n=2). These strains seem to be triploid, and cloning with subsequent sequencing of the PCR product showed that they contain three different copies of the *URA5* gene. The six-band *URA5* RFLP pattern found in Spain (n=5) and Chile (n=1), was also found in Mexico (n=1) and Argentina (n=2), possibly due to the presence of the molecular types VNII and VNIV in these countries (14). These hybrid isolates are diploid at the *URA5* locus and contain two different copies of the gene (Jackson and Meyer, unpub. data).

Further studies are needed to investigate the special relationship between isolates obtained from these two countries. The similarity in the molecular types obtained from Spanish and Chilean isolates provides further evidence, that the cryptococcal strains present today in South America could be introduced during the European colonization. This idea had been suggested by Franzot et al. (25) when investigating isolates obtained from Brazil. The authors argue that the pigeon (*Columba livia*), thought to provide a major reservoir of *C. neoformans* in pigeon excreta, is believed to have originated in southern Europe and northern Africa and has been dispersed worldwide by human travel (29).

Most of the cryptococcal isolates in this study were recovered from patients whose main risk factor was HIV infection. Overwhelming numbers of these isolates corresponded to the molecular type VNI, in accordance with previous findings, showing that isolates of this molecular type are the major source of infection in HIV-positive patients worldwide (18,19). This finding highlights the fact that most human cryptococcal infections are caused by *C. neoformans* var. *grubii,* serotype A (4,5). A distinct picture emerged in the group of isolates obtained from patients with no known risk factors, as most were *C. neoformans* var. *gattii* isolates (n=28), with the molecular types VGI (n=3), VGII (n=10), VGIII (n=14), and VGIV (n=1), compared to 23 isolates belonging to the overall most common molecular type, VNI (41.2%) of *C. neoformans* var. *grubii*. This finding supports the conclusion that variety *gattii* primarily infects immunocompetent patients as Chen et al. had found when investigating Australian isolates (30). These authors have proposed that aboriginal people living in rural areas of Australia's Northern Territory have a higher risk of cryptococcosis because they live in close proximity to the potential natural host of *C. neoformans* var. *gattii,* the eucalyptus trees (30).

Despite the fact that isolates included in this study constituted a random sampling, the results show again that HIV infection is the most important risk factor for cryptococcosis (31). This conclusion is supported by the number of isolates recovered from HIV-positive patients (n=177), the age distribution, which peaks between 20 and 40 years of age, and the date of isolation with a peak corresponding to the 1990s.

Overall, the network of mycology laboratories established in IberoAmerica provided, for the first time, a baseline knowl-

edge of *C. neoformans* variety and molecular type distribution in the participating countries, placing the IberoAmerican isolates in the global picture of cryptococcosis.

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References

1. Kwon-Chung KJ. A new genus, *Filobasidiella*, the perfect state of *Cryptococcus neoformans*. Mycologia 1975;67:1197–200.
2. Kwon-Chung KJ. A new genus, *Filobasidiella*, the sexual state of *Cryptococcus neoformans* B and C serotypes. Mycologia 1976;68:942–6.
3. Franzot SP, Salkin IF, Casadevall A. *Cryptococcus neoformans* var. *grubii*: separate varietal status for *Cryptococcus neoformans* serotype A isolates. J Clin Microbiol 1999;37:838–40.
4. Casadevall A, Perfect JR. *Cryptococcus neoformans*. Washington: ASM Press; 1998.
5. Mitchell TG, Perfect JR. Cryptococcosis in the era of AIDS—100 years after the discovery of *Cryptococcus neoformans*. Clin Microbiol Rev 1995;8:515–48.
6. Lazera MS, Pires FDA, Camillo-Coura L, Nishikawa MM, Bezerra CCF, Trilles L, et al. Natural habitat of *Cryptococcus neoformans* var. *neoformans* in decaying wood forming hollows in living trees. J Med Vet Mycol 1996;34:127–31.
7. Ellis DH, Pfeiffer TC. Natural habitat of *Cryptococcus neoformans* var. *gattii.* J Clin Microbiol 1990;28:1642–4.
8. Sorrell TC. *Cryptococcus neoformans* variety *gattii.* Med Mycol 2001;39:155–68.
9. Lazera M, Cavalcanti M, Trilles L, Nishikawa M, Wanke B. *Cryptococcus neoformans* var. *gattii*—evidence for a natural habitat related to decaying wood in a pottery tree hollow. Med Mycol 1998;36:112–9.
10. Lazera MS, Salmito MA, Londero AT, Trilles L, Nishikawa M, Wanke B. Possible primary ecological niche of *Cryptococcus neoformans*. Med Mycol 2000;38:379–83.
11. Callejas A, Ordóñez N, Rodríguez MC, Castañeda E. First isolation of *Cryptococcus neoformans* var. *gattii*, serotype C, from the environment in Colombia. Med Mycol 1998;36:341–4.
12. Brandt ME, Hutwagner LC, Kuykendall RJ, Pinner WS. Comparison of multilocus enzyme electrophoresis and random amplified polymorphic DNA analysis for molecular subtyping of *Cryptococcus neoformans*. J Clin Microbiol 1995;33:1890–5.
13. Crampin AC, Mathews RC, Hall D, Evans EG. PCR fingerprinting *Cryptococcus neoformans* by random amplification of polymorphic DNA. Journal of Medical and Veterinary Mycology 1993;31:463–5.
14. Meyer W, Marszewska K, Amirmostofina M, Igreja RP, Hardtke C, Methling K, et al. Molecular typing of global isolates of *Cryptococcus neoformans* var. *neoformans* by PCR-fingerprinting and RAPD. A pilot study to standardize techniques on which to base a detailed epidemiological survey. Electrophoresis 1999;20:1790–9.
15. Currie BP, Freundlich IF, Casadevall A. Restriction fragments length polymorphism analysis of *Cryptococcus neoformans* isolates from environmental (pigeons excreta) and clinical sources in New York City. J Clin Microbiol 1994;32:1188–92.
16. Spitzer SG, Spitzer ED. Characterization of the CNRE-1 family of repetitive the DNA elements in *Cryptococcus neoformans*. Gene 1994;144:103–6.
17. Varma A, Kwon-Chung KJ, DNA probes for strain typing of *Cryptococcus neoformans*. J Clin Microbiol 1992;30:2960–7.
18. Ellis D, Marriott D, Hajjeh RA, Warnock D, Meyer W, Barton R. Epidemiology: surveillance of fungal infections. Med Mycol 2000;38:173–82.
19. Meyer W, Kidd S, Castañeda A, Jackson S, Huynh M, Latouche GN, et al. Global molecular epidemiology offers hints towards ongoing speciation within *Cryptococcus neoformans*. In: Abstracts of the 5th International Conference on *Cryptococcus* and Cryptococcosis, Adelaide, Australia, March 3–7, 2002. Adelaide: South Australian Postgraduate Medical Education Association; 2002.
20. Kwon-Chung KJ, Bennet JE. Medical mycology. Philadelphia: Lea & Febiger Press; 1992. p. 397–446.
21. Kwon-Chung KJ, Polacheck I, Bennet JE. Improved diagnostic medium for separation of *Cryptococcus neoformans* var. *neoformans* (serotype A and D) and *Cryptococcus neoformans* var. *gattii* (serotype B and C). J Clin Microbiol 1982;15:535–7.
22. Vassart G, Georges M, Monsieur R, Brocas H, Lequarre AN, Christophe D. A sequence in M13 phage detects hypervariable minisatellites in human and animal DNA. Science 1986;246:683–4.
23. Boekhout T, Theelen B, Diaz M, Fell JW, Hop WCJ, Abeln ECA, et al. Hybrid genotypes in the pathogenic yeast *Cryptococcus neoformans*. Microbiology 2001; 147:891–907.
24. Cogliati M, Allaria M, Liberi G, Tortorano AM, Viviani MA. Sequence analysis and ploidy determination of *Cryptococcus neoformans*. J Mycol Méd 2000;10:171–6.
25. Franzot SP, Hamdan JS, Currie BP, Casadevall A. Molecular epidemiology of *Cryptococcus neoformans* in Brazil and the United States: evidence for both local genetic differences and a global clonal population structure. J Clin Microbiol 1997:35:2243–51.
26. Drommer F, Mathoulin S, Dupont B, Laporte A. Epidemiology of cryptococcosis in France: a 9 year survey (1985–1993). Clin Infect Dis 1996;23:82–90.
27. Viviani MA, Wen H, Roverselli A, Calderelli-Stefano R, Cogliati M, Ferrante P, et al. Identification by polymerase chain reaction fingerprinting of *Cryptococcus neoformans* serotype AD. Journal of Medical and Veterinary Mycology 1997;35:355–60.
28. Tortorano AM, Viviani MA, Rigoni AL, Cogliati M, Roverselli A, Pagano A. Prevalence of serotype D in *Cryptococcus neoformans* isolates from HIV positive and HIV negative patients in Italy. Mycoses 1997;40:297–302.
29. Johnston RF. Birds of North America. no 13. Philadelphia: The American Ornithologists Union and the Academy of natural Sciences of Philadelphia; 1992.
30. Chen S, Sorrell T, Nimmo G, Speed B, Currie BJ, Marriott D, et al. Epidemiology and host- and variety-dependent characterisation of infection due to *Cryptococcus neoformans* in Australia and New Zealand. Clin Infect Dis 2000;31:499–508.
31. Hajjeh RA, Conn LA, Stephens DS Baughman W, Hamill R, Graviss E, et al. Cryptococcosis: population-based multistate active surveillance and risk factors in human immunodeficiency virus-infected persons. J Infect Dis 1999;179:449–54.

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Appendix Table 1. *Cryptococcus neoformans* clinical, human, and veterinary isolates studied by country, institution characteristics, variety (serotype), and molecular type as identified by PCR fingerprinting with the primer M13 and *URA5* RFLP analysis[a]

LA code	Original code	Year	Original city	Sample	Gender	Age	Risk factor	Variety (serotype)	Molecularar type
Argentina									
Hospital Muñiz, Buenos Aires—Human Isolates									
LA 286#	135773	1999	Tucumán	CSF	F	25	HIV+	*grubii*	VNI
LA 28#	135868	1999	Buenos Aires	CSF	F	24	HIV+	*grubii*	VNI
LA 288#	136725	1999	Buenos Aires	CSF	M	31	HIV+	*grubii*	VNI
LA 289#	137235	1999	Buenos Aires	Blood	F	36	HIV+	*grubii*	VNI
LA 290#	136353	1999		CSF	M	50	PCM	*gattii*	VGIII
LA 291#	137965	1999	Córdoba	Urine	M	64	Mielodysplasia	*grubii*	VNI
LA 292#	144408	2000	Buenos Aires	Blood	M	28	HIV+	*grubii*	VNI
LA 293#	146323	2000	Buenos Aires	Blood	M	28	HIV+	*grubii*	VNI
LA 294#	146783	2000	Buenos Aires	CSF	M	28	HIV+	*grubii*	VNI
LA 295	143839	2000	Chaco	CSF	M	40	none	*gattii*	VGII
LA 296#	139856	1999	Buenos Aires	Node	M	35	HIV+	*grubii*	VNI
LA 297#	142181	1999	Buenos Aires	Blood	M	31	HIV+	*grubii*	VNI
LA 298#	136105	1999	Peru-Buenos Aires	Blood	M	40	HIV+	*grubii*	VNII
LA 299#	138493	1999	Buenos Aires	Urine	M	30	HIV+	*grubii*	VNI
LA 300#	138925	1999	Buenos Aires	CSF	M	31	HIV+	*grubii*	VNI
LA 301#	137154	1999	Buenos Aires	Bone marrow	M	37	HIV+	*grubii*	VNI
LA 302#	137141	1999	Buenos Aires	Blood	M	37	HIV+	*grubii*	VNI
LA 303#	137182	1999	Buenos Aires	Blood	M	25	HIV+	*grubii*	VNI
LA 304#	137921	1999	Buenos Aires	CSF	M	25	HIV+	*grubii*	VNI
LA 305#	145554	1999	Buenos Aires	Blood	M	32	HIV+	*grubii*	VNI
LA 306#	145535	1999	Uruguay- Buenos Aires	CSF	M	35	HIV+	*grubii*	VNI
LA 307#	145469	1999	Buenos Aires	Skin	M	49	transplant	*grubii*	VNI
LA 308#	145516	1999	Buenos Aires	Blood	M	30	HIV+	*grubii*	VNI
LA 309#	137278	1999	Adrogue	CSF	M	20	HIV+	*grubii*	VNI
LA 310#	140086	1999	Buenos Aires	CSF	F	29	HIV+	*grubii*	VNI
LA 311#	135610	1999	Buenos Aires	CSF	M	33	HIV+	*Hybrid*	VNIII*
LA 312#	138860	1999	Buenos Aires	CSF	F	31	HIV+	*grubii*	VNI
LA 313#	138205	1999	Buenos Aires	CSF	M	37	HIV+	*grubii*	VNI
LA 314#	138984	1999	Pergamino	CSF	M	23	HIV+	*grubii*	VNI
LA 315#	140102	1999	Buenos Aires	CSF	M	26	HIV+	*grubii*	VNI
LA 316#	137939	1999	Buenos Aires	CSF	F	34	HIV+	*grubii*	VNI
LA 318#	138027	1999	Buenos Aires	BAL	M	23	none	*grubii*	VNI
LA 319#	144687	2000	Buenos Aires	CSF	F	51	HIV+	*grubii*	VNI
LA 320#	144603	2000	Buenos Aires	Skin	F	50	HIV+	*grubii*	VNI
LA 321#	137332	1999	Buenos Aires	CSF	M	55	HIV+	*Hybrid*	VNIII*
LA 322#	135988	1999	Buenos Aires	Blood	M	54	HIV+	*grubii*	VNI
LA 323#	135887	1999	Buenos Aires	Blood	F	46	HIV+	*grubii*	VNI
LA 324#	136639	1999	Buenos Aires	CSF	M	29	HIV+	*grubii*	VNII
Departamento Micología, INEI, ANLIS “Dr C.G. Malbran,” Buenos Aires—Human Isolates									
LA 423#	00-381	2000	Buenos Aires	BAL	M	32	ISP	*grubii*	VNI

LA 424#	00-345	2000	Jujuy	CSF			HIV+	*grubii*	VNI
LA 425#	00-343	2000	Buenos Aires	CSF	M	58	ND	*grubii*	VNII
LA 426#	00-284	2000	Misiones	CSF	M	32	HIV+	*grubii*	VNI
LA 427#	00-236	2000	Mendoza	CSF			HIV+	*grubii*	VNI
LA 428#	00-364	2000	Buenos Aires	CSF	M	20	HIV+	*grubii*	VNII
LA 429#	962279	1996	Buenos Aires	CSF			HIV+	*grubii*	VNI
LA 430	962278	1996	Buenos Aires	CSF			HIV+	*grubii*	VNI
LA 431#	961935	1996	Buenos Aires	CSF			HIV+	*grubii*	VNI
LA 432#	972724	1998	Buenos Aires	CSF			HIV+	*grubii*	VNI
LA 433#	972756	1997	Buenos Aires	CSF	M	35	HIV+	*grubii*	VNI
LA 434#	982808	1998	Buenos Aires	CSF	M	30	HIV+	*grubii*	VNI
LA 435#	983036	1998	Buenos Aires	CSF	M		HIV+	*grubii*	VNI
LA 436#	983039	1998	Buenos Aires	CSF	M		HIV+	*grubii*	VNII
LA 437#	993126	1999	Buenos Aires	CSF	M	31	HIV+	*grubii*	VNI
Brazil									
Fundaçao Oswaldo Cruz, Rio de Janeiro—Human Isolates									
LA 55#	417	1995	Piaui State	CSF	M			*gattii*	VGII
LA 57	506	1995	Piaui State	CSF	M			*gattii*	VGII
LA 58#	512	1995	Piaui State	CSF	F			*grubii*	VNI
LA 59#	513	1995	Piaui State	CSF	M			*grubii*	VNI
LA 61#	557	1997	Piaui State	CSF	F		HIV+	*gattii*	VGII
LA 63#	567	1997	Piaui State	CSF	M			*grubii*	VNI
Universidade Estadual Paulista, Araraquara—Human Isolates									
LA 346#	481(5)	1995	São José do Rio Preto	CSF	F	24	HIV+	*grubii* (A)	VNI
LA 347#	482(5)	1995	São José do Rio Preto	CSF	F	24	HIV+	*grubii* (A)	VNI
LA 348	540(9)	1995	São José do Rio Preto	CSF	M	26	HIV+	*grubii* (A)	VNI
LA 349#	541(9)	1995	São José do Rio Preto	CSF	M	26	HIV+	*grubii* (A)	VNI
LA 350#	14(9)	1996	São José do Rio Preto	CSF	M	26	HIV+	*grubii* (A)	VNI
LA 351	13(11)	1996	São José do Rio Preto	CSF	M	26	HIV+	*grubii* (A)	VNI
LA 352#	49(11)	1996	São José do Rio Preto	CSF	M	26	HIV+	*grubii* (A)	VNI
LA 353#	98(17)	1996	São José do Rio Preto	CSF	M	40	HIV+	*grubii* (A)	VNI
LA 354#	14(17)	1996	São José do Rio Preto	CSF	M	40	HIV+	*grubii* (A)	VNI
LA 355#	221(23)	1996	São José do Rio Preto	CSF	M	42	HIV+	*grubii* (A)	VNI
LA 356#	18(23)	1997	São José do Rio Preto	CSF	M	42	HIV+	*grubii* (A)	VNI
LA 357#	222(24)	1996	São José do Rio Preto	CSF	M	30	HIV+	*grubii* (A)	VNI
LA 358	65(24)	1997	São José do Rio Preto	CSF	M	22	HIV+	*grubii* (A)	VNI
LA 359#	62(27)	1997	São José do Rio Preto	CSF	M	29	HIV+	*grubii* (A)	VNI
LA 360#	66(27)	1997	São José do Rio Preto	CSF	M	29	HIV+	*grubii* (A)	VNI
LA 361#	67(27)	1997	São José do Rio Preto	CSF	M	30	HIV+	*grubii* (A)	VNI
LA 363#	1342	1993	Araraquara	CSF	M	27	HIV+	*grubii* (A)	VNI
LA 364#	1755	1997	Araraquara	CSF	F	20	HIV+	*grubii* (A)	VNI
LA 365#	1920	1998	Araraquara	CSF	M	64	HIV+	*grubii* (A)	VNI
LA 366#	2177	2000	Araraquara	CSF	M	50	HIV+	*grubii* (A)	VNI
Universidade Estadual Paulista, Araraquara - Veterinary Isolates									
LA 362 #	Bird1	2000	Jaboticabal	Parrot liver				*gattii* (B)	VGII
Instituto Adolfo Lutz, São Paulo—Human Isolates									
LA 32#	LCS-534		São Paulo	CSF	M	30	HIV+	*grubii*	VNI
LA 328#	GFR-1505	1997	São Paulo	CSF	F	30	HIV+	*grubii*	VNI

LA 329#	AFG-34	2000	São Paulo	CSF	M	29	HIV+	*grubii*	VNI
LA 330#	AFG-14	2000	São Paulo	CSF	M	30	HIV+	*grubii*	VNI
LA 331#	CMS-1226	1996	São Paulo	CSF	M	28	HIV+	*grubii*	VNI
LA 332#	AFG-25/00	2000	São Paulo	CSF	M	35	HIV+	*grubii*	VNI
LA 333#	PRL-1133	1996	São Paulo	CSF	M	40	HIV+	*grubii*	VNI
LA 334#	JCJ-1157	1996	São Paulo	CSF	M	32	HIV+	*grubii*	VNI
LA 335#	MAAC-1866	1996	São Paulo	CSF	M	36	HIV+	*grubii*	VNI
LA 336#	VPD-25	1996	São Paulo	CSF	F	28	HIV+	*grubii*	VNI
LA 337#	AFG-09/00	1996	São Paulo	CSF	M	30	HIV+	*gattii*	VGII
LA 338#	AFG-15/00	2000	São Paulo	CSF	M	30	HIV+	*gattii*	VGII
LA 339#	LCS-394	1996	São Paulo	CSF	M	31	HIV+	*grubii*	VNI
LA 340#	IFOC-1446	1996	São Paulo	CSF	M	36	HIV+	*grubii*	VNI
LA 341#	MO-438	1996	São Paulo	CSF	M	34	HIV+	*grubii*	VNI
LA 342#	KSO-948	1996	São Paulo	CSF	M	23	HIV+	*grubii*	VNI
LA 343#	CSC-1016	1996	São Paulo	CSF	M	34	HIV+	*grubii*	VNII
LA 344#	MDS-949	1996	São Paulo	CSF	M	32	HIV+	*grubii*	VNI
LA 345#	MEM-1064	1996	São Paulo	CSF	M	33	HIV+	*grubii*	VNI
Centro de Biotechnologia, Porto Alegre—Human Isolates									
LA 755	HC2	2000	Porto Alegre	CSF	M	38	HIV+	*grubii* (A)	VNI
LA 756	HC3	2000	Porto Alegre	CSF	M	38	HIV+	*grubii* (A)	VNII
LA 757	HC4	2000	Porto Alegre	CSF	F	36	HIV+	*grubii* (A)	VNI
LA 758	HC6	2000	Porto Alegre	CSF	M	46	HIV+	*grubii* (A)	VNI
LA 759	HC8	2000	Porto Alegre	CSF	M	40	HIV+	*grubii* (A)	VNI
LA 760	HC9	2000	Porto Alegre	CSF	M	33	HIV+	*grubii* (A)	VNI
LA 761	HC10	2000	Porto Alegre	CSF	M	29	HIV+	*grubii* (A)	VNI
LA 762	HC11	2000	Porto Alegre	CSF	F	50	HIV+	*grubii* (A)	VNI
LA 763	HC13	2000	Porto Alegre	CSF	M	42	HIV+	*grubii* (A)	VNI
LA 764	HC14	2000	Porto Alegre	CSF	M	29	HIV+	*grubii* (A)	VNI
LA 765	HC15	2000	Porto Alegre	CSF	F	55	HIV+	*grubii* (A)	VNI
Chile									
Universidad de Chile, Santiago de Chile—Human Isolates									
LA 256#	CR-01	1990	Santiago	Blood	M		HIV+	*neoformans*	VNIV
LA 258	CR-03	1991	Santiago	CSF	M		HIV+	Hybrid	VNIII*
LA 259#	CR-06	1991	Santiago	Blood	M		HIV+	*neoformans*	VNIV
LA 260	CR-10	1995	Santiago	Blood	M		HIV+	Hybrid	VNIII
LA 262#	CR-12	1997	Santiago	Blood	M	33	HIV+	*neoformans*	VNIV
LA 263	CR-16	1998	Santiago	CSF	M		HIV+	Hybrid	VNIII
LA 264#	CR-21	1997	Santiago	Pleural fluid	M		ND	*grubii*	VNI
LA 267#	CR-26	2000	Santiago	Abscess	M		HIV+	*grubii*	VNII
LA 268#	CR-27	2000	Santiago	Blood	M		HIV+	*neoformans*	VNIV
LA 269#	CR-29	2000	Santiago	BAL	M	57	HIV+	*grubii*	VNI
LA 270#	CR-30	2000	Santiago	Blood	M		HIV+	*grubii*	VNI
LA 271#	CR-31	2000	Santiago	Blood	M		HIV+	*grubii*	VNI
LA 272#	CR-34	2000	Santiago	CSF	M	29	HIV+	*neoformans*	VNIV
LA 273#	CR-35	2000	Viña del Mar	CSF	M	35	HIV+	*grubii*	VNII
LA 274#	CR-36	2000	Santiago	CSF	M		ND	*grubii*	VNII
Colombia									
Instituto Nacional de Salud, Bogotá—Human Isolates									

LA 214#	H0058-I-138	1991	Tolima	CSF	F	4	None	*grubii* (A)	VNI
LA 461#	H0058-I-34	1986	Casanare	CSF	F	16	None	*gattii* (B)	VGII
LA 469#	H0058-I-43	1987	Cundinamarca	CSF	M	67	ND	*grubii* (A)	VNII
LA 472#	H0058-I-50	1988	Antioquia	CSF	F		HIV+	*grubii* (A)	VNI
LA 473#	H0058-I-57	1988	Bogotá	CSF	M	58	None	*grubii* (A)	VNI
LA 478#	H0058-I-62	1988	Bogotá	CSF	F	16	Lupus	*grubii* (A)	VNI
LA 479#	H0058-I-64	1988	Huila	CSF	M	33	None	*grubii* (A)	VNI
LA 481#	H0058-I-67	1989	N. Santander	CSF	M	46	None	*grubii* (A)	VNI
LA 487#	H0058-I-78	1989	Arauca	CSF	M	43	None	*gattii* (C)	VGIII
LA 495	H0058-I-96	1990	Cundinamarca	CSF	M		None	*grubii* (A)	VNI
LA 498	H0058-I-103	1990	Antioquia	CSF	M	65	None	*grubii* (A)	VNI
LA 499#	H0058-I-106	1990	N. Santander	CSF	M	44	None	*gattii* (B)	VGII
LA 506#	H0058-I-160	1992	Tolima	CSF	M	28	None	*grubii* (A)	VNI
LA 511	H0058-I-190	1992	Bogotá	CSF	M	34	HIV+	*grubii* (A)	VNII
LA 516#	H0058-I-212	1993	N. Santander	CSF	M	54	None	*gattii* (B)	VGII
LA 518#	H0058-I-229	1993	Bogotá	CSF	M	33	HIV+	*grubii* (A)	VNI
LA 521#	H0058-I-232	1993	Bogotá	CSF	M	30	HIV+	*grubii* (A)	VNI
LA 523#	H0058-I-238	1994	Bogotá	CSF	M	42	HIV+	*grubii* (A)	VNI
LA 525	H0058-I-246	1994	Bogotá	CSF	M	65	None	*grubii* (A)	VNI
LA 550#	H0058-I-471	1996	Nariño	CSF	M	35	HIV+	*grubii* (A)	VNI
LA 552#	H0058-I-473	1997	Bogotá	CSF	M	40	HIV+	*grubii* (A)	VNI
LA 564	H0058-I-628	1997	Bogotá	CSF	M	29	None	*gattii* (B)	VGI
LA 567#	H0058-I-638	1997	Caquetá	CSF	F	34	None	*gattii* (B)	VGII
LA 568	H0058-I-642	1997	Boyacá	CSF	M	36	None	*gattii* (B)	VGIV
LA 572#	H0058-I-653	1998	Cundinamarca	CSF	M	57	HIV+	*grubii* (A)	VNII
LA 584#	H0058-I-675	1998	Bolivar	CSF	M	23	None	*gattii* (B)	VGII
LA 590#	H0058-I-762	1998	Santander	CSF	M	48	None	*gattii* (B)	VGII
LA 593#	H0058-I-789	1998	Bogotá	CSF	M	23	HIV+	*neoformans* (D)	VNIV
LA 595#	H0058-I-828	1998	Bogotá	CSF	M	52	HIV+	*grubii* (A)	VNII
LA 599#	H0058-I-881	1999	N. Santander	CSF	M	33	None	*gattii* (B)	VGII
LA 602#	H0058-I-889	1999	Bogotá	CSF	M	42	HIV+	*grubii* (A)	VNII
LA 603#	H0058-I-902	1999	Arauca	CSF	M	50	None	*grubii* (A)	VNII
LA 615#	H0058-I-1068	2000	Antioquia	CSF	M	33	HIV+	*grubii* (A)	VNI
LA 620	H0058-I-1097	2000	Antioquia	BAL	M	19	HIV+	*grubii* (A)	VNII
LA 622#	H0058-I-1134	2000	Risaralda	CSF	F	41	ND	*gattii* (B)	VGIII
LA 628#	H0058-I-1198	2001	Bogotá	CSF	M	23	HIV+	*grubii* (A)	VNI
LA 629#	H0058-I-1199	2001	Bogotá	CSF	M	43	HIV+	*grubii* (A)	VNI
LA 752	H0058-I-1276	2001	N. Santander	CSF	M	29	None	*gattii* (C)	VGIII
LA 753#	H0058-I-1278	2001	N. Santander	CSF	M	39	None	*gattii* (B)	VGII
Guatemala									
Hospital San Juan de Dios and Universidad de San Carlos, Guatemala—Human Isolates									
LA 737#	134	2000	Guatemala City	CSF	F		HIV+	*grubii* (A)	VNI
LA 738#	188	2000	Guatemala City	CSF	M	57	HIV+	*grubii* (A)	VNI
LA 739#	415	2001	Guatemala City	Blood	M		ND	*grubii* (A)	VNI
LA 740#	600	2000	Guatemala City	CSF	M	53	HIV+	*grubii* (A)	VNI
LA 741	C.9							*grubii* (A)	VNI
LA 742#	C.9.4.67.BM(1)							*grubii* (A)	VNI
LA 743	C.9.7.17.G2(1)							*grubii* (A)	VNI

LA 744	C.9.98RM3-6							*grubii* (A)	VNI
LA 745	C.9.10.11.BG(2)							*grubii* (A)	VNI
LA 746	C.9.11.60AG1(1)							*grubii* (A)	VNI
LA 747	2591	2000		CSF	M		ND	*grubii* (A)	VNI
LA 748	3308	2000		CSF	M		ND	*grubii* (A)	VNI
LA 749#	3417	2000					ND	*grubii* (A)	VNI
LA 750#	2361	2000			F	14	ND	*gattii*	VGIII
LA 751#	193	2000		CSF	M	57	HIV+	*grubii* (A)	VNI
Mexico									
Universidad Nacional Autonoma de Mexico, Mexico City—Human Isolates									
LA 1#	116	1994	Mexico City	CSF	M	19	None	*gattii* (B)	VGI
LA 2#	117	1994	Mexico City	CSF	M	35	Tumor	*gattii* (B)	VGI
LA 3	122	1986	Mexico City	CSF	F	10	None	*gattii* (B)	VGIII
LA 4#	174	1996	Texcoco, Edo	CSF	F	15	IMS	*grubii*	VNI
LA 5	190	1995	Mexico City	CSF	F	45	None	*gattii*	VGIII
LA 6#	194	1995	Mexico City	CSF	M	27	HIV+	*grubii*	VNI
LA 7#	196	1995	Mexico City	CSF	M	27	HIV+	*grubii*	VNI
LA 8#	202	1996	Mexico City	CSF	M	38	HIV+	*grubii*	VNI
LA 9#	204	1996	Mexico City	CSF	M	39	None	*gattii*	VGI
LA 10	205	1996	Mexico City	CSF	M	67	None	*grubii*	VNI
LA 11#	207	1996	Mexico City	CSF	M	33	HIV+	*grubii*	VNI
LA 12#	209	1996	Mexico City	CSF	M	35	IMS	*grubii*	VNI
LA 13#	216	1996	Mexico City	CSF	M	46	None	*grubii*	VNI
LA 14#	218	1997	Mexico City	CSF	M	47	HIV+	Hybrid	VNIII*
LA 15#	220	1997	Mexico City	CSF	M	34	HIV+	*grubii*	VNI
LA 16#	221	1996	Mexico City	CSF	F	17	None	*gattii*	VGIII
LA 17#	223	1996	Mexico City	CSF	M	27	HIV+	*grubii*	VNI
LA 18#	224	1996	Mexico City	CSF	M	31	HIV+	*gattii*	VGIII
LA 19	225	1997	Mexico City	Blood	M	29	HIV+	*grubii*	VNI
LA 20	227	1997	Mexico City	CSF	M	36	HIV+	*gattii*	VGIII
Instituto Nacional de Diagnostica y Referencia Epidemiologicos, Mexico City—Human Isolates									
LA 390#	5604	1961	Distrito Federal	CSF	M	38	ND	*gattii*	VGIV
LA 391#	5605	1962	Distrito Federal	CSF	M	47	ND	*gattii*	VGIV
LA 392#	5606	1965	Distrito Federal	CSF	M	40	ND	*gattii*	VGIV
LA 393	5607	1965	Distrito Federal	CSF	M	52	ND	*grubii*	VNI
LA 394#	5608	1967	Distrito Federal	CSF	M	41	ND	*grubii*	VNI
LA 396#	5611	1970	Distrito Federal	CSF	M	33	ND	*grubii*	VNI
LA 397#	5613	1973	Distrito Federal	CSF	M	47	ND	*grubii*	VNI
LA 398#	5615	1978	Distrito Federal	Skin	M	59	ND	*grubii*	VNI
LA 399#	5616	1980	Distrito Federal	Lungs, CSF	M	59	ND	*gattii*	VGIII
LA 400#	5617	1980	Distrito Federal	CSF	F	32	ND	*gattii*	VGIII
LA 402#	5619	1982	Distrito Federal	CSF	M	35	ND	*gattii*	VGIII
LA 403	5620	1986	Distrito Federal	CSF	F	11	ND	*gattii*	VGIII
LA 404#	5621	1987	Michoacan	CSF	M	47	ND	*grubii*	VNII
LA 405#	5622	1988	Distrito Federal	CSF	M	43	ND	*grubii*	VNI
LA 406#	5623	1990	Distrito Federal	CSF	M	34	HIV+	*grubii*	VNI
LA 407	5624	1990	Distrito Federal	CSF	M	46	ND	*gattii*	VGIII
LA 408	5625	1991	Distrito Federal	Blood	M	49	ND	*grubii*	VNI

LA 409#	5626	1992	Distrito Federal	CSF	M	53	HIV+	*grubii*	VNI
LA 410#	5627	1992	Distrito Federal	CSF	M	37	HIV+	*grubii*	VNI
LA 411	5628	1991	Distrito Federal	CSF	M	29	ND	*gattii*	VGIII
LA 412#	5629	1997	Distrito Federal	CSF	M	58	ND	*gattii*	VGIII
LA 413#	5630	1998	Distrito Federal	CSF	M	30	HIV+	*grubii*	VNI
LA 414#	5631	1999	Distrito Federal	CSF	M	28	ND	*gattii*	VGIII
LA 415#	5632	1999	Distrito Federal	CSF	M	36	ND	*grubii*	VNI
LA 416#	5633	1999	Distrito Federal	CSF	M	42	HIV+	*grubii*	VNI
LA 417#	5634	1999	Distrito Federal	CSF	M	33	HIV+	*grubii*	VNI
Peru									
Instituto de Medicina Tropical Alexander von Humboldt, Lima—Human Isolates									
LA 153#	39401/U	1998	Lima	Urine	M	27	HIV+	*grubii*	VNI
LA 154#	39401/S	1998	Lima	Sputum	M	27	HIV+	*grubii*	VNI
LA 155#	39401/B	1998	Lima	Blood	M	27	HIV+	*grubii*	VNI
LA 156	39407/CSF	1998	Lima	CSF	M	30	HIV+	*grubii*	VNI
LA 157#	39407/B	1998	Lima	Blood	M	30	HIV+	*grubii*	VNI
LA 158#	39408/CSF	1998	Lima	CSF	M	22	HIV+	*grubii*	VNI
LA 159#	39408/U	1998	Lima	Urine	M	22	HIV+	*grubii*	VNI
LA 160#	39408/B	1998	Lima	Blood	M	22	HIV+	*grubii*	VNI
LA 162#	39410/U	1998	Lima	Urine	F	36	HIV+	*grubii*	VNI
LA 163#	39410/B	1998	Lima	Blood	F	36	HIV+	*grubii*	VNI
LA 164	39410/BM	1998	Lima	Blood marrow	F	36	HIV+	*grubii*	VNI
LA 169	8640	1988	Lima	Abscesses	M	66	None	*grubii*	VNI
LA 170#	15335	1993	Lima	CSF	M	36	None	*gattii*	VGI
Spain									
GREMEC, IMM/UAB, Barcelona—Human Isolates									
LA 180#	154c	1995	Barcelona	Blood	F		HIV+	Hybrid (A, AD)	VNIII
LA 181#	155c	1995	Barcelona	CSF	M		HIV+	Hybrid (A, AD)	VNIII
LA 182#	156c	1995	Barcelona	Blood	M		HIV+	*grubii* (A)	VNI
LA 183#	1c	1994	Valencia	CSF	M		HIV+	Hybrid (AD, D)	VNIII*
LA 184#	2c	1994	Valencia	CSF	F		HIV+	Hybrid (AD, D)	VNIII*
LA 185#	3c	1994	Valencia	CSF	M		HIV+	Hybrid (AD, D)	VNIII**
LA 186#	73c	1994	Sevilla	CSF	M		HIV+	Hybrid (AD)	VNIII
LA 187#	74c	1995	Sevilla	CSF	M		HIV+	*grubii* (A)	VNI
LA 188#	75c	1995	Sevilla	CSF	M		HIV+	Hybrid (AD)	VNIII*
GREMEC, IMM/UAB, Barcelona—Veterinary Isolates:									
LA 174#	48 A	1995	Cáceres	Goat				*gattii* (B)	VGI
LA 175#	50 A	1995	Cáceres	Goat				*gattii* (B)	VGI
LA 176#	52 A	1995	Cáceres	Goat				*gattii* (B)	VGI
LA 177#	54 A	1995	Cáceres	Goat				*gattii* (B)	VGI
LA 178#	56 A	1995	Cáceres	Goat				*gattii* (B)	VGI
LA 179#	58 A	1995	Cáceres	Goat				*gattii* (B)	VGI
Venezuela									
Universidad del Zulia, Maracaibo—Human Isolates									
LA 367#	BVC125-96	1996	Caracas	ND	F	36	HIV+	*grubii* (A)	VNII
LA 368#	BV1742	1996	Caracas	CSF	M	66	leukemia	*grubii* (A)	VNI
LA 369#	BV2086	1996	Caracas	CSF	M	34	HIV+	*grubii* (A)	VNI
LA 370#	BV2098	1996	Caracas	CSF	M	28	HIV+	*grubii* (A)	VNI

LA 371#	BV2189	1996	Caracas	CSF	M	26	HIV+	*grubii* (A)	VNI
LA 372#	BV2198	1996	Caracas	CSF	M	Adult	HIV+	*grubii* (A)	VNI
LA 373#	BV2199	1996	Caracas	CSF	M	34	HIV+	*grubii* (A)	VNI
LA 374#	BV2244	1997	Caracas	CSF	M	Adult	HIV+	*grubii* (A)	VNI
LA 375#	BV2293	1997	Caracas	CSF	M	Adult	HIV+	*grubii* (A)	VNI
LA 376#	BV2379	1997	Caracas	CSF	M	Adult	HIV+	*grubii* (A)	VNI
LA 377	BV2450	1997	Maracaibo	CSF	M	Adult	HIV+	*grubii* (A)	VNI
LA 379	BV1	1995	Maracaibo	CSF	M	28	HIV+	*grubii* (A)	VNI
LA 380	BV2	1995	Maracaibo	CSF	M	19	HIV+	*grubii* (A)	VNI
LA 381#	BV5	1996	Maracaibo	CSF	M	30	None	*gattii* (B)	VGII
LA 382#	BV19	1997	Maracaibo	CSF	M	32	None	*gattii* (C)	VGIII
LA 383#	BV201A	1997	Maracaibo	CSF	M	41	Transplant	*grubii* (A)	VNI
LA 384	BV170cli	1999	Maracaibo	CSF	M	73	None	*grubii*	VNI
LA 385#	BV1118	1999	Maracaibo	CSF	M	30	None	*grubii*	VNI
LA 386#	BV1218	1999	Maracaibo	CSF	F	42	MS	*gattii*	VGII
LA 387#	BV1297	1999	Maracaibo	CSF	F	25	Diabetes	*gattii*	VGII

[a]PCR, polymerase chain reaction; RFLP, restriction fragment length polymorphism; GREMEC, IMM/UAB,;CSF, cerebrospinal fluid; F, female; M, male; BAL, bronchoalveolar lavage; ISP, immunosupresed; MS, multiple sclerosis; ND, no data; VNIII, seven-band *URA5* RFLP pattern; VNIII*, six-band *URA5* RFLP pattern; *, strains included in the Gelcompar*II* analysis.

Appendix Table 2. *Cryptococcus neoformans* environmental isolates studied by country, institution characteristics, variety (serotype) and molecular type as identified by PCR-fingerprinting with the primer M13 and *URA*5 RFLP analysis[a]

LA Code	Original code	Year	Original city	Sample	Variety (serotype)	Molecular type
Argentina						
Departamento Micología, INEI, ANLIS "Dr C.G. Malbran," Buenos Aires						
LA 438#	Cr34Eb	2000	Buenos Aires	Trees	grubii	VNI
LA 440#	CrA29	2000	Buenos Aires	Trees	*grubii*	VNI
LA 441#	Cr10	2000	Buenos Aires	Trees	*gattii*	VGI
LA 442#	CrA32	2000	Buenos Aires	Trees	*grubii*	VNI
Brazil						
Fundaçao Oswaldo Cruz, Rio de Janeiro						
LA 44#	414	1995	Piaui State	Pottery tree hollow	*gattii* (B)	VGII
LA 45#	415	1995	Piaui State	Pink shower tree hollow	*gattii* (B)	VGII
LA 47#	434	1996	Piaui State	*E. camaldulensis* debris	*gattii* (B)	VGII
LA 52#	561	1997	Piaui State	Pink shower tree hollow	*grubii*	VNI
LA 54	564	1997	Piaui State	Pink shower tree hollow	*grubii*	VNI
LA 419#	WM1012	2000	Rio de Janeiro	Pink shower tree 4, large hollow	*grubii*	VNI
LA 420#	WM1013	2000	Rio de Janeiro	Pink shower tree 4, large hollow	*grubii*	VNI
LA 421#	WM1014	2000	Rio de Janeiro	Pink shower tree 4, large hollow	*grubii*	VNI
LA 422#	WM1015	2000	Rio de Janeiro	Pink shower tree 4, large hollow	*grubii*	VNI
Chile						
Universidad de Chile, Santiago de Chile						
LA 276#	04-A	1993	Santiago	Bird droppings	*grubii*	VNI
LA 277#	06-A	1993	Santiago	Bird droppings	*grubii*	VNI
LA 281	21-A	1993	Santiago	Bird droppings	*grubii*	VNI
LA 284#	52-A	1993	Santiago	Bird droppings	*grubii*	VNI
Colombia						
Instituto Nacional de Salud, Bogota						
LA 235#	H0058-I-737	1998	N. Santander	Almond tree (9)	*gattii* (C)	VGIII
LA 241	H0058-I-1014	1999	N. Santander	Almond tree (20)	*gattii* (C)	VGIII
LA 631#	H0058-I-556	1997	N. Santander	Almond tree (9)	*gattii* (C)	VGIII
LA 645#	H0058-I-607	1997	N. Santander	Almond tree (9)	*grubii*	VNI
LA 651	H0058-I-613	1997	N. Santander	Almond tree (9)	*gattii* (C)	VGIII
LA 658#	H0058-I-679	1998	N. Santander	Almond tree (9)	*grubii* (A)	VNI
LA 659	H0058-I-682	1998	N. Santander	Almond tree (21)	*gattii* (C)	VGIII
LA 667	H0058-I-755	1998	N. Santander	Almond tree (9)	*gattii* (C)	VGIII
LA 672	H0058-I-798	1998	N. Santander	Almond tree (9)	*gattii* (C)	VGIV
LA 674	H0058-I-818	1998	N. Santander	Almond tree (9)	*gattii* (C)	VGIII
LA 676	H0058-I-820	1998	N. Santander	Almond tree (9)	*gattii* (C)	VGIII
LA 680	H0058-I-826	1998	N. Santander	Almond tree (9)	*gattii* (C)	VGIII
LA 684#	H0058-I-908	1998	N. Santander	Almond tree (9)	*gattii* (C)	VGIII
LA 688	H0058-I-548	1996	N. Santander	House patient 3	*grubii* (A)	VNI
LA 691#	H0058-I-551	1996	N. Santander	Older oiti tree	*grubii* (A)	VNI
LA 706#	H0058-I-587	1996	N. Santander	Oiti tree	*grubii* (A)	VNI
LA 710#	H0058-I-595	1996	N. Santander	Acacia tree (1)	*grubii* (A)	VNI

LA 714	H0058-I-599	1997	N. Santander	Acacia tree (6)	*gattii*	VGIII
LA 720#	H0058-I-735	1998	Bogotá	*Eucalyptus* tree (13)	*grubii* (A)	VNI
LA 726#	H0058-I-745	1997	N. Santander	Cuji tree	*grubii* (A)	VNI
LA 731	H0058-I-752	1997	N. Santander	Seed of almond tree	*grubii* (A)	VNI
LA 733#	H0058-I-757	1997	N. Santander	Cuji, tree	*gattii*	VGIII
LA 736	H0058-I-857	1998	Bogotá	*Eucalyptus* tree	*grubii* (A)	VNI
Mexico						
Universidad Nacional Autonoma de Mexico, Mexico City						
LA 21#	88	1994	Mexico City	Green parrot droppings	*grubii*	VNI
LA 22#	89	1994	Mexico City	Budgerigar droppings	*grubii*	VNI
LA 23#	90	1994	Mexico City	Budgerigar droppings	*grubii*	VNI
LA 24#	146	1995	Mexico City	Pigeon droppings	*grubii*	VNI
LA 25#	147	1995	Mexico City	Pigeon droppings	*grubii*	VNI
LA 26#	148 A	1995	Mexico City	Pigeon droppings	*grubii*	VNI
LA 27#	148 B	1995	Mexico City	Pigeon droppings	*grubii*	VNI
LA 28#	149	1995	Mexico City	Pigeon droppings	*grubii*	VNI
LA 29#	150 A	1995	Mexico City	Pigeon droppings	*grubii*	VNI
LA 30#	150 B	1995	Mexico City	Pigeon droppings	*grubii*	VNI
LA 31#	151	1995	Mexico City	Pigeon droppings	*grubii*	VNI
LA 32#	152	1995	Mexico City	Pigeon droppings	*grubii*	VNI
LA 33	164	1995	Mexico City	Pigeon droppings	*grubii*	VNI
LA 34#	165	1995	Mexico City	Pigeon droppings	*grubii*	VNI
LA 35#	168	1995	Mexico City	Pigeon droppings	*grubii*	VNI
LA 36#	170	1995	Mexico City	Pigeon droppings	*grubii*	VNI
LA 37#	230	1998	Edo, Mexico	*E. camaldulensis* (leaves and flowers)	*grubii*	VNI
LA 38#	234	1998	Edo, Mexico	*E. camaldulensis* (leaves and flowers)	*grubii*	VNI
LA 39#	235	1998	Edo, Mexico	*E. camaldulensis* (leaves and flowers)	*grubii*	VNI
LA 40#	237	1998	Edo, Mexico	*E. camaldulensis* (leaves and flowers)	*grubii*	VNI
LA 41#	238	1998	Edo, Mexico	*E. camaldulensis* (leaves and flowers)	*grubii*	VNI
LA 42	239	1998	Edo, Mexico	*E. camaldulensis* (leaves and flowers)	*grubii*	VNI
Instituto Nacional de Diagnostica y Referencia Epidemiologicos, Mexico City						
LA 418#	5635	2000	Distrito Federal	Pigeon excreta	*grubii*	VNI
Spain						
GREMEC, IMM/UAB, Barcelona						
LA 189 #	152 A	1999	Barcelona	Pigeon droppings	*grubii* (A)	VNI
LA 190 #	76 A	1999	Barcelona	Pigeon droppings	*grubii* (A)	VNI
LA 192 #	4 A	1997	Alacant	Pigeon droppings	Hybrid (A)(AD)	VNIII
LA 193 #	6 A	1997	Alacant	Pigeon droppings	*grubii* (A)	VNI

[a]RFLP, restriction fragment length polymorphism; INEI, Instituto Nacional de Enfermedades Infecciosas; ANLIS, Administracion Nacional de Laboratorios e Institutos de Salud; GREMEC, Grup de Recerca en Micologia Experimental i Clinica; IMM, Institut Municipal d' Investigacio Medica; UAB, Universita Autonoma de Barcelona.

[b]VNIII: seven-band *URA5* RFLP pattern;VNIII*, six-band *URA5* RFLP pattern; *, strains included in the Gelcompar*II* analysis