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Assessment of genetic diversity among environmental *Cryptococcus* species using RAPD analysis

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Abstract:

Background: *Cryptococcus* species, including *C. neoformans* and *C. gattii*, are environmental yeasts of clinical importance and major agents of cryptococcosis. Environmental reservoir represents the primary source of human and animal infections therefore, characterization of genetic diversity is essential for epidemiological surveillance and understanding the pool of genotypes from which pathogenic strains may arise.
Methodology: This study evaluated the genetic diversity of 18 environmental *Cryptococcus* isolates recovered from soil samples using random amplified polymorphic DNA (RAPD) analysis. Seven primer combinations (three 20–22 mer and four 12-mer) were employed to generate reproducible banding patterns, and genetic diversity indices were calculated based on RAPD polymorphic loci.
Results: A total of 214 bands, ranging from 330-3800 bp, were amplified from 17 isolates with 10-13 polymorphic bands per isolate; one isolate failed to amplify with all primers and was excluded from further analysis. The isolates were resolved into five haplotypes (A-E), with haplotype B being most prevalent (61.1%). Primer-specific bands enabled diagnostic differentiation of certain haplotypes, demonstrating the discriminating capacity of the selected RAPD primers. Diversity indices indicated high genetic variability among the soil isolates, with a Shannon–Wiener index (H') of 2.83, Simpson's index of diversity (1-D) of 0.941, and evenness (E) of 0.999.
Conclusion: These results demonstrate substantial genetic heterogeneity among environmental *Cryptococcus* isolates, even within a restricted locale. Although no direct comparisons were made with clinical or animal isolates, the observed diversity highlights the complexity of environmental reservoirs that serve as potential sources of infection. This study underscores the utility of RAPD markers for preliminary molecular characterization and epidemiological studies, providing baseline data relevant to environmental and public health surveillance in Nigeria.

Keywords: *Cryptococcus* Species; Genetic Diversity; RAPD; Polymorphic bands

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Évaluation de la diversité génétique des espèces de *Cryptococcus* environnementales par analyse RAPD

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Résumé:

Contexte: Les espèces de *Cryptococcus*, notamment C. *neoformans* et *C. gattii*, sont des levures environnementales d'importance clinique et des agents majeurs de la cryptococcose. Le réservoir environnemental représente la principale source d'infections humaines et animales. Par conséquent, la caractérisation de la diversité génétique est essentielle à la surveillance épidémiologique et à la compréhension du pool de génotypes à partir duquel des souches pathogènes peuvent émerger.
Méthodologie: Cette étude a évalué la diversité génétique de 18 isolats environnementaux de *Cryptococcus*, obtenus à partir d'échantillons de sol, par analyse RAPD (ADN polymorphe amplifié aléatoirement). Sept combinaisons d'amorces (trois de 20 à 22 nucléotides et quatre de 12 nucléotides) ont été utilisées pour générer des profils de bandes reproductibles, et les indices de diversité génétique ont été calculés à partir des loci

polymorphes RAPD.
Résultats: Au total, 214 bandes, de 330 à 3 800 pb, ont été amplifiées à partir de 17 isolats, avec 10 à 13 bandes polymorphes par isolat. Un isolat n'a pas pu être amplifié avec toutes les amorces et a été exclu des analyses ultérieures. Les isolats ont été classés en cinq haplotypes (A à E), l'haplotype B étant le plus fréquent (61,1 %). Les bandes spécifiques aux amorces ont permis la différenciation diagnostique de certains haplotypes, démontrant ainsi le pouvoir discriminant des amorces RAPD sélectionnées. Les indices de diversité ont révélé une forte variabilité génétique parmi les isolats de sol, avec un indice de Shannon-Wiener (H') de 2,83, un indice de diversité de Simpson (1-D) de 0,941 et un indice d'équitabilité (E) de 0,999.
Conclusion: Ces résultats démontrent une hétérogénéité génétique substantielle parmi les isolats environnementaux de *Cryptococcus,* même au sein d'une zone géographique restreinte. Bien qu'aucune comparaison directe n'ait été effectuée avec des isolats cliniques ou animaux, la diversité observée souligne la complexité des réservoirs environnementaux qui constituent des sources potentielles d'infection. Cette étude met en évidence l'utilité des marqueurs RAPD pour la caractérisation moléculaire préliminaire et les études épidémiologiques, fournissant des données de référence pertinentes pour la surveillance environnementale et de santé publique au Nigéria.

Mots-clés: Espèces de *Cryptococcus*; Diversité génétique; RAPD; Bandes polymorphes

Introduction:

The genus *Cryptococcus* comprises a group of encapsulated, basidiomycetous yeasts of significant medical importance, particularly *Cryptococcus neoformans* and *Cryptococcus gattii* (1,2), which are recognized globally as major etiological agents of cryptococcosis (3, 4). As a consequence of their pathogenic potential and environmental persistence, infections caused by these species manifest clinically as cryptococcosis, a life-threatening systemic fungal disease that primarily affects immuno-compromised individuals, especially patients with HIV/AIDS, organ transplant recipients, and those receiving immunosuppressive therapy (5,6). The disease burden is especially high in sub-Saharan Africa, where cryptococcal meningitis remains a leading cause of AIDS-related mortality (5,7).

Generally, *Cryptococcus* species exhibit remarkable genetic and phenotypic diversity, which contributes to variations in virulence, antifungal susceptibility, environmental adaptation, and geographic distribution (8,9,10). Thus, molecular epidemiological studies have revealed that *C. neoformans* and *C. gattii* are species complexes composed of multiple molecular types and genotypes, and reflect extensive evolutionary divergence (11,12). This pronounced genetic heterogeneity underscores the importance of understanding the population structure, genetic diversity, and evolutionary relationships within and between these species complexes as a basis for epidemiological and ecological investigations rather than direct inference of transmission pathways or outbreak dynamics which is beyond the scope of the present study (13,14).

Traditional phenotypic identification methods, including capsule visualization, biochemical profiling, and serotyping, are limited in their ability to discriminate among closely related *Cryptococcus* strains (3). Consequently, molecular techniques have become indispensable tools for strain differentiation and genetic characterization. Among these techniques, random amplified polymorphic DNA (RAPD) analysis has been widely employed due to its simplicity, cost-effectiveness, and ability to detect genome-wide polymorphisms without prior sequence information (15,16, 17). RAPD analysis relies on the amplification of random DNA segments using short, arbitrary primers under low stringency conditions, generating unique banding patterns that reflect genetic variation among isolates (17,18). In *Cryptococcus*, RAPD has been successfully used to assess genetic diversity, differentiate strains, and infer phylogenetic relationships across clinical, veterinary, and environmental isolates (19,20,21), while this study is providing a comparative and preliminary level of genetic resolution.

The technique has proven particularly useful in resource-limited settings, where access to more advanced genotyping platforms such as multilocus sequence typing (MLST) or whole-genome sequencing may be constrained. Several studies have demonstrated significant genetic heterogeneity among *Cryptococcus* isolates using RAPD markers, revealing correlations between RAPD profiles and serotypes, molecular types, geographic origin, and ecological niches (22,23,24). This genetic variability has important epidemiological and clinical implications, as certain genotypes have been associated with increased virulence, altered host specificity, and differences in antifungal response (25,26). However, such clinical associations are derived from studies incorporating non-environmental isolates and were not directly evaluated in the present investigation. Despite the availability of newer high-resolution molecular tools, RAPD remains a valuable preliminary and comparative method for genetic diversity studies, particularly in developing regions where cryptococcosis poses a substantial public health challenge. Its application provides essential baseline data that can support surveillance programs, inform infection control strategies, and guide further molecular investigations.

Therefore, this study aims to determine the genetic diversity among *Cryptococ-*

cus species recovered from soil samples. Using RAPD analysis, thereby contributing to a deeper understanding of their population structure and genetic variability. Such insights are relevant for characterizing environmental, clinical and animal isolates useful and for diagnostic resolution, and advancing knowledge of the evolutionary dynamics of fungal pathogens of public health significance.

Materials and method:

Source of *Cryptococcus* strains:

The *Cryptococcus* strains used in this study were isolated from soil samples as part of an ongoing PhD research project (Coker, unpublished data). Soil sampling and primary isolation were conducted using standard mycological procedures and the isolates were subsequently sub-cultured on Sabouraud Dextrose agar (SDA; HiMedia, India) and incubated at 37°C for 48 h to ensure viability and purity prior to downstream analyses.

Random amplified polymorphic DNA analysis

DNA extraction:

Genomic DNA was extracted from *Cryptococcus* isolates using modified alkaline lysis-silica binding protocol adapted from Ruma et al., (22). Approximately 250–500 mg (wet weight) of *Cryptococcal* cells were harvested and suspended in lysis buffer containing 0.2 M NaOH, 1% (w/v) sodium dodecyl sulfate (SDS), and 10 mM EDTA. The suspension was incubated in a boiling water bath for 30 min to facilitate disruption of the rigid cryptococcal cell wall and polysaccharide capsule, followed by centrifugation at 12,000 × g for 5 min.

The supernatant was neutralized by the addition of potassium acetate–acetic acid solution, prepared to yield final concentrations of 3 M potassium acetate and 2 M acetic acid, and centrifuged under the same conditions to remove cellular debris. The clarified supernatant was subsequently incubated with 10% (w/v) Celite 545 resin (Fluka Chemical Corp., USA), pre-equilibrated in guanidine hydrochloride (GuHCl) binding buffer (pH 5.5), at 60°C for 60 min on a rotary mixer to allow adsorption of DNA to the silica matrix.

The DNA–resin mixture was transferred into a column and washed three times with wash buffer containing 100 mM NaCl, 10 mM Tris-HCl (pH 7.6), and 2.5 mM EDTA in 50% (v/v) ethanol. The wash solution was allowed to drain by gravity, after which the column was centrifuged briefly to remove residual wash buffer. Genomic DNA was eluted twice using preheated Tris–EDTA buffer (10 mM Tris-HCl, pH 7.6; 1 mM EDTA) at 55°C. DNA concentration and purity were determined spectrophotometrically by measuring A260/A280 ratios, and DNA preparations were stored at −20 °C until further analysis.

Primer screening and PCR amplification:

Seven primer combinations were selected for RAPD analysis following preliminary optimization for reproducibility and polymerphism among the *Cryptococcus* isolates (22). Three 20–22 mer primers viz; CN1 (5′-TACC CCCGCCCATATTCCAT-3′), MYC1 (5′-GAGGAA GGTGGGGATGACGT-3′), and 5SOR (5′-ATGG GAATACGACGTGCTGTAG-3′) were obtained from laboratory stocks and used in paired combinations. In addition, six commercially synthesized 12-mer primers from FPK1-FAPD kit (Bresatec, Thebarton, SA, Australia) — FPK1-01 (5′-ACACGGACGTCA-3′), FPK1-05 (5′-ACTTG GCGGCCT-3′), FPK1-07 (5′-ACCCTGCTCATC-3′), FPK1-08 (5′-CAGGCGAAGGTT-3′), FPK1-13 (5′-ACGGCTGGTTCC-3′), and FPK1-20 (5′-CAACAGCCCCCA-3′) were screened individually and in paired combinations to evaluate their discriminatory potential across isolates. Based on reproducibility, clarity, and polymerphism of amplification profiles, four 12-mer primer combinations (FPK1-01–FPK1-05, FPK1-05–FPK1-07, FPK1-05–FPK1-13, and FPK1-08–FPK1-20) and three 20–22 mer combinations were selected for further analysis as they produced the most consistent and informative RAPD patterns.

PCR amplification was performed in 25 µL reaction volumes containing 10 ng of genomic DNA, 200 µM of each deoxynucleoside triphosphate, 0.2 µM of each 12-mer primer or 0.4 µM of each 20–22 mer primer, 0.8 U of Taq DNA polymerase, and $MgCl_2$ at a final concentration of 3 mM for 12-mer primers or 6 mM for 20–22 mer primers, under the manufacturer's recommended buffer conditions. Amplifications were carried out in a thermal cycler (Bio-Rad, Hercules, CA, USA) with an initial denaturation at 93°C for 3 min, followed by 10 low-stringency cycles (denaturation at 93°C for 1 min, annealing at 35°C for 1 min, extension at 72°C for 1 min), and then 20 high-stringency cycles (denaturation at 93°C for 1 min, annealing at 55°C for 1 min, extension at 72°C for 1 min), with a final extension at 72 °C for 5 min. For the primer pair 5SOR–CN1, the annealing temperature during the initial 10 cycles was increased to 40°C, and the number of high-stringency cycles was increased to 30.

Amplification products were resolved on 8% polyacrylamide gels and visualized by silver staining using a modified Bassam and Caetano-Anollés (27) method, including 0.6 mg/mL sodium thiosulfate in the development step. RAPD assays were repeated at least once per DNA preparation, and DNA extracted from two independent cultures of each isolate was analyzed in parallel to ensure reproducibility.

Amplified bands were scored manually based on presence (+) or absence (−) irrespective of intensity. RAPD profiles were considered distinct and classified into major haplotypes only when reproducible, polymorphic banding patterns were obtained with all the seven primer combinations.

Genetic diversity analysis:

The genetic diversity of the *Cryptococcus* isolates was assessed using the number of polymorphic bands generated by RAPD profiling as a measure of genomic variation among isolates. Several diversity indices were calculated, including species richness (S), Simpson's index (D), index of similarity (1 − D), reciprocal index (1/D), Shannon–Wiener index (H), and evenness (E) to comprehensively describe population structure and diversity. Calculations were performed using the online biodiversity calculator available at https://www.virtue.gmbl.se/english-content/biodiversity-calculator.

RAPD-derived genetic diversity indices:

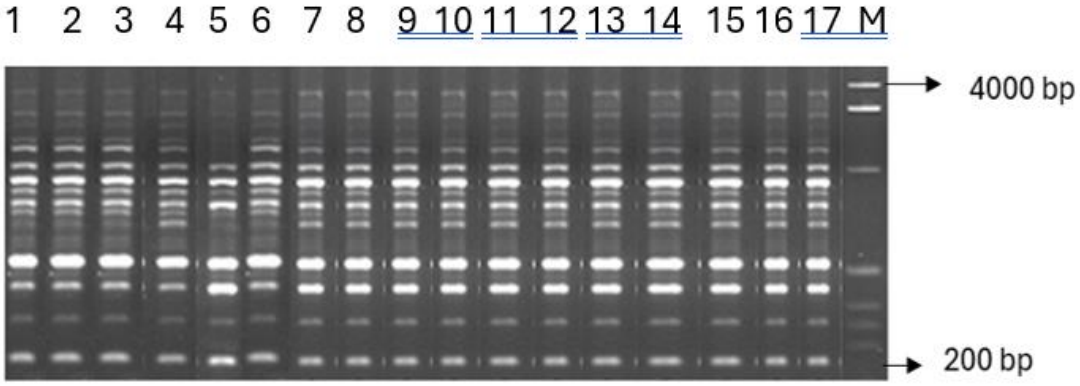
RAPD banding patterns were scored as binary data and used to calculate diversity indices based on band frequency distributions. Species richness (S), Simpson's index (D), Simpson's index of diversity (1−D), Simpson's reciprocal index (1/D), Shannon–Wiener index (H), and evenness (E) were employed to comprehensively assess genome-wide genetic diversity among *Cryptococcus* isolates. These indices collectively capture the extent of polymorphism, dominance of common RAPD band, effective diversity, and distribution balance, and are well suited for dominant RAPD markers where allele-based estimates are unreliable.

Results:

RAPD profile and genetic diversity of *Cryptococcus* isolates:

The RAPD profiles of the eighteen (18) *Cryptococcus* isolates analysed revealed clear and reproducible amplification patterns across multiple loci, indicating substantial polymorphism and genetic diversity within the population (Table 1, Plate 1). Most isolates produced 10–13 bands, with RAPD loci ranging from 330 to 3800 bp, whereas isolate 18 failed to amplify with all primer combinations and was excluded from further analysis. The banding pat terns demonstrated that not all isolates shared identical profiles, reflecting significant genetic variation. For example, the 330 bp locus was present only in isolates 5 and 7, whereas loci ranging from 1332 bp to 3800 bp were conserved across most isolates. Other loci, including 333, 932, 934, 2031, and 2329 bp, exhibited greater variability among isolates.

Based on the presence or absence of DNA fragments across 17 polymorphic loci, the isolates were classified into five haplotypes (A–E) (Table 1). Haplotype B was the most prevalent, encompassing 11 isolates (61.1%), whereas isolates 5, 6, and 7 displayed unique banding patterns and were assigned to haplotypes C, D, and E, respectively. Haplotype A included isolates 1–3, which shared identical profiles across all loci. Overall, the RAPD analysis demonstrates both conserved and variable genomic regions, reflecting the genetic heterogeneity of the *Cryptococcus* population.



M – 200 bp molecular marker; 1: SB6 = *C. neoformans*; 2: SD1 = *C. neoformans*; 3: SD2 = *C. neoformans*; 4: SE3 = *C. neoformans*; 5: SG1 = *C. neoformans*; 6: SG3 = *C. neoformans*; 7: SG7 = *C. neoformans*; 8: SG8 = *C. neoformans*; 9: SH1 = *C. neoformans*; 10: SH2 = *C. gattii*; 11: SJ1 = *C. neoformans*; 12: SL1 = *C. neoformans*; 13: SM1 = *C. neoformans*; 14: SP1 = *C. neoformans*; 15: SQ1 = *C. neoformans*; 16: SQ2 = *C. gattii*; 17: SR1 = *C. neoformans*; 18: SR2 = *C. neoformans*.

Plate 1: RAPD profile of *Cryptococcus* isolates

Table 1: RAPD profile and haplotype assignment of *Cryptococcus* isolates

RAPD Loci (bp)	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
330	-	-	-	-	+	-	+	-	-	-	-	-	-	-	-	-	-	-
333	+	+	+	+	-	+	-	+	+	+	+	+	+	+	+	+	+	-
932	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	-
934	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-
1332	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-
1665	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-
2031	-	-	-	+	-	-	+	+	+	+	+	+	+	+	+	+	+	-
2198	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	-
2329	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-
2331	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	-
2531	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-
2664	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-
2797	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-
3064	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	-
3400	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-
3800	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-
Haplotype	**A**	**A**	**A**	**B**	**C**	**D**	**E**	**B**	**B**	**B**	**B**	**B**	**B**	**B**	**B**	**B**	**B**	**-**

+ = presence, – = absence; Haplotypes A–E correspond to distinct RAPD profiles. 1: SB6 = *C. neoformans*; 2: SD1 = *C. neoformans*; 3: SD2 = *C. neoformans*; 4: SE3 = *C. neoformans*; 5: SG1 = *C. neoformans*; 6: SG3 = *C. neoformans*; 7: SG7 = *C. neoformans*; 8: SG8 = *C. neoformans*; 9: SH1 = *C. neoformans*; 10: SH2 = *C. gattii*; 11: SJ1 = *C. neoformans*; 12: SL1 = *C. neoformans*; 13: SM1 = *C. neoformans*; 14: SP1 = *C. neoformans*; 15: SQ1 = *C. neoformans*; 16: SQ2 = *C. gattii*; 17: SR1 = *C. neoformans*; 18: SR2 = *C. neoformans*.

Table 2: Population-level genetic diversity of *Cryptococcus* isolates

Diversity index	Value
Total polymorphic bands (TNOPB)	214
Species richness (S)	17
Simpson's index (D)	0.059
Simpson's index of diversity (1−D)	0.941
Simpson's reciprocal index (1/D)	16.95
Shannon–Wiener index (H')	2.83
Evenness (E)	0.999

NOPB- number of polymorphic bands; TNOPB- total number of polymorphic bands; G.D- genetic diversity; S- species richness; D- Simpson's index; 1-D- Simpson's index of diversity; 1/D- Simpson's reciprocal index; H- Shannon Weiner index; E- evenness;

The genetic diversity of the isolates was further quantified using multiple diversity indices (Table 2). Across the population, a total of 214 polymorphic bands were identified. Most isolates (n=12, 66.7%) exhibited 13 polymorphic bands each, isolate 5 produced 10 bands, and one isolate (isolate 18) did not yield amplification products. The species richness (S) was 17, indicating the number of distinct genetic variants observed among the analysed isolates.

The Simpson's index (D) was 0.059, reflecting a low probability that two randomly selected isolates share the same genotype, while the Simpson's index of diversity (1 − D) was 0.941, underscoring high genetic variability. The Simpson's reciprocal index (1/D) was 16.95, supporting the presence of multiple genetically distinct individuals.

The Shannon–Wiener diversity index (H') was 2.83, indicating moderate-to-high genetic variation, and the evenness value (E) was 0.999, suggesting an almost uniform distribution of genetic variants across isolates. Collectively, these indices confirm the high *g*enetic diversity of the *Cryptococcus* population examined in this study and the broad distribution of polymorphic loci.

Primer-specific RAPD polymorphism:

The RAPD analysis of the *Cryptococcus* isolates revealed substantial genetic polymorphism across the primer combinations tested (Table 3). A total of 20 to 40 polymorphic bands were amplified per primer pair, with band sizes ranging from 330 to 3800 bp, indicating high variability within the population analysed. Several primer combinations demonstrated both resolving and diagnostic capacity within the analysed dataset. For example, the CN1–5SOR primer pair produced a 934 bp band that was diagnostic for haplotype D, while FPK1-05–FPK1-07 amplified bands that were diagnostic for haplotypes B and D. Other primer combinations, such as CN1–MYC1 and MYC1–5SOR, resolved multiple haplotypes, distinguishing groups such as SG1, SG3, SH2, and others, although no single band was uniquely diagnostic across all isolates.

The FPK1-01–FPK1-05 combination amplified the highest number of polymorphic loci, facilitating the differentiation of closely related haplotypes SB6, SD1, and SD2. Simi-

larly, FPK1-08–FPK1-20 produced distinct bands that aided in differentiating SQ1, SQ2, and SR1 isolates. These results collectively indicate that the selected RAPD primers are effective both in distinguishing most *C. neoformans* isolates and in identifying specific haplotypes through diagnostic bands, highlighting the utility of RAPD profiling for molecular characterization of environmental isolates rather than direct inference of clinical or epidemiological relationships.

Discussion:

Random amplified polymorphic DNA (RAPD) markers are widely recognized as effective tools for assessing genetic diversity and population structure in fungal pathogens, including *Cryptococcus* species (17,23,24,28). In the present study, RAPD analysis of 17 *Cryptococcus* isolates generated a total of 214 bands, with an average of 10–13 polymorphic bands per isolate. Each band represents a putative genetic locus that may be present or absent, and the resulting patterns revealed substantial genetic heterogeneity, resolving the isolates into five distinct haplotypes (A–E). Haplotype B was the most prevalent, encompassing 11 isolates (61.1%), while isolates 5, 6, and 7 were assigned to unique haplotypes C, D, and E, respectively, and haplotype A included isolates 1–3. The high proportion of polymorphic loci, even though all isolates originating from a single environmental source underscores the ability of RAPD markers to uncover fine-scale genetic variation within apparently homogeneous populations.

The primer-specific RAPD profiles also contributed significantly to resolving this diversity. For example, the CN1–MYC1 primer pair (20–22 mer) produced 30 polymorphic bands (330–3063 bp), differentiating haplotypes A, B, and C. The CN1–5SOR combination (20–22 mer) amplified a 934 bp band that was diagnostic for haplotype D, while FPK1-05–FPK1-07 (12-mer) produced bands at 2197.8 and 2530.8 bp that were diagnostic for haplotypes B and D. Other primer combinations, such as MYC1–5SOR and FPK1-05–FPK1-13, resolved multiple haplotypes, including SG1, SG3, SH2, and SG7, whereas FPK1-01–FPK1-05 amplified the greatest number of polymorphic loci, thereby facilitating discrimination of closely related isolates (SB6, SD1, SD2). FPK1-08–FPK1-20 further aided differentiation of SQ1, SQ2, and SR1 isolates.

These results indicate that the selected primers were consistently effective in capturing both intra- and inter-haplotype variation with certain bands serving as diagnostic markers for specific haplotypes.

The quantitative diversity indices further support the high level of genetic variability observed. The Shannon–Wiener index (H' = 2.83) indicated moderate-to-high genotype diversity, reflecting both the number of haplotypes and their distribution among isolates (29). Simpson's index (D=0.059) and its complement (1-D=0.941) indicate a low probability that two randomly selected isolates share the same genotype (30), while the reciprocal index (1/D=16.95) confirms the presence of multiple genetically distinct individuals. The evenness value (E=0.999) suggests an almost uniform distribution of haplotypes across the population. Collectively, these indices corroborate the RAPD patterns, demonstrating that genetic diversity is widely distributed rather than dominated by a single genotype (31,32).

The observed diversity aligns with previous RAPD-based studies of *Cryptococcus* (23,24,33,34,35), which report stable and reproducible RAPD profiles that reflect genuine genetic differences rather than methodological artifacts. Unlike some studies in which isolates cluster strictly by clinical or environmental origin (23,33), the recovery of multiple haplotypes and the absence of strong clustering in this study suggest that microenvironmental complexity within the sampling site may drive diversification, even in geographically restricted niches. Such localized ecological pressures likely contribute to the extensive polymorphism observed, reinforcing the idea that *Crypto coccus* populations can be genetically heterogeneous within small environmental regions.

At a broader scale, these findings are consistent with global trends in *Cryptococcus* diversity. Strains from sub-Saharan Africa, particularly southern Africa, exhibit remarkable genetic heterogeneity (36), with populations described as geographically restricted yet genetically diverse, likely due to long-term endemicity, complex environmental reservoirs and historical recombination events. Genome-based analyses further support this, showing higher nucleotide diversity in African isolates relative to other populations (37). Although RAPD markers offer lower resolution than whole-genome sequencing, the diversity observed in this study mirrors these global patterns, suggesting that the isolates analyzed represent part of a broader, genetically diverse regional gene pool. From biological and clinical perspectives, this variability is significant because it underpins microevolutionary processes that can generate phenotypic differences.

Table 3: Primer-wise RAPD bands and haplotype resolution in *Cryptococcus* isolates

Primer combination	**Primer type**	**Number of polymorphic bands**	**Band size range (bp)**	**Representative bands (bp)**	**Key haplotypes resolved**	**Haplotype differentiation**
CN1–MYC1	20–22 mer	30	330–3063	330, 333, 932.4, 1665, 2530.8	A, B, C	Differentiates most *C. neoformans* isolates
CN1–5SOR	20–22 mer	20	334–934	334, 934	B, D	Band at 934 bp diagnostic for haplotype D
MYC1–5SOR	20–22 mer	26	1332–2031	1332, 2031.3	C, E	Resolves SG1, SG3, SH2
FPK1-01–FPK1-05	12-mer	40	330–3800	330, 333, 932.4, 3400, 3800	A	Highest number of polymorphic loci; distinguishes SB6, SD1, SD2
FPK1-05–FPK1-07	12-mer	35	2197–2531	2197.8, 2530.8	B, D	Diagnostic bands for haplotypes B and D
FPK1-05–FPK1-13	12-mer	35	932–2797	932.4, 2797.2	C, E	Resolves SG3, SG7, SH2
FPK1-08–FPK1-20	12-mer	28	2664–3800	2664, 2797.2, 3800	B, C	Distinct bands facilitate differentiation of SQ1, SQ2, SR1

In this study, no clear correlation was observed between RAPD genotypes and phenotypic traits, consistent with previous reports (23,38). Nevertheless, the extensive genetic heterogeneity suggests the presence of underlying variation that may influence virulence, antifungal susceptibility, and environmental persistence. Previous studies have demonstrated that genetic diversity within *Cryptococcus* populations can affect disease severity, therapeutic outcomes, and even the coexistence of multiple species within a single host (39,40).

Finally, it is plausible that the observed diversity reflects not only nucleotide-level polymorphisms, but it is reasonable to hypothesize that the observed diversity could also be influenced by epigenetic mechanisms. Processes such as DNA methylation, histone modification, and non-coding RNA regulation are known to modulate gene expression without changing the DNA sequence (41,42). Previous studies in *C. neoformans* and *C. gattii* have linked epigenetic variation to capsule production and melanin synthesis (43,44,45), however confirming such contributions in this context will require targeted epigenetic analysis.

Conclusion:

In conclusion, RAPD analysis of environmental *Cryptococcus* isolates showed substantial genetic diversity and multiple distinct haplotypes, demonstrating the utility of RAPD markers in capturing population-level variation. Primer-specific amplification patterns highlighted diagnostic bands that distinguish key haplotypes, while quantitative diversity indices confirmed high heterogeneity across the population. These findings underscore the ecological, biological, and potential clinical significance of *Cryptococcus* genetic diversity within restricted environmental niches.

Contributions of authors:

MOC and BTT were involved in the study conceptualization and design; MOC, BTT and ODP were involved in the methodology and data analysis; MOC wrote the original draft; BTT, ODP and MOE reviewed and edited the manuscript. All authors read and approved the final version of the manuscript.

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Conflict of interest:

No conflict of interest is declared.

Availability of data and materials:

All data generated or analysed during this study are included in this manuscript.

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