

**Supplementary Table S1. Reference strains with AFLP (A) and/or MLST (M) genotypes of *Cryptococcus neoformans* var. *grubii***

<b>Molecular type</b>	<b>Reference strains</b>	<b>Genotype</b>	<b>References</b>
VNI Global	A1-35-8	A1/M1	(1)
	H99	A1/M1b	(1)
	A4-1-12	A4/M4e	(1)
	UG2471; GB118; GB118-2; RTC9	A2/M3b	(1,2)
	MAL9	A3/M3a	(1)
	TU239	M1c	(2)
	A3-1-1; D16-1; PR284	A3/M3	(1,2)
	125.91	A4/M4f	(1)
	A5-35-17; D16-6; D16-11; JO278	A5/M5	(1,2)
	TH84	A10/M10	(1)
	TH104	A10/M10a	(1)
	TU259	M10c	(2)
	BR795; RTC1	A4/M4	(1,2)
	A4-34-6; C27	A4/M4a	(1)
	A2-102-5	A2/M2	(1)
	D16-16	M2a	(2)
VNI African	BT9	A1/M43	(1)
	BT15	M32	(1)
	BT100	A1/M31	(1)
	BT121	M41	(1)
	BT150	A3/M28	(1)
VNB African	BT1	M11	(1)
	BT24	M19	(1)
	BT27	M42	(1)
	BT31	M12	(1)
	BT33	M26	(1)

	BT35	M24	(1)
	BT63	M27	(1)
	BT85	M34	(1)
	BT88	M35	(1)
	BT206	M20	(1)
VNII	A7-35-23; C19; 8-1	A7/M7a	(1)
Global	C44	A8/M8a	(1)
	C45	A6/M7c	(1)

### References

1. Litvintseva, AP, R Thakur, RJ Vilgalys, TG Mitchell. 2006. Multilocus sequence typing reveals three genetic subpopulations of *Cryptococcus neoformans* var. *grubii* (serotype A), including a unique population in Botswana. *Genetics* 172:2223-2238.
2. Litvintseva, AP, I Carbone, J Rossouw, R Thakur, NP Govender, TG Mitchell. 2011. Evidence that the human pathogenic fungus *Cryptococcus neoformans* var. *grubii* may have evolved in Africa. *PLoS ONE* 6:e19688.

**Supplementary Figure S1. A.** Allelic profiles of incident and serial isolates of *C. neoformans* var. *grubii*. Clinical cases are numbered on the left; two or three isolates were analyzed from each case. Patients from whom strains with different genotypes were isolated are noted in red. The second column lists the laboratory numbers of the isolates, which correspond to the numbers on the Neighbor-Joining (NJ) tree (Fig. 2B). The eight MLST loci are shown on the top. Under each locus, identical haplotypes are denoted with the same number and color. The last column indicates the molecular type of each strain, VNI (blue), VNII (green) or VNB (red). Strains of *C. gattii* are shown with deep purple (VGI, cases 69 & 91) or brown (VGIV, cases 31 & 67) colors. **B.** Genetic relationships of MLST genotypes among isolates of *C. neoformans* var. *grubii* visualized by NJ dendrogram. DNA sequences of eight loci were concatenated totaling 4,408 base pairs and analyzed with the NJ method using mean number of pairwise character distances. Major molecular types are color-coded and labeled. Typing strains from the global population sample are included in the dendrogram (19); geographic and ecological origin of the typing strains are indicated in parentheses (“clin” for clinical; “env” for environmental; RSA = Republic of South Africa). The labels, A4/M4, A5/M5, and SA, denote the predominant clades of VNI genotypes among the isolates analyzed (see Table 3); A4/M4 and A5/M5 are globally prevalent, but the large clade of SA strains may be unique to Africa. As indicated in this NJ analysis and Table 3, most of the genotyped isolates were identical to previously characterized strains (18-21). As indicated in Supplementary Table S1, the analysis included 22 global strains of molecular type VNI (A1-35-8, A2-105-5, A3-1-1, A4-1-12, A4-34-6, A5-35-17, BR795, C27, D16-6, D16-11, D16-16, GB118, GB118-2, JO278, M9, RTC1, RTC9, TH84,

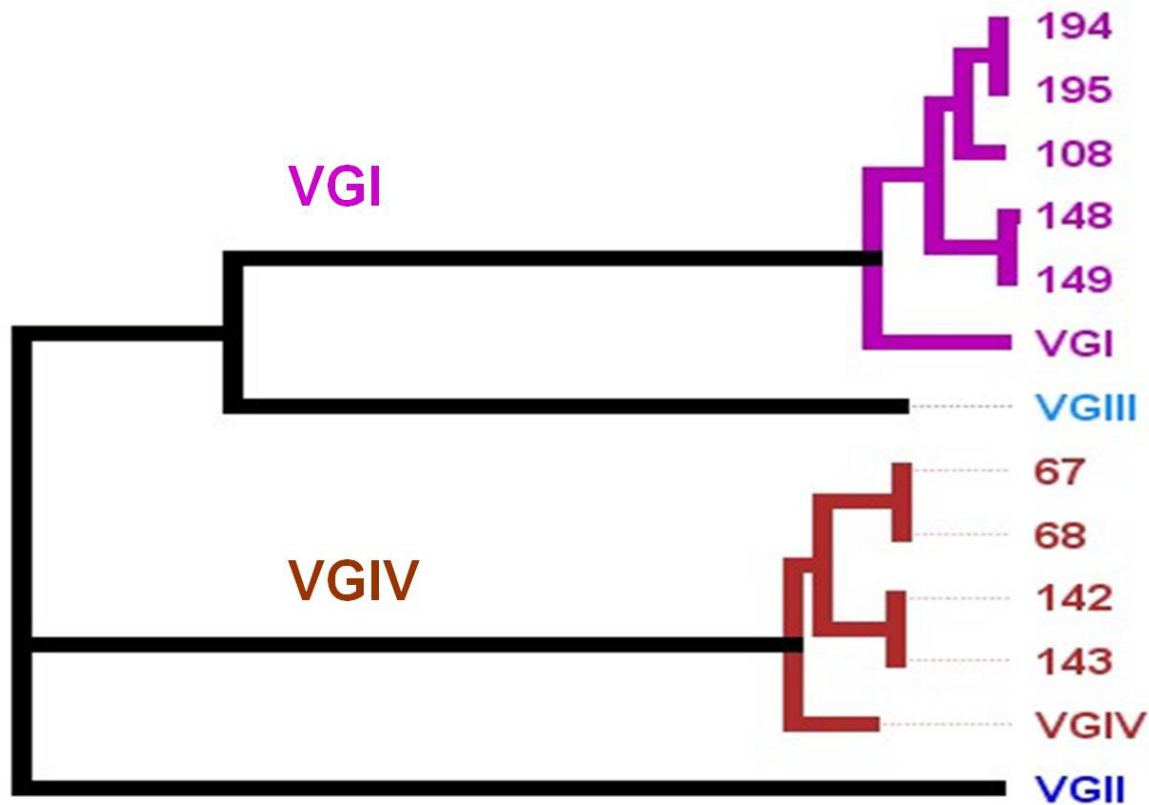
TH104, TH259, UG2471, 125.91), 10 strains of molecular type VNI as yet found only in southern Africa (BT9, BT15, BT100, BT121, BT150, D16-1, GB159, PR284, PR284-1, TU239); 21 strains of molecular type VNB as yet found only in southern Africa (BT1, BT24, BT27, BT31, BT33, BT35, BT63, BT85, BT88, BT206, RTC2, RTC3, TU229, TU248, TU360, TU369, TU401, TU406, TU416, TU422, ZE93 ); and five strains of molecular type VNII (A7-35-23, 8-1, C19, C44, C45).

Note: Reference numbers refer to reference list in the paper.

Case No	Strain No	CAP59	GPDI	LACI	PLB1	SOD1	URAS	IGS1	TEF1	TYPE	
1	1	1	1	1	2	1	1	1	7	1	VNI
1	2	1	1	1	2	1	1	1	7	1	VNI
2	3	2	1	2	3	1	1	1	2	VNI	
2	4	2	1	2	3	1	1	1	2	VNI	
3	5	1	1	1	2	1	1	1	7	1	VNI
3	6	6	5	7	9	1	9	16	1	VNB	
4	7	1	1	1	1	1	1	1	7	5	VNI
4	8	1	1	1	1	1	1	1	7	5	VNI
5	9	9	12	6	13	18	13	26	19	VNII	
5	10	9	12	6	13	18	13	26	19	VNII	
6	11	9	12	6	13	18	13	26	19	VNII	
6	12	1	3	5	2	1	1	1	1	VNI	
7	13	6	5	7	7	1	9	16	10	VNB	
7	14	6	5	7	7	1	9	16	10	VNB	
8	15	1	1	1	1	1	1	1	7	5	VNI
8	16	1	1	1	1	1	1	1	7	5	VNI
9	17	9	12	6	13	18	13	26	19	VNII	
9	18	9	12	6	13	18	13	26	19	VNII	
10	19	1	1	2	3	1	2	1	2	VNI	
10	20	1	1	2	3	1	2	1	2	VNI	
11	21	2	1	3	3	1	2	1	2	VNI	
11	22	2	1	3	3	1	2	1	2	VNI	
12	23	1	3	5	2	1	1	1	1	VNI	
12	24	1	3	5	2	1	1	1	1	VNI	
13	25	1	1	1	2	1	1	1	7	1	VNI
13	27	1	1	1	2	1	1	1	7	1	VNI
14	28	2	1	2	3	20	1	1	2	VNI	
14	29	2	1	2	3	20	1	1	2	VNI	
15	30	1	3	5	2	1	1	1	1	VNI	
15	31	1	3	5	2	1	1	1	1	VNI	
15	32	1	3	5	2	1	1	1	1	VNI	
16	33	1	7	1	4	1	1	1	21	VNI	
16	34	1	7	1	4	1	1	1	21	VNI	
17	35	1	7	1	4	1	1	1	21	VNI	
17	36	1	3	5	2	1	1	1	1	VNI	
18	37	1	7	1	4	1	1	1	21	VNI	
18	38	1	7	1	4	1	1	1	21	VNI	
19	39	9	12	6	13	18	13	26	19	VNII	
19	40	9	12	6	13	18	13	26	19	VNII	
21	45	1	3	5	2	1	1	1	1	VNI	
21	46	1	3	5	2	1	1	1	1	VNI	
22	47	2	1	14	3	1	2	1	2	VNI	
22	48	2	1	14	3	1	2	1	2	VNI	
23	49	2	1	3	3	1	2	1	22	VNI	
23	50	2	1	3	3	1	2	1	22	VNI	
24	51	2	1	3	3	1	2	1	2	VNI	
24	52	2	1	3	3	1	2	1	2	VNI	
25	53	1	3	5	2	1	1	1	1	VNI	
25	54	1	3	5	2	1	1	1	1	VNI	
26	55	2	7	2	1	1	1	1	21	VNI	
26	56	2	7	2	1	1	1	1	21	VNI	
26	57	2	7	2	1	1	1	1	21	VNI	
27	58	1	1	1	1	1	1	1	7	5	VNI
27	59	1	1	1	1	1	1	1	7	5	VNI
28	60	6	5	7	1	8	9	16	23	VNB	
28	61	6	5	7	1	8	9	16	23	VNB	
29	62	1	7	1	4	1	1	1	21	VNI	
29	63	1	7	1	4	1	1	1	21	VNI	
29	64	1	7	1	4	1	1	1	21	VNI	
30	65	1	1	1	1	1	1	1	7	1	VNI
30	66	1	1	1	1	1	1	1	7	1	VNI
31	67									VGIV	
31	68									VGIV	
32	69	1	7	1	4	1	1	1	21	VNI	
32	70	1	3	5	2	1	1	1	1	VNI	
33	71	1	7	2	1	1	1	1	3	VNI	
33	72	1	7	2	1	1	1	1	3	VNI	
33	73	1	7	2	1	1	1	1	3	VNI	
34	74	1	3	5	2	1	1	1	1	VNI	
34	75	1	3	5	2	1	1	1	1	VNI	
35	76	1	3	5	2	1	1	1	1	VNI	
35	77	1	3	5	2	1	1	1	1	VNI	
36	78	1	1	2	3	1	2	1	2	VNI	
36	79	1	1	2	3	1	2	1	2	VNI	
36	80	1	1	2	3	1	2	1	2	VNI	
37	81	1	1	1	2	1	1	1	7	1	VNI
37	82	1	1	1	2	1	1	1	7	1	VNI
38	83	1	1	1	1	1	1	1	7	5	VNI
38	84	1	1	1	1	1	1	1	7	5	VNI
39	85	1	1	1	1	1	1	1	7	5	VNI
39	86	2	1	3	3	1	2	1	22	VNI	
40	87	9	12	6	13	21	13	26	19	VNII	
40	88	9	12	6	13	21	13	26	19	VNII	
41	89	2	1	3	3	1	2	1	22	VNI	
41	90	2	1	3	3	1	2	1	22	VNI	
43	91	1	3	5	2	1	1	1	1	VNI	
43	92	6	5	7	7	8	9	16	10	VNB	
44	93	1	3	5	2	1	1	1	1	VNI	
44	94	1	3	5	2	1	1	1	1	VNI	

Case No	Strain No	CAP59	GPDI	LACI	PLB1	SOD1	URAS	IGS1	TEF1	TYPE	
45	99	9	12	6	13	18	13	26	19	VNII	
45	100	9	12	6	13	18	13	26	19	VNII	
46	101	1	1	1	1	1	1	1	7	5	VNI
46	102	1	3	5	2	1	1	1	1	VNI	
47	103	1	1	1	2	1	1	1	7	1	VNI
47	104	1	1	1	2	1	1	1	7	1	VNI
55	120	1	3	5	2	1	1	1	1	VNI	
55	121	1	3	5	2	1	1	1	1	VNI	
56	122	1	7	1	4	1	1	1	21	VNI	
56	123	1	7	1	4	1	1	1	21	VNI	
57	124	1	7	1	4	1	1	1	21	VNI	
57	125	1	7	1	4	1	1	1	21	VNI	
58	126	1	7	1	4	1	1	1	21	VNI	
58	127	1	7	1	4	1	1	1	21	VNI	
59	128	1	1	1	1	1	1	1	7	1	VNI
59	129	1	1	1	1	1	1	1	7	1	VNI
61	132	1	1	1	1	1	1	1	7	5	VNI
61	133	1	1	1	1	1	1	1	7	5	VNI
62	134	1	3	5	2	1	1	1	1	VNI	
62	135	1	3	5	2	1	1	1	1	VNI	
63	136	1	1	1	1	1	1	1	7	5	VNI
63	137	1	1	1	1	1	1	1	7	5	VNI
64	138	9	12	6	13	18	13	26	19	VNII	
64	139	1	1	1	2	1	1	1	7	1	VNI
66	140	1	1	1	1	1	1	1	7	5	VNI
66	141	1	1	1	1	1	1	1	7	5	VNI
67	142									VGIV	
67	143									VGIV	
68	144	2	1	3	1	1	2	1	2	VNI	
68	147	2	1	3	3	1	2	1	2	VNI	
69	148									VGI	
69	149									VGI	
70	150	6	5	7	7	9	15	11	25	VNB	
70	151	1	1	1	2	1	1	1	7	1	VNI
70	152	1	1	1	2	1	1	1	7	1	VNI
71	153	1	1	1	1	1	1	1	7	5	VNI
71	154	1	1	1	1	1	1	1	7	5	VNI
72	155	1	3	5	2	1	1	1	1	VNI	
72	156	1	3	5	2	1	1	1	1	VNI	
74	159	2	1	3	3	1	2	1	2	VNI	
74	160	2	1	3	3	1	2	1	2	VNI	
75	161	1	7	1	4	1	1	1	1	21	VNI
75	162	1	7	1	4	1	1	1	1	21	VNI
76	163	1	7	1	4	1	1	1	1	21	VNI
76	164	1	7	1	4	1	1	1	1	21	VNI
77	165	10	13	6	14	17	14	27	20	VNII	
77	166	10	13	6	14	17	14	27	20	VNII	
77	167	10	13	6	14	17	14	27	20	VNII	
78	168	1	7	1	4	1	1	1	1	21	VNI
78	169	1	7	1	4	1	1	1	1	21	VNI
79	170	1	1	2	3	1	2	1	2	VNI	
79	171	1	1	2	3	1	2	1	2	VNI	
80	172	1	3	5	2	1	1	1	1	VNI	
80	173	1	3	5	2	1	1	1	1	VNI	
81	174	6	5	16	7	8	9	16	10	VNB	
81	175	6	5	16	7	8	9	16	10	VNB	
82	176	1	7	2	1	1	1	22	3	VNI	
82	177	1	7	2	1	1	1	22	3	VNI	
83	178	1	3	5	2	1	1	1	1	VNI	
83	179	1	3	5	2	1	1	1	1	VNI	
84	180	1	3	5	2	1	1	1	1	VNI	
84	181	1	3	5	2	1	1	1	1	VNI	
85	182	1	1	1	1	1	1	1	7	5	VNI
85	183	1	1	1	1	1	1	1	7	5	VNI
86	184	1	3	5	2	1	1	1	1	VNI	
86	185	1	3	5	2	1	1	1	1	VNI	
87	186	1	1	1	2	1	1	1	28	1	VNI
87	187	1	1	1	2	1	1	1	28	1	VNI
88	188	1	3	5	2	1	1	1	1	VNI	
88	189	1	3	5	2	1	1	1	1	VNI	
90	192	9	12	6	13	19	13	26	19	VNII	
90	193	9									

**Supplementary Figure S2.** Genetic relationships of MLST genotypes among isolates of *C. gattii* visualized by the NJ dendrogram. DNA sequences of the eight MLST loci were concatenated totaling 4,130 base pairs. The dataset was analyzed with the NJ method using the mean number of pairwise character distances. Reference strains for the major molecular types are color-coded and labeled; the clinical isolates from this investigation are numbered (see Table 2).



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