Burning issues for *Cryptococcus*: taxonomy, ecology and clinical characteristics

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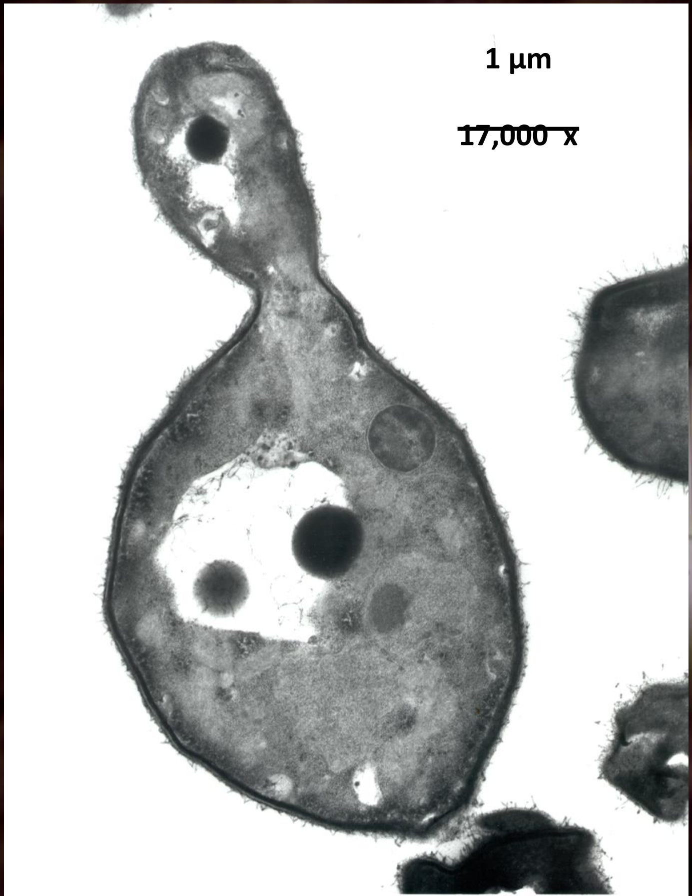
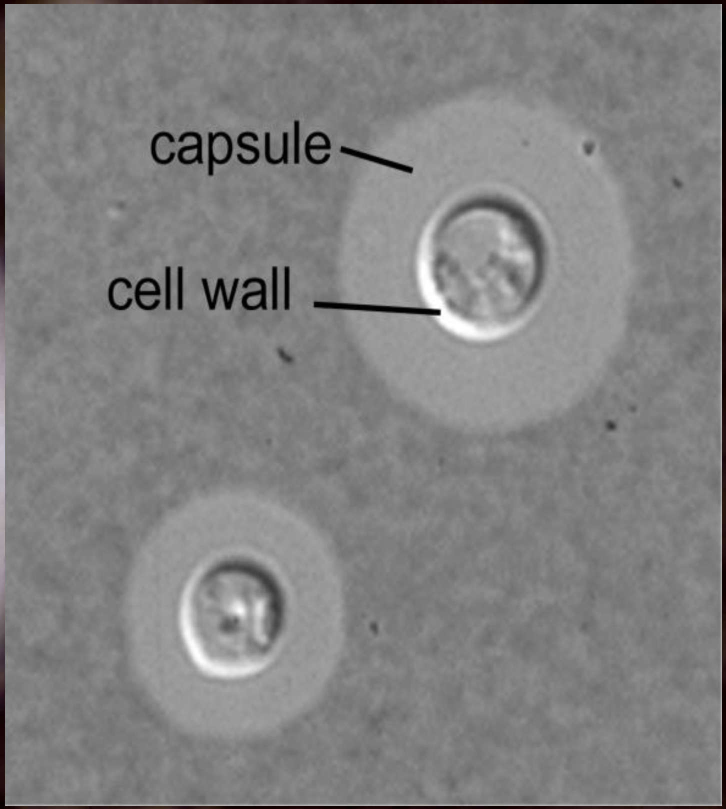
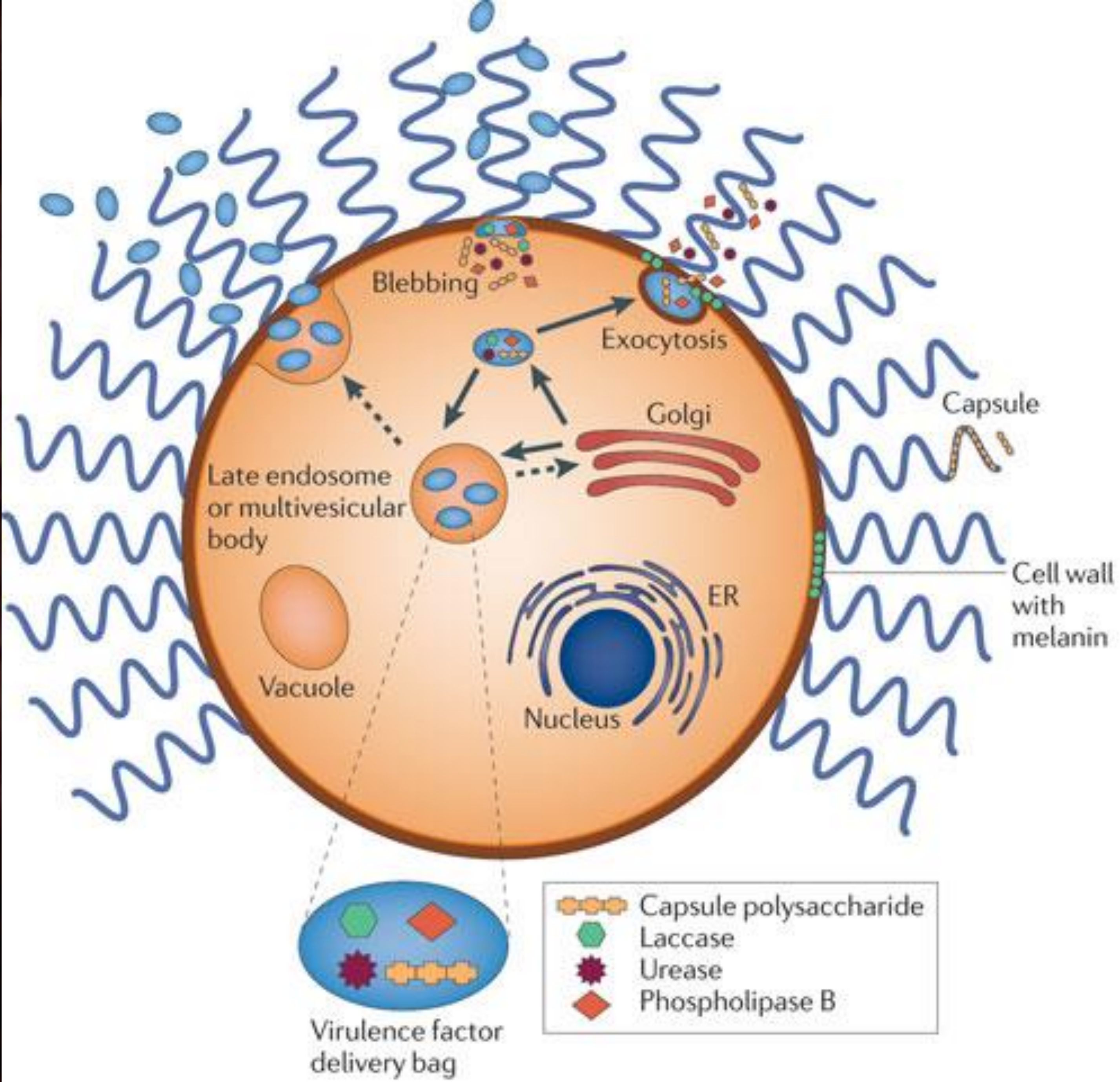


GXM (glucouronxylomannan)

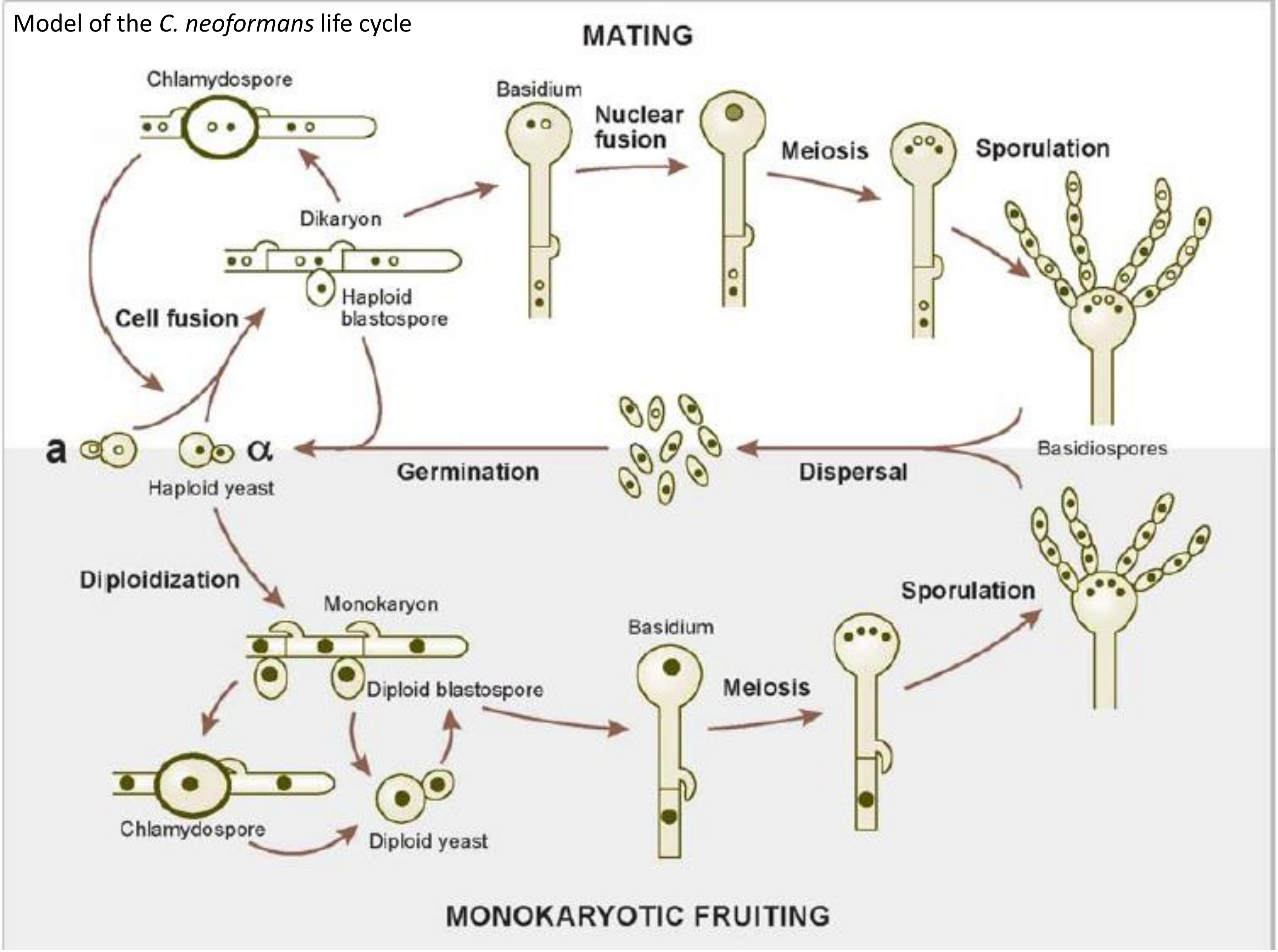


Nature [illegible] Microbiology 9, 193-203



Nature Reviews Microbiology 9, 193-203

Model of the *C. neoformans* life cycle



Lin X, Heitman J. The biology of the *Cryptococcus neoformans* species complex. *Annu. Rev. Microbiol. 2006; 60: 69-105.*

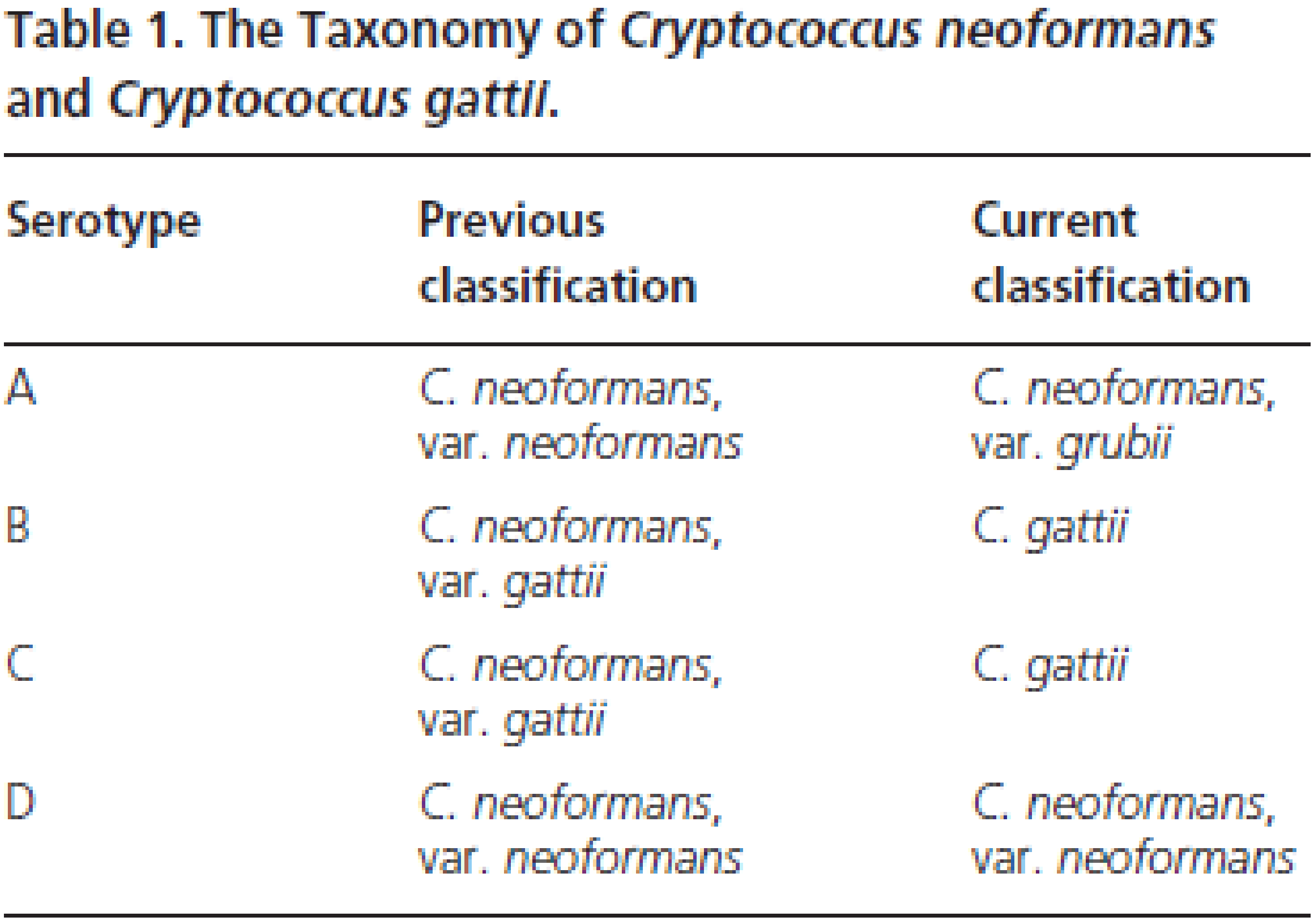


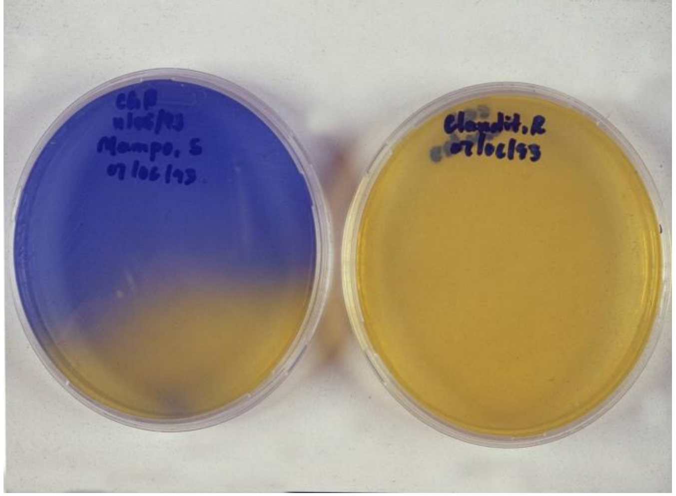
Table 1. The Taxonomy of *Cryptococcus neoformans* and *Cryptococcus gattII*.

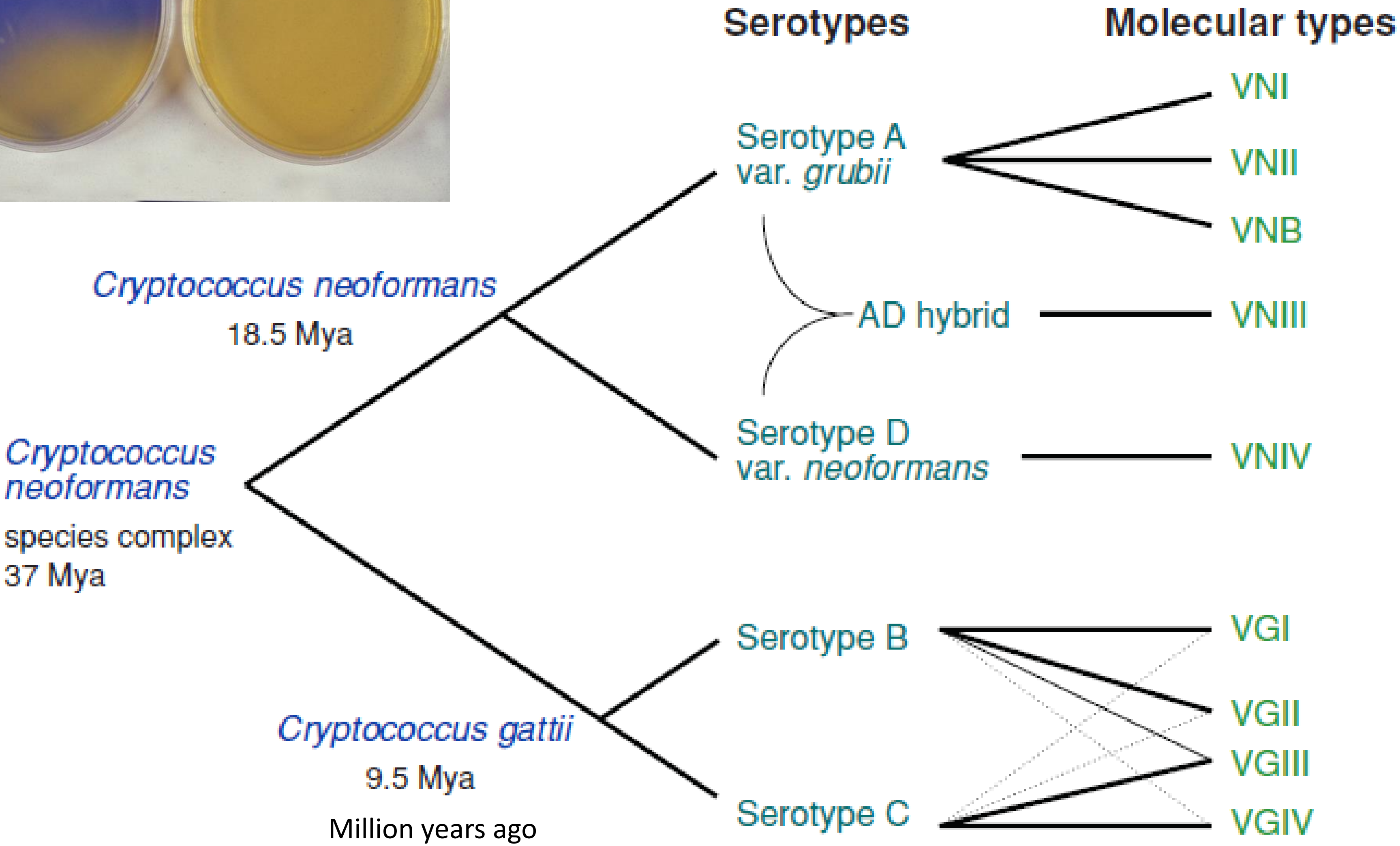
Serotype	Previous classification	Current classification
A	C. *neoformans*, var. *neoformans*	C. *neoformans*, var. *grubii*
B	C. *neoformans*, var. *gattii*	C. *gattii*
C	C. *neoformans*, var. *gattii*	C. *gattii*
D	C. *neoformans*, var. *neoformans*	C. *neoformans*, var. *neoformans*

Pukkila-Worley R, Mylonakis E. *Expert opinion on pharmacotherapy 2008; 9(4): 551-60.*

Typing Methods **mating type: α (mostly) ; a (rare)**

Variety	Serotype	Molecular Genotype
L-Canavanine-Glycine-Bromothymol blue (CGB) medium	Commercial antibodies to capsule: Iatron serotyping kit	PCR fingerprinting, URA5-Restriction Fragment Length Polymorphism (RFLP), Amplified Fragment Length Polymorphism (AFLP)
C. neoformans variety grubii	Serotype A	VN I, VN II (AFLP 1, 1a)
C. neoformans* var. *gattii	**Serotype B**	**VG I, VG II, VG III (AFLP 4, 6, 5)**
	Serotype C	**VG IV (AFLP 7)**
C. neoformans var. *neoformans*	Serotype D	VN IV (AFLP 2)
	Serotype AD	VN III (AFLP 3)



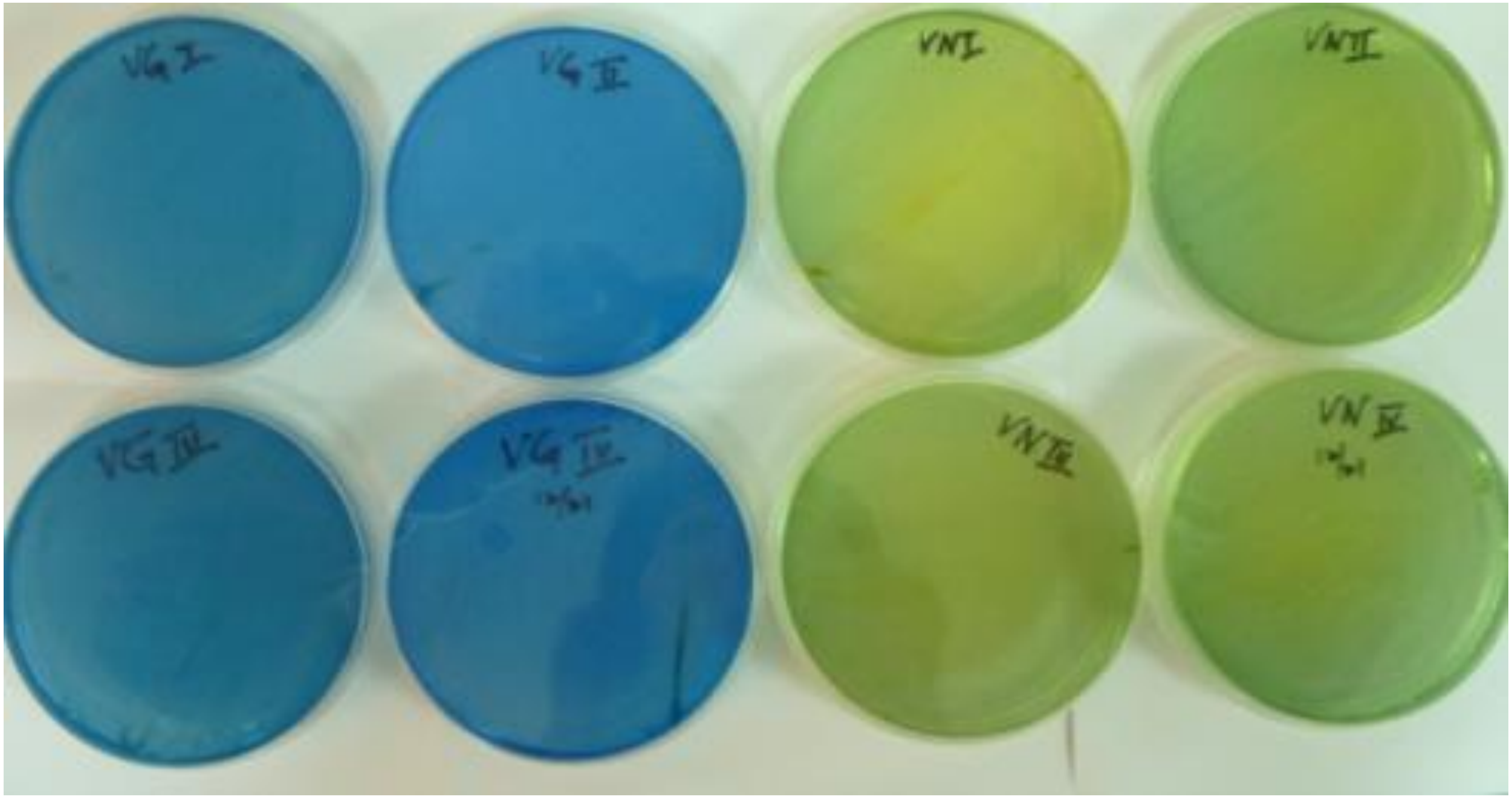


Lin X, Heitman J. *Annu. Rev. Microbiol. 2006; 60: 69-105.*

Genotype Reference Strains

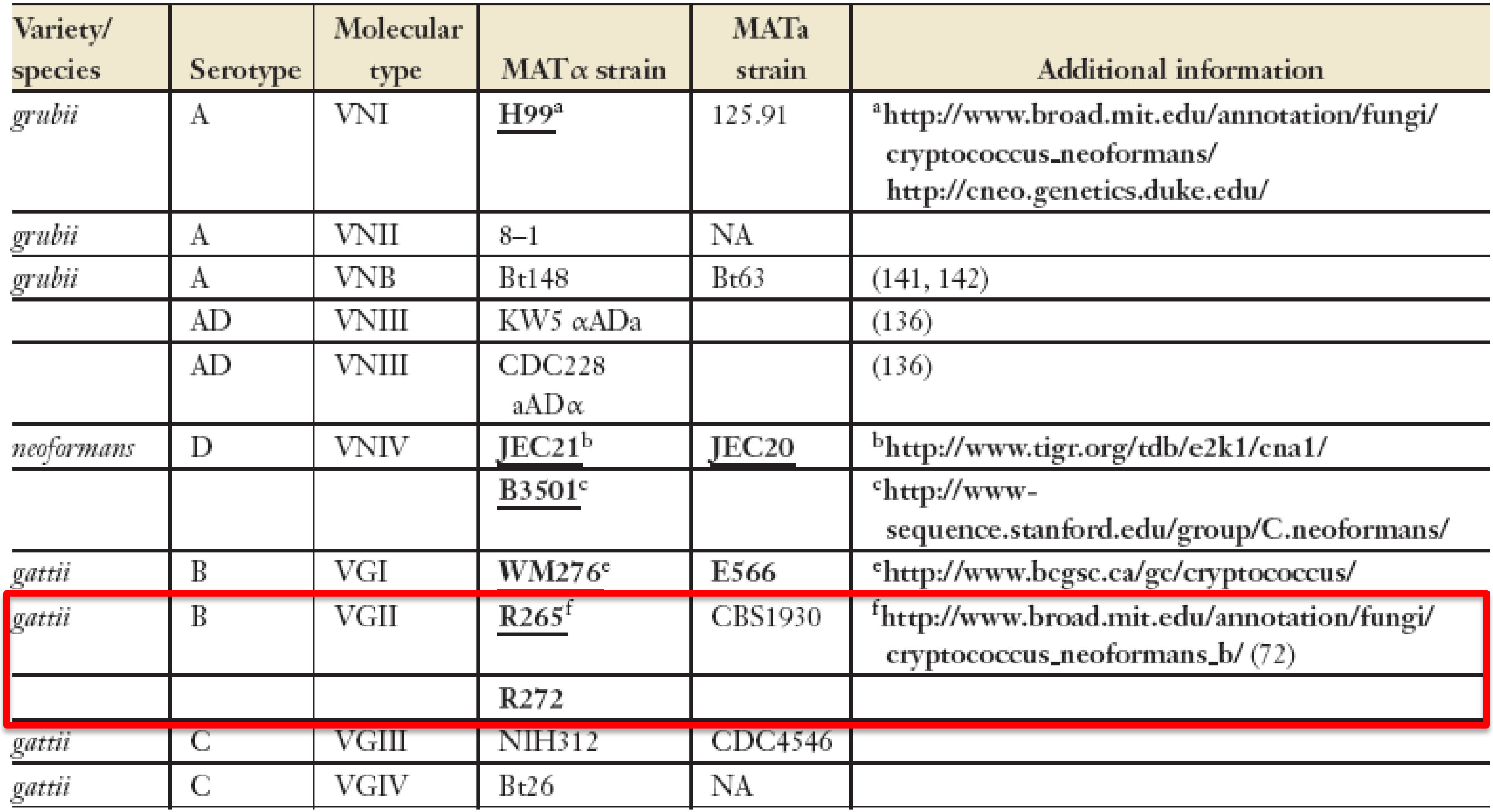
Strain	Serotype	Genotype	Geographical origin	Source
WM148	A	VNI	Sydney, Australia	Clinical
WM626	A	VNII	Sydney, Australia	Clinical
WM628	AD	VNIII	Melbourne, Australia	Clinical
WM629	D	VNIV	Melbourne, Australia	Clinical
WM179	B*	VGI	Sydney, Australia	Clinical
WM178	B*	VGII	Sydney, Australia	Clinical
WM161	B*	VGIII	San Diego, USA	Woody debris
WM779	C*	VGIV	Johannesburg, South Africa	Cheetah

Meyer W, et al. Electrophoresis **1999 Jun;20(8):1790-9.** Meyer W, et al. Med Mycol **2009 May 22:1-14.**



CGB Medium with Genotype Reference Strains of *C. neoformans* (VNI, VNII, VNIII, VNIV) and *C. gattii* (VGI, VGII, VGIII, VGIV) from Duke University Medical Center

Cryptococcus neoformans Reference Strain List



Variety/ species	Serotype	Molecular type	MATα strain	MATa strain	Additional information
grubii	A	VNI	**H99**[a]	125.91	[a]http://www.broad.mit.edu/annotation/fungi/cryptococcus_neoformans/ http://cneo.genetics.duke.edu/
grubii	A	VNII	8–1	NA	
grubii	A	VNB	Bt148	Bt63	(141, 142)
	AD	VNIII	KW5 αADa		(136)
	AD	VNIII	CDC228 aADα		(136)
neoformans	D	VNIV	**JEC21**[b]	**JEC20**	[b]http://www.tigr.org/tdb/e2k1/cna1/
			B3501[c]		[c]http://www-sequence.stanford.edu/group/C.neoformans/
gattii	B	VGI	**WM276**[e]	E566	[e]http://www.bcgsc.ca/gc/cryptococcus/
gattii	B	VGII	**R265**[f]	CBS1930	[f]http://www.broad.mit.edu/annotation/fungi/cryptococcus_neoformans_b/ (72)
			R272		
gattii	C	VGIII	NIH312	CDC4546	
gattii	C	VGIV	Bt26	NA	

Lin, X., and J. Heitman. 2006. Annual Review of Microbiology 60:69-105.

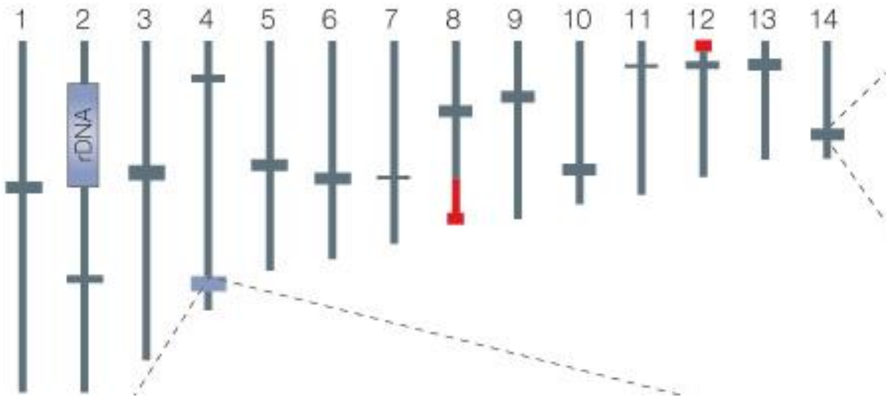
Genome and 3 Major Virulence Phenotypes

a) 14 chromosomes;
~ 6,500 genes

(Loftus, et al., 2005. Science 307, 1321-4.)

a) ~ 190 targeted gene reported , 1994 – 2008

(Toffaletti, D. L., et al., 1993. J Bacteriol. 175:1405-11.)

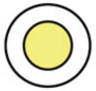


MAT α or MAT a locus

1) Polysaccharide capsule

2) Melanin formation

3) Growth in high temperature

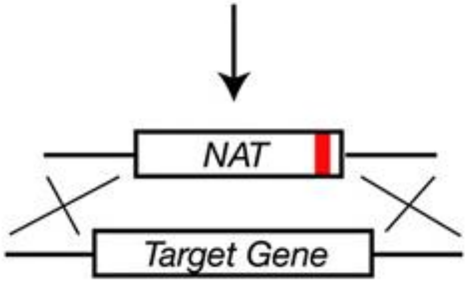

Capsule


Melanin


Growth at 37°

Idnurm A, et al. *Nature Reviews Microbiology . 2005,* 3, 753-764

Optimized high-throughput biolistic transformation of *C. neoformans*



Generated 1,201 targeted mutants using 48 DNA signature tags

Liu, O. W., et al. 2008. Cell 135:174-88.

Genome Statistics

	Size	Chrs	%GC	Genes	tRNAs	rRNAs
C. neoformans var. *grubii* H99 (CNA3)	18.89 Mb	14	48.23	**6,962**	148	4
C. neoformans var. *grubii* H99 mitochondria	24.92 Kb	1	34.98	13	18	1

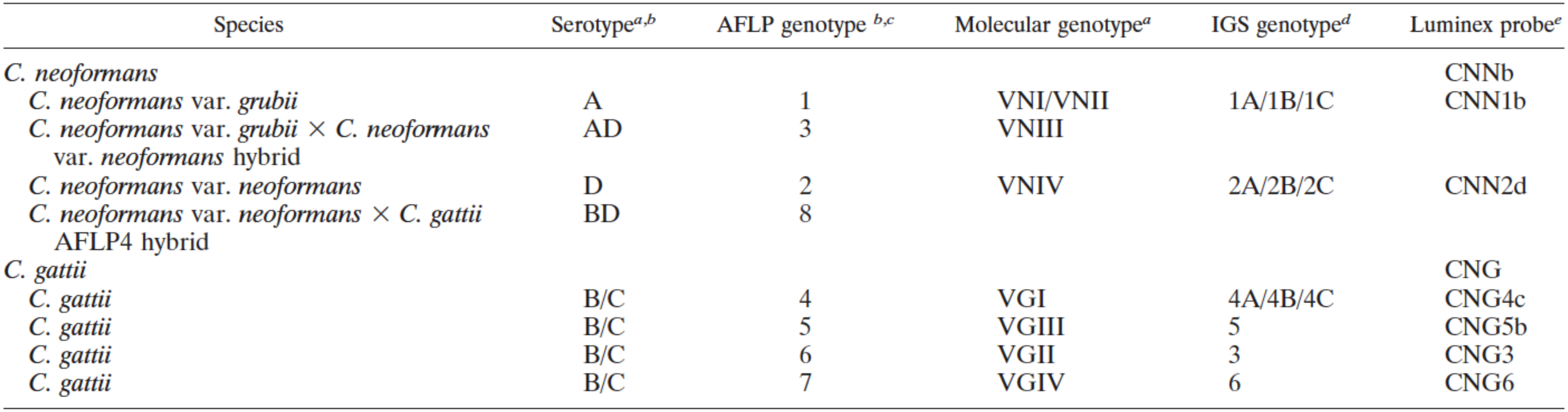
https://data.broadinstitute.org/

Do **major species concepts** support one, two or more species within *Cryptococcus neoformans*?

- Considered a homogeneous species until 1949 when the existence of **four serotypes** based on its polysaccharide capsule
- **Two-species system**: Two teleomorphs discovered
  - *Filobasidiella neoformans* by serotype A and D in 1975,1976
  - *Filobasidiella bacillispora* by serotype B and C in 1976
- **Three major species concepts**:
  - Phenetic species concept (morphologic, phenotypic)
  - Biologic species concept (interbreeding)
  - Cladistic species concept (evolutionary, phylogenetic)

Kwon-Chung, K. J., & Varma, A. (2006). FEMS Yeast Res, 6(4), 574-587.

Identification of genotypically diverse *Cryptococcus neoformans* and *Cryptococcus gattii* isolates by Luminex xMAP technology

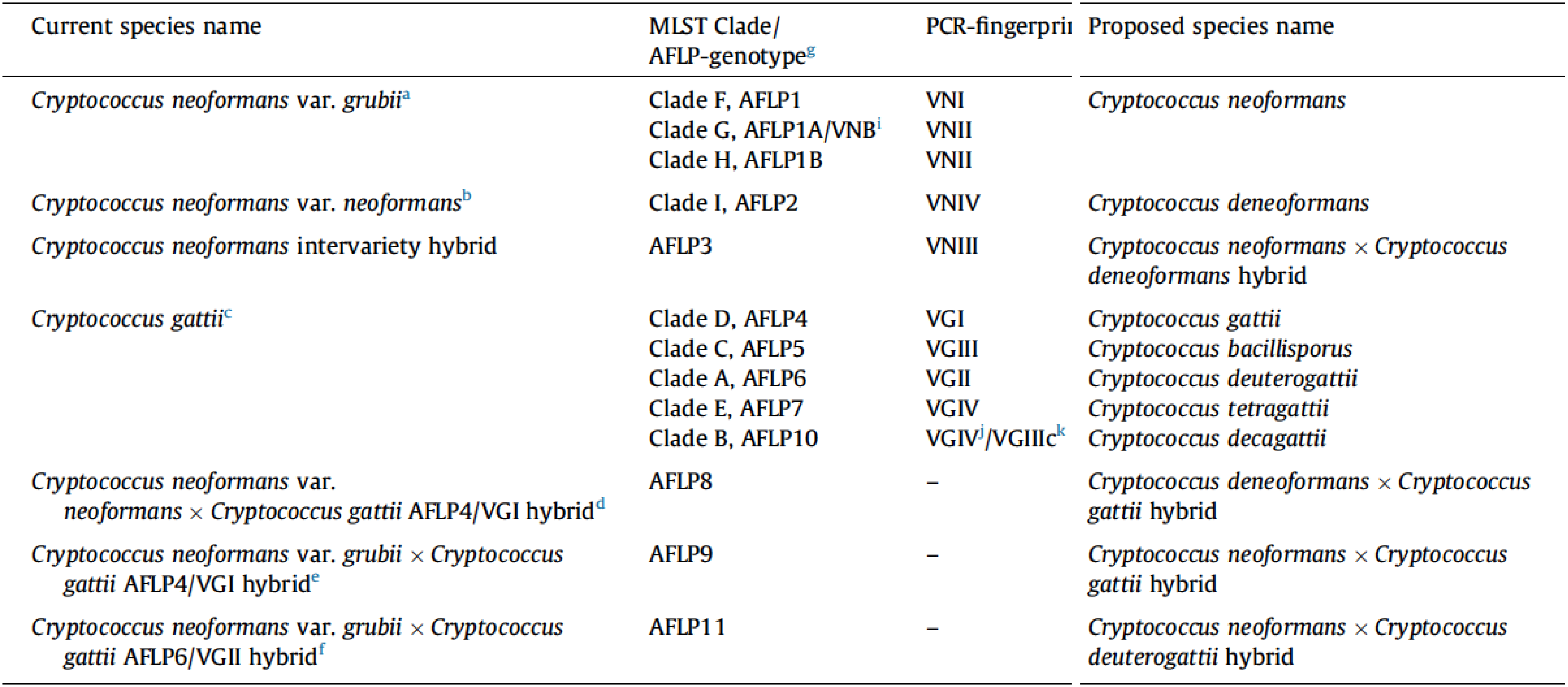


Species	Serotype[a,b]	AFLP genotype [b,c]	Molecular genotype[a]	IGS genotype[d]	Luminex probe[e]
C. neoformans					CNNb
C. neoformans var. *grubii*	A	1	VNI/VNII	1A/1B/1C	CNN1b
C. neoformans var. *grubii* × *C. neoformans* var. *neoformans* hybrid	AD	3	VNIII		
C. neoformans var. *neoformans*	D	2	VNIV	2A/2B/2C	CNN2d
C. neoformans var. *neoformans* × *C. gattii* AFLP4 hybrid	BD	8			
C. gattii					CNG
C. gattii	B/C	4	VGI	4A/4B/4C	CNG4c
C. gattii	B/C	5	VGIII	5	CNG5b
C. gattii	B/C	6	VGII	3	CNG3
C. gattii	B/C	7	VGIV	6	CNG6

- The xMAP technology is based on uniquely color-coded microspheres, which allows as many as one hundred different species to be detected in a single reaction.
- The Luminex suspension array, which detects 5 biotin-labeled PCR amplicons hybridized to specific capture probes.

Bovers, M., et al. (2007). J Clin Microbiol, 45(6), 1874-1883.
Diaz and Fell. (2005). J Clin Microbiol, 43(8), 3662-3672.

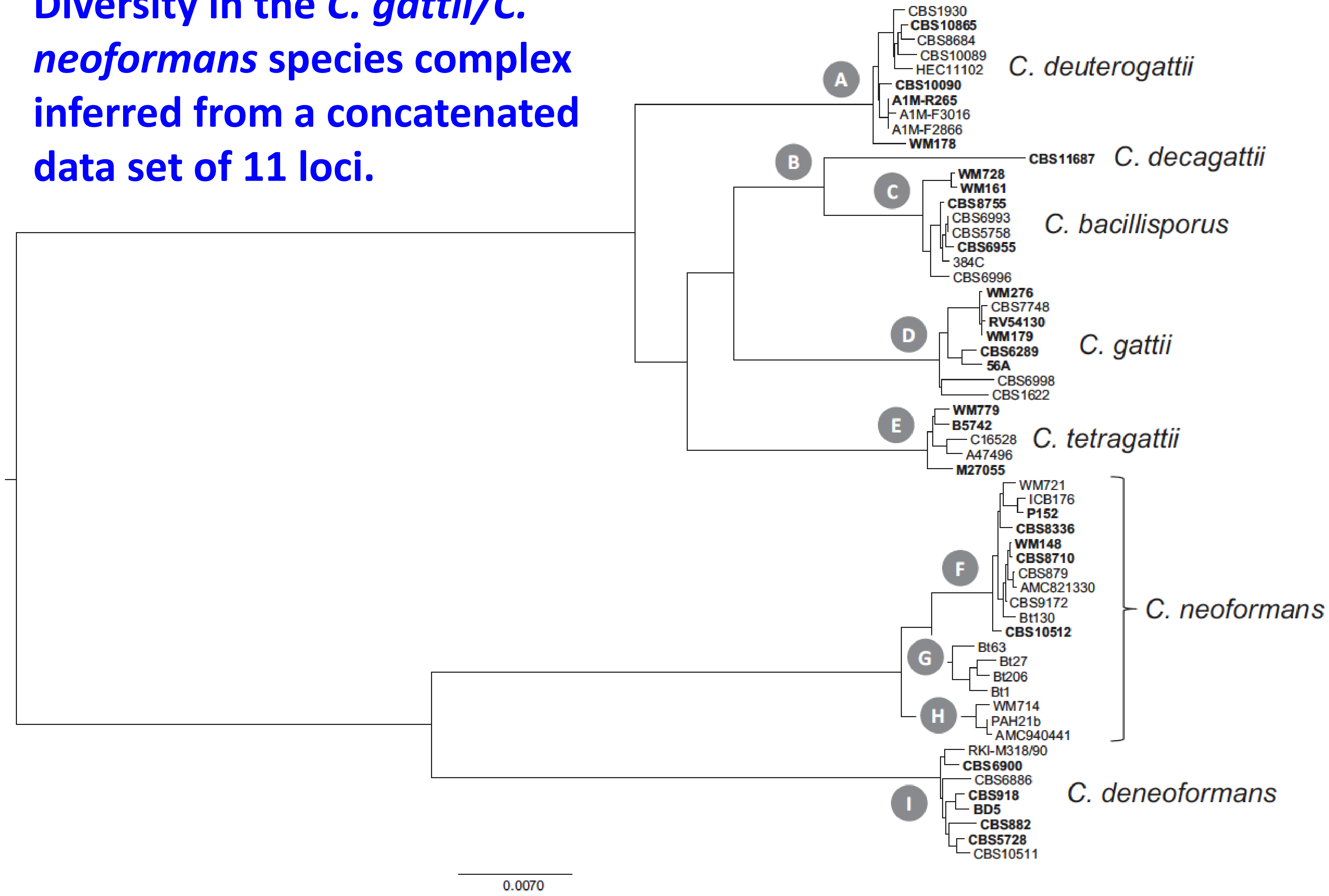
Recognition of seven species in the *Cryptococcus gattii/Cryptococcus neoformans* species complex



Current species name	MLST Clade/ AFLP-genotype[g]	PCR-fingerpri	Proposed species name
Cryptococcus neoformans var. *grubii*[a]	Clade F, AFLP1 Clade G, AFLP1A/VNB[i] Clade H, AFLP1B	VNI VNII VNII	*Cryptococcus neoformans*
Cryptococcus neoformans var. *neoformans*[b]	Clade I, AFLP2	VNIV	*Cryptococcus deneoformans*
Cryptococcus neoformans intervariety hybrid	AFLP3	VNIII	*Cryptococcus neoformans* × *Cryptococcus deneoformans* hybrid
Cryptococcus gattii[c]	Clade D, AFLP4 Clade C, AFLP5 Clade A, AFLP6 Clade E, AFLP7 Clade B, AFLP10	VGI VGIII VGII VGIV VGIV[j]/VGIIIc[k]	*Cryptococcus gattii* *Cryptococcus bacillisporus* *Cryptococcus deuterogattii* *Cryptococcus tetragattii* *Cryptococcus decagattii*
Cryptococcus neoformans var. *neoformans* × *Cryptococcus gattii* AFLP4/VGI hybrid[d]	AFLP8	–	*Cryptococcus deneoformans* × *Cryptococcus gattii* hybrid
Cryptococcus neoformans var. *grubii* × *Cryptococcus gattii* AFLP4/VGI hybrid[e]	AFLP9	–	*Cryptococcus neoformans* × *Cryptococcus gattii* hybrid
Cryptococcus neoformans var. *grubii* × *Cryptococcus gattii* AFLP6/VGII hybrid[f]	AFLP11	–	*Cryptococcus neoformans* × *Cryptococcus deuterogattii* hybrid

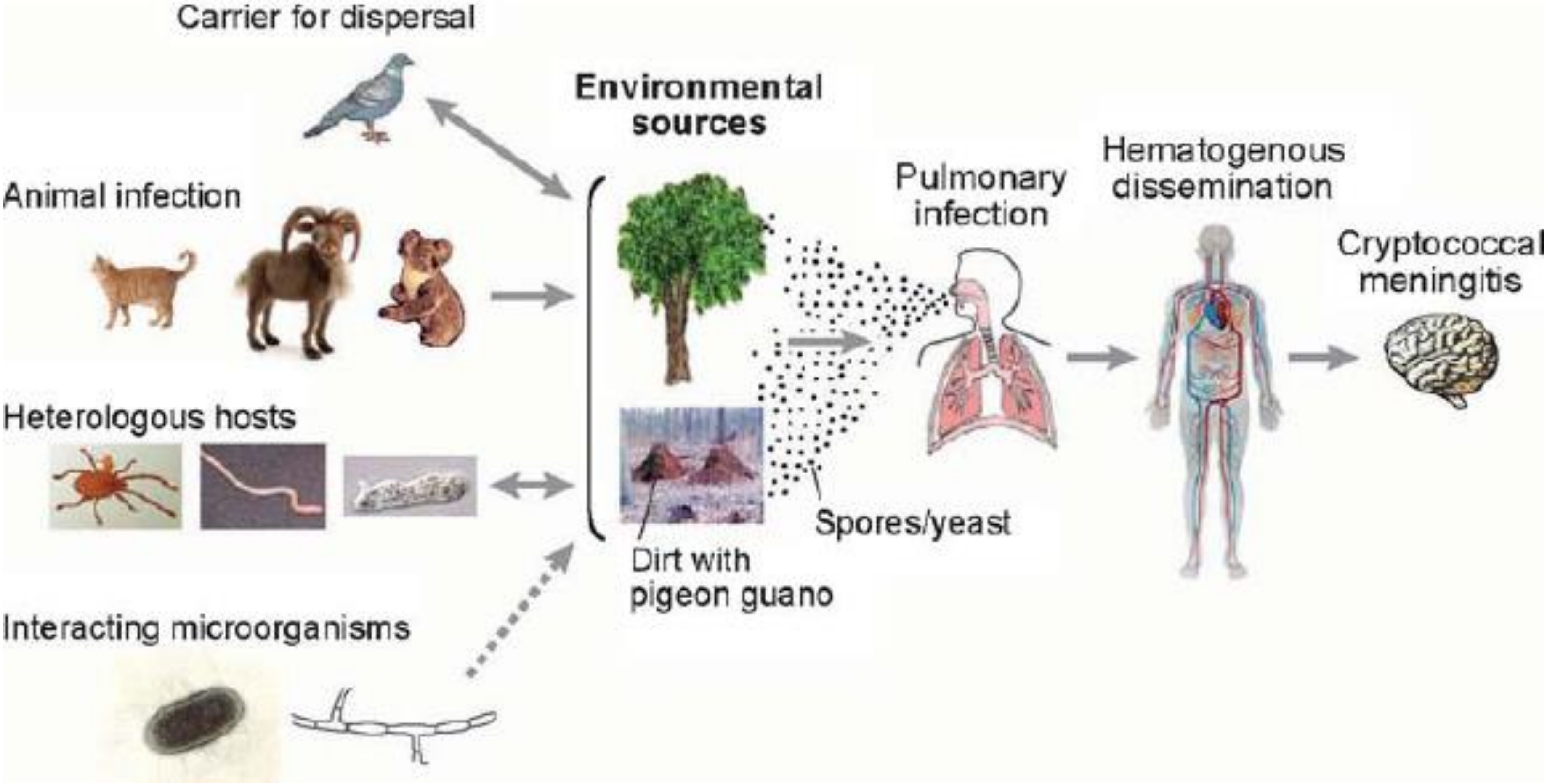
Hagen, F., et al. (2015). Fungal Genetics and Biology, 78, 16-48.

Diversity in the *C. gattii/C. neoformans* species complex inferred from a concatenated data set of 11 loci.



Hagen, F., et al. (2015). Fungal Genetics and Biology, 78, 16-48.

Infection cycle of *Cryptococcus* (environment and host)



Lin, X., and J. Heitman. 2006. Annual Review of Microbiology 60:69-105.

Primary cutaneous infection

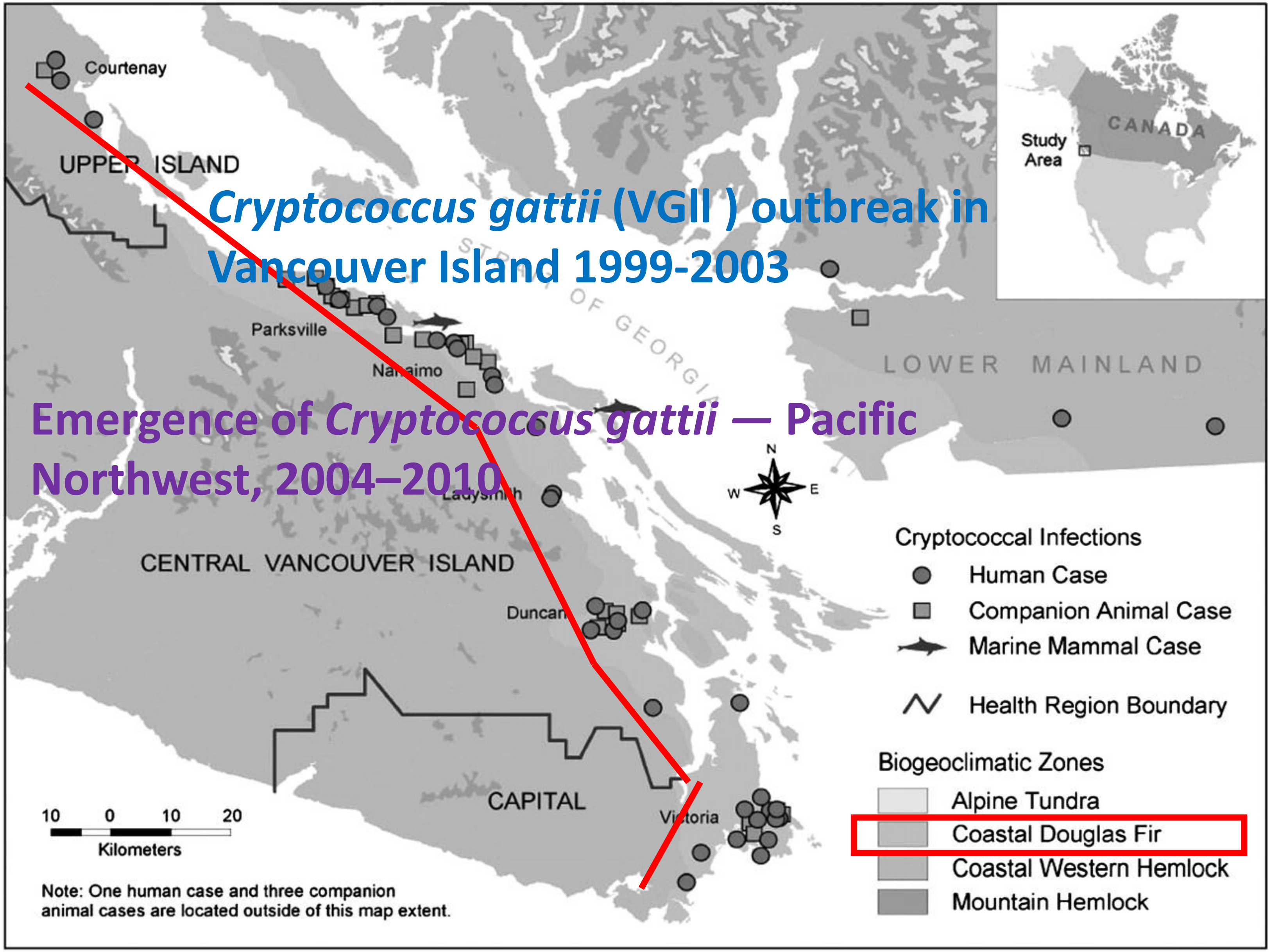
- History of trauma and direct inoculation
- Most often occurs in immunosuppressed patients but may occur in nonimmunosuppressed patients
- Single lesion develops at the site of infection
- Solitary nodules that may ulcerate
- Cellulitis; Ulcers; Abscesses; Panniculitis
- Whitlow

Patel P, et al. J Am Acad Dermatol 2000; 43: 344-345.

Allegue F, et al. Acta Derm Venereol 2007; 87: 443-444.

Vancouver island: 32,284 km^2.
Population: 0. 697 million, 2/3 of which live in the southern part
Temperate climate zone
2.7°C in winter and 17.6°C in summer
the mean precipitation is 136 mm/month in winter and 40 mm/month in summer.

Taiwan island: 36,000 km^2.
Population: 23 million
Subtropical climate zone
above 25°C between June and September
over 15°C between November and March.



Cryptococcus gattii (VGII) outbreak in Vancouver Island 1999-2003

Emergence of *Cryptococcus gattii* — Pacific Northwest, 2004–2010

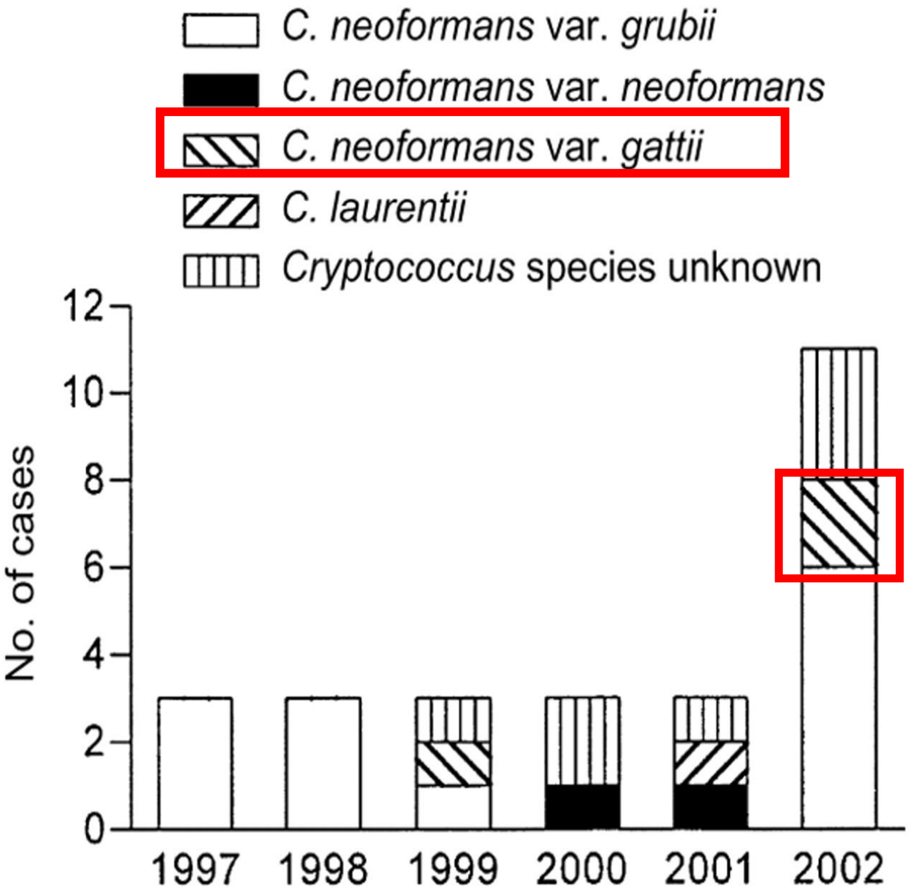
Emergence of *Cryptococcus gattii*-- Pacific Northwest, 2004-2010. MMWR Morb Mortal Wkly Rep 2010 Jul 23;59(28):865-8.

GLOBAL DISTRIBUTION OF *C. neoformans var. gattii* in 1984

- Less than 15% of the isolates from Argentina, **Canada**, the United Kingdom, and the United States (except southern California) were ***C. neoformans var. gattii.***
- There was an unusually high (35-100%) prevalence of ***C. neoformans* var. *gattii*** in Australia, Brazil, Cambodia, Hawaii, southern California, Mexico, Paraguay, Thailand, Vietnam, Nepal, and countries in central Africa.
- These findings indicated that ***C. neoformans var. gattii*** is prevalent only in tropical and subtropical regions.

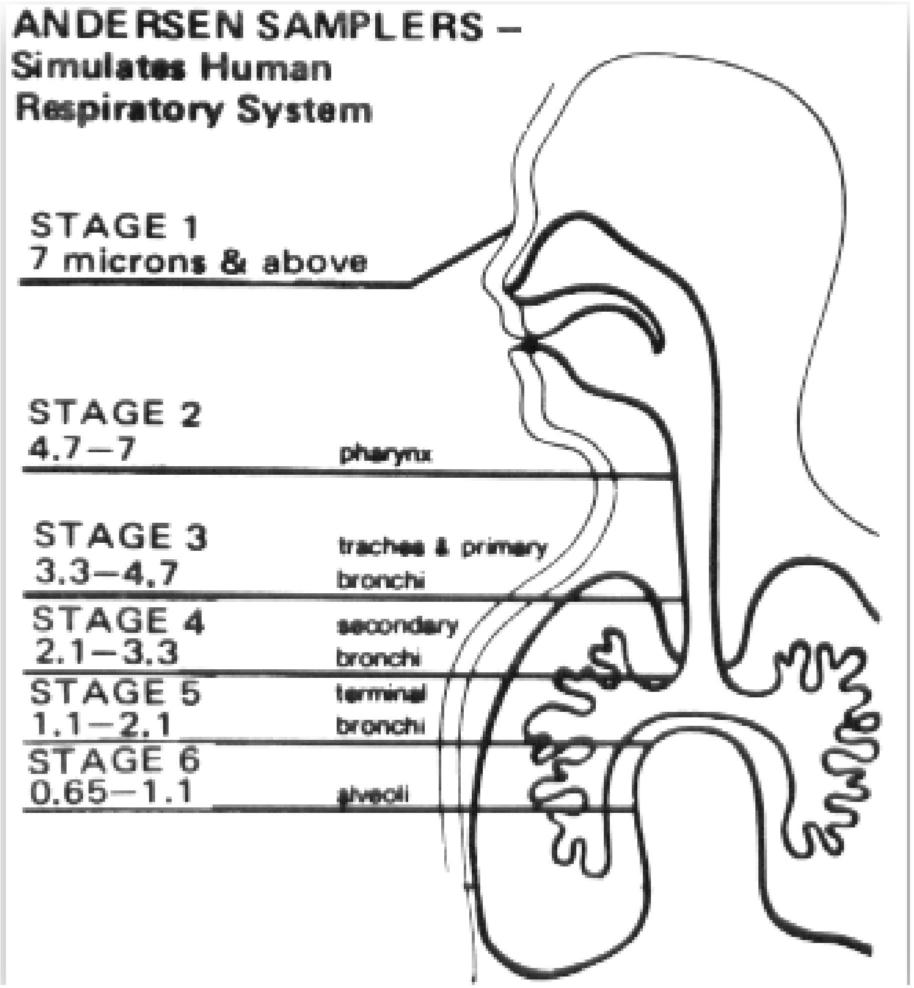
Kwon-Chung, et al (1984) *Am. J. Epidemiol.* **120,** 123–130.

A total of 26 cases of cryptococcosis were definitively diagnosed at the Vancouver Hospital and Health Sciences Centre between 1997 and 2002



Hoang et al. (2004) *J. Med. Microbiol.* **53,** 935–940

The Andersen six-stage air sampling head was connected to an Air-Con2 pump



11 air samples were taken to test for the existence of airborne cryptococcal propagules in the environs of the trees where swabs had been taken.

Outbreak isolates VGIIa and VGIIb

- **PCR-fingerprinting** divided all outbreak and environmental VGII isolates into 2 subgroups, designated VGIIa and VGIIb.
- The PCR-fingerprint profiles of the 2 subgroups were 98% similar, distinguishable by a single band of 1,218 bp.
- **AFLP analysis** identified the majority of outbreak isolates as AFLP6/VGII, whereas 2 isolates were AFLP4/VGI.
- The overall similarity of the AFLP profiles of the clinical isolates was 84.6% using UPGMA (unweighted pair group method using arithmetic means) and 94.9% using single linkage.

Rathtrevor Beach Provincial Park (RBPP)

Kidd SE, et al. Proc Natl Acad Sci U S A. 2004;101:17258-17263.

Environmental isolates

- Of the 732 environmental samples taken until June 2002 across 16 different sites in BC, 58 *C. gattii* isolates were obtained from 25 trees in 2 different locations on Vancouver Island.
- These included 57 isolates from 24 trees at Rathtrevor Beach Provincial Park (RBPP), located in Parksville, and 1 isolate from MacMillan Park, located in Cathedral Grove, 31 km west of Parksville. Both parks are located in the CDF of Vancouver Island.
- *C. gattii* was isolated from the following 5 native tree species: alder (*n* = 5), cedar (*n* = 1), Douglas fir (*n* = 16), grand fir (*n* = 1), and Garry oak (*n* = 2). There were no positive samples from eucalypts.

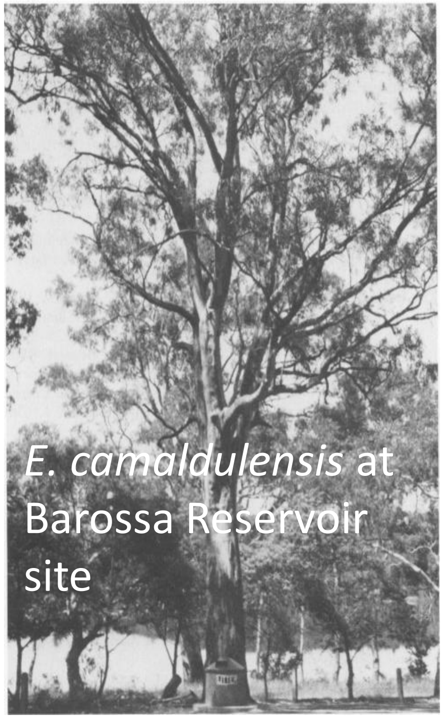
Rathtrevor Beach Provincial Park (RBPP)

Kidd SE, et al. Proc Natl Acad Sci U S A. 2004;101:17258-17263.

Plant Niches

Arbutus 楊梅樹 (岩梨)

Douglas fir
洋松



E. camaldulensis at Barossa Reservoir site

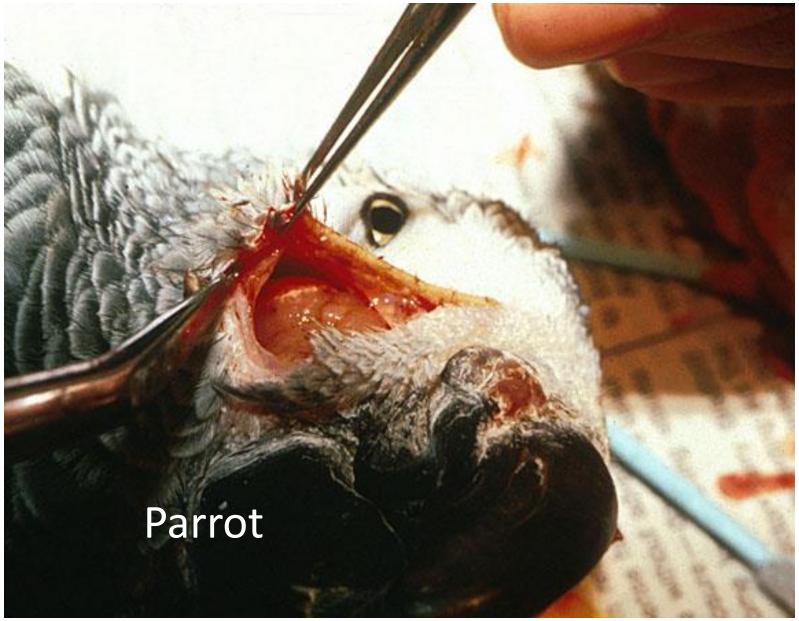
Cedar 西洋杉 (香柏)

Grand fir
樅樹

Garry oak (橡樹)

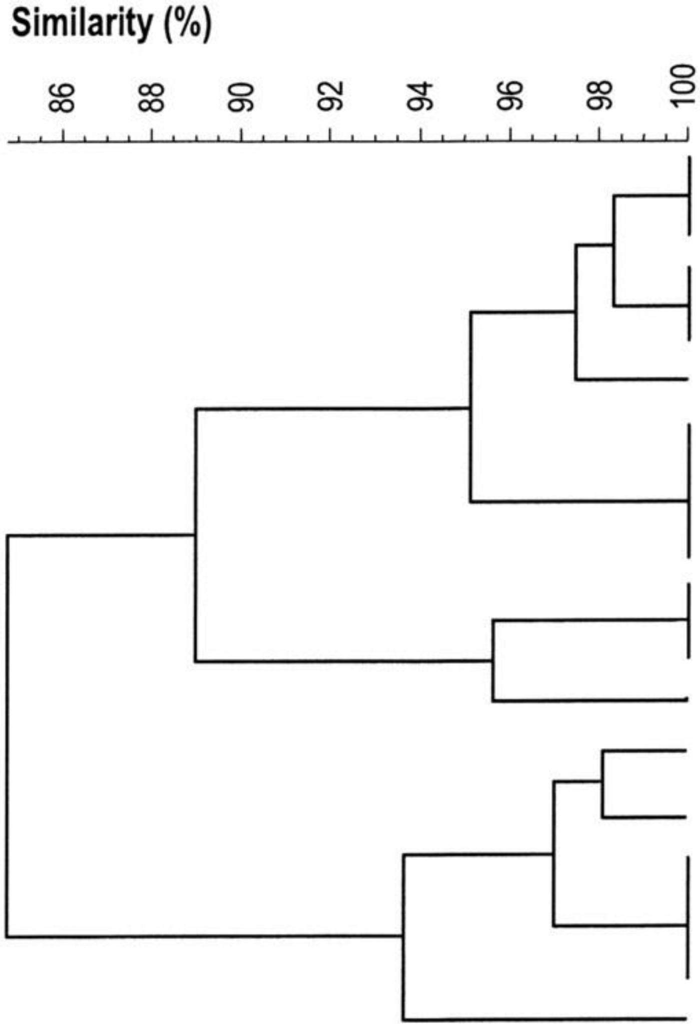
1. Ellis DH, Pfeiffer TJ. *J Clin Microbiol. 1990;28:1642-1644.*
2. Kidd SE, et al. Proc Natl Acad Sci U S A. 2004;101:17258-17263.

Animal Cryptococcosis



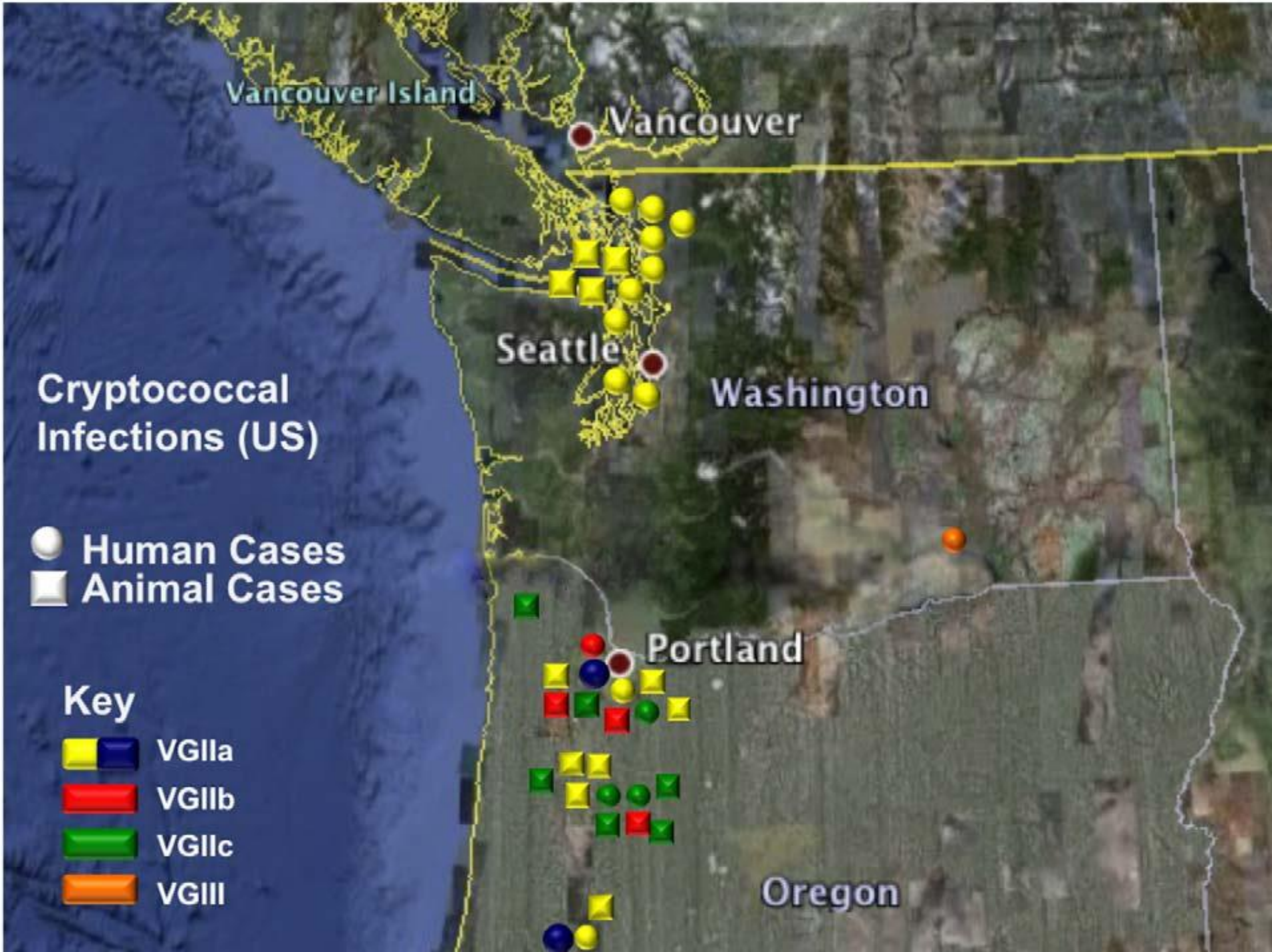
Kidd SE, et al. Proc Natl Acad Sci U S A. 2004;101:17258-17263.
And from Textbook of Infectious Diseases

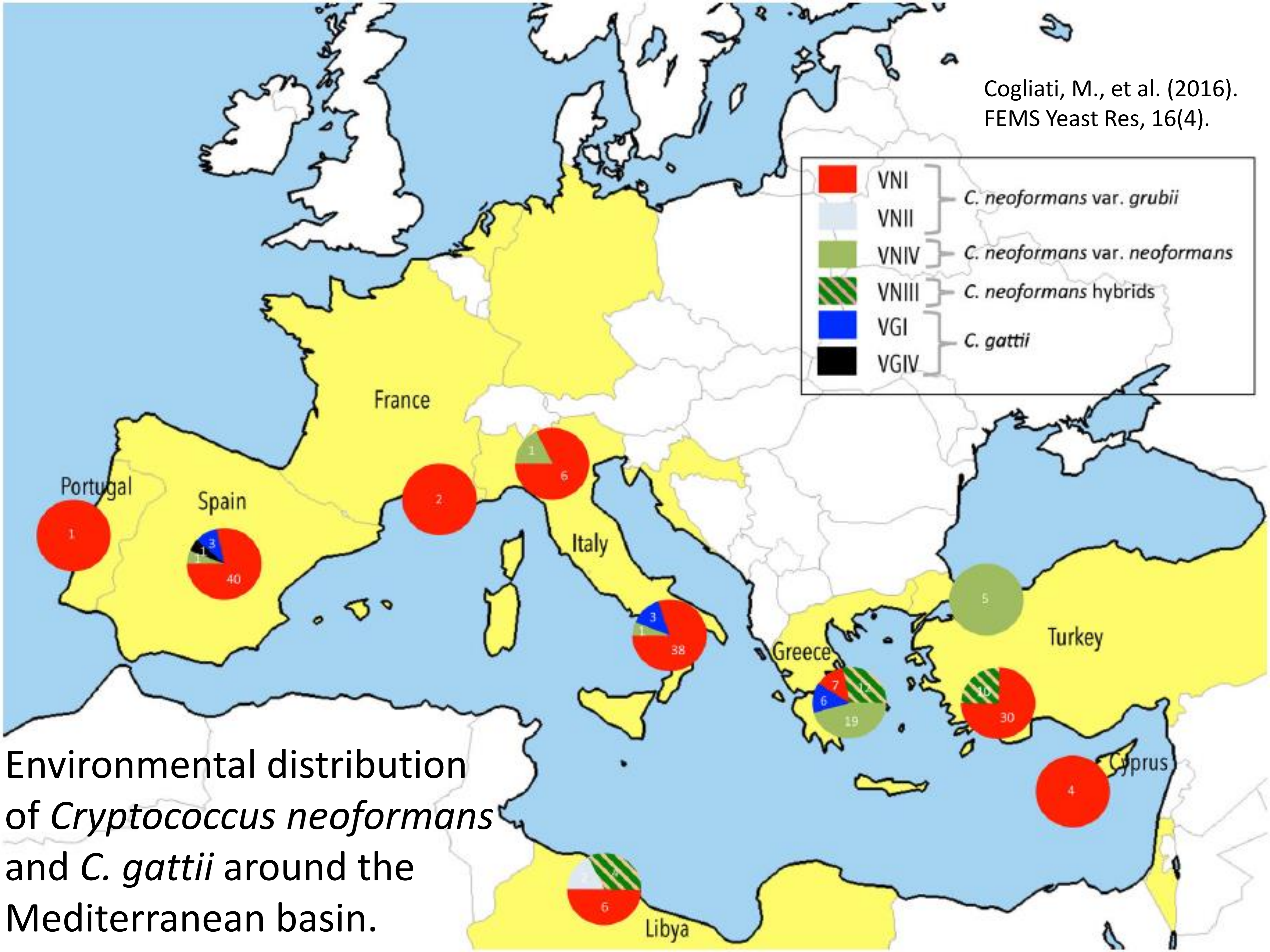
Comparison of PCR-fingerprint similarity between selected outbreak isolates and previously studied global VGI and VGII isolates.



Strain ID	Molecular type	Geographical Origin
RB31	VGIIb	Vancouver Island, Canada
A1M R272	VGIIb	Vancouver Island, Canada
ENV 130	VGIIa	Vancouver Island, Canada
A1M R265	VGIIa	Vancouver Island, Canada
WM1008	VGII	Sydney, Australia
Ram005	VGII	Arnhemland, Australia
Ram002	VGII	Arnhemland, Australia
ARN001	VGII	Arnhemland, Australia
CBS 7750	VGII	San Francisco, USA
ATCC 32609	VGII	Washington, USA
WM178	VGII Standard	Sydney, Australia
CBS 7748	VGI	Balranald, Australia
WM830	VGI	Papua New Guinea
A1M F2863	VGI	Vancouver Island, Canada
WM179	VGI Standard	Sydney, Australia
A1M R794	VGI	Vancouver Island, Canada
WM1009	VGI	Sydney, Australia

Kidd SE, et al. Proc Natl Acad Sci U S A. 2004;101:17258-17263.

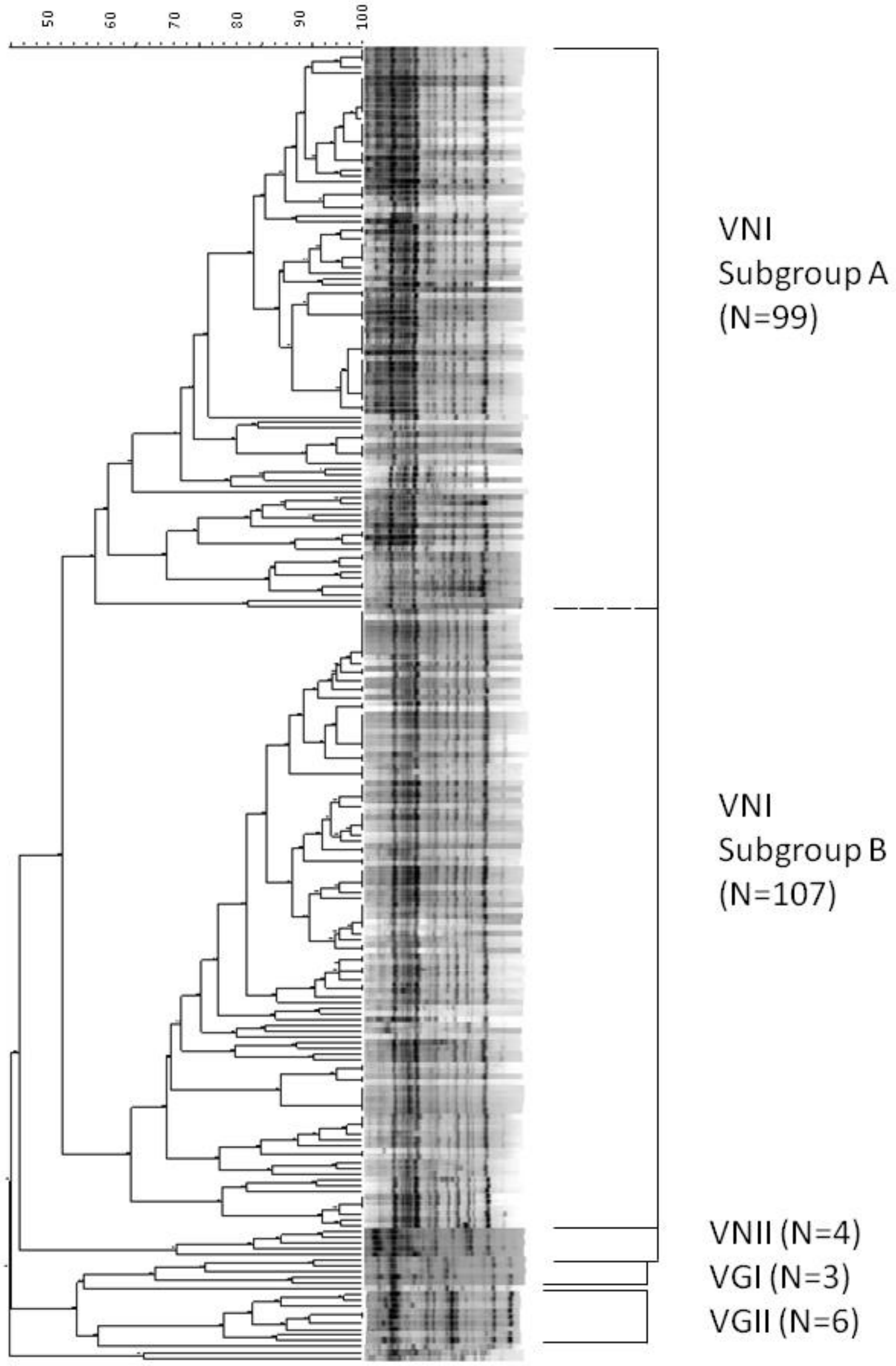

Vancouver Island
Vancouver
Seattle
Washington
Portland
Oregon
Cryptococcal Infections (US)
Human Cases
Animal Cases
Key
VGIIa
VGIIb
VGIIc
VGIII



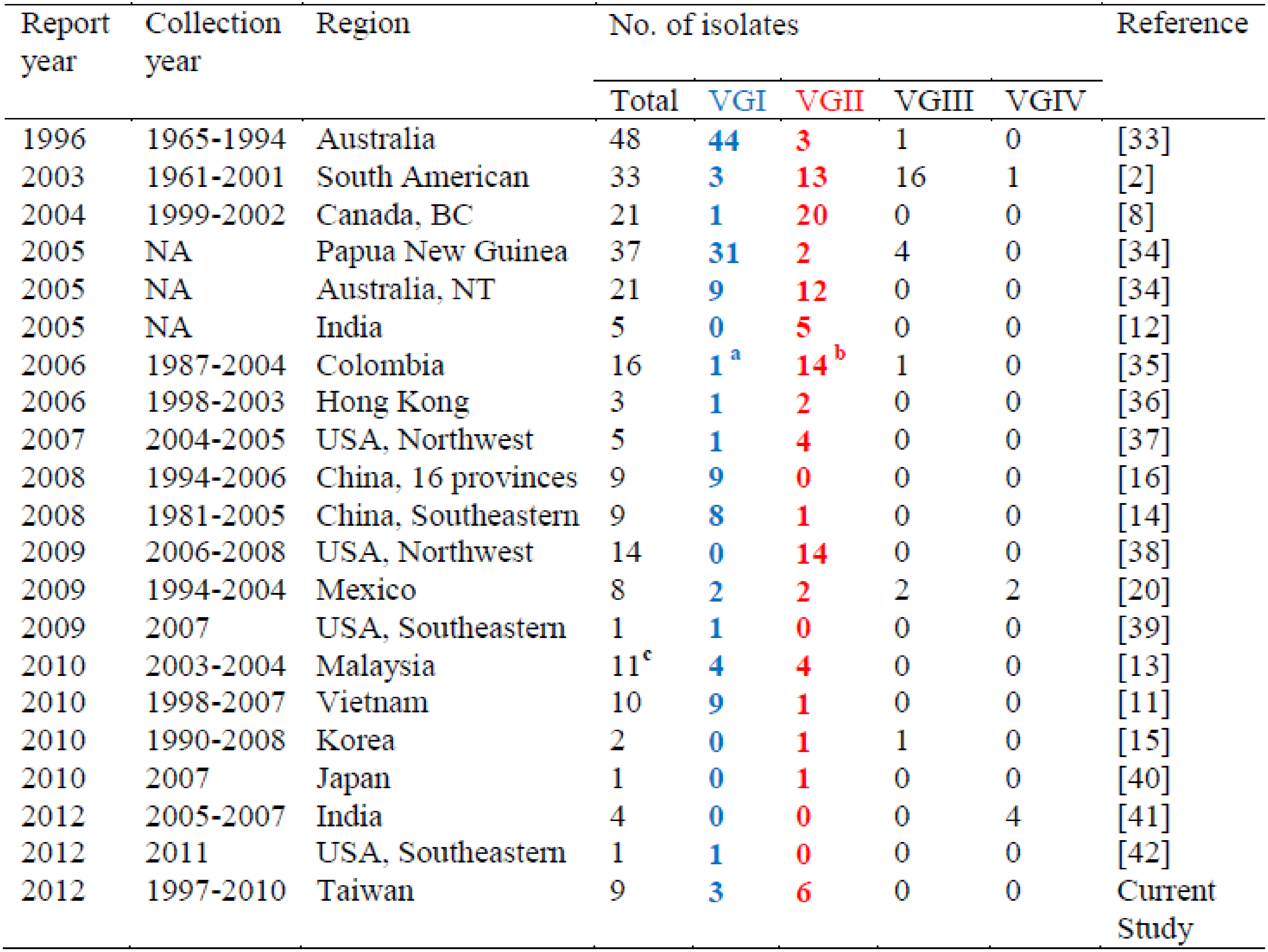
Cogliati, M., et al. (2016).
FEMS Yeast Res, 16(4).

Environmental distribution of *Cryptococcus neoformans* and *C. gattii* around the Mediterranean basin.

Dendrogram of M13 PCR fingerprinting analysis of 219 clinical isolates collected in Taiwan during 1997-2010



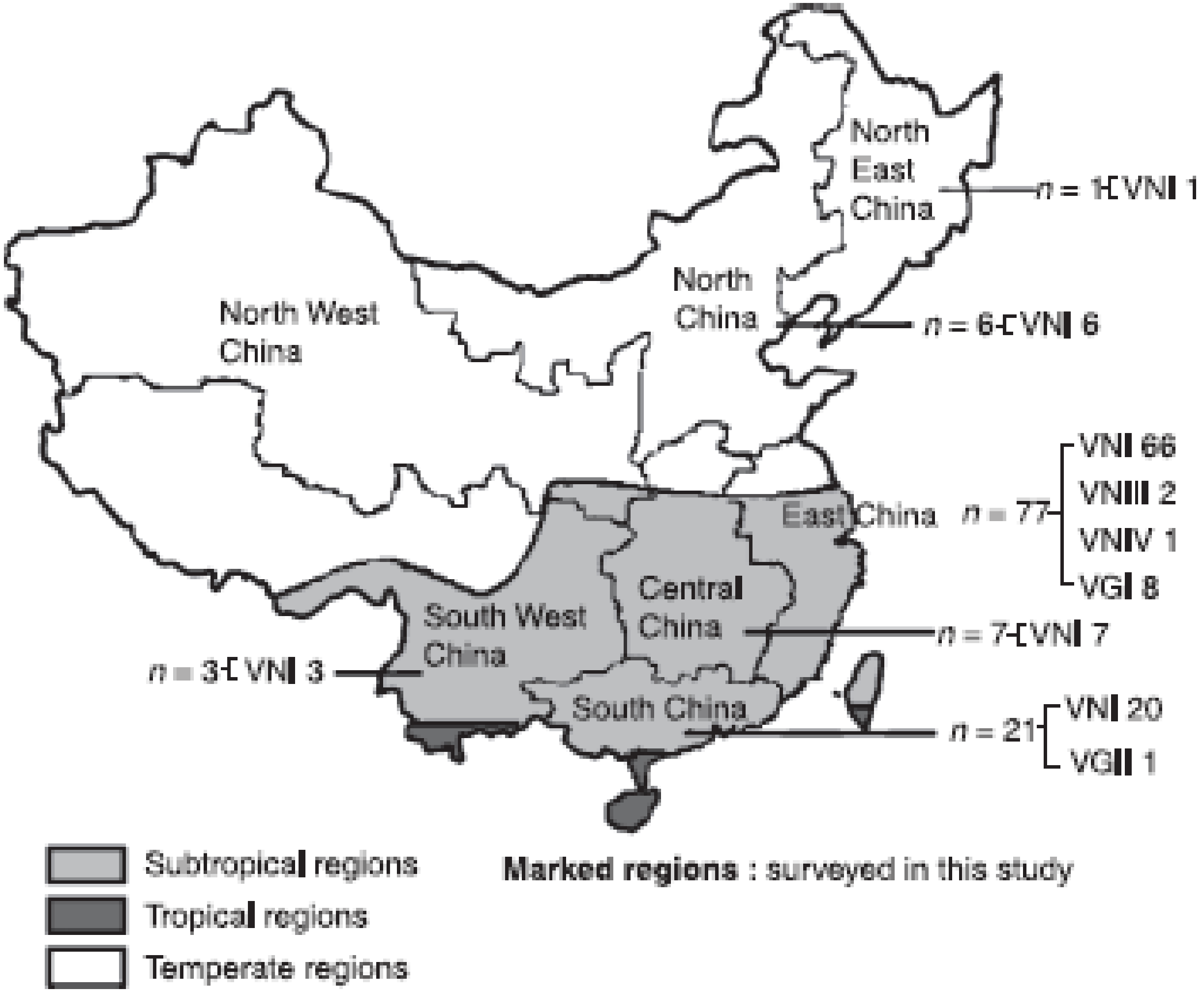
Tseng, H. K., et al. (2013). PloS One, 8(4), e61921.



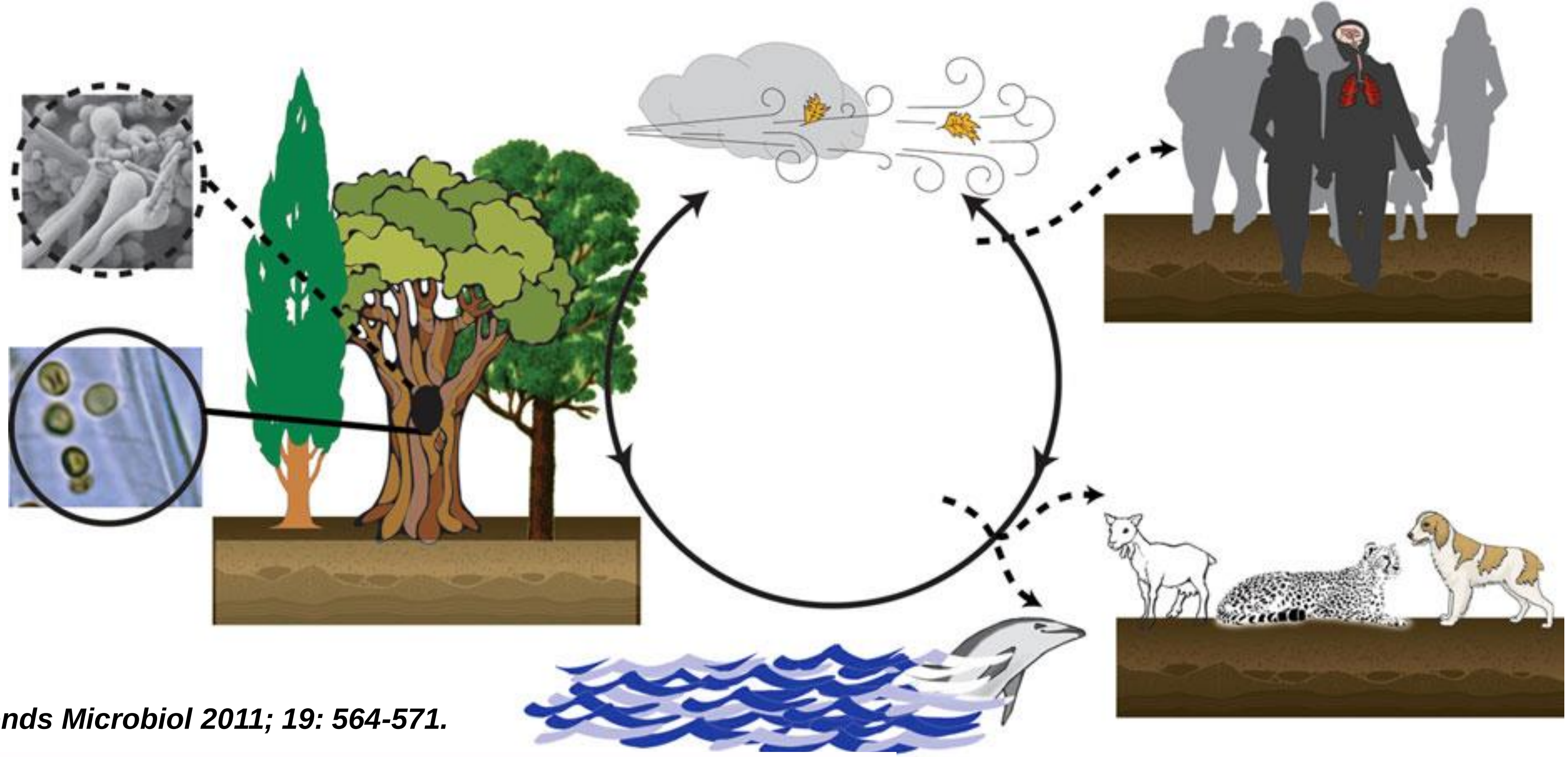
Report year	Collection year	Region	No. of isolates					Reference
			Total	VGI	VGII	VGIII	VGIV	
1996	1965-1994	Australia	48	**44**	**3**	1	0	[33]
2003	1961-2001	South American	33	**3**	**13**	16	1	[2]
2004	1999-2002	Canada, BC	21	**1**	**20**	0	0	[8]
2005	NA	Papua New Guinea	37	**31**	**2**	4	0	[34]
2005	NA	Australia, NT	21	**9**	**12**	0	0	[34]
2005	NA	India	5	**0**	**5**	0	0	[12]
2006	1987-2004	Colombia	16	**1** [a]	**14** [b]	1	0	[35]
2006	1998-2003	Hong Kong	3	**1**	**2**	0	0	[36]
2007	2004-2005	USA, Northwest	5	**1**	**4**	0	0	[37]
2008	1994-2006	China, 16 provinces	9	**9**	**0**	0	0	[16]
2008	1981-2005	China, Southeastern	9	**8**	**1**	0	0	[14]
2009	2006-2008	USA, Northwest	14	**0**	**14**	0	0	[38]
2009	1994-2004	Mexico	8	**2**	**2**	2	2	[20]
2009	2007	USA, Southeastern	1	**1**	**0**	0	0	[39]
2010	2003-2004	Malaysia	11[c]	**4**	**4**	0	0	[13]
2010	1998-2007	Vietnam	10	**9**	**1**	0	0	[11]
2010	1990-2008	Korea	2	**0**	**1**	1	0	[15]
2010	2007	Japan	1	**0**	**1**	0	0	[40]
2012	2005-2007	India	4	**0**	**0**	0	4	[41]
2012	2011	USA, Southeastern	1	**1**	**0**	0	0	[42]
2012	1997-2010	Taiwan	9	**3**	**6**	0	0	Current Study

Tseng, H. K., et al. (2013). PloS One, 8(4), e61921.

The distribution of cryptococcal isolates from China and the constitution of molecular types from six regions of China



Feng, X., et al. (2008) *FEMS Yeast Res*, 8(6), 930-938.



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