



# Importance of Resolving Fungal Nomenclature: the Case of Multiple Pathogenic Species in the *Cryptococcus* Genus

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 Resolving *Cryptococcus* nomenclature

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**ABSTRACT** Cryptococcosis is a major fungal disease caused by members of the *Cryptococcus gattii* and *Cryptococcus neoformans* species complexes. After more than 15 years of molecular genetic and phenotypic studies and much debate, a proposal for a taxonomic revision was made. The two varieties within *C. neoformans* were raised to species level, and the same was done for five genotypes within *C. gattii*. In a recent perspective (K. J. Kwon-Chung et al., mSphere 2:e00357-16, 2017, <https://doi.org/10.1128/mSphere.00357-16>), it was argued that this taxonomic proposal was premature and without consensus in the community. Although the authors of the perspective recognized the existence of genetic diversity, they preferred the use of the informal nomenclature “*C. neoformans* species complex” and “*C. gattii* species complex.” Here we highlight the advantage of recognizing these seven species, as ignoring these species will impede deciphering further biologically and clinically relevant differences between them, which may in turn delay future clinical advances.

**KEYWORDS** *Cryptococcus*, cryptococcosis, diagnostics, species delimitation, taxonomy

This perspective concerns the revision of the genus *Cryptococcus* in 2015 to recognize seven new species in what had been considered to be two species complexes of this important human-pathogenic fungus (1) and the more recent perspective (2) criticizing the 2015 revision. The following three main issues were raised (2). (i) The taxonomic proposal is premature. (ii) The new species cannot be identified using phenotypic tests alone. (iii) The new species names are confusing. The “2015 taxonomy paper” (1) has been highly cited, indicating that it fulfills a role in the scientific discussions on the taxonomy of the species complexes. At the recently held 10th International Conference on *Cryptococcus* and Cryptococcosis (ICCC10) (Foz do Iguaçu,

Brazil, 26 to 30 March 2017), this matter was once more discussed, and ample evidence was provided that at least seven, and likely even more, species exist.

Cryptococcosis is an important fungal infection, globally affecting immunocompromised and immunocompetent humans and animals (3, 4). Annually more than 200,000 HIV-positive individuals develop cryptococcal meningitis with approximately 180,000 casualties (5). The phenotypic heterogeneity within the *Cryptococcus neoformans* species complex has been known for many years, beginning with the identification of four serotypes, serotypes A to D (6, 7). The discovery of an atypical clinical cryptococcal isolate led to the designation of a new variety named *C. neoformans* var. *gattii* (serotypes B and C) next to *C. neoformans* var. *neoformans* (serotypes A and D) (8, 9). The observation of the sexual cycle led to the description of *Filobasidiella neoformans* and *Filobasidiella bacillispora* (10–12). A third variety, *C. neoformans* var. *grubii*, was introduced in 1999 for serotype A strains, thus the variety *neoformans* became restricted to serotype D strains (13). In 2002, *C. neoformans* var. *gattii* was raised to species level, and the name *C. gattii* was given nomenclatural priority over the older name *C. bacillisporus* (14). At this stage, two species, *C. gattii* and *C. neoformans*, were recognized with the latter comprising two varieties, *neoformans* and *grubii*. The presence of diploid and aneuploid serotype A and serotype D hybrids (*C. neoformans* × *C. deneoformans*) has been known for a long time (7, 15–18), and they constitute 19 to 36% of the cryptococcal agents in southern Europe (19, 20). It is noteworthy that from a nomenclatural point of view, the type strain of *C. neoformans* CBS132 is a serotype AD hybrid (1, 17).

Morphology is a poor predictor to infer phylogenetic relationships of fungal isolates and particularly so for yeasts (21–27). Recently, the earlier name used to refer to the yeast morphology of *Cryptococcus* isolates was given priority over the teleomorphic name *Filobasidiella* (21, 22). The genus *Cryptococcus* in its current concept contains the dimorphic yeasts *C. amyloletus*, *C. bacillisporus*, *C. decagattii*, *C. deneoformans*, *C. deuterogattii*, *C. neoformans*, *C. gattii*, and *C. tetragattii* (21, 22) and the filamentous species *C. depauperatus* and *C. luteus* (8, 22, 28, 29).

Molecular data revealed that the *C. neoformans* and *C. gattii* species complexes were unexpectedly genetically diverse (30). On the basis of four genes, it was calculated that *C. neoformans*/*C. deneoformans* separated from the *C. gattii* species complex 37 million years ago, *C. neoformans* and *C. deneoformans* separated 18.5 million years ago, and *C. gattii* and *C. bacillisporus* separated 9.5 million years ago (31). These divergence times might be older, as recent calculations based on genomic data fine-tuned the divergence time of the *C. neoformans*/*C. deneoformans* and the *C. gattii* species complex to 80 to 100 million years ago (32). The genomes of *C. deneoformans* and *C. neoformans* differ at ~10% of nucleotide positions (33). This difference is so large that the same phylogenetic groups have been found no matter which particular isolates were used and despite the increasing resolution of molecular typing tools, such as PCR-fingerprinting, amplified fragment length polymorphism (AFLP) fingerprinting, multi-locus sequence typing (MLST), and whole-genome sequencing (WGS) (15, 30, 34–42).

Phenotypic, ecological, and geographical variation also supports creating species-level taxa in the *C. gattii* and *C. neoformans* species complexes (Table 1) (1, 43–67). For example, a recent study on virulence attributes such as capsule and melanin of members of the *C. gattii* species complex concluded with “These findings argue for increased acceptance of the new species and may be useful for informing diagnosis and prognosis in clinical infection” (50).

Genetic methods revealed that intraspecies crosses between *C. neoformans* and *C. deneoformans* isolates showed a higher spore viability compared to *C. deneoformans* × *C. neoformans* interspecies crosses (33). Twenty-three quantitative trait loci were identified from the analysis of interspecific crosses involved in virulence-associated and azole-resistant phenotype differences between both species (61), and the observed postzygotic isolation mechanisms were explained by Bateson-Dobzhansky-Muller incompatibility affecting basidiospore viability in interspecific crosses (62). Mitotic recombination, causing chromosomal loss and crossing over, seems a further genetic separation mechanism

**TABLE 1** Characteristics of pathogenic *Cryptococcus* species<sup>a</sup>

Characteristic	<i>C. neoformans</i> AFLP1/VNI, AFLP1A/VNB/ VNI1, and AFLP1B/VNI1	<i>C. deaneoformans</i> AFLP2/VNIV	<i>C. gattii</i> AFLP4/VGI	<i>C. bacillisporus</i> AFLP5/VGIII	<i>C. deuterogattii</i> AFLP6/VGII	<i>C. tetrageattii</i> AFLP7/VGIV	<i>C. decagattii</i> AFLP10
Genotype							
Geographical distribution <sup>b</sup>	Worldwide (↑ AFR)	Global (↑ EUR)	Worldwide (↑ Asia, AUS, EUR)	Global (↑ California)	Worldwide (↑ AUS, NAM, SAM)	Sub-Saharan Africa and India	Latin America
Ecological preference	Bird droppings, soil, trees (1, 51–55)	Bird droppings, soil, trees (1, 51–55)	Trees (1)	Trees	Trees	?	?
Colonization	↑ in <i>Arabidopsis thaliana</i> compared to <i>C. deaneoformans</i> (54)	↓ in <i>Arabidopsis thaliana</i> compared to <i>C. neoformans</i> (54)	ND	ND	ND	ND	ND
Animal infection	↑ Birds	?	↑ Mammals	Mammals	↑ Mammals	?	?
Susceptibility to antifungal drugs <sup>c</sup>	↑ GM MICs for AMB than <i>C. deaneoformans</i> and interspecies hybrids (19, 48); ↑ GM MICs for 5FC compared to <i>C. tetrageattii</i> (152)	↑ GM MICs for 5FC than <i>C. neoformans</i> and interspecies hybrids (48)	↑ GM MICs for FLZ, ITZ, and VCZ than <i>C. neoformans</i> (49)	No specific determinants	↑ GM MICs for 5FC, FLZ, VCZ, ITZ, PSZ, and ISA than <i>C. gattii</i> (44–46)	↓ GM MICs for 5FC compared to <i>C. neoformans</i> (152)	?
Clinical/host immune status	Mainly immunocompromised (↑ HIV), but subgenotype VNI1y from immunocompetent subjects (84). ↑ meningitis	Immunocompromised and immunocompetent, ↑ cutaneous and elderly (153)	↑ Apparently healthy subjects, ↑ cryptococcoma	↑ HIV-positive subjects	↑ Apparently healthy subjects, ↑ pulmonary infections	↑ HIV-positive subjects	HIV-positive subjects
Capsule properties	↓ compared to <i>C. gattii sensu lato</i> (154)	ND	↑ compared to <i>C. neoformans</i> (154); ↑ compared to <i>C. bacillisporus</i> , <i>C. deuterogattii</i> , and <i>C. tetrageattii</i> (50)	↑ compared to <i>C. neoformans</i> and <i>C. deuterogattii</i> (154)	↑ compared to <i>C. neoformans</i> (154); ↓ compared to <i>C. bacillisporus</i> , <i>C. gattii</i> , and <i>C. tetrageattii</i> (48)	↑ compared to <i>C. neoformans</i> (154)	ND
Cell volume	ND	ND	↓ compared to <i>C. bacillisporus</i> , <i>C. deuterogattii</i> , and <i>C. tetrageattii</i> ; absence of giant cells (50)	ND	↑ compared to <i>C. bacillisporus</i> , <i>C. gattii</i> , and <i>C. tetrageattii</i> ; ↑ giant cells (50)	↑ Giant cells (50)	ND

(Continued on next page)

**TABLE 1** (Continued)

Characteristic	<i>C. neoformans</i>	<i>C. deeneoformans</i>	<i>C. gattii</i>	<i>C. bacillisporus</i>	<i>C. deuterogattii</i>	<i>C. tetragattii</i>	<i>C. decagattii</i>
Thermotolerance	↑ Growth rate at 37°C (154)	↓ Growth rate at 37°C (154)	↓ Growth rate at 37°C (154); intermediate compared to <i>C. bacillisporus</i> , <i>C. deuterogattii</i> , and <i>C. tetragattii</i> (50)	↓ Growth rate at 37°C (154); ↓ compared to <i>C. gattii</i> , <i>C. deuterogattii</i> , and <i>C. tetragattii</i> (50)	↓ Growth rate at 37°C compared to <i>C. neoformans</i> (154); ↑ compared to <i>C. gattii</i> , <i>C. bacillisporus</i> , and <i>C. tetragattii</i> (50)	↓ compared to <i>C. gattii</i> , <i>C. bacillisporus</i> , and <i>C. deuterogattii</i> (50)	ND
Melanin	↑ compared to <i>C. gattii sensu lato</i> (154)	ND	↓ compared to <i>C. neoformans</i> (154)	↓ compared to <i>C. neoformans</i> (154)	↓ compared to <i>C. neoformans</i> (154)	↓ compared to <i>C. neoformans</i> (154)	ND
Virulence in <i>Drosophila melanogaster</i> model	ND	ND	↓ compared to <i>C. bacillisporus</i> (154)	↑ compared to <i>C. gattii</i> , <i>C. deuterogattii</i> , and <i>C. tetragattii</i> (154)	↓ compared to <i>C. bacillisporus</i> (154)	↓ compared to <i>C. bacillisporus</i> (154)	ND
RNAi pathway <sup>d</sup>	Present (65)	Present (65)	Present (65)	Present (65)	Lost (65)	Present (65)	ND
Mycophenolic acid	Sensitive (66)	Sensitive (66)	Sensitive (66)	Sensitive (66)	Sensitive (66)	Not sensitive (66)	ND
Growth on the following medium:							
CGB	Yellowish	Yellowish	Blue	Blue	Blue	Blue	Blue
CDBT	Pale colonies with no apparent color effect on the medium (155)	Colonies bright red, medium bright orange (155)	ND	ND	ND	ND	ND

<sup>a</sup>Overview of characteristics of the pathogenic *Cryptococcus* species, using data from Hagen et al. (1) and updated where indicated with reference numbers. See reference 1, including its supplemental data, for more detailed phenotypic information. A question mark indicates that the specific item is unknown. ↑, higher or increase in; ↓, lower or decrease in; ND, not determined.

<sup>b</sup>Abbreviations: AFR, Africa; EUR, Europe; AUS, Australia; NAM, North America; SAM, South America.

<sup>c</sup>Abbreviations: GM, geometric mean; AMB, amphotericin B; 5FC, 5-fluorocytosine; FLZ, fluconazole; ISA, isavuconazole; ITZ, itraconazole; PSZ, posaconazole; VCZ, voriconazole.

<sup>d</sup>RNAi, RNA interference.



between both species (63). One study indicated that *C. neoformans* (cited as serotype A strains) reproduced mainly clonally, whereas *C. deneoformans* (cited as serotype D strains) showed recombination. Moreover, genomic differences and MLST analysis separated both species (64).

Cryptococcosis is usually diagnosed by microscopy, histopathology, culture, and serology, including lateral flow assays, and by molecular assays (Table 1) (68–92), all of which allow straightforward identification of unknown environmental and clinical cryptococcal isolates. Importantly, the matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS) approach can reliably identify the recognized species of *Cryptococcus* (that may have been cited as genotypes) (1, 93, 94). Kwon-Chung and coworkers (2) questioned the usefulness of MALDI-TOF MS for the separation of the new species and the hybrids, suggesting that only score values of  $\geq 2.0$  indicate a reliable species identification. However, several studies show that yeast and even filamentous fungal isolates can be reliably identified with a score value of  $\geq 1.7$  (95–97), and this is acknowledged in the current Bruker guidelines. The identification of *Cryptococcus* isolates by MALDI-TOF MS yields comparable results or even outperforms the identification methods used for *Candida*, *Geotrichum*, *Malassezia*, and *Trichosporon* isolates.

Kwon-Chung and coworkers (2) questioned the phylogenetic methods that were used to delimit the seven species. Yeast biodiversity research has changed from a discipline driven mainly by phenotype to a discipline based largely on molecular variation (98, 99). Molecular phylogenetic analyses of many species complexes of fungi have resulted in the recognition of new species based on molecular variation. An early example was the recognition and description of the human-pathogenic genus *Coccidioides* based solely on molecular variation (100). New, molecularly defined species are common in yeasts and include the recognition of many “cryptic,” “sibling,” and “sister” species. Examples are *Saccharomyces eubayanus*/*S. uvarum* (101), *Candida albicans*/*C. africana*/*C. stellatoidea* (102–106), *Candida auris*/*C. haemulonii*/*C. duobushaemulonii* (107–112), *Candida glabrata*/*C. nivariensis*/*C. bracarensis* (103, 113–115), *Candida parapsilosis*/*C. orthopsilosis*/*C. metapsilosis* (103, 116), *Malassezia furfur* that now comprises 16 species (117–119), *Trichosporon cutaneum* with at least 10 species (120, 121), the *Aspergillus fumigatus* complex (122–124), *Coccidioides immitis*/*C. posadasii* (100), and *Paracoccidioides brasiliensis*/*P. lutzii* (125). Although this listing is far from complete, it underlines the impact of molecular taxonomic studies for clinically important yeasts and molds.

Kwon-Chung and coworkers (2) suggested that methods employed in the 2015 taxonomic proposal are not appropriate because they have been developed for sexually reproducing organisms. One of the first applications of molecular recognition of species was with a fungus that has yet to reveal its sexual morphology, *Coccidioides* (100). Furthermore, *Cryptococcus* has a sexual cycle and clearly can reproduce both sexually and asexually. Moreover, the methods used have been applied to identify species-level lineages in asexual taxa (126–134). Methods using branch length differences to identify thresholds between intra- and interspecific distances (such as the coalescence-based general mixed Yule coalescent method) potentially underestimate species diversity in asexual species, since sexual species are separated by larger genetic gaps than asexual species (135). Individual methods for species delimitation based on molecular data have been shown to either oversplit or underestimate species diversity under specific circumstances (136); understanding the performance of each method is still in its infancy given the recent and rapid development of this field of research. Therefore, three independent approaches were used to delimit species boundaries within the *C. neoformans*/*C. gattii* species complexes. In addition, DNA-based approaches were congruent with, for example, MALDI-TOF MS-based data. Sampling of additional loci would certainly be useful, as well as the addition of further genomic data sets. However, studies of other microorganisms repeatedly show that additional loci will either confirm clades found or reveal the presence of new ones. Thus, species delimitation for the seven etiologic agents of cryptococcosis was minimal and conservative

(1). Most, if not all, studies that used whole-genome data published before the 2015 taxonomy paper (cited in reference 1), and thereafter, e.g., Farrer and coworkers (36) and those presented at ICC10 (42, 43, 137–139) identified the same species clades.

The insights that resulted in the 2015 taxonomy proposal (1) were elaborated, presented, and discussed at several related meetings from ICC4 (London, United Kingdom, 1999) to ICC10 (Foz do Iguaçu, Brazil, 2017). At ICC6 (Boston, MA, USA, 2005), a debate entitled “*Cryptococcus neoformans*: one, two or more species” was held. Two different opinions were presented, namely, for two species or multiple species (at that time, six species). The community strongly supported the name *C. neoformans* for serotype A strains that are clinically important. The type strain of *C. nasalis* belongs to serotype D (15); hence, it had nomenclatural priority. However, the community leaders present at ICC6 to ICC8 were strongly against the use of this name. Therefore, *C. deneoformans* was proposed for this clade at ICC6, as it shows affinity with the epithet *neoformans* and serotype D (*de-neoformans*). The name *C. gattii* received renewed attention, as it was reported as the cause of a number of major outbreaks (35, 140, 141). The rules of fungal nomenclature do not allow this name to be used for a clade other than the one containing the type strain (and ex-type strain). The clade referred to as AFLP4/VGI represents *C. gattii*, and the AFLP5/VGIII clade is *C. bacillisporus*. Three other consistently observed clades in the *C. gattii* species complex were named using “*gattii*” in part of the epithet in order to keep reference to the name “*gattii*.”

The taxonomy of the species complexes is complicated by various interspecies hybrids (16, 20, 142–147). Hybrids occur among many yeast genera, such as *Saccharomyces*, where well-recognized species form hybrids and even triple hybrids (147–150). For *Saccharomyces* hybrids, a conventional nomenclature has been proposed (150). The species that contribute to the hybrid will be given in alphabetic order, and in cases where the genomic contribution is known, this will be indicated. For instance, the type strain of *S. bayanus* CBS380 is written as *S. cerevisiae* <1% × *S. eubayanus* 37% × *S. uvarum* 63%. This convention is also applicable to the genus *Cryptococcus*. The hybrid type strain of *C. neoformans* can be thus described as *C. deneoformans* × *C. neoformans*.

#### **FOLLOWING THE RULES OF THE INTERNATIONAL CODE OF NOMENCLATURE**

The naming of fungi is governed by the *International Code of Nomenclature for Algae, Fungi, and Plants*, and naming fungi is based on a number of principles (151). Among them, the priority principle implies that the oldest validly given name should be applied to an organism and that the phylogenetic position of the type that determines the name has to be given to a certain clade at a specific taxonomic level. Thus, when a validly described species name exists for a certain species, that name must be used. This was the case for the species that were reinstated as *C. gattii*, *C. bacillisporus*, and in fact also for *C. deneoformans* (see above).

#### **SUMMARY**

The main advantage of recognizing seven species rather than just two “species complexes” (*viz.*, *C. gattii sensu lato* and *C. neoformans sensu lato*) is that researchers and clinicians will be stimulated to search for further phenotypic and genetic differences and similarities between the recognized species. This stimulation of research has already yielded new genetic, molecular, and phenotypic features, including differences in drug susceptibility (Table 1). The recognized species can be identified using a diverse array of molecular diagnostics and MALDI-TOF MS, and some of them can already be identified by phenotypic means. Ignoring the species impedes deciphering the differences among them, which may delay future clinical advances. Finally, it is apparent that more species seem to occur within *Cryptococcus*, e.g., the Botswana lineage within *C. neoformans* (18, 137–139).

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## REFERENCES

- Hagen F, Khayhan K, Theelen B, Kolecka A, Polacheck I, Sionov E, Falk R, Parnmen S, Lumbsch HT, Boekhout T. 2015. Recognition of seven species in the *Cryptococcus gattii*/*Cryptococcus neoformans* species complex. *Fungal Genet Biol* 78:16–48. <https://doi.org/10.1016/j.fgb.2015.02.009>.
- Kwon-Chung KJ, Bennett JE, Wickes BL, Meyer W, Cuomo CA, Wollenburg KR, Bicanic TA, Castañeda E, Chang YC, Chen J, Cogliati M, Dromer F, Ellis D, Filler SG, Fisher MC, Harrison TS, Holland SM, Kohno S, Kronstad JW, Lazera M, Levitz SM, Lionakis MS, May RC, Ngamskulrongsroj P, Pappas PG, Perfect JR, Rickerts V, Sorrell TC, Walsh TJ, Williamson PR, Xu JP, Zelazny AM, Casadevall A. 2017. The case for adopting the “species complex” nomenclature for the etiologic agents of cryptococcosis. *mSphere* 2:e00357-16. <https://doi.org/10.1128/mSphere.00357-16>.
- Chaturvedi V, Chaturvedi S. 2011. *Cryptococcus gattii*: a resurgent fungal pathogen. *Trends Microbiol* 19:564–571. <https://doi.org/10.1016/j.tim.2011.07.010>.
- Lin X, Heitman J. 2006. The biology of the *Cryptococcus neoformans* species complex. *Annu Rev Microbiol* 60:69–105. <https://doi.org/10.1146/annurev.micro.60.080805.142102>.
- Rajasingham R, Smith RM, Park BJ, Jarvis JN, Govender NP, Chiller TM, Denning DW, Loyse A, Boulware DR. 2017. Global burden of disease of HIV-associated cryptococcal meningitis: an updated analysis. *Lancet Infect Dis* 17:873–881. [https://doi.org/10.1016/S1473-3099\(17\)30243-8](https://doi.org/10.1016/S1473-3099(17)30243-8).
- Evans EE. 1950. The antigenic composition of *Cryptococcus neoformans*. I. A serologic classification by means of the capsular and agglutination reactions. *J Immunol* 64:423–430.
- Wilson DE, Bennett JE, Bailey JW. 1968. Serologic grouping of *Cryptococcus neoformans*. *Proc Soc Exp Biol Med* 127:820–823. <https://doi.org/10.3181/00379727-127-32812>.
- Kwon-Chung KJ. 1998. Chapter 82. *Filobasidiella* Kwon-Chung, p 656–662. In Kurtzman CP, Fell JW (ed), *The yeasts, a taxonomic study*, 4th ed. Elsevier Science BV, Amsterdam, The Netherlands.
- Vanbreuseghem R, Takashio M. 1970. An atypical strain of *Cryptococcus neoformans* (San Felice) Vuillemin 1894. II. *Cryptococcus neoformans* var. *gattii* var. nov. *Ann Soc Belges Med Trop Parasitol Mycol* 50:695–702.
- Kwon-Chung KJ. 1975. A new genus, *Filobasidiella*, the perfect state of *Cryptococcus neoformans*. *Mycologia* 67:1197–1200. <https://doi.org/10.2307/3758842>.
- Kwon-Chung KJ. 1976. A new species of *Filobasidiella*, the sexual state of *Cryptococcus neoformans* B and C serotypes. *Mycologia* 68:943–946. <https://doi.org/10.2307/3758813>.
- Kwon-Chung KJ, Bennett JE, Rhodes JC. 1982. Taxonomic studies on *Filobasidiella* species and their anamorphs. *Antonie Van Leeuwenhoek* 48:25–38. <https://doi.org/10.1007/BF00399484>.
- Franzot SP, Salkin IF, Casadevall A. 1999. *Cryptococcus neoformans* var. *grubii*: separate varietal status for *Cryptococcus neoformans* serotype A isolates. *J Clin Microbiol* 37:838–840.
- Kwon-Chung KJ, Boekhout T, Fell JW, Diaz M. 2002. Proposal to con-
- serve the name *Cryptococcus gattii* against *C. honduricus* and *C. bacillisporus* (Basidiomycota, Hymenomycetes, Tremellomycetidae). *Taxon* 51:804–806. <https://doi.org/10.2307/1555045>.
- Boekhout T, Theelen B, Diaz M, Fell JW, Hop WC, Abeln EC, Dromer F, Meyer W. 2001. Hybrid genotypes in the pathogenic yeast *Cryptococcus neoformans*. *Microbiology* 147:891–907. <https://doi.org/10.1099/00221287-147-4-891>.
- Ikeda R, Shinoda T, Fukazawa Y, Kaufman L. 1982. Antigenic characterization of *Cryptococcus neoformans* serotypes and its application to serotyping of clinical isolates. *J Clin Microbiol* 16:22–29.
- Lengeler KB, Cox GM, Heitman J. 2001. Serotype AD strains of *Cryptococcus neoformans* are diploid or aneuploid and are heterozygous at the mating-type locus. *Infect Immun* 69:115–122. <https://doi.org/10.1128/IAI.69.1.115-122.2001>.
- Litvintseva AP, Lin X, Templeton I, Heitman J, Mitchell TG. 2007. Many globally isolated AD hybrid strains of *Cryptococcus neoformans* originated in Africa. *PLoS Pathog* 3:e114. <https://doi.org/10.1371/journal.ppat.0030114>.
- Guinea J, Hagen F, Peláez T, Boekhout T, Tahoune H, Torres-Narbona M, Bouza E. 2010. Antifungal susceptibility, serotyping, and genotyping of clinical *Cryptococcus neoformans* isolates collected during 18 years in a single institution in Madrid, Spain. *Med Mycol* 48:942–948. <https://doi.org/10.3109/13693781003690067>.
- Viviani MA, Cogliati M, Esposto MC, Lemmer K, Tintelnot K, Colom Valiente MF, Swinne D, Velegraki A, Velho R, European Confederation of Medical Mycology (ECMM) Cryptococcosis Working Group. 2006. Molecular analysis of 311 *Cryptococcus neoformans* isolates from a 30-month ECMM survey of cryptococcosis in Europe. *FEMS Yeast Res* 6:614–619. <https://doi.org/10.1111/j.1567-1364.2006.00081.x>.
- Liu XZ, Wang QM, Göker M, Groenewald M, Kachalkin AV, Lumbsch HT, Millanes AM, Wedin M, Yurkov AM, Boekhout T, Bai FY. 2015. Towards an integrated phylogenetic classification of the Tremellomycetes. *Stud Mycol* 81:85–147. <https://doi.org/10.1016/j.simyco.2015.12.001>.
- Liu XZ, Wang QM, Theelen B, Groenewald M, Bai FY, Boekhout T. 2015. Phylogeny of tremellomycetous yeasts and related dimorphic and filamentous basidiomycetes reconstructed from multiple gene sequence analyses. *Stud Mycol* 81:1–26. <https://doi.org/10.1016/j.simyco.2015.08.001>.
- James TY, Kauff F, Schoch CL, Matheny PB, Hofstetter V, Cox CJ, Celio G, Gueidan C, Fraker E, Miadlikowska J, Lumbsch HT, Rauhut A, Reeb V, Arnold AE, Amtoft A, Stajich JE, Hosaka K, Sung GH, Johnson D, O'Rourke B, Crockett M, Binder M, Curtis JM, Slot JC, Wang Z, Wilson AW, Schüssler A, Longcore JE, O'Donnell K, Mozley-Standridge S, Porter D, Letcher PM, Powell MJ, Taylor JW, White MM, Griffith GW, Davies DR, Humber RA, Morton JB, Sugiyama J, Rossman AY, Rogers JD, Pfister DH, Hewitt D, Hansen K, Hambleton S, Shoemaker RA, Kohlmeyer J, Volkman-Kohlmeyer B, Spotts RA, Serdani M, et al. 2006. Reconstructing the early evolution of Fungi using a six-gene phylogeny. *Nature* 443:818–822. <https://doi.org/10.1038/nature05110>.



24. Kurtzman CP, Mateo RQ, Kolecka A, Theelen B, Robert V, Boekhout T. 2015. Advances in yeast systematics and phylogeny and their use as predictors of biotechnologically important metabolic pathways. *FEMS Yeast Res* 15:fov050. <https://doi.org/10.1093/femsyr/fov050>.
25. Wang QM, Begerow D, Groenewald M, Liu XZ, Theelen B, Bai FY, Boekhout T. 2015. Multigene phylogeny and taxonomic revision of yeasts and related fungi in the Ustilaginomycotina. *Stud Mycol* 81: 55–83. <https://doi.org/10.1016/j.simyco.2015.10.004>.
26. Wang QM, Groenewald M, Takashima M, Theelen B, Han PJ, Liu XZ, Boekhout T, Bai FY. 2015. Phylogeny of yeasts and related filamentous fungi within Pucciniomycotina determined from multigene sequence analyses. *Stud Mycol* 81:27–53. <https://doi.org/10.1016/j.simyco.2015.08.002>.
27. Wang QM, Yurkov AM, Göker M, Lumbsch HT, Leavitt SD, Groenewald M, Theelen B, Liu XZ, Boekhout T, Bai FY. 2015. Phylogenetic classification of yeasts and related taxa within Pucciniomycotina. *Stud Mycol* 81:149–189. <https://doi.org/10.1016/j.simyco.2015.12.002>.
28. Findley K, Sun S, Fraser JA, Hsueh YP, Averette AF, Li W, Dietrich FS, Heitman J. 2012. Discovery of a modified tetrapolar sexual cycle in *Cryptococcus amylolentus* and the evolution of MAT in the *Cryptococcus* species complex. *PLoS Genet* 8:e1002528. <https://doi.org/10.1371/journal.pgen.1002528>.
29. Kwon-Chung KJ. 2011. Chapter 114. *Filobasidiella* Kwon-Chung (1975), p 1443–1455. In Kurtzman CP, Fell JW, Boekhout T (ed), *The yeasts, a taxonomic study*, 5th ed. Elsevier, Amsterdam, The Netherlands. <https://doi.org/10.1016/B978-0-444-52149-1.00114-2>.
30. Meyer W, Gilgado F, Ngamskulrungraj P, Trilles L, Hagen F, Castañeda E, Boekhout T. 2011. Chapter 24. Molecular typing of the *Cryptococcus neoformans*/*C. gattii* species complex, p 327–357. In Heitman J, Kozel TR, Kwon-Chung KJ, Perfect JR, Casadevall A (ed), *Cryptococcus: from human pathogen to model yeast*. ASM Press, Washington, DC. <https://doi.org/10.1128/9781555816858.ch24>.
31. Xu J, Vilgalys R, Mitchell TG. 2000. Multiple gene genealogies reveal recent dispersion and hybridization in the human pathogenic fungus *Cryptococcus neoformans*. *Mol Ecol* 9:1471–1481. <https://doi.org/10.1046/j.1365-294x.2000.01021.x>.
32. Casadevall A, Freij JB, Hann-Soden C, Taylor J. 2017. Continental drift and speciation of the *Cryptococcus neoformans* and *Cryptococcus gattii* species complexes. *mSphere* 2:e00103-17. <https://doi.org/10.1128/mSphere.00103-17>.
33. Forsythe A, Vogan A, Xu J. 2016. Genetic and environmental influences on the germination of basidiospores in the *Cryptococcus neoformans* species complex. *Sci Rep* 6:33828. <https://doi.org/10.1038/srep33828>.
34. Bovers M, Hagen F, Kuramae EE, Boekhout T. 2008. Six monophyletic lineages identified within *Cryptococcus neoformans* and *Cryptococcus gattii* by multi-locus sequence typing. *Fungal Genet Biol* 45:400–421. <https://doi.org/10.1016/j.fgb.2007.12.004>.
35. Engelthaler DM, Hicks ND, Gillette JD, Roe CC, Schupp JM, Driebe EM, Gilgado F, Carriconde F, Trilles L, Firacative C, Ngamskulrungraj P, Castañeda E, Lazera Mdos S, Melhem MS, Pérez-Bercoff A, Huttley G, Sorrell TC, Voelz K, May RC, Fisher MC, Thompson GR, III, Lockhart SR, Keim P, Meyer W. 2014. *Cryptococcus gattii* in North American Pacific Northwest: whole-population genome analysis provides insights into species evolution and dispersal. *mBio* 5:e01464-14. <https://doi.org/10.1128/mBio.01464-14>.
36. Farrer RA, Desjardins CA, Sakthikumar S, Gujja S, Saif S, Zeng Q, Chen Y, Voelz K, Heitman J, May RC, Fisher MC, Cuomo CA. 2015. Genome evolution and innovation across the four major lineages of *Cryptococcus gattii*. *mBio* 6:e00868-15. <https://doi.org/10.1128/mBio.00868-15>.
37. Fraser JA, Giles SS, Wenink EC, Geunes-Boyer SG, Wright JR, Diezmann S, Allen A, Stajich JE, Dietrich FS, Perfect JR, Heitman J. 2005. Same-sex mating and the origin of the Vancouver Island *Cryptococcus gattii* outbreak. *Nature* 437:1360–1364. <https://doi.org/10.1038/nature04220>.
38. Meyer W, Aanensen DM, Boekhout T, Cogliati M, Diaz MR, Esposto MC, Fisher M, Gilgado F, Hagen F, Kaocharoen S, Litvintseva AP, Mitchell TG, Simwami SP, Trilles L, Viviani M, Kwon-Chung J. 2009. Consensus multi-locus sequence typing scheme for *Cryptococcus neoformans* and *Cryptococcus gattii*. *Med Mycol* 47:561–570. <https://doi.org/10.1080/13693780902953886>.
39. Meyer W, Castañeda A, Jackson S, Huynh M, Castañeda E, IberoAmerican Cryptococcal Study Group. 2003. Molecular typing of IberoAmerican *Cryptococcus neoformans* isolates. *Emerg Infect Dis* 9:189–195. <https://doi.org/10.3201/eid0902.020246>.
40. Meyer W, Mitchell TG, Freedman EZ, Vilgalys R. 1993. Hybridization probes for conventional DNA fingerprinting used as single primers in the polymerase chain reaction to distinguish strains of *Cryptococcus neoformans*. *J Clin Microbiol* 31:2274–2280.
41. Ngamskulrungraj P, Gilgado F, Faganello J, Litvintseva AP, Leal AL, Tsui KM, Mitchell TG, Vainstein MH, Meyer W. 2009. Genetic diversity of the *Cryptococcus* species complex suggests that *Cryptococcus gattii* deserves to have varieties. *PLoS One* 4:e5862. <https://doi.org/10.1371/journal.pone.0005862>.
42. Desjardins CA, Giamberardino C, Sykes SM, Yu CH, Tenor JL, Chen Y, Yang T, Jones AM, Sun S, Haverkamp MR, Heitman J, Litvintseva AP, Perfect JR, Cuomo CA. 2017. Population genomics and the evolution of virulence in the fungal pathogen *Cryptococcus neoformans*. *Genome Res* 27:1207–1219. <https://doi.org/10.1101/gr.218727.116>.
43. Firacative C, Roe CC, Malik R, Ferreira-Paim K, Escandón P, Sykes JE, Castañón-Olivares LR, Contreras-Peres C, Samayoa B, Sorrell TC, Castañeda E, Lockhart SR, Engelthaler DM, Meyer W. 2017. Novel insights in the molecular epidemiology of *Cryptococcus gattii* VGIII. In 10th International Conference on Cryptococcus and Cryptococcosis, Foz do Iguaçu, Brazil, 26 to 30 2017.
44. Hagen F, Illnait-Zaragozi MT, Bartlett KH, Swinnee D, Geertsens E, Klaassen CH, Boekhout T, Meis JF. 2010. In vitro antifungal susceptibilities and amplified fragment length polymorphism genotyping of a worldwide collection of 350 clinical, veterinary, and environmental *Cryptococcus gattii* isolates. *Antimicrob Agents Chemother* 54:5139–5145. <https://doi.org/10.1128/AAC.00746-10>.
45. Iqbal N, DeBess EE, Wohrle R, Sun B, Nett RJ, Ahlquist AM, Chiller T, Lockhart SR, Cryptococcus gattii Public Health Working Group. 2010. Correlation of genotype and in vitro susceptibilities of *Cryptococcus gattii* strains from the Pacific Northwest of the United States. *J Clin Microbiol* 48:539–544. <https://doi.org/10.1128/JCM.01505-09>.
46. Trilles L, Meyer W, Wanke B, Guarro J, Lazéra M. 2012. Correlation of antifungal susceptibility and molecular type within the *Cryptococcus neoformans*/*C. gattii* species complex. *Med Mycol* 50:328–332. <https://doi.org/10.3109/13693786.2011.602126>.
47. Perfect JR, Dismukes WE, Dromer F, Goldman DL, Graybill JR, Hamill RJ, Harrison TS, Larsen RA, Lortholary O, Nguyen MH, Pappas PG, Powderly WG, Singh N, Sobel JD, Sorrell TC. 2010. Clinical practice guidelines for the management of cryptococcal disease: 2010 update by the Infectious Diseases Society of America. *Clin Infect Dis* 50:291–322. <https://doi.org/10.1086/649858>.
48. Hagen F, Illnait-Zaragozi MT, Meis JF, Chew WH, Curfs-Breuker I, Mouton JW, Hoepelman AI, Spanjaard L, Verweij PE, Kampinga GA, Kuijper EJ, Boekhout T, Klaassen CH. 2012. Extensive genetic diversity within the Dutch clinical *Cryptococcus neoformans* population. *J Clin Microbiol* 50:1918–1926. <https://doi.org/10.1128/JCM.06750-11>.
49. Chowdhary A, Randhawa HS, Sundar G, Kathuria S, Prakash A, Khan Z, Sun S, Xu J. 2011. In vitro antifungal susceptibility profiles and genotypes of 308 clinical and environmental isolates of *Cryptococcus neoformans* var. *grubii* and *Cryptococcus gattii* serotype B from northwestern India. *J Med Microbiol* 60:961–967. <https://doi.org/10.1099/jmm.0.029025-0>.
50. Fernandes KE, Dwyer C, Campbell LT, Carter DA. 2016. Species in the *Cryptococcus gattii* complex differ in capsule and cell size following growth under capsule-inducing conditions. *mSphere* 1:e00350-16. <https://doi.org/10.1128/mSphere.00350-16>.
51. Criseo G, Bolognani MS, De Leo F, Staib F. 1995. Evidence of canary droppings as an important reservoir of *Cryptococcus neoformans*. *Zentralbl Bakteriol* 282:244–254.
52. Nweze EI, Kechia FA, Dibua UE, Eze C, Onoja US. 2015. Isolation of *Cryptococcus neoformans* from environmental samples collected in Southeastern Nigeria. *Rev Inst Med Trop Sao Paulo* 57:295–298. <https://doi.org/10.1590/S0036-46652015000400004>.
53. Spina-Tensini T, Muro MD, Queiroz-Telles F, Strozzi I, Moraes ST, Petterle RR, Vettorello M, Staudacher C, Miguez LA, de Almeida SM. 2017. Geographic distribution of patients affected by *Cryptococcus neoformans*/*Cryptococcus gattii* species complexes meningitis, pigeon and tree populations in Southern Brazil. *Mycoses* 60:51–58. <https://doi.org/10.1111/myc.12550>.
54. Springer DJ, Mohan R, Heitman J. 2017. Plants promote mating and dispersal of the human pathogenic fungus *Cryptococcus*. *PLoS One* 12:e0171695. <https://doi.org/10.1371/journal.pone.0171695>.
55. Springer DJ, Ren P, Raina R, Dong Y, Behr MJ, McEwen BF, Bowser SS, Samsonoff WA, Chaturvedi S, Chaturvedi V. 2010. Extracellular fibrils of pathogenic yeast *Cryptococcus gattii* are important for ecological niche,

- murine virulence and human neutrophil interactions. *PLoS One* 5:e10978. <https://doi.org/10.1371/journal.pone.0010978>.
56. Cogliati M, Chandrashekar N, Esposto MC, Chandramuki A, Petrini B, Viviani MA. 2012. *Cryptococcus gattii* serotype-C strains isolated in Bangalore, Karnataka, India. *Mycoses* 55:262–268. <https://doi.org/10.1111/j.1439-0507.2011.02082.x>.
  57. Nyazika TK, Hagen F, Meis JF, Robertson VJ. 2016. *Cryptococcus tetragattii* as a major cause of cryptococcal meningitis among HIV-infected individuals in Harare, Zimbabwe. *J Infect* 72:745–752. <https://doi.org/10.1016/j.jinf.2016.02.018>.
  58. Chen J, Varma A, Diaz MR, Litvintseva AP, Wollenberg KK, Kwon-Chung KJ. 2008. *Cryptococcus neoformans* strains and infection in apparently immunocompetent patients, China. *Emerg Infect Dis* 14:755–762. <https://doi.org/10.3201/eid1405.071312>.
  59. Choi YH, Ngamskulrungraj P, Varma A, Sionov E, Hwang SM, Carriconde F, Meyer W, Litvintseva AP, Lee WG, Shin JH, Kim EC, Lee KW, Choi TY, Lee YS, Kwon-Chung KJ. 2010. Prevalence of the VNlc genotype of *Cryptococcus neoformans* in non-HIV-associated cryptococcosis in the Republic of Korea. *FEMS Yeast Res* 10:769–778. <https://doi.org/10.1111/j.1567-1364.2010.00648.x>.
  60. Pan W, Khayhan K, Hagen F, Wahyuningsih R, Chakrabarti A, Chowdhary A, Ikeda R, Taj-Aldeen SJ, Khan Z, Imran D, Sjam R, Sriburee P, Liao W, Chaicumpar K, Ingviya N, Mouton JW, Curfs-Breuker I, Boekhout T, Meis JF, Klaassen CH. 2012. Resistance of Asian *Cryptococcus neoformans* serotype A is confined to few microsatellite genotypes. *PLoS One* 7:e32868. <https://doi.org/10.1371/journal.pone.0032868>.
  61. Vogan AA, Khankhet J, Samarasinghe H, Xu J. 2016. Identification of QTLs associated with virulence related traits and drug resistance in *Cryptococcus neoformans*. *G3 (Bethesda)* 6:2745–2759. <https://doi.org/10.1534/g3.116.029595>.
  62. Vogan AA, Xu J. 2014. Evidence for genetic incompatibilities associated with post-zygotic reproductive isolation in the human fungal pathogen *Cryptococcus neoformans*. *Genome* 57:335–344. <https://doi.org/10.1139/gen-2014-0077>.
  63. Vogan AA, Khankhet J, Xu J. 2013. Evidence for mitotic recombination within the basidia of a hybrid cross of *Cryptococcus neoformans*. *PLoS One* 8:e62790. <https://doi.org/10.1371/journal.pone.0062790>.
  64. Desnos-Ollivier M, Patel S, Raoux-Barbot D, Heitman J, Dromer F, French Cryptococcosis Study Group. 2015. Cryptococcosis serotypes impact outcome and provide evidence of *Cryptococcus neoformans* speciation. *mBio* 6:e00311. <https://doi.org/10.1128/mBio.00311-15>.
  65. Feretzaki M, Billmyre RB, Clancey SA, Wang X, Heitman J. 2016. Gene network polymorphism illuminates loss and retention of novel RNAi silencing components in the *Cryptococcus* pathogenic species complex. *PLoS Genet* 12:e1005868. <https://doi.org/10.1371/journal.pgen.1005868>.
  66. Morrow CA, Valkov E, Stamp A, Chow EW, Lee IR, Wronski A, Williams SJ, Hill JM, Djordjevic JT, Kappler U, Kobe B, Fraser JA. 2012. De novo GTP biosynthesis is critical for virulence of the fungal pathogen *Cryptococcus neoformans*. *PLoS Pathog* 8:e1002957. <https://doi.org/10.1371/journal.ppat.1002957>.
  67. Chang YC, Khanal Lamichhane A, Bradley J, Rodgers L, Ngamskulrungraj P, Kwon-Chung KJ. 2015. Differences between *Cryptococcus neoformans* and *Cryptococcus gattii* in the molecular mechanisms governing utilization of D-amino acids as the sole nitrogen source. *PLoS One* 10:e0131865. <https://doi.org/10.1371/journal.pone.0131865>.
  68. Arvanitis M, Anagnostou T, Fuchs BB, Caliendo AM, Mylonakis E. 2014. Molecular and nonmolecular diagnostic methods for invasive fungal infections. *Clin Microbiol Rev* 27:490–526. <https://doi.org/10.1128/CMR.00091-13>.
  69. Nalintya E, Kiggundu R, Meya D. 2016. Evolution of cryptococcal antigen testing: what is new? *Curr Fungal Infect Rep* 2016:1–6. <https://doi.org/10.1007/s12281-016-0256-3>.
  70. Dufait R, Velho R, De Vroey C. 1987. Rapid identification of the two varieties of *Cryptococcus neoformans* by D-proline assimilation. *Mykosen* 30:483.
  71. Martínez Machín G, Barrial de la Rosa L, Illnait Zaragoza MT, Valdés Hernández Idel C, Fernández Andreu CM, Perurena Lancha MR, Polo Leal JL, Mendoza Llanes D. 2004. Usefulness of D-proline in the differentiation of varieties of *Cryptococcus neoformans*. *Rev Cuba Med Trop* 56:77–79. (In Spanish).
  72. Chaskes S, Frases S, Cammer M, Gerfen G, Casadevall A. 2008. Growth and pigment production on D-tryptophan medium by *Cryptococcus gattii*, *Cryptococcus neoformans*, and *Candida albicans*. *J Clin Microbiol* 46:255–264. <https://doi.org/10.1128/JCM.01721-07>.
  73. Nyazika TK, Robertson VJ, Nherera B, Mapondera PT, Meis JF, Hagen F. 2016. Comparison of biotyping methods as alternative identification tools to molecular typing of pathogenic *Cryptococcus* species in sub-Saharan Africa. *Mycoses* 59:151–156. <https://doi.org/10.1111/myc.12444>.
  74. Veron V, Simon S, Blanchet D, Aznar C. 2009. Real-time polymerase chain reaction detection of *Cryptococcus neoformans* and *Cryptococcus gattii* in human samples. *Diagn Microbiol Infect Dis* 65:69–72. <https://doi.org/10.1016/j.diagmicrobio.2009.05.005>.
  75. Gago S, Esteban C, Valero C, Zaragoza O, Puig de la Bellacasa J, Buitrago MJ. 2014. A multiplex real-time PCR assay for identification of *Pneumocystis jirovecii*, *Histoplasma capsulatum*, and *Cryptococcus neoformans*/*Cryptococcus gattii* in samples from AIDS patients with opportunistic pneumonia. *J Clin Microbiol* 52:1168–1176. <https://doi.org/10.1128/JCM.02895-13>.
  76. Leber AL, Everhart K, Balada-Llasat JM, Cullison J, Daly J, Holt S, Lephart P, Salimnia H, Schreckenberger PC, DesJarlais S, Reed SL, Chapin KC, LeBlanc L, Johnson JK, Soliven NL, Carroll KC, Miller JA, Dien Bard J, Mestas J, Bankowski M, Enomoto T, Hemmert AC, Bourzac KM. 2016. Multicenter evaluation of BioFire FilmArray meningitis/encephalitis panel for detection of bacteria, viruses, and yeast in cerebrospinal fluid specimens. *J Clin Microbiol* 54:2251–2261. <https://doi.org/10.1128/JCM.00730-16>.
  77. Satoh K, Maeda M, Umeda Y, Miyajima Y, Makimura K. 2011. Detection and identification of probable endemic fungal pathogen, *Cryptococcus gattii*, and worldwide pathogen, *Cryptococcus neoformans*, by real-time PCR. *Microbiol Immunol* 55:454–457. <https://doi.org/10.1111/j.1348-0421.2011.00324.x>.
  78. Tavares ER, Azevedo CS, Panagio LA, Pelisson M, Pinge-Filho P, Venancio EJ, Barros TF, Yamada-Ogata SF, Yamauchi LM. 2016. Accurate and sensitive real-time PCR assays using intergenic spacer 1 region to differentiate *Cryptococcus gattii sensu lato* and *Cryptococcus neoformans sensu lato*. *Med Mycol* 54:89–96. <https://doi.org/10.1093/mmy/myv078>.
  79. Arsic Arsenijevic V, Pekmezovic MG, Meis JF, Hagen F. 2014. Molecular epidemiology and antifungal susceptibility of Serbian *Cryptococcus neoformans* isolates. *Mycoses* 57:380–387. <https://doi.org/10.1111/myc.12171>.
  80. Feng X, Fu X, Ling B, Wang L, Liao W, Pan W, Yao Z. 2013. Rapid differentiation of cryptic species within *Cryptococcus gattii* by a duplex PCR assay. *J Clin Microbiol* 51:3110–3112. <https://doi.org/10.1128/JCM.01455-13>.
  81. Feng X, Yao Z, Ren D, Liao W. 2008. Simultaneous identification of molecular and mating types within the *Cryptococcus* species complex by PCR-RFLP analysis. *J Med Microbiol* 57:1481–1490. <https://doi.org/10.1099/jmm.0.2008/003665-0>.
  82. Katsu M, Kidd S, Ando A, Moretti-Branchini ML, Mikami Y, Nishimura K, Meyer W. 2004. The internal transcribed spacers and 5.8S rRNA gene show extensive diversity among isolates of the *Cryptococcus neoformans* species complex. *FEMS Yeast Res* 4:377–388. [https://doi.org/10.1016/S1567-1356\(03\)00176-4](https://doi.org/10.1016/S1567-1356(03)00176-4).
  83. Kelley EJ, Driebe EM, Etienne K, Brandt ME, Schupp JM, Gillette JD, Trujillo JS, Lockhart SR, Deak E, Keim PS, Engelthaler DM. 2014. Real-time PCR assays for genotyping of *Cryptococcus gattii* in North America. *BMC Microbiol* 14:125. <https://doi.org/10.1186/1471-2180-14-125>.
  84. Day JN, Hoang TN, Duong AV, Hong CT, Diep PT, Campbell JJ, Sieu TP, Hien TT, Bui T, Boni MF, Laloo DG, Carter D, Baker S, Farrar JJ. 2011. Most cases of cryptococcal meningitis in HIV-uninfected patients in Vietnam are due to a distinct amplified fragment length polymorphism-defined cluster of *Cryptococcus neoformans* var. *grubii* VN1. *J Clin Microbiol* 49:658–664. <https://doi.org/10.1128/JCM.01985-10>.
  85. Illnait-Zaragozi MT, Martínez-Machín GF, Fernández-Andreu CM, Boekhout T, Meis JF, Klaassen CH. 2010. Microsatellite typing of clinical and environmental *Cryptococcus neoformans* var. *grubii* isolates from Cuba shows multiple genetic lineages. *PLoS One* 5:e9124. <https://doi.org/10.1371/journal.pone.0009124>.
  86. Ferreira-Paim K, Andrade-Silva L, Fonseca FM, Ferreira TB, Mora DJ, Andrade-Silva J, Khan A, Dao A, Reis EC, Almeida MT, Maltos A, Junior VR, Trilles L, Rickerts V, Chindamporn A, Sykes JE, Cogliati M, Nielsen K, Boekhout T, Fisher M, Kwon-Chung J, Engelthaler DM, Lazéra M, Meyer W, Silva-Vergara ML. 2017. MLST-based population genetic analysis in a global context reveals clonality amongst *Cryptococcus neoformans*

- var. *grubii* VNI isolates from HIV patients in Southeastern Brazil. *PLoS Negl Trop Dis* 11:e0005223. <https://doi.org/10.1371/journal.pntd.0005223>.
87. Khayhan K, Hagen F, Pan W, Simwami S, Fisher MC, Wahyuningsih R, Chakrabarti A, Chowdhary A, Ikeda R, Taj-Aldeen SJ, Khan Z, Ip M, Imran D, Sjam R, Sriburee P, Liao W, Chaicumpar K, Vuddhakul V, Meyer W, Trilles L, van Iersel LJ, Meis JF, Klaassen CH, Boekhout T. 2013. Geographically structured populations of *Cryptococcus neoformans* variety *grubii* in Asia correlate with HIV status and show a clonal population structure. *PLoS One* 8:e72222. <https://doi.org/10.1371/journal.pone.0072222>.
  88. Wiesner DL, Moskalenko O, Corcoran JM, McDonald T, Rolfs MA, Meya DB, Kajumbula H, Kambugu A, Bohjanen PR, Knight JF, Boulware DR, Nielsen K. 2012. Cryptococcal genotype influences immunologic response and human clinical outcome after meningitis. *mBio* 3:e00196-12. <https://doi.org/10.1128/mBio.00196-12>.
  89. Bovers M, Diaz MR, Hagen F, Spanjaard L, Duim B, Visser CE, Hoogveld HL, Scharringa J, Hoepelman IM, Fell JW, Boekhout T. 2007. Identification of genotypically diverse *Cryptococcus neoformans* and *Cryptococcus gattii* isolates by Luminex xMAP technology. *J Clin Microbiol* 45:1874–1883. <https://doi.org/10.1128/JCM.00223-07>.
  90. Diaz MR, Fell JW. 2005. Use of a suspension array for rapid identification of the varieties and genotypes of the *Cryptococcus neoformans* species complex. *J Clin Microbiol* 43:3662–3672. <https://doi.org/10.1128/JCM.43.8.3662-3672.2005>.
  91. Trilles L, Wang B, Firacative C, Lazéra Mdos S, Wanke B, Meyer W. 2014. Identification of the major molecular types of *Cryptococcus neoformans* and *C. gattii* by hyperbranched rolling circle amplification. *PLoS One* 9:e94648. <https://doi.org/10.1371/journal.pone.0094648>.
  92. Billmyre RB, Croll D, Li W, Mieczkowski P, Carter DA, Cuomo CA, Kronstad JW, Heitman J. 2014. Highly recombinant VGII *Cryptococcus gattii* population develops clonal outbreak clusters through both sexual macroevolution and asexual microevolution. *mBio* 5:e01494-14. <https://doi.org/10.1128/mBio.01494-14>.
  93. Firacative C, Trilles L, Meyer W. 2012. MALDI-TOF MS enables the rapid identification of the major molecular types within the *Cryptococcus neoformans/C. gattii* species complex. *PLoS One* 7:e37566. <https://doi.org/10.1371/journal.pone.0037566>.
  94. Posteraro B, Vella A, Cogliati M, De Carolis E, Florio AR, Posteraro P, Sanguinetti M, Tortorano AM. 2012. Matrix-assisted laser desorption/ionization-time of flight mass spectrometry-based method for discrimination between molecular types of *Cryptococcus neoformans* and *Cryptococcus gattii*. *J Clin Microbiol* 50:2472–2476. <https://doi.org/10.1128/JCM.00737-12>.
  95. Normand AC, Cassagne C, Gautier M, Becker P, Ranque S, Hendrickx M, Piarroux R. 2017. Decision criteria for MALDI-TOF MS-based identification of filamentous fungi using commercial and in-house reference databases. *BMC Microbiol* 17:25. <https://doi.org/10.1186/s12866-017-0937-2>.
  96. Van Herendael BH, Bruynseels P, Bensaïd M, Boekhout T, De Baere T, Surmont I, Mertens AH. 2012. Validation of a modified algorithm for the identification of yeast isolates using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS). *Eur J Clin Microbiol Infect Dis* 31:841–848. <https://doi.org/10.1007/s10096-011-1383-y>.
  97. Vlek A, Kolecka A, Khayhan K, Theelen B, Groenewald M, Boel E, Multicenter Study Group, Boekhout T. 2014. Interlaboratory comparison of sample preparation methods, database expansions, and cutoff values for identification of yeasts by matrix-assisted laser desorption/ionization-time of flight mass spectrometry using a yeast test panel. *J Clin Microbiol* 52:3023–3029. <https://doi.org/10.1128/JCM.00563-14>.
  98. Kurtzman CP. 2014. Use of gene sequence analyses and genome comparisons for yeast systematics. *Int J Syst Evol Microbiol* 64:325–332. <https://doi.org/10.1099/ijso.0.054197-0>.
  99. Kurtzman CP, Fell JW, Boekhout T. 2011. Chapter 10 - Gene sequence analyses and other DNA-based methods for yeast species recognition, p 137–144. *In* The yeasts, a taxonomic study, 5th ed. Elsevier, Amsterdam, The Netherlands. <https://doi.org/10.1016/B978-0-444-52149-1.00010-0>.
  100. Fisher MC, Koenig GL, White TJ, Taylor JW. 2002. Molecular and phenotypic description of *Coccidioides posadasii* sp. nov., previously recognized as the non-California population of *Coccidioides immitis*. *Mycologia* 94:73–84. <https://doi.org/10.1080/15572536.2003.11833250>.
  101. Libkind D, Hittinger CT, Valério E, Gonçalves C, Dover J, Johnston M, Gonçalves P, Sampaio JP. 2011. Microbe domestication and the identification of the wild genetic stock of lager-brewing yeast. *Proc Natl Acad Sci U S A* 108:14539–14544. <https://doi.org/10.1073/pnas.1105430108>.
  102. Chowdhary A, Hagen F, Sharma C, Al-Hatmi AMS, Giuffrè L, Giosa D, Fan S, Badali H, Felice MR, de Hoog S, Meis JF, Romeo O. 2017. Whole genome-based amplified fragment length polymorphism analysis reveals genetic diversity in *Candida africana*. *Front Microbiol* 8:556. <https://doi.org/10.3389/fmicb.2017.00556>.
  103. Criseo G, Scordino F, Romeo O. 2015. Current methods for identifying clinically important cryptic *Candida* species. *J Microbiol Methods* 111:50–56. <https://doi.org/10.1016/j.mimet.2015.02.004>.
  104. Ngouana TK, Krasteva D, Drakulovski P, Toghueo RK, Kouanfack C, Ambe A, Reynes J, Delaporte E, Boyom FF, Mallié M, Bertout S. 2015. Investigation of minor species *Candida africana*, *Candida stellatoidea* and *Candida dubliniensis* in the *Candida albicans* complex among Yaoundé (Cameroon) HIV-infected patients. *Mycoses* 58:33–39. <https://doi.org/10.1111/myc.12266>.
  105. Romeo O, Criseo G. 2008. First molecular method for discriminating between *Candida africana*, *Candida albicans*, and *Candida dubliniensis* by using *HWP1* gene. *Diagn Microbiol Infect Dis* 62:230–233. <https://doi.org/10.1016/j.diagmicrobio.2008.05.014>.
  106. Tietz HJ, Hopp M, Schmalreck A, Sterry W, Czaika V. 2001. *Candida africana* sp. nov., a new human pathogen or a variant of *Candida albicans*? *Mycoses* 44:437–445. <https://doi.org/10.1046/j.1439-0507.2001.00707.x>.
  107. Cendejas-Bueno E, Kolecka A, Alastruey-Izquierdo A, Theelen B, Groenewald M, Kostrzewa M, Cuenca-Estrella M, Gómez-López A, Boekhout T. 2012. Reclassification of the *Candida haemulonii* complex as *Candida haemulonii* (C. haemulonii group I), *C. duobushaemulonii* sp. nov. (C. haemulonii group II), and *C. haemulonii* var. *vulnera* var. nov.: three multiresistant human pathogenic yeasts. *J Clin Microbiol* 50:3641–3651. <https://doi.org/10.1128/JCM.02248-12>.
  108. Kathuria S, Singh PK, Sharma C, Prakash A, Masih A, Kumar A, Meis JF, Chowdhary A. 2015. Multidrug-resistant *Candida auris* misidentified as *Candida haemulonii*: characterization by matrix-assisted laser desorption/ionization-time of flight mass spectrometry and DNA sequencing and its antifungal susceptibility profile variability by Vitek 2, CLSI broth microdilution, and Etest method. *J Clin Microbiol* 53:1823–1830. <https://doi.org/10.1128/JCM.00367-15>.
  109. Kumar A, Prakash A, Singh A, Kumar H, Hagen F, Meis JF, Chowdhary A. 2016. *Candida haemulonii* species complex: an emerging species in India and its genetic diversity assessed with multilocus sequence and amplified fragment-length polymorphism analyses. *Emerg Microbes Infect* 5:e49. <https://doi.org/10.1038/emi.2016.49>.
  110. Prakash A, Sharma C, Singh A, Kumar Singh P, Kumar A, Hagen F, Govender NP, Colombo AL, Meis JF, Chowdhary A. 2016. Evidence of genotypic diversity among *Candida auris* isolates by multilocus sequence typing, matrix-assisted laser desorption/ionization time-of-flight mass spectrometry and amplified fragment length polymorphism. *Clin Microbiol Infect* 22:277.e1–277.e9. <https://doi.org/10.1016/j.cmi.2015.10.022>.
  111. Satoh K, Makimura K, Hasumi Y, Nishiyama Y, Uchida K, Yamaguchi H. 2009. *Candida auris* sp. nov., a novel ascomycetous yeast isolated from the external ear canal of an inpatient in a Japanese hospital. *Microbiol Immunol* 53:41–44. <https://doi.org/10.1111/j.1348-0421.2008.00083.x>.
  112. Schelenz S, Hagen F, Rhodes JL, Abdolrasouli A, Chowdhary A, Hall A, Ryan L, Shackleton J, Trimlett R, Meis JF, Armstrong-James D, Fisher MC. 2016. First hospital outbreak of the globally emerging *Candida auris* in a European hospital. *Antimicrob Resist Infect Control* 5:35. <https://doi.org/10.1186/s13756-016-0132-5>.
  113. Alcoba-Flórez J, Méndez-Alvarez S, Cano J, Guarro J, Pérez-Roth E, del Pilar Arévalo M. 2005. Phenotypic and molecular characterization of *Candida nivariensis* sp. nov., a possible new opportunistic fungus. *J Clin Microbiol* 43:4107–4111. <https://doi.org/10.1128/JCM.43.8.4107-4111.2005>.
  114. Correia A, Sampaio P, James S, Pais C. 2006. *Candida bracarenensis* sp. nov., a novel anamorphic yeast species phenotypically similar to *Candida glabrata*. *Int J Syst Evol Microbiol* 56:313–317. <https://doi.org/10.1099/ijso.0.64076-0>.
  115. Lockhart SR, Messer SA, Gherna M, Bishop JA, Merz WG, Pfaller MA, Diekema DJ. 2009. Identification of *Candida nivariensis* and *Candida bracarenensis* in a large global collection of *Candida glabrata* isolates:



- comparison to the literature. *J Clin Microbiol* 47:1216–1217. <https://doi.org/10.1128/JCM.02315-08>.
116. Tavanti A, Davidson AD, Gow NA, Maiden MC, Odds FC. 2005. *Candida orthopsilosis* and *Candida metapsilosis* spp. nov. to replace *Candida parapsilosis* groups II and III. *J Clin Microbiol* 43:284–292. <https://doi.org/10.1128/JCM.43.1.284-292.2005>.
  117. Cabañes FJ. 2014. *Malassezia* yeasts: how many species infect humans and animals? *PLoS Pathog* 10:e1003892. <https://doi.org/10.1371/journal.ppat.1003892>.
  118. Cabañes FJ, Coutinho SD, Puig L, Bragulat MR, Castellá G. 2016. New lipid-dependent *Malassezia* species from parrots. *Rev Iberoam Micol* 33:92–99. <https://doi.org/10.1016/j.riam.2016.03.003>.
  119. Honnavar P, Prasad GS, Ghosh A, Dogra S, Handa S, Rudramurthy SM. 2016. *Malassezia arunalokei* sp. nov., a novel yeast species isolated from seborrheic dermatitis patients and healthy individuals from India. *J Clin Microbiol* 54:1826–1834. <https://doi.org/10.1128/JCM.00683-16>.
  120. Colombo AL, Padovan AC, Chaves GM. 2011. Current knowledge of *Trichosporon* spp. and trichosporonosis. *Clin Microbiol Rev* 24:682–700. <https://doi.org/10.1128/CMR.00003-11>.
  121. Guého E, de Hoog GS, Smith MT. 1992. Neotypification of the genus *Trichosporon*. *Antonie Van Leeuwenhoek* 61:285–288. <https://doi.org/10.1007/BF00713937>.
  122. Barrs VR, van Doorn TM, Houbraken J, Kidd SE, Martin P, Pinheiro MD, Richardson M, Varga J, Samson RA. 2013. *Aspergillus felis* sp. nov., an emerging agent of invasive aspergillosis in humans, cats, and dogs. *PLoS One* 8:e64871. <https://doi.org/10.1371/journal.pone.0064871>.
  123. Houbraken J, Weig M, Groß U, Meijer M, Bader O. 2016. *Aspergillus oerlinghausenensis*, a new mould species closely related to *A. fumigatus*. *FEMS Microbiol Lett* 363:fnv236. <https://doi.org/10.1093/femsle/fnv236>.
  124. Masih A, Singh PK, Kathuria S, Agarwal K, Meis JF, Chowdhary A. 2016. Identification by molecular methods and matrix-assisted laser desorption/ionization-time of flight mass spectrometry and antifungal susceptibility profiles of clinically significant rare *Aspergillus* species in a referral chest hospital in Delhi, India. *J Clin Microbiol* 54:2354–2364. <https://doi.org/10.1128/JCM.00962-16>.
  125. Teixeira Mde M, Theodoro RC, Oliveira FF, Machado GC, Hahn RC, Bagagli E, San-Blas G, Soares Felipe MS. 2014. *Paracoccidioides lutzii* sp. nov.: biological and clinical implications. *Med Mycol* 52:19–28. <https://doi.org/10.3109/13693786.2013.794311>.
  126. Birky CW, Ricci C, Melone G, Fontaneto D. 2011. Integrating DNA and morphological taxonomy to describe diversity in poorly studied microscopic animals: new species of the genus *Abrochtha* Bryce, 1910 (Rotifera: Bdelloidea: Philodinavidae). *Zool J Linn Soc* 161:723–734. <https://doi.org/10.1111/j.1096-3642.2010.00674.x>.
  127. Del-Prado R, Divakar PK, Lumbsch HT, Crespo AM. 2016. Hidden genetic diversity in an asexually reproducing lichen forming fungal group. *PLoS One* 11:e0161031. <https://doi.org/10.1371/journal.pone.0161031>.
  128. Henk DA, Eagle CE, Brown K, Van Den Berg MA, Dyer PS, Peterson SW, Fisher MC. 2011. Speciation despite globally overlapping distributions in *Penicillium chrysogenum*: the population genetics of Alexander Fleming's lucky fungus. *Mol Ecol* 20:4288–4301. <https://doi.org/10.1111/j.1365-294X.2011.05244.x>.
  129. O'Donnell K, Ward TJ, Geiser DM, Kistler HC, Aoki T. 2004. Genealogical concordance between the mating type locus and seven other nuclear genes supports formal recognition of nine phylogenetically distinct species within the *Fusarium graminearum* clade. *Fungal Genet Biol* 41:600–623. <https://doi.org/10.1016/j.fgb.2004.03.003>.
  130. Peterson SW, Jurjević Ž, Frisvad JC. 2015. Expanding the species and chemical diversity of *Penicillium* section *Cinnamopurpurea*. *PLoS One* 10:e0121987. <https://doi.org/10.1371/journal.pone.0121987>.
  131. Pringle A, Baker DM, Platt JL, Wares JP, Latgé JP, Taylor JW. 2005. Cryptic speciation in the cosmopolitan and clonal human pathogenic fungus *Aspergillus fumigatus*. *Evolution* 59:1886–1899. <https://doi.org/10.1111/j.0014-3820.2005.tb01059.x>.
  132. Stewart JE, Timmer LW, Lawrence CB, Pryor BM, Peever TL. 2014. Discord between morphological and phylogenetic species boundaries: incomplete lineage sorting and recombination results in fuzzy species boundaries in an asexual fungal pathogen. *BMC Evol Biol* 14:38. <https://doi.org/10.1186/1471-2148-14-38>.
  133. Taylor J, Jacobson D, Fisher M. 1999. The evolution of asexual fungi: reproduction, speciation and classification. *Annu Rev Phytopathol* 37:197–246. <https://doi.org/10.1146/annurev.phyto.37.1.197>.
  134. Widhalm TJ, Egan RS, Bertoletti FR, Asztalos MJ, Kraichak E, Leavitt SD, Lumbsch HT. 2016. Picking holes in traditional species delimitations: an integrative taxonomic reassessment of the *Parmotrema perforatum* group (Parmeliaceae, Ascomycota). *Bot J Linn Soc* 182:868–884. <https://doi.org/10.1111/boj.12483>.
  135. Tang CQ, Obertegger U, Fontaneto D, Barraclough TG. 2014. Sexual species are separated by larger genetic gaps than asexual species in rotifers. *Evolution* 68:2901–2916. <https://doi.org/10.1111/evo.12483>.
  136. Carstens BC, Pelletier TA, Reid NM, Satler JD. 2013. How to fail at species delimitation. *Mol Ecol* 22:4369–4383. <https://doi.org/10.1111/mec.12413>.
  137. Desjardins CA, Sykes SM, Rhodes J, Giamberardino C, Yu C, Tenor JL, Chen Y, Yang T, Jones AM, Sun S, Haverkamp MR, Heitman J, Litvintseva AP, Fisher MC, Perfect JR, Cuomo CA. 2017. Population genomics and the evolution of virulence traits in *Cryptococcus neoformans*. In 10th International Conference on Cryptococcus and Cryptococcosis, Foz do Iguaçu, Brazil, 26 to 30 March 2017.
  138. Engelthaler DM. 2017. A phylogenomic view of the *Cryptococcus* species complexes. In 10th International Conference on Cryptococcus and Cryptococcosis, Foz do Iguaçu, Brazil, 26 to 30 March 2017.
  139. Rhodes J, Desjardins CA, Harrison T, Bicanic T, Fisher MC, Cuomo CA. 2017. On the origin and dispersal of *Cryptococcus neoformans* var. *grubii*. In 10th International Conference on Cryptococcus and Cryptococcosis, Foz do Iguaçu, Brazil, 26 to 30 March 2017.
  140. Hagen F, Ceresini PC, Polacheck I, Ma H, van Nieuwerburgh F, Gabaldón T, Kagan S, Pursall ER, Hoogveld HL, van Iersel LJ, Klau GW, Kelk SM, Stougie L, Bartlett KH, Voelz K, Pryszcz LP, Castañeda E, Lazera M, Meyer W, Deforce D, Meis JF, May RC, Klaassen CH, Boekhout T. 2013. Ancient dispersal of the human fungal pathogen *Cryptococcus gattii* from the Amazon rainforest. *PLoS One* 8:e71148. <https://doi.org/10.1371/journal.pone.0071148>.
  141. Kidd SE, Hagen F, Tschärke RL, Huynh M, Bartlett KH, Fyfe M, Macdougall L, Boekhout T, Kwon-Chung KJ, Meyer W. 2004. A rare genotype of *Cryptococcus gattii* caused the cryptococcosis outbreak on Vancouver Island (British Columbia, Canada). *Proc Natl Acad Sci U S A* 101:17258–17263. <https://doi.org/10.1073/pnas.0402981101>.
  142. Aminnejad M, Diaz M, Arabatzis M, Castañeda E, Lazera M, Velegraki A, Marriott D, Sorrell TC, Meyer W. 2012. Identification of novel hybrids between *Cryptococcus neoformans* var. *grubii* VNI and *Cryptococcus gattii* VGII. *Mycopathologia* 173:337–346. <https://doi.org/10.1007/s11046-011-9491-x>.
  143. Bovers M, Hagen F, Kuramae EE, Diaz MR, Spanjaard L, Dromer F, Hoogveld HL, Boekhout T. 2006. Unique hybrids between the fungal pathogens *Cryptococcus neoformans* and *Cryptococcus gattii*. *FEMS Yeast Res* 6:599–607. <https://doi.org/10.1111/j.1567-1364.2006.00082.x>.
  144. Bovers M, Hagen F, Kuramae EE, Hoogveld HL, Dromer F, St-Germain G, Boekhout T. 2008. AIDS patient death caused by novel *Cryptococcus neoformans* × *C. gattii* hybrid. *Emerg Infect Dis* 14:1105–1108. <https://doi.org/10.3201/eid1407.080122>.
  145. Chaturvedi V, Fan J, Stein B, Behr MJ, Samsonoff WA, Wickes BL, Chaturvedi S. 2002. Molecular genetic analyses of mating pheromones reveal intervariety mating or hybridization in *Cryptococcus neoformans*. *Infect Immun* 70:5225–5235. <https://doi.org/10.1128/IAI.70.9.5225-5235.2002>.
  146. Hagen F, Hare Jensen R, Meis JF, Arendrup MC. 2016. Molecular epidemiology and in vitro antifungal susceptibility testing of 108 clinical *Cryptococcus neoformans sensu lato* and *Cryptococcus gattii sensu lato* isolates from Denmark. *Mycoses* 59:576–584. <https://doi.org/10.1111/myc.12507>.
  147. Groth C, Hansen J, Piskur J. 1999. A natural chimeric yeast containing genetic material from three species. *Int J Syst Bacteriol* 49:1933–1938. <https://doi.org/10.1099/00207713-49-4-1933>.
  148. Gabaldón T, Naranjo-Ortiz MA, Marcet-Houben M. 2016. Evolutionary genetics of yeast pathogens in the Saccharomycotina. *FEMS Yeast Res* 16:fow064. <https://doi.org/10.1093/femsyr/fow064>.
  149. Morales L, Dujon B. 2012. Evolutionary role of interspecies hybridization and genetic exchanges in yeasts. *Microbiol Mol Biol Rev* 76:721–739. <https://doi.org/10.1128/MMBR.00022-12>.
  150. Nguyen HV, Boekhout T. 2017. Characterization of *Saccharomyces uvarum* (Beijerinck, 1898) and related hybrids: assessment of molecular markers that predict the parent and hybrid genomes and a proposal to name yeast hybrids. *FEMS Yeast Res* 17:fox014. <https://doi.org/10.1093/femsyr/fox014>.
  151. McNeill J, Turland NJ, Barrie FR, Buck WR, Greuter W, Wiersema JH. 2012. International code of nomenclature for algae, fungi, and plants. Koeltz Scientific Books, Königstein, Germany.
  152. Nyazika TK, Herkert PF, Hagen F, Mateveke K, Robertson VJ, Meis JF.

2016. In vitro antifungal susceptibility profiles of *Cryptococcus* species isolated from HIV-associated cryptococcal meningitis patients in Zimbabwe. *Diagn Microbiol Infect Dis* 86:289–292. <https://doi.org/10.1016/j.diagmicrobio.2016.08.004>.
153. Dromer F, Mathoulin S, Dupont B, Letenneur L, Ronin O, French Cryptococcosis Study Group. 1996. Individual and environmental factors associated with infection due to *Cryptococcus neoformans* serotype D. *Clin Infect Dis* 23:91–96. <https://doi.org/10.1093/clinids/23.1.91>.
154. Thompson GR, III, Albert N, Hodge G, Wilson MD, Sykes JE, Bays DJ, Firacative C, Meyer W, Kontoyiannis DP. 2014. Phenotypic differences of *Cryptococcus* molecular types and their implications for virulence in a *Drosophila* model of infection. *Infect Immun* 82:3058–3065. <https://doi.org/10.1128/IAI.01805-14>.
155. Irokanulo EA, Akueshi CO, Makinde AA. 1994. Differentiation of *Cryptococcus neoformans* serotypes A and D using creatinine dextrose bromothymol blue thymine medium. *Br J Biomed Sci* 51:100–103.