

S5.1 Genus and species concepts.

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Biological diversity is an important aspect to understand fungal disease. This is true at different taxonomic levels, ranging from order to population. Within the Basidiomycota, three classes are distinguished, viz. the Hymenomycetes, Ustilaginomycetes and Urediniomycetes, with the latter two comprising mainly plant pathogens.

Human pathogenic Basidiomycetes were derived from many different ancestors. Within the mainly plant pathogenic smut fungi, a unique clade occurs with human pathogens, namely the order Malasseziales with the genus *Malassezia* (Begerow et al., 2000). Within the Hymenomycetes, the genus *Trichosporon* (except *T. pullulans*) is classified in the order Trichosporonales (Fell et al., 2000), and contains several human pathogens. *Filobasidiella neoformans* (including the anamorph *Cryptococcus neoformans*), which belongs to the Hymenomycetes as well, is distantly related to the Trichosporonales. Molecular data suggest that *F. neoformans* belongs to the Tremellales or jelly fungi (Fell et al., 2000), which mostly comprise saprophytic or parasitic fungi occurring on wood or other fungi. The same data showed that *Filobasidiella* is not closely related to morphologically similar genera like *Filobasidium* and *Chionosphaera*.

A complicating factor in mycology is the dual system of nomenclature. So far, asexual (anamorphic or mitosporic) fungi are classified separately from the sexual (teleosporic or meiosporic) ones. The use of two names for the same organisms, which is confusing for many users, seems irrelevant from an evolutionary point of view. Currently, an intense debate is going on whether the Linnaean system of nomenclature should be changed in favor of a system based on molecular phylogeny (phylocode), and a second discussion focuses on the dual nomenclatural system currently used in mycology.

However, at present *Cryptococcus neoformans* and *Filobasidiella neoformans* are the names in use for the asexual and sexual stages, respectively. In case only one name will be used in the future, that of the sexual state may be preferred. However, the possible abandonment of the name *Cryptococcus neoformans* will cause understandable turmoil in the scientific community, including medical, biological and pharmaceutical sciences. Therefore, future nomenclatural changes need not only be judged on their theoretical nomenclatural aspects, but should also consider the practical consequences and implications for the scientific and clinical communities. A similar plea for careful judgment may be held when new names are being introduced. It is strongly recommended that before doing so, all possible synonyms are critically compared, particularly those represented by living authentic isolates preserved in culture collections.

A long and 'stormy' debate on the nature of taxa, such as genus and species, is going on since the acceptance of evolution as the driving force for biological diversity. For instance, 22 species concepts (SCs) have been distinguished (Mayden, 1997), which more recently have

been separated in operational and theoretical SCs (Taylor et al., 2000). Important parameters for SCs are universality, applicability and theoretical significance (Hull, 1997). However, the same author stated that attempts to make the SCs more operational may undermine the theoretical aspects. Three SCs, namely the morphological (MSC), the biological (BSC) and the phylogenetic species concepts (PSC) have been applied to understand the biodiversity within *C. neoformans*. The MSC, which is based on phenotypic discrepancies occurring between populations, is widely used in mycology. The BSC has as the main attribute the occurrence of gene flow between populations, and the PSC has been defined as the smallest aggregate of populations with a common lineage that share unique, diagnosable phenotypic characteristics (Harrington & Rizzo, 1999). The MSC has been used to define the two currently recognized varieties *C. neoformans* var. *neoformans* and var. *gattii*. After the observation of mating and gene flow in the species complex, the BSC dominated our views on biological variation in *C. neoformans*. This resulted in the description of a separate genus, *Filobasidiella*, for the sexual state. The initially recognized two species in the complex were reduced to varietal level because of the observation of an interspecific hybridization event (Kwon-Chung et al., 1982). Recently, many molecular studies, e.g. those on the intergenic and internally transcribed spacers of the rDNA, the URA5 gene, the laccase gene, and the mitochondrial large subunit rRNA (Diaz et al., 2000; Xu et al., 2000) and AFLP genotypes showed that the two varieties belong to different phylogenetic lineages and most likely represent distinct species (Boekhout et al., 2001). This two species hypothesis is sustained by the many physiological, biochemical, genetical, clinical, morphological, ecological and geographical differences known for a long time. To test the two species hypothesis, AFLP patterns and IGS sequences of descendants resulting from a mating between isolates B-3502 (*C. neoformans*) and B-3939 (*C. bacillisporus*) were investigated. Most of the descendants possessed the genotypes of either parental isolate, except isolate sb-18 which showed some variation in its banding pattern. Gene flow could not be demonstrated under natural circumstances. Extensive AFLP typing of a number of Brazilian isolates belonging to both species, which were isolated from a single tree, did not give any indication of the occurrence of interspecific hybridization events in nature. Therefore, we propose to recognize two different species, *C. neoformans* and *C. bacillisporus*. A proposal to conserve the latter name against an older validly published name is underway.

The MSC, traditionally classified species in the anamorph genus *Cryptococcus*, is gradually being replaced by the PSC, because many molecular studies contributed to a better understanding of species boundaries and their phylogenetic relationships (e.g. Fell et al., 2000). Moreover, the studies by Fell and coworkers clearly indicated that the genus *Cryptococcus* is highly polyphyletic, because the members occurred in almost all lineages of the Hymenozymetes and even in the Urediniomycetes (e.g. *C. yarrowii* = *Rhodotorula yarrowii*). Due to the conservation of *C. neoformans* as the type species of the genus, this heterogeneity will not afflict the naming of *C. neoformans* when the genus will be split into smaller monophyletic genera in the future. Contrary to the anamorph genus *Cryptococcus*, the teleomorph genus *Filobasidiella* is monophyletic.

A more detailed AFLP and IGS analysis of the genomic diversity within *C. bacillisporus* resulted in the discovery of three genotypes within the species (genotypes 4, 5 and 6) (Diaz et al., 2000; Boekhout et al., 2001). Interestingly, the borders between these genotypes did not follow serotype boundaries but rather followed geographical lines. The American populations seem rather different from the African, Australian, Asian and Southern European ones. The genotypes do not differ significantly in their chromosomal make up. Many of the Brazilian tree isolates studied, as well as some clinical isolates were found to belong to genotype 6, from which until recently only 4 isolates were known. All known isolates of genotypes 5 and 6 came from the Americas. The available phylogenetic trees however, suggest a considerable distance between the three genotypes of *C. bacillisporus*, which may

represent distinct varieties. However, genotype 6 may have originated from a hybridization event between the genotypes 4 and 5 (Boekhout et al., 2001). Future genomic research may support a hybrid origin of genotype 6, and also may clarify the taxonomic position of the three genotypes.

We investigated a number of virulence related phenotypic traits, as well the susceptibility against several antifungal drugs in all 6 genotypes of *C. neoformans* and *C. bacillisporus*. The latter species showed slower melanization rates, and somewhat less protease and phospholipase activities, whereas the capsule sizes tend to be bigger. Virulence as analyzed in a mouse model, showed considerable differences between isolates belonging to the same genotype. Within *C. bacillisporus*, high values of virulence were observed in clinical isolates. The most virulent strain was isolated from CSF (genotype 5) and a slightly less virulent strain was isolated from sputum (genotype 6), both originating from the U.S.A. Resistance against fluconazole was observed in several environmental isolates of genotypes 4 and 6, thus suggesting the presence of an innate resistance against the drug.

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