Phylogenetics and phylogenomics to understand fungal diversity

João F. Moreira Salgado[1], C. Alisha Quandt[2], and Jason E. Stajich[3]

[1]RG Insect Microbiology and Symbiosis, Max Planck Institute for Terrestrial Microbiology, 35043 Marburg, Germany. Email: joao.salgado@mpi-marburg.mpg.de
[2]Ecology and Evolutionary Biology Department, University of Colorado Boulder, Boulder, CO 80309 USA
[3]Department of Microbiology and Plant Pathology, University of California Riverside, Riverside, CA, 92521 USA

March 10, 2023

Abstract

Fungi are ubiquitous in the environment and some of the basal lineages found within the kingdom date back to the earliest known divergences in the eukaryotic tree of life. Such ubiquity is manifested in a myriad of different lifestyles and morphologies which are ultimately an expression of their genetic diversity. Advances in technology and molecular biology supporting high-throughput sequencing and bioinformatics have allowed us to develop a robust phylogenetic framework for fungal systematics improving upon previous, more simplistic, morphological classifications. Despite the obvious benefits reaped from such advances, the relationships among earlier diverging phyla remain largely unresolved, mostly due to a lack of extensive sequencing of species in these clades. Furthermore, inherent biases, as well as different types of methodological or computational errors may cause misleading assumptions in phylogenetic hypotheses for this highly diverse kingdom. In this chapter, we review molecular mechanisms that are responsible for the evolution and diversification of the fungi, with special remarks to the varied ecological niches occupied by its members. We then consider the impact of genetic and genomic-scale studies in fungal systematics, elucidating classic methods and strategies employed in these studies and their current limitations. Finally, we discuss how these phylogenetic methods can be integrated into phylogenomics to find and resolve accurate species placements and thus shed light on the biodiversity of these fascinating organisms.

On the diversity of the kingdom Fungi

Fungi are one of the most diverse groups of organisms, with species richness estimates of about 1.5 to 5 million of species worldwide. This unique kingdom has representatives that may be unicellular, in the form of yeasts, multicellular, with mycelia composed of masses of filamentous cells known as hyphae, or both at different stages of development. Fungi are amongst the most versatile organisms in terms of exploitation of different ecological niches and are recognized by their capability of recycling organic matter as free-living saprotrophs in a range of climates that can comprise terrestrial or aquatic environments. They can also be important pathogens, parasites, and symbionts of other fungi, algae, bacteria, protists, plants, and animals. Studies of fungal diversity and systematics date back to the 18th century, with new species being described in the Americas and Europe by botanists and early mycologists (Berkeley, 1874; Cooke, 1893; Hennings, 1896). This knowledge soon raised awareness to the perception that fungi could have significant

contributions in biogeochemical cycles and even represent economic losses in agriculture, with decomposition of crops and other goods (Cobb, 1892; Horne, 1925; Kidd, 1932; Ryakhovsky, 1931). Nonetheless, despite its ubiquitous distribution around the globe, the described richness of fungi comprises representatives that have very specific biogeographical patterns and optimal environmental conditions (Meiser et al., 2014). Generally, warmer temperatures and higher humidity contribute towards higher diversity of fungal species, although the advances in sequencing and metagenomic techniques have allowed the study of extremophiles in poorly-studied hot, hypersaline, and even glacial environments (Berka et al., 2011; Gonçalves et al., 2012; Peidro-Guzmán et al., 2020; Tsuji et al., 2017; Zalar et al., 2007).

1.1. Roles of the fungal diversity in ecosystems

Fungi are heterotrophs and, among many of the niches they can occupy, their decomposition of organic matter, including lignocellulose, has been thoroughly studied. In the soil, specifically, fungi are considered to represent the largest portion of biomass and are known to have intrinsic participation in the recycling of carbon (Malik et al., 2016) along with bacteria and other microorganisms with whom fungi compete for resources. Several fungal genera are known for their capability of producing powerful antibiotic substances that can impact both micro and macro ecological communities they are found in. On the other hand, the synergistic association of these microorganisms may be essential for maintaining soil ecosystems The study of fungal-bacterial interactions in soil ecology is still a vastly open field for investigation, and the lack of knowledge in these associations has been attributed to the difficulty in implementing more holistic methodologies, given the spatial-temporal and taxonomic specificity of the interactions (de Menezes et al., 2017).

In the rhizosphere, some fungi in the Ascomycota, Basidiomycota, and Glomeromycota phyla form mycorrhizae with a wide range of host plants (Merckx et al., 2009). These associations are promoted by specialized hyphal projections, known as hyphopodium, which penetrate the outermost layer of the host's cells, providing a source of photosynthetically fixed carbon to the fungus in exchange of enhanced uptake of non-soluble inorganic ions, such as phosphate for the plant (Begum et al., 2019). Because of the usually complex networks and cycling of nutrients promoted by the association of fungal mycelia and the plant hosts' roots, mycorrhiza can shape the landscape of the whole ecosystem they are found in (Smith and Read, 2008). Arbuscular mycorrhizal fungi are also observed to increase the resistance of the host to root colonization by pathogens with different mechanisms and in different levels, in a fungal species-specific fashion. For this reason, such a niche may be a hub for the diversity of obligate mycorrhizal fungi (Wehner et al., 2010). Genomic studies have elucidated that the establishment of such symbiotic association with the host involves secretion of effectors that trigger innate immunological responses in the plant, with involvement of signaling mechanisms that are paradoxically similar, if not identical, to those observed in early stages of colonization by phytopathogenic fungi (Corradi and Bonfante, 2012).

The origins of pathogenicity in fungi are yet to be fully uncovered, given that only most of the studies are focused on human pathogens (Boddy, 2016). Still, the occurrence of a parasitic lifestyle in a broad range of hosts is widespread across the kingdom, implying that this is a trait that has been gained and, in some cases, lost multiple times during its natural history. Further comparative genomic studies have concluded that this evolutionary threshold represents convergent and divergent pathogenic traits in clades that have plant, human, and insect hosts, arising from saprotrophic and even mutualistic lifestyles (Redman et al., 2001; Shang et al., 2016). This implies that the reasons underlying these thresholds may be completely unrelated for each specific pathogenic clade, thus complicating the study of speciation and evolution in pathogenic fungi. Nonetheless, phylogenomic studies on species with different host specificities may elucidate some of these processes. For instance, the pathogenesis of some fungi is associated with speciation through expansion of protein families, such as toxins (Joneson et al., 2011) and the emergence of generalists from specialist taxa may be due to positive selection and development of asexual life-cycles through transitory species with increasingly broader host specificities (Hu et al., 2014; Thomma, 2003).

The aforementioned examples attempt to show the plasticity and diversity of fungi yet, are far from depicting the real variety of ecological niches occupied by these organisms. Historically, it is clear that studying the diversity of fungi and their associations with other organisms can be greatly benefitted by genetic and genomic studies, where the estimation of phylogenetic trees to infer their evolutionary relationships becomes paramount. In *vivo* , environmental pressure in fungal populations is responsible for their diversity and the mechanisms that are intrinsically associated with the systematic positioning of taxa are better elucidated by molecular methods with the possibility of unveiling shifts on a genetic level.

1.2. Mechanisms that contribute to the fungal genetic variability

What makes living organisms unique is an intriguing question that has been posed in the natural sciences from its early developments. While the characteristics that differentiate organisms may be explained by biotic and abiotic interactions with the environment, the underlying genetic shifts selected in populations by such interactions are the main drivers of evolution in these organisms. Nowadays, it is known that these shifts may be responsible for altering major biochemical and physiological processes that will ultimately culminate in the observed richness of different biological traits, allowing for the exploration of new ecological niches, for instance. Diversity can, thus, be considered a classification of the genotypic and phenotypic variability of organisms, which can be applied to different taxonomic levels and ecological scales. Because fungi reproduce at considerably faster rates than macroorganisms, it is theoretically easier to tie together microevolutionary molecular processes with their population dynamics of variability and track speciation events. Population genetics of these organisms is, thus, mostly explained by and reliant upon their varied modes of reproduction, ranging from exclusively sexual cycles to anywhere in between infrequent asexuality and parasexuality in different clades. We will give a brief overview of these cycles in the best studied clades, linking their occurrence to genetic processes that generate variability, or the lack thereof, and, further on, their applications to phylogenetic and phylogenomic studies.

The phylum Ascomycota is one of the best studied and, to date, most diverse groups of fungi. Most asexual filamentous ascomycetes are known to be haploid for most of their lifecycle, which begins with the germination of dispersal units, or spores, known as conidia. The spores grow to form mycelia, which can develop into specialized structures named conidiophores, to reproduce asexually through mitotic divisions. Because there is no crossover of genetic information between homologous chromosomes during mitosis, it has been parsimoniously assumed that genetic discrepancies in clonal populations of predominantly asexual fungi could only be attributed to inheritance independent processes, such as mutations or via lateral-gene transfer (LGT). Indeed, the latter can represent prominent causes of evolutionary transitions (Gabaldón, 2020), being associated, for example, with increased virulence in different fungal pathogens (McDonald et al., 2019; Slot and Rokas, 2011; van der Does and Rep, 2012; Vlaardingerbroek et al., 2016). However, it is now clear that several lineages of Ascomycetes with no known teleomorphs have either cryptic sexual or anomalous reproductive cycles, inferred from high recombination rates in populations. In fact, the occurrence of recombination can be the primary driver of genetic variability in fungi that don't necessarily reproduce sexually (reviewed in Taylor et al., 2015).

Although the possibility of genetic diversity that meiosis provides confers advantages in adapting to variable environments , sexual reproduction has been associated with the proliferation of repetitive sequences, particularly that of transposable elements (TEs) (Bakkeren et al., 2006; Hickey, 1982). Much has been discussed on the ability of TEs to directly impact the diversity and evolution of eukaryotic organisms based on its property to promote insertions and deletions on restriction sites that can virtually take place throughout the whole genome of the host, potentially promoting changes in reading frames or causing genes to be truncated. Although this may seem as an ominously remote possibility in fungi given the usually large size of the eukaryotic genome, Muszewska et al. (2019) found that older non-autonomous TE insertions or remnants are largely found in coding regions. Furthermore, animal pathogenic fungi have more TEs inserted in genes than other fungi, contributing to the hypothesis that these mobile selfish tandem repeats can have great contributions to the host's gene expression and diversification (Muszewska et al., 2017). To protect their ge-

nomes against potential deleterious effects of transposition events, fungi use several mechanisms to recognize and silence high repetitive content regions during the sexual cycle, such as the interference RNA (RNAi), methylation induced premeiotically (MIP), meiotic silencing by unpaired DNA (MSUD) and repeat-induced point mutation (RIP) pathways (Irelan and Selker, 1996; Lax et al., 2020; Shiu et al., 2001; Wang et al., 2016). The RIP pathway, which only occurs in Ascomycetes may be of particular interest from an evolutionary perspective because of its direct mutation of G:C to T:A pairs following genome duplication, which arises the possibility of generating functional paralogs after meiotic duplication (Galagan and Selker, 2004).

In some fungi, sexual reproduction may occur alternatively every other generation or after many generations of asexual cycles, depending on factors such as nutrient availability and other environmental cues (Wilson et al., 2019). In the case of heterothallic isolates, two individuals that present opposite mating type identities will need to sense and find each other, while primary homothallic isolates possess both identities and can start the mating event independently or with another isolate of either type. The reproduction then proceeds with fusion of cells, subsequent karyogamy forming diploids and further meiotic divisions, during which several rounds of recombinations can occur. Mating-type identities are a result of which mating type idiomorph (MAT) - or combination thereof, in the case of some Basidiomycetes - is harbored by the isolate (Maia et al., 2015; Wilson et al., 2019). A particular type of secondary homothallism, called pseudothallism, has been described, where nuclei carrying compatible mating types coexist in the same hyphae or yeast cell and allow mating-type switching, culminating in self mating. Additionally, and more recently, a novel type of homothallism, known as unisexual cycle, has been discovered. This reproductive cycle enables an isolate to sexually reproduce possessing a heterothallic genotype (Wilson et al., 2018). Consequently, for the meiotic cycle to be completed, the isolate must undergo diploidization by either endoreplication or karyogamy events. It has been shown that such a cycle represents adaptive advantages beyond the capability of recombination in the basidiomycete yeast *Cryptococcus neoformans* species complex, allowing for a phenotype of dimorphic transition to hyphal growth and enhancing sexual mating competition between isolates of the opposite mating type (Fu et al., 2019; Phadke et al., 2013). Furthermore, the ability to mate genetically identical cells, adding a limited amount of genetic diversity, mostly given by chromosomal-size mutations, SNPs and aneuploidy in these cases, results in a controlled phenotypically diverse progeny and, thereby, more fitness in response to environmental pressure (Ni et al., 2013).

Alternatively, some fungi present parasexual cycles, in which haploid nuclei may fuse within growing mycelia of an isolate, forming diploid and subsequently haploid recombinants by mitotic crossing-over and chromosome loss, respectively. Schoustra et al. (2007) define the diploid state as an accumulator of mutations that, although majoritarily neutral or potentially deleterious when on their own, may become advantageous when combined. As an outcome, however, chromosome segregation or loss may eventually fail to complete, generating progenies with aberrant, yet stable, ploidy numbers, as it has been observed in a independently convergent fashion in different species of the filamentous ascomycete entomopathogen *Metarhizium*spp (Kepler et al., 2016; Nielsen et al., 2021). It is not known yet to what extent such loci of these genomes can accumulate mutations and originate new paralogs with divergent functions assuming i) homozygous origin and ii) that they will not be subjected to haploidization in the course of their evolution. Certainly some of these mechanisms can have an impactful part in the genetic and possibly phenotypic diversity of these organisms, but much is still left to be uncovered by the increasingly powerful sequencing technologies and computational resources.

The state of art in the fungal tree of life

The great plasticity of fungal morphologies, ecological niches, reproductive life cycles, and genetic variation has made it a difficult job for mycologists to classify these organisms using observable macroscopic features. Because of that, sequence similarity approaches have lately become part of the foundation for fungal systematics due to its accuracy and reliability in estimating the relationships within the kingdom. Numerous taxonomic studies have been conducted on Ascomycota and Basidiomycota and, although there is still some

degree of uncertainty of paraphyletic topologies in lower taxonomic groups within the phyla, there is strong evidence to conclude phylum-level monophyly (Hibbett et al., 2018; Robbertse et al., 2006; Schoch et al., 2009; Zhao et al., 2017). They form a subkingdom named Dikarya and are considered the latest diverging lineage hence being positioned as the sister group to all other major lineages. The subkingdom has not undergone dramatic changes since it was first proposed, except by the conclusion that the previously Basidiomycete genus *Entorrhiza* formed a sister group with Dikarya, hence being proposed as its new phylum Entorrhizomycota (Fig. 1; Bauer et al. 2015).

Basal clades in the kingdom are, however, still largely unresolved. Among other inconsistencies with previous single gene phylogenies, multilocus phylogenies revealed i) the polyphyletic nature of previously well accepted phylum Zygomycota, ii) the placement of *Rozella allomycis* as sister to Microsporidia and iii) the phylum Glomeromycota forming a clade with Dikarya (Blackwell et al., 2006; Hibbett et al., 2007; James et al., 2006a). Similar studies concluded that Chytridiomycota was paraphyletic confirming the divergence of Blastocladiales from other chytrids, elevating it to its own phylum, Blastocladiomycota (James et al., 2006b). Later, two phyla were proposed by Spatafora et al. (2017) from the formerly Zygomycetes, using genome-scale phylogenies. The first one, Mucoromycota, with saprobes and soil colonizers, including endophytes and arbuscular mycorrhizal forming fungi forming the subphyla Mucoromycotina, Mortierellomycotina and now a newly-resolved Glomeromycotina (Fig. 1). The second phylum, Zoopagomycota, comprised Entomophthoromycota, Kickxellomycotina and Zoopagomycotina, which are mostly symbionts and pathogens of animals, saprobes, and mycoparasites. However, the placements of these new subphyla classifications are contested to the rank of phyla, whose divergence-times and monophyly are taken into account along with the proposed Olpidiomycota and Basidiobolomycota in further work (Tedersoo et al., 2018). Moreover, it also recognizes a new phylum named Calcarisporiellomycota in the now superphylum Mucoromycetes, which were well accepted in further publications (da Silva et al., 2021; Wijayawardene et al., 2018).

More recently, a new well supported phylum, Sanchytriomycota, composed of ameboid zoosporic fungi, has been proposed as a sister group to Blastocladiomycota (Galindo et al., 2021) using a large fungal genomic dataset and corroborating with the previous classifications on other groups. Regarding Chytrids, much has been discussed on Neocallimastigomycota and Monoblepharomycota forming a paraphyletic clade with the now monophyletic phylum Chytridiomycota, thus being considered phyla in the superphylum Chytridiomyceta, (Tedersoo et al., 2018, 2017), although it does not yet seem to be a consensus (James et al., 2020). Lastly, Microsporidia are regarded as the most basal in the kingdom after splitting with the proposed protist kingdom Nucleariae (Park and Poulin, 2021), sister to Fungi. Target of a number of taxonomic replacements over the last decades and once thought to be protists (Edlund et al., 1996), Microsporidian intracellular parasites of animals and their relationship to the Cryptomycota/Rozellomycota phylum have been better studied in phylogenomic studies (James et al., 2013). Despite proteomic trees suggesting a prostitan origin of their protein sequences (Choi and Kim, 2017), nucleotide based phylogenomic approaches agree with the well supported topology of the subkingdom Opisthosporidia as the deepest-branching clade of Fungi, containing Aphellidiomycota as a sister groups of a well-supported clade comprised of Rozellomycota/Cryptomycota and Microsporidia (Fig. 1; Li et al., 2021; Park and Poulin, 2021). Bass et al. (2018) propose that these two are actually members of a same phylum, where the genera included in what is traditionally known as "Microsporidia", are but highly divergent and specialized long branching (LB) taxa, whereas what is known as "Rozellomycota/Cryptomycota" are its short branching (SB) representatives. It is, nonetheless, still unclear as to whether or not Ophistosporidia form a paraphyletic group, since there is no consensus on the split of Aphelids before or after the emergence of other lineages of Fungi (Letcher and Powell, 2019).

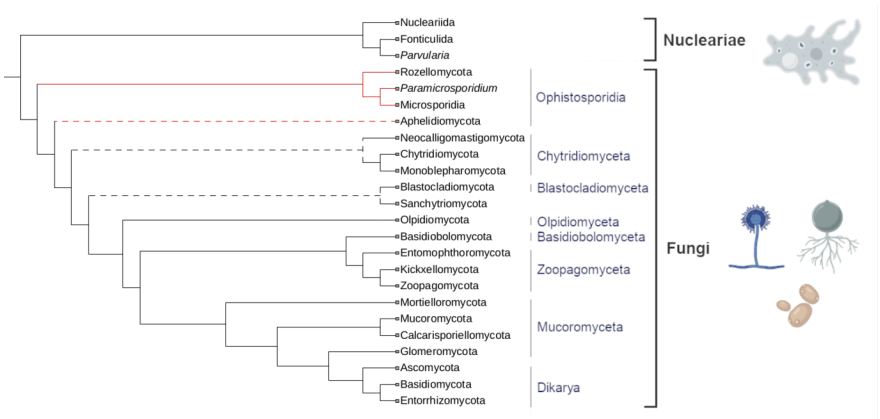

Figure 1. The current phylum-level phylogeny of the Holomycota. Terminal nodes of the tree show Holomycota phyla and the genera Parvularia and Paramicrosporidium, the latter of which represent short branch Microsporidia taxa, but are formally described as Rozellomycota (for a discussion on short and long branch Microsporidia, see Bass et al., 2018; Wadi and Reike, 2020). Names in blue indicate the highest taxonomic classification (Superphylum or Subkingdom) in their resective phyla belonging to one of the proposed kingdoms Fungi or Nucleariae. Branches colored in red indicate potentially paraphyletic clades. Dotted branches indicate phyla whose topologies are still contested. The tree compiles taxonomies from Bauer et al. (2015), Capella-Gutiérrez et al. (2012), Chang et al. (2021), Galindo et al. (2021), Li et al. (2021), Quandt et al. (2017), Tedersoo et al. (2018, 2017) and Wijayawardene et al. (2018).

2.1. Fungal systematics based on single markers

Fungi have traditionally been described and identified based on morphological features provided observation of spores, mycelia, fruiting bodies, cellular structures under light microscope or even the preferential substrate of growth for each particular specimen. Although structuring the foundation of fungal taxonomy up to this day, these characters proved to be misleading on the basis of systematics given the extraordinary plasticity of observable features in closely related specimens, further challenging the concept of species within the kingdom. Therefore, the study of fungal diversity has been greatly benefitted and propelled by the advances in molecular biology, providing an unprecedented surge in the description of fungal taxa. This is due to the possibility of studying conserved genomic regions, increasing our capacity to unveil the richness of this kingdom on the scale of genotypes, while also achieving a better resolution in their taxonomic relationships. By integrating genotypic information with the already existing phenotypic and biochemical characters in a more holistic manner, polyphasic approaches have been elucidating, with good molecular reliability, major inconsistencies in taxonomic placements of several groups of fungi, revisiting conventional classifications and in several cases replacing them in new monophyletic taxa of their own.

The most common molecular markers used for reconstructing fungal phylogenies are standardized barcodes known as internal transcribed spacer (ITS) regions, as well as the large and small subunits of the ribosomal RNA gene (respectively, 28S and 18S rRNA) among others, each of which represent different optimal usages for different groups of fungi. These markers can be employed either by themselves or in combination for better resolution of species taxonomic relationships and phylogenetic inference (Tekpinar and Kalmer, 2019). Alternatively, gene trees can be reconstructed using virtually any gene of interest to understand the evolutionary relationships between homologs. All this is possible because, unlike phenotype-based systematics, phylogenetics accounts for accumulated variations in homologous nucleotide or protein sequences of organisms to infer their taxonomic relationships, being such macromolecules considered molecular clocks given the universal nature of the genetic code and their high rate of conservation (homology). Objectively, this concept

postulates it so that we are able to stochastically determine taxa relatedness by treating amino-acid and nucleotide sequences as strings that can be analyzed computationally to generate phylogenetic hypotheses.

Currently, 10 major fungal clades, including "GS01" (Tedersoo et al., 2017) and comprising 20 phyla are recognized by the UNITE database (12/11/2020; Nilsson et al., 2019) based on the conservation of eukaryotic nuclear ribosomal ITS regions. The vast majority of phyla account for a sum of only 9% of the total described fungal diversity, all of which are early-diverging branches, with the exception of Entorrizhomycetes. Ascomycota and Basidiomycota account for 52% and 39%, respectively. This illustrates that the lack of descriptive studies makes the positioning of these taxa difficult to resolve and suggests that most of the still hidden fungal diversity is probably concentrated in such early diverging branches. Nonetheless, efforts on scrutinizing for the cryptic diversity of these understudied groups seem to experience a promising dawn, as the usage of molecular techniques such as DNA metabarcoding and metagenomics become more powerful and easily accessible, which can certainly be used along with computational phylogenomics to shed light onto divergence processes in the fungal tree of life and the evolution of early eukaryotes.

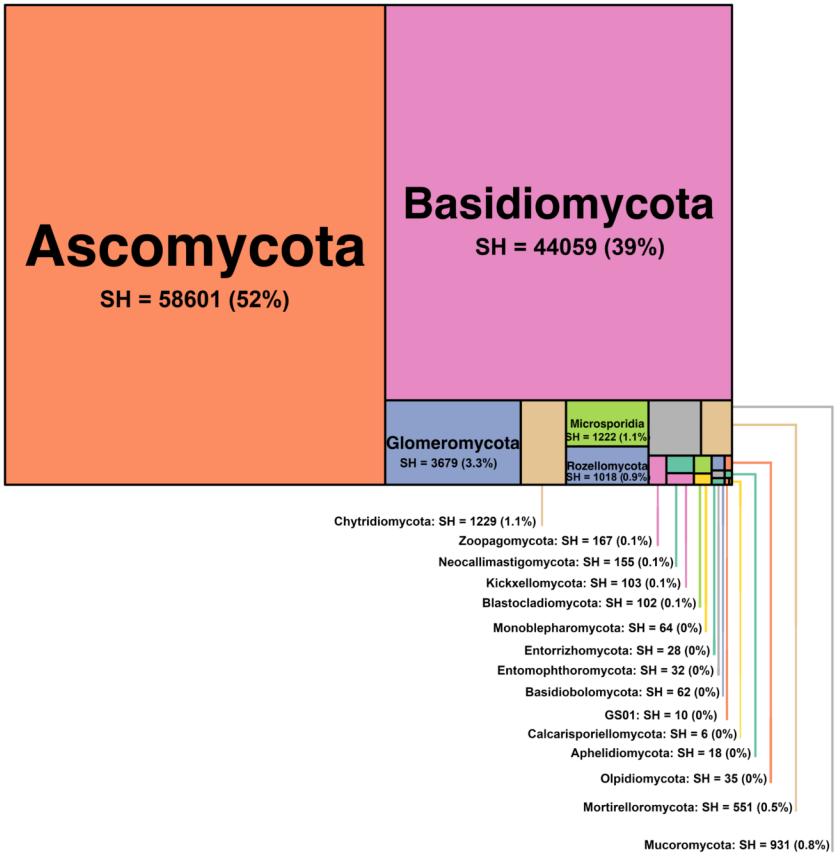

Figure 2. Number and percentage of species hypotheses (SH) available for fungal phyla. With the exception of Microsporidia, all 9 major fungal clades, comprising 19 phyla are recognized by the UNITE database (*https://unite.ut.ee/*) (12/11/2020; Nilsson et al., 2019) based on the conservation of eukaryotic nuclear ribosomal ITS regions. Data for Microsporidia was retrieved from the Catalogue of Life database (*http://www.catalogueoflife.org/annual-checklist/2019/*) (Roskov et al., 2019).

2.2. Impacts and challenges of genomic-scale fungal phylogenies

Information from single or few genes can be insufficient to provide statistical support (Delsuc et al., 2005) and, as new technologies arise providing a growing capacity of sequencing output, the possibility of increasing the sampling of genes to whole genomes has become possible in large-scale phylogenomics projects, thus averaging out stochastic errors in a larger, genomic pool. Such projects have been successfully employed over the last decades on several groups of fungi, providing insights into their evolutionary history from early diverging fungal taxa and related non-fungal Eukaryotes to more complex multicellular Ascomycota and Basiodiomycota (Capella-Gutiérrez et al., 2012; Chang et al., 2019; Kuramae et al., 2006; Y. Liu et al., 2008; Robbertse et al., 2006).

These advances are, however, not without controversies. Even though genomic-scale approaches indeed theoretically increase the resolution of systematics in fungi and reduce stochastic noise in the phylogeny methods so widely used, these approaches are still susceptible to sequencing and systematic errors due to compositional bias or incorrect choice of reconstruction methods (Jeffroy et al. 2006). It is, thus, necessary to understand the advantages and consequences of the usage of each approach in order to leverage the currently available resources for the investigation of fungal diversity (See section 3). Studies that aimed to assess the accuracy of phylogenetic methods to genome-scale datasets have come to the conclusion that, unlike single-gene phylogenetics, where stochastic errors are of main concern, non-phylogenetic signal such as G+C content, within-codon nucleotide position, and rates of evolution among genes may create biases that result in nodal incongruences with high support and bootstrap values (Jeffroy et al., 2006). Diverse Basidiomycete subphyla represent some of such clades whose relationships remained recalcitrant even to genome-scale analyses, according to Prasanna et al. (2020). The authors demonstrate that various unidentified sources of systematic errors are pervasive in genomic datasets, thereby rendering the position of basal clades in the phylum unresolved. Taking into consideration that Basidiomycetes is, in the present moment, one of the phyla with the most species hypotheses (39%; Figure 1), it becomes clear how fragile the interpretation of phylogenomic reconstructions in understudied deep branching phyla can be to even the most innovative sequencing and reconstruction methods.

In addition to the biases inherently introduced by the natural history of the genes, systematic errors also include the possibility of introduced data errors caused by sequencing, missing data, accidental assembly of chimaeras, contaminations, as well as incompleteness and lack of data, which are cumulative in genome-scale projects, given the large amount of genes analyzed and the unpractical manual curation of such. For fungi, these errors must be acknowledged accordingly, considering that the genomic scale information available for the kingdom is not yet nearly as close to the diversity currently described for the kingdom, and to an even lesser extent to its projected diversity (Lofgren and Stajich, 2021). A comparison of the volume of fungal sequences for barcodes deposited in curated databases to the current availability of fungal genomes in Figure 2 shows that, presently, the number of fungal ITS are on the order of approximately 100x larger than the number of fungal genomes. Generation of genome-scale data has also been inhibited by the lack of cultivability of many taxa including obligate symbionts and many species in early diverging lineages. Efforts are being made to work around this impediment using techniques such as metagenomics (e.g. Quandt et al. 2015, Nguyen et al. 2017, Chang et al. 2019) and single cell genomics (Ahrendt et al. 2018, Davis et al. 2019). Nevertheless, the current lack of genomic information for the kingdom, in a predominantly homology and/or clustering based model of phylogenomics, represents a methodological fragility in projects that aim to unveil relationships in the fungal tree of life, demonstrating that not only robust new methods accounting for systematic errors in genome scale datasets, but also more genome-wide species sampling is required.

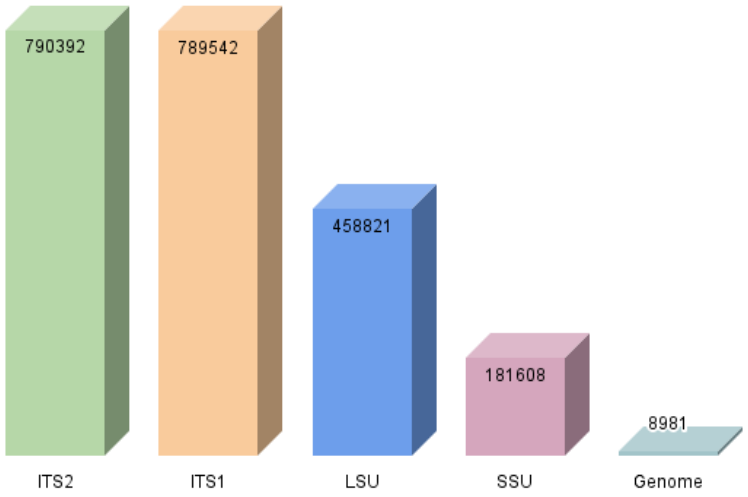

Figure 3. Comparison between the number of publicly available genetic barcodes ITS (Internal Transcribed Spacers) and rRNA (LSU = Large Subunit rRNA; SSU = Small Subunit), as well as genomes. Data were compiled respectively from the UNITED ITS rRNA database (updated in 12/11/2020; Available at *https://unite.ut.ee/*), SILVA rRNA database (updated in 08/27/20; Available at *https://www.arb-silva.de/*) and the NCBI Datasets Genome feature (accessed in 09/13/21; Available at *https://www.ncbi.nlm.nih.gov/datasets/*).

2.3. Seeing the forest for the phylogenomic trees

Largely employed on the basis of whole-genome based analyses, the term phylogenomics does not necessarily mean all genes of a given species pool will be sampled to infer their phylogeny. This is the case because, in order to compare genomes of different organisms, especially when aiming for a complete tree of life, resorting to multilocus approaches to build trees and infer phylogenies is necessary. More specifically, 1:1 homologs, also called Single Copy Orthologs (SCOs), are commonly used for such analyses, as the use of paralogs, duplicated genes of any sort or pseudogene may cause phylogenetic noise in the data (Dutilh et al., 2007). This leads to two main problems: The first being that the selection of gene sets itself scales down the resolution of the "phylogenomic" sampling. Especially in the case of SCOs, distantly related clades with lower levels of homology will drastically reduce the number of genes compared. A special case of this scenario is the utilization of outgroups, commonly employed to root trees. However, maybe even more concerning is the second problem, which is a function of the first: the evolutionary history of the chosen subset of genes is likely not to fully depict the evolutionary history of the species itself (Dagan and Martin, 2006; Nagy and Szöllősi, 2017). The result of the combination of these problems is a non-negligible distortion of the topologies caused by the bias that the incomplete computation of the evolutionary distances between such sequences introduces.

Using a strategy where either single genes or a subset thereof from a genome are used to reconstruct species phylogenies can be, therefore, misleading, as it implies that the species tree (ST) coincides perfectly with the gene trees (GT) analysed. The consequence of that is the assumption that speciation events depicted in the ST are but gene coalescence events. This can raise issues in scenarios where the evolutionary history of the genes is detached from that of the species, creating discordance between ST and GT, phenomenon denominated deep coalescence or Incomplete Lineage Sorting (ILS). The underlying reason is that in the genetics of a population, different alleles or idiomorphs may coexist through speciation processes, causing new species to have different paralogs and making it difficult to coalesce either to a common ancestral gene (Nagy

and Szöllősi, 2017). Common genetic mechanisms in fungi, such as introgression, hybrid speciation, gene duplication, transfer and loss (DTL) and gene flow are included in such scenarios and have been discussed in previous sections of this chapter. These microevolutionary events commonly attributed in large scale to microorganisms, coupled with a typically complex eukaryotic genome architecture and a still incipient genomic-level sampling of the kingdom's diversity hinder the advance of high-resolution fungal systematics. Furthermore, commonly used *ad hoc* methods in multilocus phylogenetics, such as concatenation, fail to take such phenomena into consideration, thus harboring incongruent results. With that in mind, novel ILS-aware phyletic methods modeled by the Coalescent Theory (CT; Bansal et al., 2013; Mallo and Posada, 2016; Rosenberg and Nordborg, 2002) have been deployed. These methods and their interplay with the main molecular evolution algorithms used for inferring species relationships will be discussed further into this chapter.

Phyletic approaches in fungal systematics

Most phylogenetic reconstructions are operated upon an alignment of the sequences of interest and subsequent employment of either distance or character based methods. The former relies on Markov Chains to find the accumulated number of substitutions (transitions or transversions), or evolutionary distance in the lineages since their last common ancestor, to build a distance matrix. Most well known distance algorithms include UPGMA and Neighbor-Joining (NJ). On the other hand, character-based methods such as Maximum Parsimony (MP) and Maximum Likelihood (ML) compare all sequences simultaneously considering one character/site at a time. Additionally parametric stochastic models such as Bayesian Markov Chain Monte Carlo (BMCMC) have also been adopted. The evolution of the deployment of all the aforementioned molecular evolution methods has, however, focused on the reconstruction of either species or gene trees based on sequence alignments, and not necessarily on how the evolutionary history of the processes of gene flow and evolution within the species' genomes converge to their actual relationships. On this matter, solving incongruences between gene and species trees has been the reason for a decades long debate among systematists on what the best approach for dealing with incongruences would be.

3.1. Testing for incongruences

Mickevich (1978) introduced the concept of taxonomic congruence, where independent datasets should be separated in different subsets for phylogenetic analyses and the resulting topologies would be analyzed and summarized combining the trees by its consensus topologies. Although originally applicable to single-gene analyses, this concept has been extrapolated to multi-locus phylogenies by becoming the foundation for the supertree phylogenomic analyses (Leigh et al., 2011; Pisani et al., 2007). Supertrees can be assembled from a combination of independent phylogenetic trees from different datasets using average scores for consensus trees or MP. In a multigene study for the reconstruction of a fungal tree of life, Fitzpatrick et al (2006) found that the former suffers from Long Branch Attraction (LBA), where clades with higher evolutionary rates are inferred to be closely-related, regardless of their true phylogenetic relationship - while techniques based on MP seemed not to be affected. Regardless of methodological artifacts, a common critique to supertree based phylogenomics is the lack of statistical support of evolutionary change (von Haeseler, 2012). More sophisticated approaches using ML, in which an optimum tree state is inferred asymptotically from a set of general theoretic estimates, often referred to as *a priori* criteria (Akaike, 1998; Cam, 1990) have, therefore, been explored.

Other systematists argued that incongruences should be handled by statistically testing the multilocus dataset in order to assess heterogeneity of the produced phylogeny (not to be confused with *heterotachy*), that is, whether or not there are clades with different evolutionary hypotheses along the same tree, and only should the results of such tests be non-significant, the data could then be combined, otherwise partitions should be analyzed individually (Farris et al., 1995; Huelsenbeck and Bull, 1996; Waddell et al., 2000). This line of

thought, known as conditional data combinatory attempts to confirm whether incongruence in a particular partition is given by chance or by violation of the method's assumptions and most of them test against a congruent, or statistically non-significant, null-hypothesis. Nevertheless, although such strategy may be useful to point out problematic partitions in a dataset, it is not nearly enough to solve the incongruence problem, as it still fails to recognize the causes of divergent partitions with non-Mendelian inheritances such as LGT, gene duplication or loss, insertions, mobile elements, hybridization and ILS (Huelsenbeck et al., 1996; Planet, 2006). Ultimately, the topological disagreements found by incongruence tests can only be speculated to be caused by any of the reasons aforementioned, whereas the agreement between phylogenomic species trees generated by supertree/supermatrix or even by phylogenetic trees depicting classifications based on barcodes and gene trees are usually taken as evidence of a conventional inheritance-driven evolutionary history.

3.2. Supermatrix

Perhaps the most widely adopted principle in phylogenomics, postulates that total evidence derived from datasets, that is, all available independent characters, should be combined and analyzed using Parsimony (Kluge, 1989). One practical illustration of how this principle is applied to phylogenomics is the concatenation of gene samples into a supermatrix in order to infer what should be their shared phylogenies. Traditionally a phyletic method intended to improve phylogenetic estimations from those harbored by Supertrees by including all possible characters in the analysis, the Supermatrix, or Concatenation approach is, however, still naive to the possibility of heterotachy and implicitly assumes that all characters have undergone similar branching patterns along the tree (de Queiroz and Gatesy, 2007).

Particularly, Unpartitioned ML in concatenation analyses have been inconsistent under the Multi-Species Coalescent model (MSC) using symmetric models of DNA evolution, such as Jukes-Cantor (J&C) (Roch and Steel, 2015). Thus, much has been discussed on the differences of performance of probabilistic algorithms in comparison to previous methods, especially those who use the Minimum Evolution principle. This principle assumes that evolution is driven randomly in populations, irrespectively of the microevolutionary events which must be accounted for in incongruence studies, thus representing the exception rather than the norm (See section 1.1 for an in depth discussion of such mechanisms in Fungi; Kidd and Sgaramella-Zonta, 1971; Rzhetsky and Nei, 1993). Clearly, parametric phylogenetic estimates will be equal or very similar to those inferred by MP provided that either the assumed *priori* probability of evolutionary change is minimal or the number of loci is large enough. In other words, concatenation analyses using unpartitioned data in ML will converge to MP, which, in turn, is not sensible to neither taxonomic heterogeneity nor heterotachy, hence not accommodating MSC. Furthermore, in homologous gene trees, it tends to estimate topologies in which character synapomorphy is accepted more often than not over homoplasy, as those are obviously the most parsimonious genealogical hypotheses (Farris, 1983).

As it happens, such generalizations seldom correspond to a real distribution of evolutionary rates and hypotheses and may, thus, underestimate the capacity of genotypic and, subsequently, phenotypic plasticity of the organisms analyzed. Specifically in fungi, for example, important genes associated with distinctive phenotypes, such as multicellularity and hyphal/yeast morphotypes, appear to have become dominant independently in different clades by convergent evolution (Kiss et al., 2019; Nagy et al., 2014), where horizontal gene transfer appears to be key, representing cases where Maximum Parsimony methods in general are not optimal. Nevertheless, Kolaczkowski and Thornton (2004) argue that ML and BMCMC can become biased with heterogeneous data, leading to scenarios where MP can even outperform them, which was contested in further study accounting for different proportions of sites affected by heterotachy (Gadagkar and Kumar, 2005).

3.3. Modeling species trees under the coalescent

If instead of applying concepts such as taxonomic congruence or total evidence using traditional models of inheritance in order to handle phylogenetic heterogeneity, rather the history of genomes is modeled as a series of gene duplication, transfer or losses (DTL) and/or allelic population genetics events (represented by ILS), then gene lineages can be correctly inferred and, as a consequence, species relationships will be easily distinguishable (Nagy and Szöllősi, 2017). This allows nodes from gene trees representing ancestral genes to be assigned to extant or extinct species, resulting in reconciliations that explain gene-species tree incongruences by pin-pointing exactly where DTL and ILS have occurred accross divergence events. Algorithms that model DTL using ML of birth-and-death phylogenetic profiles (Csűrös and Miklós, 2009) - where birth events correspond to gene paralogy/orthology and death events to loss; reviewed in Szöllősi et al. (2015) - allow for the generation of gene trees inside a species tree, in a similar fashion to approaches that tackle processes of lineage speciation and extinction (Morlon et al., 2010). Other approaches for modeling DTL include MP reconciliation between incomplete/undated datasets (Jacox et al., 2016) and BMCMC for co-estimation of multiple gene trees embedded in a species tree (Heled and Drummond, 2010; Liu et al., 2009; L. Liu et al., 2008). Alternatively, MSC-aware algorithms have successfully modeled ILS separately using pseudo-ML to draw coalescent units into species trees (Liu et al., 2010). Nowadays, it has been made possible to integrate DTL and ILS (DTLI) in non-binary tree models that may be caused by incongruence (Rasmussen and Kellis, 2012; Stolzer et al., 2012; Vernot et al., 2008). On the other hand, it is also possible to model coalescence with implicit DTL information contained in paralogs, which have been proven to contain phylogenetic signals strong enough to provide accurate topology inference (Hellmuth et al., 2015).

In fungi, these approaches have rendered novel advances into understanding transitions from saprotrophy to ectomycorrhizal relationships by lineage-specific diversification of symbiotic genes and general losses of lignocellulolytic enzymes in Ascomycota, Basidiomycota and Mucoromycota (Miyauchi et al., 2020). Furthermore they made it possible to date the speciation events of plant pathogen *Pucciniales* , correlating it with the divergence times of gymnosperms and angiosperms (Aime et al., 2018). Other reconciliation studies established a common ancestry between pectinases found in *Rozella,* Chytridiomycota, Zoopagoycota, Mucoromycota and Dikarya, giving estimations for their time-divergences and suggesting early marine fungal lineages relied on the pectin degradation from algal sources (Chang et al., 2015). While some of these discoveries might not be revolutionary, it is undeniable that analyses modeling evolutionary processes other than simply vertical inheritance represent powerful tools for fungal systematics, not only by their increased accuracy in recovering relationships (Liu et al., 2009) and, sometimes, divergence times, but also by uncovering DTL events that have generally not been regarded as major players in the evolutionary history of fungi. For example, massive LGT events involving pectin genes in *Trichoderma* from its plant-associated hosts have been found to be responsible for generating a lineage of aggressive mycoparasites with cellulolytic capabilities from non-cellulolytic ancestors (Druzhinina et al., 2018). In fact, a DTL-oriented MSC model in phylogenomic scale of both Fungi and Cyanobacteria revealed that both present similar rates of LGT throughout their evolutionary histories (Szöllősi et al., 2015a).

Concluding remarks

The era of molecular biology has revolutionized the study of fungal systematics not only by revealing an extraordinary cryptic diversity within the kingdom, but also by elucidating mechanisms that lead to this diversification in detail, allowing for studies of the most complex life-cycles these organisms can exhibit. Albeit new sequencing technologies over the last two decades have impacted the amount of genetic information that can be generated, publicly available genome-scale datasets are still scarce for a number of fungal lineages, which may make up for most of the still undiscovered fungal diversity that is estimated to exist. Furthermore, it is unclear how further taxonomy must continue to make efforts in order to reach a satisfactory level of resolution on its tree of life if the estimation methods in phylogenomics don't advance at the same pace as our capability of generating new data. It becomes, therefore, of extreme importance that, in order for the

fungal diversity to be better studied, larger sampling efforts be made in deep-branching fungal clades with a critical choice of evolutionary models accounting for both inheritance-based and unconventional genetic mechanisms.

Acknowledgments

We thank Danny Haelewaters for the kind invitation to write this chapter. JFMS is a doctoral fellow in the program 'International Max Planck Research School: Principles of Microbial Life', supported by funding from the Max Planck Institute for Terrestrial Microbiology, Marburg, Germany. CAQ is supported by the National Science Foundation (grants no. DEB-2018215 and DEB-2127291). JES is a CIFAR Fellow in the program 'Fungal Kingdom: Threats and Opportunities' and was supported by funding from the National Science Foundation (grants no. DEB-1441715 and DEB-1557110).

References

1. Aime, M.C., Bell, C.D., Wilson, A.W., 2018. Deconstructing the evolutionary complexity between rust fungi (Pucciniales) and their plant hosts. Stud. Mycol., Leading women in fungal biology 89, 143–152. https://doi.org/10.1016/j.simyco.2018.02.002https://doi.org/10.1186/s13059-019-1755-7
2. Ahrendt, S. R., Quandt, C. A., Ciobanu, D., Clum, A., Salamov, A., Andreopoulos, B., et al., 2018. Leveraging single-cell genomics to expand the fungal tree of life. Nat. Microbiol. 3, 1417–1428. doi: 10.1038/s41564-018-0261-260
3. Akaike, H., 1998. Information Theory and an Extension of the Maximum Likelihood Principle, in: Parzen, E., Tanabe, K., Kitagawa, G. (Eds.), Selected Papers of Hirotugu Akaike. Springer New York, New York, NY, pp. 199–213. https://doi.org/10.1007/978-1-4612-1694-0_15
4. Bakkeren, G., Jiang, G., Warren, R.L., Butterfield, Y., Shin, H., Chiu, R., Linning, R., Schein, J., Lee, N., Hu, G., Kupfer, D.M., Tang, Y., Roe, B.A., Jones, S., Marra, M., Kronstad, J.W., 2006. Mating factor linkage and genome evolution in basidiomycetous pathogens of cereals. Fungal Genet. Biol. FG B 43, 655–666. https://doi.org/10.1016/j.fgb.2006.04.002
5. Bansal, M.S., Alm, E.J., Kellis, M., 2013. Reconciliation revisited: handling multiple optima when reconciling with duplication, transfer, and loss. J. Comput. Biol. J. Comput. Mol. Cell Biol. 20, 738–754. https://doi.org/10.1089/cmb.2013.0073
6. Bass, D., Czech, L., Williams, B.A.P., Berney, C., Dunthorn, M., Mahé, F., Torruella, G., Stentiford, G.D., Williams, T.A., 2018. Clarifying the Relationships between Microsporidia and Cryptomycota. J. Eukaryot. Microbiol. 65, 773–782. https://doi.org/10.1111/jeu.12519
7. Bauer, R., Garnica, S., Oberwinkler, F., Riess, K., Weiss, M., Begerow, D., 2015. Entorrhizomycota: A New Fungal Phylum Reveals New Perspectives on the Evolution of Fungi. PloS One 10, e0128183. https://doi.org/10.1371/journal.pone.0128183
8. Begum, N., Qin, C., Ahanger, M.A., Raza, S., Khan, M.I., Ashraf, M., Ahmed, N., Zhang, L., 2019. Role of Arbuscular Mycorrhizal Fungi in Plant Growth Regulation: Implications in Abiotic Stress Tolerance. Front. Plant Sci. 0. https://doi.org/10.3389/fpls.2019.01068
9. Berka, R.M., Grigoriev, I.V., Otillar, R., Salamov, A., Grimwood, J., Reid, I., Ishmael, N., John, T., Darmond, C., Moisan, M.-C., Henrissat, B., Coutinho, P.M., Lombard, V., Natvig, D.O., Lindquist, E., Schmutz, J., Lucas, S., Harris, P., Powlowski, J., Bellemare, A., Taylor, D., Butler, G., de Vries, R.P., Allijn, I.E., van den Brink, J., Ushinsky, S., Storms, R., Powell, A.J., Paulsen, I.T., Elbourne, L.D.H., Baker, S.E., Magnuson, J., LaBoissiere, S., Clutterbuck, A.J., Martinez, D., Wogulis, M., de Leon, A.L., Rey, M.W., Tsang, A., 2011. Comparative genomic analysis of the thermophilic biomass-degrading fungi Myceliophthora thermophila and Thielavia terrestris. Nat. Biotechnol. 29, 922–927. https://doi.org/10.1038/nbt.1976
10. Berkeley, M.J., 1874. Notices of North American Fungi. Grevillea 3, 1–17.

11. Blackwell, M., Hibbett, D.S., Taylor, J.W., Spatafora, J.W., 2006. Research Coordination Networks: a phylogeny for kingdom Fungi (Deep Hypha). Mycologia 98, 829–837. https://doi.org/10.1080/15572536.2006.11832613
12. Boddy, L., 2016. Chapter 9 - Interactions with Humans and Other Animals, in: Watkinson, S.C., Boddy, L., Money, N.P. (Eds.), The Fungi (Third Edition). Academic Press, Boston, pp. 293–336. https://doi.org/10.1016/B978-0-12-382034-1.00009-8
13. Cam, L.L., 1990. Maximum Likelihood: An Introduction. Int. Stat. Rev. Rev. Int. Stat. 58, 153. https://doi.org/10.2307/1403464
14. Capella-Gutiérrez, S., Marcet-Houben, M., Gabaldón, T., 2012. Phylogenomics supports microsporidia as the earliest diverging clade of sequenced fungi. BMC Biol. 10, 47. https://doi.org/10.1186/1741-7007-10-47
15. Chang, Y., Desirò, A., Na, H., Sandor, L., Lipzen, A., Clum, A., Barry, K., Grigoriev, I.V., Martin, F.M., Stajich, J.E., Smith, M.E., Bonito, G., Spatafora, J.W., 2019. Phylogenomics of Endogonaceae and evolution of mycorrhizas within Mucoromycota. New Phytol. 222, 511–525. https://doi.org/10.1111/nph.15613
16. Chang, Y., Rochon, D., Sekimoto, S., Wang, Y., Chovatia, M., Sandor, L., Salamov, A., Grigoriev, I.V., Stajich, J.E., Spatafora, J.W., 2021. Genome-scale phylogenetic analyses confirm Olpidium as the closest living zoosporic fungus to the non-flagellated, terrestrial fungi. Sci. Rep. 11, 3217. https://doi.org/10.1038/s41598-021-82607-4
17. Chang, Y., Wang, S., Sekimoto, S., Aerts, A.L., Choi, C., Clum, A., LaButti, K.M., Lindquist, E.A., Yee Ngan, C., Ohm, R.A., Salamov, A.A., Grigoriev, I.V., Spatafora, J.W., Berbee, M.L., 2015. Phylogenomic Analyses Indicate that Early Fungi Evolved Digesting Cell Walls of Algal Ancestors of Land Plants. Genome Biol. Evol. 7, 1590–1601. https://doi.org/10.1093/gbe/evv090
18. Choi, J., Kim, S.-H., 2017. A genome Tree of Life for the Fungi kingdom. Proc. Natl. Acad. Sci. 114, 9391–9396. https://doi.org/10.1073/pnas.1711939114
19. Cobb, N.A., 1892. Contributions to an economic knowledge of the Australian rusts (Uredineae). Agric. Gaz. New South Wales 3, 44–48.
20. Cooke, M.C., 1893. British Fungus Flora. Nature 49, 195–196. https://doi.org/10.1038/049195a0
21. Corradi, N., Bonfante, P., 2012. The Arbuscular Mycorrhizal Symbiosis: Origin and Evolution of a Beneficial Plant Infection. PLOS Pathog. 8, e1002600. https://doi.org/10.1371/journal.ppat.1002600
22. Csűrös, M., Miklós, I., 2009. Streamlining and Large Ancestral Genomes in Archaea Inferred with a Phylogenetic Birth-and-Death Model. Mol. Biol. Evol. 26, 2087–2095. https://doi.org/10.1093/molbev/msp123
23. da Silva, T.H., Câmara, P.E.A.S., Pinto, O.H.B., Carvalho-Silva, M., Oliveira, F.S., Convey, P., Rosa, C.A., Rosa, L.H., 2021. Diversity of Fungi Present in Permafrost in the South Shetland Islands, Maritime Antarctic. Microb. Ecol. https://doi.org/10.1007/s00248-021-01735-6
24. Dagan, T., Martin, W., 2006. The tree of one percent. Genome Biol. 7, 118. https://doi.org/10.1186/gb-2006-7-10-118
25. Davis, W.J., Amses, K.R., Benny, G.L., Carter-House, D., Chang, Y., Grigoriev, I., Smith, M.E., Spatafora, J.W., Stajich, J.E. and James, T.Y., 2019. Genome-scale phylogenetics reveals a monophyletic Zoopagales (Zoopagomycota, Fungi). Molecular phylogenetics and evolution, 133, pp.152-163.
26. de Menezes, A.B., Richardson, A.E., Thrall, P.H., 2017. Linking fungal–bacterial co-occurrences to soil ecosystem function. Curr. Opin. Microbiol. 37, 135–141. https://doi.org/10.1016/j.mib.2017.06.006
27. de Queiroz, A., Gatesy, J., 2007. The supermatrix approach to systematics. Trends Ecol. Evol. 22, 34–41. https://doi.org/10.1016/j.tree.2006.10.002
28. Delsuc, F., Brinkmann, H., Philippe, H., 2005. Phylogenomics and the reconstruction of the tree of life. Nat. Rev. Genet. 6, 361–375. https://doi.org/10.1038/nrg1603
29. Druzhinina, I.S., Chenthamara, K., Zhang, J., Atanasova, L., Yang, D., Miao, Y., Rahimi, M.J., Grujic, M., Cai, F., Pourmehdi, S., Salim, K.A., Pretzer, C., Kopchinskiy, A.G., Henrissat, B., Kuo, A., Hundley, H., Wang, M., Aerts, A., Salamov, A., Lipzen, A., LaButti, K., Barry, K., Grigoriev, I.V., Shen, Q., Kubicek, C.P., 2018. Massive lateral transfer of genes encoding plant cell wall-degrading enzymes

to the mycoparasitic fungus Trichoderma from its plant-associated hosts. PLoS Genet. 14, e1007322. https://doi.org/10.1371/journal.pgen.1007322
30. Dutilh, B.E., van Noort, V., van der Heijden, R.T.J.M., Boekhout, T., Snel, B., Huynen, M.A., 2007. Assessment of phylogenomic and orthology approaches for phylogenetic inference. Bioinformatics 23, 815–824. https://doi.org/10.1093/bioinformatics/btm015
31. Edlund, T.D., Li, J., Visvesvara, G.S., Vodkin, M.H., McLaughlin, G.L., Katiyar, S.K., 1996. Phylogenetic Analysis of β-Tubulin Sequences from Amitochondrial Protozoa. Mol. Phylogenet. Evol. 5, 359–367. https://doi.org/10.1006/mpev.1996.0031
32. Farris, J., 1983. The logical basis of phylogenetic analysis.
33. Farris, J.S., Kallersjo, M., Kluge, A.G., Bult, C., 1995. Constructing a Significance Test for Incongruence. Syst. Biol. 44, 570. https://doi.org/10.2307/2413663
34. Fitzpatrick, D.A., Logue, M.E., Stajich, J.E., Butler, G., 2006. A fungal phylogeny based on 42 complete genomes derived from supertree and combined gene analysis. BMC Evol. Biol. 6, 99. https://doi.org/10.1186/1471-2148-6-99
35. Fu, C., Thielhelm, T.P., Heitman, J., 2019. Unisexual reproduction promotes competition for mating partners in the global human fungal pathogen Cryptococcus deneoformans. PLOS Genet. 15, e1008394. https://doi.org/10.1371/journal.pgen.1008394
36. Gabaldón, T., 2020. Patterns and impacts of nonvertical evolution in eukaryotes: a paradigm shift. Ann. N. Y. Acad. Sci. 1476, 78–92. https://doi.org/10.1111/nyas.14471
37. Gadagkar, S.R., Kumar, S., 2005. Maximum Likelihood Outperforms Maximum Parsimony Even When Evolutionary Rates Are Heterotachous. Mol. Biol. Evol. 22, 2139–2141. https://doi.org/10.1093/molbev/msi212
38. Galagan, J.E., Selker, E.U., 2004. RIP: the evolutionary cost of genome defense. Trends Genet. 20, 417–423. https://doi.org/10.1016/j.tig.2004.07.007
39. Galindo, L.J., López-García, P., Torruella, G., Karpov, S., Moreira, D., 2021. Phylogenomics of a new fungal phylum reveals multiple waves of reductive evolution across Holomycota. Nat. Commun. 12, 4973. https://doi.org/10.1038/s41467-021-25308-w
40. Gonçalves, V.N., Vaz, A.B.M., Rosa, C.A., Rosa, L.H., 2012. Diversity and distribution of fungal communities in lakes of Antarctica. FEMS Microbiol. Ecol. 82, 459–471. https://doi.org/10.1111/j.1574-6941.2012.01424.x
41. Heled, J., Drummond, A.J., 2010. Bayesian Inference of Species Trees from Multilocus Data. Mol. Biol. Evol. 27, 570–580. https://doi.org/10.1093/molbev/msp274
42. Hellmuth, H., Wieseke, N., Lechner, M., Lenhof, H.P, Middendorf, M., Stadler, P.F. Phylogenomics with paralogs. Proceedings of the National Academy of Sciences Feb 2015, 112 (7) 2058-2063; DOI: 10.1073/pnas.1412770112
43. Hennings, P., 1896. Contributions to the fungal flora of South America. Hedwigia 35.
44. Hibbett, D.S., Binder, M., Bischoff, J.F., Blackwell, M., Cannon, P.F., Eriksson, O.E., Huhndorf, S., James, T., Kirk, P.M., Lücking, R., Thorsten Lumbsch, H., Lutzoni, F., Matheny, P.B., McLaughlin, D.J., Powell, M.J., Redhead, S., Schoch, C.L., Spatafora, J.W., Stalpers, J.A., Vilgalys, R., Aime, M.C., Aptroot, A., Bauer, R., Begerow, D., Benny, G.L., Castlebury, L.A., Crous, P.W., Dai, Y.-C., Gams, W., Geiser, D.M., Griffith, G.W., Gueidan, C., Hawksworth, D.L., Hestmark, G., Hosaka, K., Humber, R.A., Hyde, K.D., Ironside, J.E., Kõljalg, U., Kurtzman, C.P., Larsson, K.-H., Lichtwardt, R., Longcore, J., Miadlikowska, J., Miller, A., Moncalvo, J.-M., Mozley-Standridge, S., Oberwinkler, F., Parmasto, E., Reeb, V., Rogers, J.D., Roux, C., Ryvarden, L., Sampaio, J.P., Schüssler, A., Sugiyama, J., Thorn, R.G., Tibell, L., Untereiner, W.A., Walker, C., Wang, Z., Weir, A., Weiss, M., White, M.M., Winka, K., Yao, Y.-J., Zhang, N., 2007. A higher-level phylogenetic classification of the Fungi. Mycol. Res. 111, 509–547. https://doi.org/10.1016/j.mycres.2007.03.004
45. Hibbett, D.S., Blackwell, M., James, T.Y., Spatafora, J.W., Taylor, J.W., Vilgalys, R., 2018. Phylogenetic taxon definitions for Fungi, Dikarya, Ascomycota and Basidiomycota. IMA Fungus 9, 291–298. https://doi.org/10.5598/imafungus.2018.09.02.05
46. Hickey, D.A., 1982. Selfish Dna: A Sexually-Transmitted Nuclear Parasite. Genetics 101, 519–531.

47. Horne, A.S., 1925. Fungal diseases of stored Apples. Fungal Dis. Stored Apples.
48. Hu, X., Xiao, G., Zheng, P., Shang, Y., Su, Y., Zhang, X., Liu, X., Zhan, S., Leger, R.J.S., Wang, C., 2014. Trajectory and genomic determinants of fungal-pathogen speciation and host adaptation. Proc. Natl. Acad. Sci. 111, 16796–16801. https://doi.org/10.1073/pnas.1412662111
49. Huelsenbeck, J.P., Bull, J.J., 1996. A Likelihood Ratio Test to Detect Conflicting Phylogenetic Signal. Syst. Biol. 45, 92–98. https://doi.org/10.1093/sysbio/45.1.92
50. Huelsenbeck, J.P., Bull, J.J., Cunningham, C.W., 1996. Combining data in phylogenetic analysis. Trends Ecol. Evol. 11, 152–158. https://doi.org/10.1016/0169-5347(96)10006-9
51. Irelan, J.T., Selker, E.U., 1996. Gene silencing in filamentous fungi: RIP, MIP and quelling. J. Genet. 75, 313–324. https://doi.org/10.1007/BF02966311
52. Jacox, E., Chauve, C., Szöllősi, G.J., Ponty, Y., Scornavacca, C., 2016. ecceTERA: comprehensive gene tree-species tree reconciliation using parsimony. Bioinformatics 32, 2056–2058. https://doi.org/10.1093/bioinformatics/btw105
53. James, T.Y., Kauff, F., Schoch, C.L., Matheny, P.B., Hofstetter, V., Cox, C.J., Celio, G., Gueidan, C., Fraker, E., Miadlikowska, J., Lumbsch, H.T., Rauhut, A., Reeb, V., Arnold, A.E., Amtoft, A., Stajich, J.E., Hosaka, K., Sung, G.-H., Johnson, D., O'Rourke, B., Crockett, M., Binder, M., Curtis, J.M., Slot, J.C., Wang, Z., Wilson, A.W., Schüßler, A., Longcore, J.E., O'Donnell, K., Mozley-Standridge, S., Porter, D., Letcher, P.M., Powell, M.J., Taylor, J.W., White, M.M., Griffith, G.W., Davies, D.R., Humber, R.A., Morton, J.B., Sugiyama, J., Rossman, A.Y., Rogers, J.D., Pfister, D.H., Hewitt, D., Hansen, K., Hambleton, S., Shoemaker, R.A., Kohlmeyer, J., Volkmann-Kohlmeyer, B., Spotts, R.A., Serdani, M., Crous, P.W., Hughes, K.W., Matsuura, K., Langer, E., Langer, G., Untereiner, W.A., Lücking, R., Büdel, B., Geiser, D.M., Aptroot, A., Diederich, P., Schmitt, I., Schultz, M., Yahr, R., Hibbett, D.S., Lutzoni, F., McLaughlin, D.J., Spatafora, J.W., Vilgalys, R., 2006a. Reconstructing the early evolution of Fungi using a six-gene phylogeny. Nature 443, 818–822. https://doi.org/10.1038/nature05110
54. James, T.Y., Letcher, P.M., Longcore, J.E., Mozley-Standridge, S.E., Porter, D., Powell, M.J., Griffith, G.W., Vilgalys, R., 2006b. A molecular phylogeny of the flagellated fungi (Chytridiomycota) and description of a new phylum (Blastocladiomycota). Mycologia 98, 860–871. https://doi.org/10.1080/15572536.2006.11832616
55. James, T.Y., Pelin, A., Bonen, L., Ahrendt, S., Sain, D., Corradi, N., Stajich, J.E., 2013. Shared Signatures of Parasitism and Phylogenomics Unite Cryptomycota and Microsporidia. Curr. Biol. 23, 1548–1553. https://doi.org/10.1016/j.cub.2013.06.057
56. James, T.Y., Stajich, J.E., Hittinger, C.T., Rokas, A., 2020. Toward a Fully Resolved Fungal Tree of Life. Annu. Rev. Microbiol. 74, 291–313. https://doi.org/10.1146/annurev-micro-022020-051835
57. Jeffroy, O., Brinkmann, H., Delsuc, F., Philippe, H., 2006. Phylogenomics: the beginning of incongruence? Trends Genet. 22, 225–231. https://doi.org/10.1016/j.tig.2006.02.003
58. Joneson, S., Stajich, J.E., Shiu, S.-H., Rosenblum, E.B., 2011. Genomic Transition to Pathogenicity in Chytrid Fungi. PLOS Pathog. 7, e1002338. https://doi.org/10.1371/journal.ppat.1002338
59. Kepler, R.M., Chen, Y., Kilcrease, J., Shao, J., Rehner, S.A., 2016. Independent origins of diploidy in Metarhizium. Mycologia 108, 1091–1103. https://doi.org/10.3852/15-270
60. Kidd, F., 1932. Influence of fungal invasion and mechanical injury upon the rate of carbon-dioxide production of Apples. Influ. Fungal Invasion Mech. Inj. Rate Carbon-Dioxide Prod. Apples.
61. Kidd, K.K., Sgaramella-Zonta, L.A., 1971. Phylogenetic analysis: concepts and methods. Am. J. Hum. Genet. 23, 235–252.
62. Kiss, E., Hegedüs, B., Virágh, M., Varga, T., Merényi, Z., Kószó, T., Bálint, B., Prasanna, A.N., Krizsán, K., Kocsubé, S., Riquelme, M., Takeshita, N., Nagy, L.G., 2019. Comparative genomics reveals the origin of fungal hyphae and multicellularity. Nat. Commun. 10, 4080. https://doi.org/10.1038/s41467-019-12085-w
63. Kluge, A.G., 1989. A Concern for Evidence and a Phylogenetic Hypothesis of Relationships Among Epicrates (Boidae, Serpentes). Syst. Zool. 38, 7–25. https://doi.org/10.2307/2992432
64. Kolaczkowski, B., Thornton, J.W., 2004. Performance of maximum parsimony and likelihood phylogenetics when evolution is heterogeneous. Nature 431, 980–984. https://doi.org/10.1038/nature02917

65. Kuramae, E.E., Robert, V., Snel, B., WeiÃ, M., Boekhout, T., 2006. Phylogenomics reveal a robust fungal tree of life. FEMS Yeast Res. 6, 1213–1220. https://doi.org/10.1111/j.1567-1364.2006.00119.x
66. Lax, C., Tahiri, G., Patiño-Medina, J.A., Cánovas-Márquez, J.T., Pérez-Ruiz, J.A., Osorio-Concepción, M., Navarro, E., Calo, S., 2020. The Evolutionary Significance of RNAi in the Fungal Kingdom. Int. J. Mol. Sci. 21, 9348. https://doi.org/10.3390/ijms21249348
67. Leigh, J.W., Lapointe, F.-J., Lopez, P., Bapteste, E., 2011. Evaluating Phylogenetic Congruence in the Post-Genomic Era. Genome Biol. Evol. 3, 571–587. https://doi.org/10.1093/gbe/evr050
68. Letcher, P.M., Powell, M.J., 2019. A taxonomic summary of Aphelidiaceae. IMA Fungus 10, 4. https://doi.org/10.1186/s43008-019-0005-7
69. Li, Y., Steenwyk, J.L., Chang, Y., Wang, Y., James, T.Y., Stajich, J.E., Spatafora, J.W., Groenewald, M., Dunn, C.W., Hittinger, C.T., Shen, X.-X., Rokas, A., 2021. A genome-scale phylogeny of the kingdom Fungi. Curr. Biol. 31, 1653-1665.e5. https://doi.org/10.1016/j.cub.2021.01.074
70. Liu, L., Pearl, D.K., Brumfield, R.T., Edwards, S.V., 2008. Estimating Species Trees Using Multiple-Allele Dna Sequence Data. Evolution 62, 2080–2091. https://doi.org/10.1111/j.1558-5646.2008.00414.x
71. Liu, L., Yu, L., Edwards, S.V., 2010. A maximum pseudo-likelihood approach for estimating species trees under the coalescent model. BMC Evol. Biol. 10, 302. https://doi.org/10.1186/1471-2148-10-302
72. Liu, L., Yu, L., Kubatko, L., Pearl, D.K., Edwards, S.V., 2009. Coalescent methods for estimating phylogenetic trees. Mol. Phylogenet. Evol. 53, 320–328. https://doi.org/10.1016/j.ympev.2009.05.033
73. Liu, Y., Leigh, J.W., Brinkmann, H., Cushion, M.T., Rodriguez-Ezpeleta, N., Philippe, H., Lang, B.F., 2008. Phylogenomic Analyses Support the Monophyly of Taphrinomycotina, including Schizosaccharomyces Fission Yeasts. Mol. Biol. Evol. 26, 27–34. https://doi.org/10.1093/molbev/msn221
74. Lofgren, L.A., Stajich, J.E., 2021. Fungal biodiversity and conservation mycology in light of new technology, big data, and changing attitudes. Curr. Biol. CB 31, R1312–R1325. https://doi.org/10.1016/j.cub.2021.06.083
75. Maia, T.M., Lopes, S.T., Almeida, J.M.G.C.F., Rosa, L.H., Sampaio, J.P., Gonçalves, P., Coelho, M.A., 2015. Evolution of Mating Systems in Basidiomycetes and the Genetic Architecture Underlying Mating-Type Determination in the Yeast Leucosporidium scottii. Genetics 201, 75–89. https://doi.org/10.1534/genetics.115.177717
76. Malik, A.A., Chowdhury, S., Schlager, V., Oliver, A., Puissant, J., Vazquez, P.G.M., Jehmlich, N., von Bergen, M., Griffiths, R.I., Gleixner, G., 2016. Soil Fungal:Bacterial Ratios Are Linked to Altered Carbon Cycling. Front. Microbiol. 7, 1247. https://doi.org/10.3389/fmicb.2016.01247
77. Mallo, D., Posada, D., 2016. Multilocus inference of species trees and DNA barcoding. Philos. Trans. R. Soc. B Biol. Sci. 371, 20150335. https://doi.org/10.1098/rstb.2015.0335
78. McDonald, M.C., Taranto, A.P., Hill, E., Schwessinger, B., Liu, Z., Simpfendorfer, S., Milgate, A., Solomon, P.S., 2019. Transposon-Mediated Horizontal Transfer of the Host-Specific Virulence Protein ToxA between Three Fungal Wheat Pathogens. mBio 10, e01515-19. https://doi.org/10.1128/mBio.01515-19
79. Meiser, A., Bálint, M., Schmitt, I., 2014. Meta-analysis of deep-sequenced fungal communities indicates limited taxon sharing between studies and the presence of biogeographic patterns. New Phytol. 201, 623–635. https://doi.org/10.1111/nph.12532
80. Merckx, V., Bidartondo, M.I., Hynson, N.A., 2009. Myco-heterotrophy: when fungi host plants. Ann. Bot. 104, 1255–1261. https://doi.org/10.1093/aob/mcp235
81. Mickevich, M.F., 1978. Taxonomic Congruence. Syst. Biol. 27, 143–158. https://doi.org/10.2307/2412969
82. Milgroom, M.G., Jiménez-Gasco, M. del M., Olivares García, C., Drott, M.T., Jiménez-Díaz, R.M., 2014. Recombination between clonal lineages of the asexual fungus Verticillium dahliae detected by genotyping by sequencing. PloS One 9, e106740. https://doi.org/10.1371/journal.pone.0106740
83. Miyauchi, S., Kiss, E., Kuo, A., Drula, E., Kohler, A., Sánchez-García, M., Morin, E., Andreopoulos, B., Barry, K.W., Bonito, G., Buée, M., Carver, A., Chen, C., Cichocki, N., Clum, A., Culley, D., Crous, P.W., Fauchery, L., Girlanda, M., Hayes, R.D., Kéri, Z., LaButti, K., Lipzen, A., Lombard, V., Magnuson, J., Maillard, F., Murat, C., Nolan, M., Ohm, R.A., Pangilinan, J., Pereira, M. de F., Perotto, S., Peter, M., Pfister, S., Riley, R., Sitrit, Y., Stielow, J.B., Szöllősi, G., Žifčáková, L.,

Štursová, M., Spatafora, J.W., Tedersoo, L., Vaario, L.-M., Yamada, A., Yan, M., Wang, P., Xu, J., Bruns, T., Baldrian, P., Vilgalys, R., Dunand, C., Henrissat, B., Grigoriev, I.V., Hibbett, D., Nagy, L.G., Martin, F.M., 2020. Large-scale genome sequencing of mycorrhizal fungi provides insights into the early evolution of symbiotic traits. Nat. Commun. 11, 5125. https://doi.org/10.1038/s41467-020-18795-w

84. Morlon, H., Potts, M.D., Plotkin, J.B., 2010. Inferring the Dynamics of Diversification: A Coalescent Approach. PLOS Biol. 8, e1000493. https://doi.org/10.1371/journal.pbio.1000493
85. Muszewska, A., Steczkiewicz, K., Stepniewska-Dziubinska, M., Ginalski, K., 2019. Transposable elements contribute to fungal genes and impact fungal lifestyle. Sci. Rep. 9, 4307. https://doi.org/10.1038/s41598-019-40965-0
86. Muszewska, A., Steczkiewicz, K., Stepniewska-Dziubinska, M., Ginalski, K., 2017. Cut-and-Paste Transposons in Fungi with Diverse Lifestyles. Genome Biol. Evol. 9, 3463–3477. https://doi.org/10.1093/gbe/evx261
87. Smith, S., Read, D., 2008. Mycorrhizal Symbiosis - 3rd Edition. URL https://www.elsevier.com/books/mycorrhizal-symbiosis/smith/978-0-12-370526-6.
88. Nagy, L.G., Ohm, R.A., Kovács, G.M., Floudas, D., Riley, R., Gácser, A., Sipiczki, M., Davis, J.M., Doty, S.L., de Hoog, G.S., Lang, B.F., Spatafora, J.W., Martin, F.M., Grigoriev, I.V., Hibbett, D.S., 2014. Latent homology and convergent regulatory evolution underlies the repeated emergence of yeasts. Nat. Commun. 5, 1–8. https://doi.org/10.1038/ncomms5471
89. Nagy, L.G., Szöllősi, G., 2017. Chapter Two - Fungal Phylogeny in the Age of Genomics: Insights Into Phylogenetic Inference From Genome-Scale Datasets, in: Townsend, J.P., Wang, Z. (Eds.), Advances in Genetics, Fungal Phylogenetics and Phylogenomics. Academic Press, pp. 49–72. https://doi.org/10.1016/bs.adgen.2017.09.008
90. Nguyen, T. A., Cissé, O. H., Wong, J. Y., Zheng, P., Hewitt, D., Nowrousian, M., et al. (2017). Innovation and constraint leading to complex multicellularity in the Ascomycota. Nat. Commun. 8:14444. doi: 10.1038/ncomms14444
91. Ni, M., Feretzaki, M., Li, W., Floyd-Averette, A., Mieczkowski, P., Dietrich, F.S., Heitman, J., 2013. Unisexual and Heterosexual Meiotic Reproduction Generate Aneuploidy and Phenotypic Diversity De Novo in the Yeast Cryptococcus neoformans. PLOS Biol. 11, e1001653. https://doi.org/10.1371/journal.pbio.1001653
92. Nielsen, K.N., Salgado, J.F.M., Natsopoulou, M.E., Kristensen, T., Stajich, J.E., De Fine Licht, H.H., 2021. Diploidy within a haploid genus of entomopathogenic fungi. Genome Biol. Evol. https://doi.org/10.1093/gbe/evab158
93. Nilsson, R.H., Larsson, K.-H., Taylor, A.F.S., Bengtsson-Palme, J., Jeppesen, T.S., Schigel, D., Kennedy, P., Picard, K., Glöckner, F.O., Tedersoo, L., Saar, I., Kõljalg, U., Abarenkov, K., 2019. The UNITE database for molecular identification of fungi: handling dark taxa and parallel taxonomic classifications. Nucleic Acids Res. 47, D259–D264. https://doi.org/10.1093/nar/gky1022
94. Park, E., Poulin, R., 2021. Revisiting the phylogeny of microsporidia. Int. J. Parasitol. https://doi.org/10.1016/j.ijpara.2021.02.005
95. Peidro-Guzmán, H., Pérez-Llano, Y., González-Abradelo, D., Fernández-López, M.G., Dávila-Ramos, S., Aranda, E., Hernández, D.R.O., García, A.O., Lira-Ruan, V., Pliego, O.R., Santana, M.A., Schnabel, D., Jiménez-Gómez, I., Mouriño-Pérez, R.R., Aréchiga-Carvajal, E.T., Sánchez-Carbente, M. del R., Folch-Mallol, J.L., Sánchez-Reyes, A., Vaidyanathan, V.K., Cabana, H., Gunde-Cimerman, N., Batista-García, R.A., 2020. Transcriptomic analysis of polyaromatic hydrocarbon degradation by the halophilic fungus Aspergillus sydowii at hypersaline conditions. Environ. Microbiol. https://doi.org/10.1111/1462-2920.15166
96. Phadke, S.S., Feretzaki, M., Heitman, J., 2013. Unisexual Reproduction Enhances Fungal Competitiveness by Promoting Habitat Exploration via Hyphal Growth and Sporulation. Eukaryot. Cell 12, 1155–1159. https://doi.org/10.1128/EC.00147-13
97. Pisani, D., Cotton, J.A., McInerney, J.O., 2007. Supertrees Disentangle the Chimerical Origin of Eukaryotic Genomes. Mol. Biol. Evol. 24, 1752–1760. https://doi.org/10.1093/molbev/msm095

98. Planet, P.J., 2006. Tree disagreement: Measuring and testing incongruence in phylogenies. J. Biomed. Inform., Phylogenetic Inferencing: Beyond Biology 39, 86–102. https://doi.org/10.1016/j.jbi.2005.08.008
99. Prasanna, A.N., Gerber, D., Kijpornyongpan, T., Aime, M.C., Doyle, V.P., Nagy, L.G., 2020. Model Choice, Missing Data, and Taxon Sampling Impact Phylogenomic Inference of Deep Basidiomycota Relationships. Syst. Biol. 69, 17–37. https://doi.org/10.1093/sysbio/syz029
100. Quandt, C.A., Beaudet, D., Corsaro, D., Walochnik, J., Michel, R., Corradi, N., James, T.Y., 2017. The genome of an intranuclear parasite, Paramicrosporidium saccamoebae, reveals alternative adaptations to obligate intracellular parasitism. eLife 6, e29594. https://doi.org/10.7554/eLife.29594
101. Quandt, C. A., Kohler, A., Hesse, C. N., Sharpton, T. J., Martin, F., and Spatafora, J. W., 2015. Metagenome sequence of Elaphomyces granulatus from sporocarp tissue reveals Ascomycota ectomycorrhizal fingerprints of genome expansion and a Proteobacteria-rich microbiome. Environ. Microbiol. 17, 2952–2968. doi: 10.1111/1462-2920.12840
102. Rasmussen, M.D., Kellis, M., 2012. Unified modeling of gene duplication, loss, and coalescence using a locus tree. Genome Res. 22, 755–765. https://doi.org/10.1101/gr.123901.111
103. Redman, R.S., Dunigan, D.D., Rodriguez, R.J., 2001. Fungal symbiosis from mutualism to parasitism: who controls the outcome, host or invader? New Phytol. 151, 705–716. https://doi.org/10.1046/j.0028-646x.2001.00210.x
104. Robbertse, B., Reeves, J.B., Schoch, C.L., Spatafora, J.W., 2006. A phylogenomic analysis of the Ascomycota. Fungal Genet. Biol. 43, 715–725. https://doi.org/10.1016/j.fgb.2006.05.001
105. Roch, S., Steel, M., 2015. Likelihood-based tree reconstruction on a concatenation of aligned sequence data sets can be statistically inconsistent. Theor. Popul. Biol. 100C, 56–62. https://doi.org/10.1016/j.tpb.2014.12.005
106. Rosenberg, N.A., Nordborg, M., 2002. Genealogical trees, coalescent theory and the analysis of genetic polymorphisms. Nat. Rev. Genet. 3, 380–390. https://doi.org/10.1038/nrg795
107. Roskov Y., Ower G., Orrell T., Nicolson D., Bailly N., Kirk P.M., Bourgoin T., DeWalt R.E., Decock W., Nieukerken E. van, Zarucchi J., Penev L., eds. (2019). Species 2000 & ITIS Catalogue of Life, 2019 Annual Checklist. Digital resource at www.catalogueoflife.org/annual-checklist/2019. Species 2000: Naturalis, Leiden, the Netherlands. ISSN 2405-884X.
108. Ryakhovsky, N., 1931. Key to the fungal diseases of Anise and Coriander. Plant Prot. 8.
109. Rzhetsky, A., Nei, M., 1993. Theoretical foundation of the minimum-evolution method of phylogenetic inference. Mol. Biol. Evol. 10, 1073–1095. https://doi.org/10.1093/oxfordjournals.molbev.a040056
110. Schoch, C.L., Sung, G.-H., López-Giráldez, F., Townsend, J.P., Miadlikowska, J., Hofstetter, V., Robbertse, B., Matheny, P.B., Kauff, F., Wang, Z., Gueidan, C., Andrie, R.M., Trippe, K., Ciufetti, L.M., Wynns, A., Fraker, E., Hodkinson, B.P., Bonito, G., Groenewald, J.Z., Arzanlou, M., Sybren de Hoog, G., Crous, P.W., Hewitt, D., Pfister, D.H., Peterson, K., Gryzenhout, M., Wingfield, M.J., Aptroot, A., Suh, S.-O., Blackwell, M., Hillis, D.M., Griffith, G.W., Castlebury, L.A., Rossman, A.Y., Lumbsch, H.T., Lücking, R., Büdel, B., Rauhut, A., Diederich, P., Ertz, D., Geiser, D.M., Hosaka, K., Inderbitzin, P., Kohlmeyer, J., Volkmann-Kohlmeyer, B., Mostert, L., O'Donnell, K., Sipman, H., Rogers, J.D., Shoemaker, R.A., Sugiyama, J., Summerbell, R.C., Untereiner, W., Johnston, P.R., Stenroos, S., Zuccaro, A., Dyer, P.S., Crittenden, P.D., Cole, M.S., Hansen, K., Trappe, J.M., Yahr, R., Lutzoni, F., Spatafora, J.W., 2009. The Ascomycota Tree of Life: A Phylum-wide Phylogeny Clarifies the Origin and Evolution of Fundamental Reproductive and Ecological Traits. Syst. Biol. 58, 224–239. https://doi.org/10.1093/sysbio/syp020
111. Schoustra, S.E., Debets, A.J.M., Slakhorst, M., Hoekstra, R.F., 2007. Mitotic Recombination Accelerates Adaptation in the Fungus Aspergillus nidulans. PLOS Genet. 3, e68. https://doi.org/10.1371/journal.pgen.0030068
112. Shang, Y., Xiao, G., Zheng, P., Cen, K., Zhan, S., Wang, C., 2016. Divergent and Convergent Evolution of Fungal Pathogenicity. Genome Biol. Evol. 8, 1374–1387. https://doi.org/10.1093/gbe/evw082
113. Shiu, P.K.T., Raju, N.B., Zickler, D., Metzenberg, R.L., 2001. Meiotic Silencing by Unpaired DNA. Cell 107, 905–916. https://doi.org/10.1016/S0092-8674(01)00609-2

114. Slot, J.C., Rokas, A., 2011. Horizontal Transfer of a Large and Highly Toxic Secondary Metabolic Gene Cluster between Fungi. Curr. Biol. 21, 134–139. https://doi.org/10.1016/j.cub.2010.12.020
115. Spatafora, J.W., Chang, Y., Benny, G.L., Lazarus, K., Smith, M.E., Berbee, M.L., Bonito, G., Corradi, N., Grigoriev, I., Gryganskyi, A., James, T.Y., O'Donnell, K., Roberson, R.W., Taylor, T.N., Uehling, J., Vilgalys, R., White, M.M., Stajich, J.E., 2017. A phylum-level phylogenetic classification of zygomycete fungi based on genome-scale data. Mycologia. https://doi.org/10.3852/16-042
116. Stolzer, M., Lai, H., Xu, M., Sathaye, D., Vernot, B., Durand, D., 2012. Inferring duplications, losses, transfers and incomplete lineage sorting with nonbinary species trees. Bioinformatics 28, i409–i415. https://doi.org/10.1093/bioinformatics/bts386
117. Szöllősi, G.J., Davín, A.A., Tannier, E., Daubin, V., Boussau, B., 2015a. Genome-scale phylogenetic analysis finds extensive gene transfer among fungi. Philos. Trans. R. Soc. B Biol. Sci. 370, 20140335. https://doi.org/10.1098/rstb.2014.0335
118. Szöllősi, G.J., Tannier, E., Daubin, V., Boussau, B., 2015b. The Inference of Gene Trees with Species Trees. Syst. Biol. 64, e42–e62. https://doi.org/10.1093/sysbio/syu048
119. Taylor, J.W., Hann-Soden, C., Branco, S., Sylvain, I., Ellison, C.E., 2015. Clonal reproduction in fungi. Proc. Natl. Acad. Sci. 112, 8901–8908. https://doi.org/10.1073/pnas.1503159112
120. Tedersoo, L., Bahram, M., Puusepp, R., Nilsson, R.H., James, T.Y., 2017. Novel soil-inhabiting clades fill gaps in the fungal tree of life. Microbiome 5, 42. https://doi.org/10.1186/s40168-017-0259-5
121. Tedersoo, L., Sánchez-Ramírez, S., Kõljalg, U., Bahram, M., Döring, M., Schigel, D., May, T., Ryberg, M., Abarenkov, K., 2018. High-level classification of the Fungi and a tool for evolutionary ecological analyses. Fungal Divers. 90, 135–159. https://doi.org/10.1007/s13225-018-0401-0
122. Tekpinar, A.D., Kalmer, A., 2019. Utility of various molecular markers in fungal identification and phylogeny. Nova Hedwig. 109, 187–224. https://doi.org/10.1127/nova_hedwigia/2019/0528
123. Thomma, B.P.H.J., 2003. Alternaria spp.: from general saprophyte to specific parasite. Mol. Plant Pathol. 4, 225–236. https://doi.org/10.1046/j.1364-3703.2003.00173.x
124. Tsuji, M., Tsujimoto, M., Imura, S., 2017. Cystobasidium tubakii and Cystobasidium ongulense, new basidiomycetous yeast species isolated from East Ongul Island, East Antarctica. Mycoscience 58, 103–110. https://doi.org/10.1016/j.myc.2016.11.002
125. van der Does, H.C., Rep, M., 2012. Horizontal Transfer of Supernumerary Chromosomes in Fungi, in: Bolton, M.D., Thomma, B.P.H.J. (Eds.), Plant Fungal Pathogens: Methods and Protocols, Methods in Molecular Biology. Humana Press, Totowa, NJ, pp. 427–437. https://doi.org/10.1007/978-1-61779-501-5_26
126. Vernot, B., Stolzer, M., Goldman, A., Durand, D., 2008. Reconciliation with Non-Binary Species Trees. J. Comput. Biol. 15, 981–1006. https://doi.org/10.1089/cmb.2008.0092
127. Vlaardingerbroek, I., Beerens, B., Rose, L., Fokkens, L., Cornelissen, B.J.C., Rep, M., 2016. Exchange of core chromosomes and horizontal transfer of lineage-specific chromosomes in Fusarium oxysporum. Environ. Microbiol. 18, 3702–3713. https://doi.org/10.1111/1462-2920.13281
128. von Haeseler, A., 2012. Do we still need supertrees? BMC Biol. 10, 13. https://doi.org/10.1186/1741-7007-10-13
129. Waddell, P.J., Kishino, H., Ota, R., 2000. Rapid Evaluation of the Phylogenetic Congruence of Sequence Data Using Likelihood Ratio Tests. Mol. Biol. Evol. 17, 1988–1992. https://doi.org/10.1093/oxfordjournals.molbev.a026300
130. Wadi L, Reinke AW (2020) Evolution of microsporidia: An extremely successful group of eukaryotic intracellular parasites. PLOS Pathogens 16(2): e1008276. https://doi.org/10.1371/journal.ppat.1008276
131. Wang, Q., Li, T., Xu, K., Zhang, W., Wang, X., Quan, J., Jin, W., Zhang, M., Fan, G., Wang, M.-B., Shan, W., 2016. The tRNA-Derived Small RNAs Regulate Gene Expression through Triggering Sequence-Specific Degradation of Target Transcripts in the Oomycete Pathogen Phytophthora sojae. Front. Plant Sci. 0. https://doi.org/10.3389/fpls.2016.01938
132. Wehner, J., Antunes, P.M., Powell, J.R., Mazukatow, J., Rillig, M.C., 2010. Plant pathogen protection by arbuscular mycorrhizas: A role for fungal diversity? Pedobiologia 53, 197–201. https://doi.org/10.1016/j.pedobi.2009.10.002

133. Wijayawardene, N.N., Pawłowska, J., Letcher, P.M., Kirk, P.M., Humber, R.A., Schüßler, A., Wrzosek, M., Muszewska, A., Okrasińska, A., Istel, Ł., Gesiorska, A., Mungai, P., Lateef, A.A., Rajeshkumar, K.C., Singh, R.V., Radek, R., Walther, G., Wagner, L., Walker, C., Wijesundara, D.S.A., Papizadeh, M., Dolatabadi, S., Shenoy, B.D., Tokarev, Y.S., Lumyong, S., Hyde, K.D., 2018. Notes for genera: basal clades of Fungi (including Aphelidiomycota, Basidiobolomycota, Blastocladiomycota, Calcarisporiellomycota, Caulochytriomycota, Chytridiomycota, Entomophthoromycota, Glomeromycota, Kickxellomycota, Monoblepharomycota, Mortierellomycota, Mucoromycota, Neocallimastigomycota, Olpidiomycota, Rozellomycota and Zoopagomycota). Fungal Divers. 92, 43–129. https://doi.org/10.1007/s13225-018-0409-5

134. Wilson, A.M., Nest, M.A. van der, Wilken, P.M., Wingfield, M.J., Wingfield, B.D., 2018. Pheromone expression reveals putative mechanism of unisexuality in a saprobic ascomycete fungus. PLOS ONE 13, e0192517. https://doi.org/10.1371/journal.pone.0192517

135. Wilson, A.M., Wilken, P.M., van der Nest, M.A., Wingfield, M.J., Wingfield, B.D., 2019. It's All in the Genes: The Regulatory Pathways of Sexual Reproduction in Filamentous Ascomycetes. Genes 10, 330. https://doi.org/10.3390/genes10050330

136. Zalar, P., de Hoog, G.S., Schroers, H.-J., Crous, P.W., Groenewald, J.Z., Gunde-Cimerman, N., 2007. Phylogeny and ecology of the ubiquitous saprobe Cladosporium sphaerospermum, with descriptions of seven new species from hypersaline environments. Stud. Mycol., The genus and similar dematiaceous hyphomycetes 58, 157–183. https://doi.org/10.3114/sim.2007.58.06

137. Zhao, R.-L., Li, G.-J., Sánchez-Ramírez, S., Stata, M., Yang, Z.-L., Wu, G., Dai, Y.-C., He, S.-H., Cui, B.-K., Zhou, J.-L., Wu, F., He, M.-Q., Moncalvo, J.-M., Hyde, K.D., 2017. A six-gene phylogenetic overview of Basidiomycota and allied phyla with estimated divergence times of higher taxa and a phyloproteomics perspective. Fungal Divers. 84, 43–74. https://doi.org/10.1007/s13225-017-0381-5