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Fungal Diversity – An Overview

Sara Branco

¹*Biodiversity and Climate Research Center*

²*Mountain Research Center (CIMO), ESA – Polytechnic Institute of Braganca*

¹*Germany*

²*Portugal*

1. Introduction

Fungi are cryptic, understudied and hyperdiverse organisms. In this chapter I address the wonders of fungal diversity, including recent advances on the understanding of the evolution of the kingdom *Fungi*, approaches to documenting and interpreting fungal diversity, and current efforts concerning fungal conservation.

Fungi are eukaryotic organisms that cannot produce their own energy and depend on enzymatic processes to break down biopolymers that are then absorbed for nutrition. The kingdom *Fungi* encompasses tremendous biological diversity, with members spanning a wide array of lifestyles, forms, habitats, and sizes. Fungi are sister to animals (fig. 1) and include thousands of lineages, from the mushroom forming fungi, to yeasts, rusts, smuts, molds, and more or less conspicuous critters with interesting morphologies. Fungi complete indispensable ecological roles, most notably decomposition processes, but are also involved in important symbiotic associations and are known to include noteworthy parasites (Alexopoulos, 1996).

Fungi have been known and used by humans for centuries, but mycology (the scientific study of fungi) traces its beginnings to the 18th century, with the development of the microscope (Ainsworth, 1976). While much has been discovered since then, fungi remain today a cryptic and understudied group of organisms. Recent estimates point to 1.5 million fungal species on the planet (Hawksworth, 2001) of which only ~7% have been described (Kirk et al, 2008). Furthermore, fungi assemble in very species-rich communities, making the full documentation of fungal diversity in targeted sites a particularly challenging task. Given the important roles fungi play in the maintenance and functioning of ecosystems, such documentation is often combined with functional perspectives, aimed at understanding the ecology of fungi. Advances in molecular techniques have formed the base for a boost in studies concerning fungal diversity, and the fast development of next-generation sequencing technologies promises further progress towards a more thorough understanding of fungal diversity and function.

Our current limited knowledge of fungal diversity and biology complicates an assessment of the conservation status of fungal species and has hindered the development of conservation tools and efforts. Furthermore, the absence of expedite and adequate methods to document fungal demographics has made it extremely difficult to fit fungi into the efforts to currently established IUCN conservation categories. There have been, however,

recent concerted efforts to bring fungi to conservation debates, such as the newly created Society for the Conservation of Fungi.

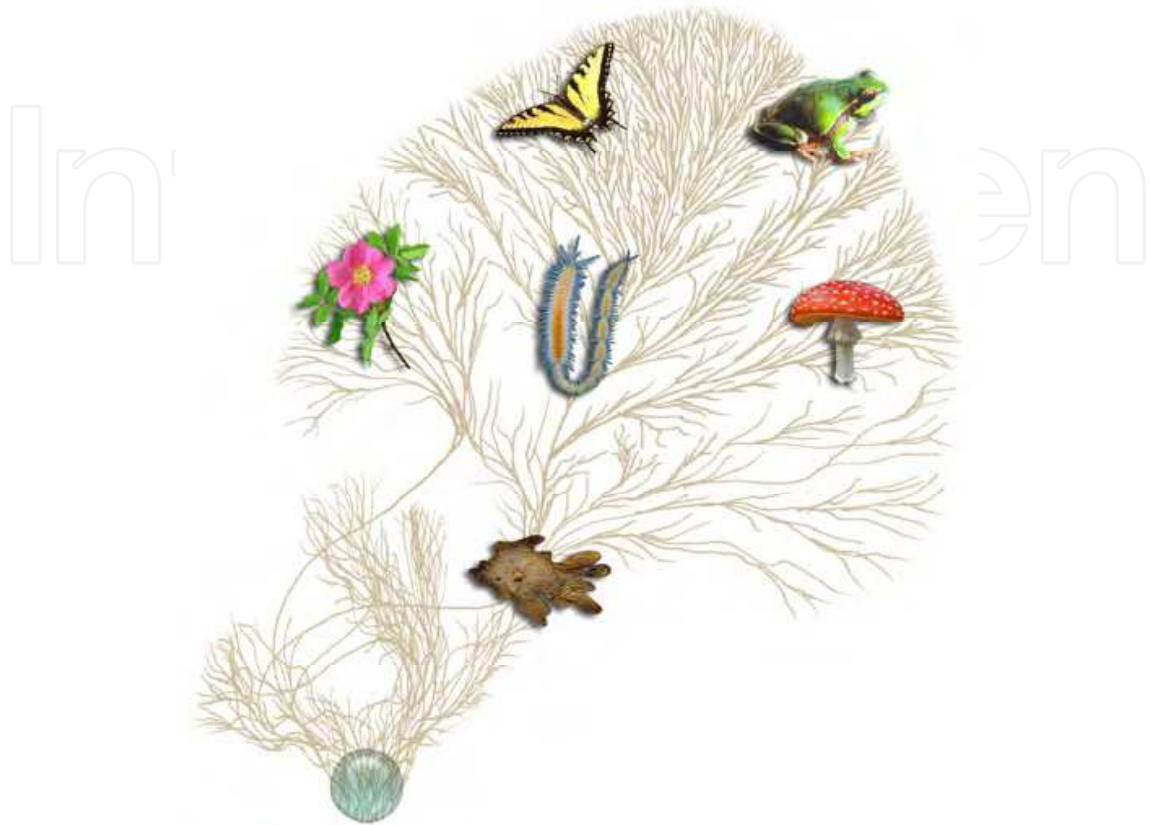


Fig. 1. A simplified tree of life, showing the relationships between three eukaryotic groups: fungi and animals are sister groups, with plants as their next closest relative. Taken from the tree of life web project (<http://tolweb.org/tree/>).

2. Fungi

2.1 The fungal tree of life

Fungi are poorly documented organisms and the phylogenetic relationships within the kingdom are not yet fully understood, but recent efforts have been shedding light on the evolutionary history of the *Fungi* (James et al., 2006, Hibbet et al., 2007). Traditionally, fungi were classified based on morphological, chemical, and anatomical characters mainly associated with spore-bearing structures (McLaughlin et al 2009). However, molecular approaches revealed the existence of repeated trait evolution and thus the prevalence of artificial groupings in these traditional classifications. Molecular data sets have permitted the development of a more natural classification and a better understanding of the fungal relationships.

In the last decades the mycological community has invested heavily in developing the field of fungal systematics. Two National Science Foundation (NSF) projects contributed to this endeavor: the Deep Hypha Research Coordination Network and the Assembling the Fungal Tree of Life project (AFTOL1). This funding allowed for the sharing of information across the mycological community and the generation of molecular data for seven loci from ~1500

species belonging to all groups of fungi (McLaughlin et al 2009). The result was the most recent comprehensive classification of the fungal kingdom to date, based on well-supported monophyletic groups (Hibbet et al 2007, fig. 2). This fungal tree of life includes only true fungi, and does not consider non-fungal groups traditionally studied by mycologists, such as Oomycetes and slime molds. It does, however, include microsporidians (unicellular obligate endoparasitic organisms with highly reduced genomes and mitochondria (Peyretailade et al., 2008)), several lineages of chytrids (flagelatted fungi) and zygomycetes, including the Glomeromycota (obligate symbionts of photoautotrophs that are suggested to have been crucial to the process of land colonization by plants (Pirozynski and Malloch, 1975)).

Around 98% of all described fungal species belong to the subkingdom *Dikarya* composed of *Basidiomycota* and *Ascomycota* (fig. 2). The former includes subphyla *Pucciniomycotina* (rusts, pathogens specialized in infecting plants), *Ustilagomycotina* (true smuts and some yeasts, mostly plant pathogens), and *Agaricomycotina* (including the vast majority of mushroom-forming fungi). *Ascomycota*, is also comprised of three subphyla, *Taphrinomycotina* (yeast-like

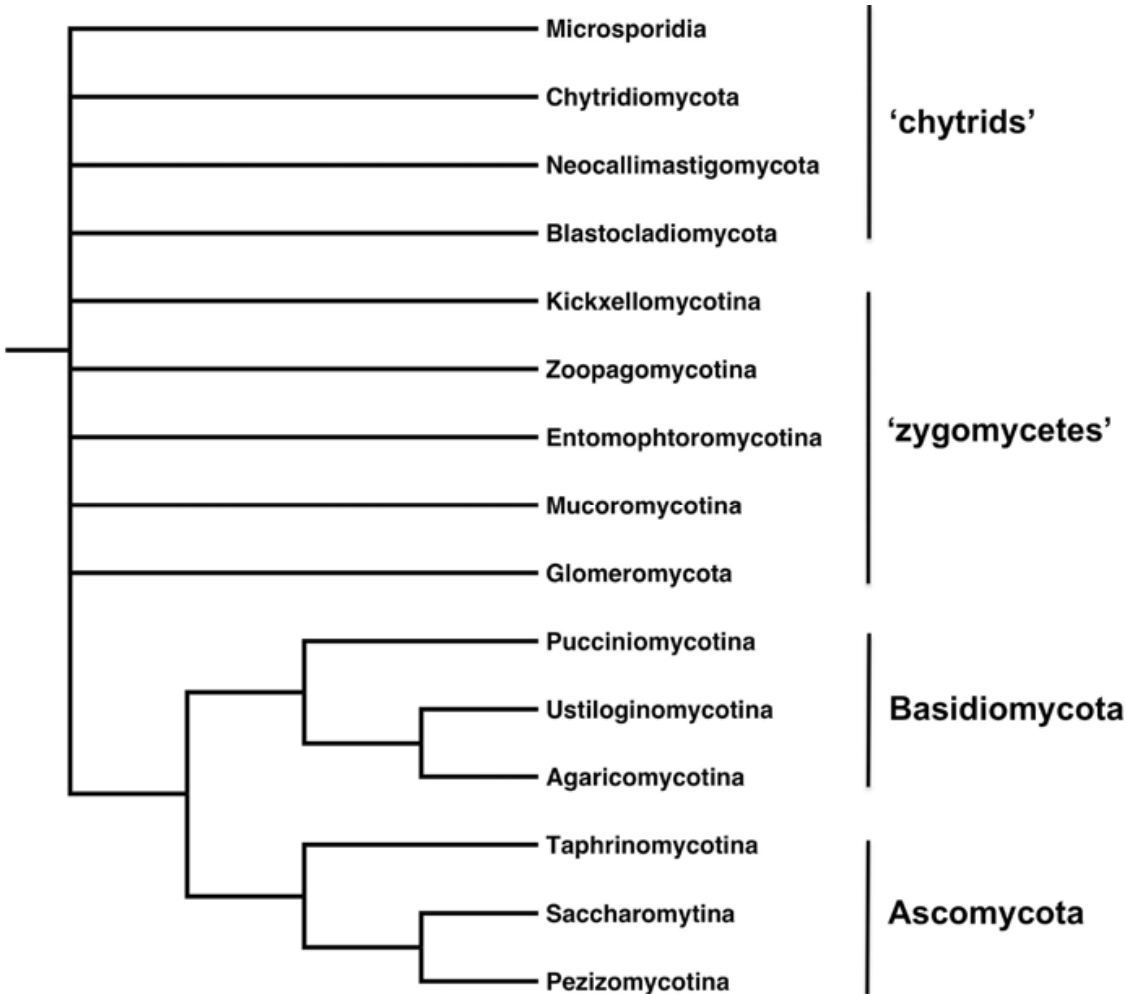


Fig. 2. The fungal tree of life (adapted from Hibbett et al., 2007 and McLaughlin et al., 2009), showing the higher level clades and the unresolved basal polytomy. The terminations - mycota refer to phyla and -mycotina to subphyla; 'chytrids' and 'zygomycetes' are informal non-monophyletic groups.

and some filamentous fungi), *Saccharomycotina* (the true yeasts), and *Pezizomycotina* (with most of the filamentous and fruit-body producing ascomycetes). There has also been extensive work to understand the arrangement taxa within these higher-level clades, a task complicated by the large numbers of fungal taxa described. As evidenced by fig. 2, the base of the tree is a large polytomy, indicating uncertainty on the resolution of the earliest branching events.

The results of these initiatives were a big step forward for mycological research. They provided not only a rigorous overview of the main fungal monophyletic groups, but also a framework for understanding and appreciating the evolution of fungi. Although much has been achieved, accurately reconstructing the fungal tree of life is not an easy task and much research effort must be still gathered in order to resolve the early branching history of this group in order to have a clear view on how different groups of fungi relate to each other. AFTOL2, an NSF funded sequel to AFTOL1, is ongoing and targeting the unresolved issues and hypotheses raised during the first phase of the project. These include resolving the basal fungal lineages, including the placement of *Microsporidia* and *Glomeromycota*, as well as resolving key lineages within the *Ascomycota* and *Basidiomycota* needed for understanding the evolution of fungal morphology and ecology (McLaughlin et al., 2009).

The availability of an accurate fungal tree of life allows for not only an appreciation of fungal diversity and evaluation of the fundamental differences across groups, but also an understanding of the evolutionary histories of different lineages that gave rise to the diversity of fungi we see today. For example, estimates point to the split between the *Ascomycota* and *Basidiomycota* having occurred ~400 million years ago (Taylor and Berbee, 2006), revealing the ancient nature of the fungal phyla. Reconstructing the timing of such evolutionary events can be particularly interesting, allowing for comparisons with diversification patterns in other biological groups and ultimately a more thorough understanding of how life evolves.

2.2 Lessons learned from fungal phylogenetics

The use of phylogenetic approaches to reconstruct the fungal tree of life enabled a much better understanding of the evolution of fungi and made testing hypotheses on trait evolution and diversification across the kingdom possible. Two examples of such approaches are discussed below: exploring the evolution of fruit body morphology and the evolution of fungal symbioses.

2.2.1 The evolution of fruit body morphology

As mentioned above, traditional fungal classifications were based on morphology, anatomy, and biochemistry. For basidiomycete mushroom-forming fungi, the fruit body shape was traditionally one of the most important characters and as such was used for a long time as the central principle of classification. This approach gave rise to groups such as the *Hymenomycetes* (fungi with exposed hymenium, such as agaricoid species with a cap and stem, fig. 3) and the *Gasteromycetes* (fungi with gasteroid fruit bodies, that is, with internal spores and a truffle-like shape, fig. 3) that we know now are not monophyletic. Detailed anatomical investigations lead to some skepticism about these non-natural groupings, but only with the advent of early molecular studies were these suspicions confirmed: morphological dissimilar taxa could actually be very closely related. The gasteroid and agaricoid habit were shown to occur in very closely related genera, such as *Rhizopogon* and *Suillus* (Bruns et al. 1989) and *Hydnangium* and *Laccaria* (Mueller & Pine, 1994), implying that



Fig. 3 Examples of agaricoid and gasteroid fruit body morphologies. *Amanita muscaria* (upper left corner), *Armillaria* sp. (upper right corner) and *Macrolepiota* sp. (lower right corner) all agaricoid, showing exposed hymenium and *Astraeus hygrometricus* (lower left corner with internal spores). Courtesy of J. Vicente.

overall fruit body morphology has not been a stable character across fungal evolution. Soon after this discovery came the realization that monophyletic groups contain multiple morphologies and that these morphologies appear scattered across clades (Hibbett and Thorn, 2001), indicating that certain fruit body forms evolved multiple times independently (see Hibbett, 2007 for a review on the topic).

This phenomenon of labile fruit body morphology is not exclusive to the basidiomycetes. Another interesting example comes from a well-preserved fossil ascomycete fruit body. This flask-shaped specimen was named *Paloepyrenomyces devonicus* and classified as a pyrenomycete (*Sordariomycetes*, within the subphylum *Pezizomycotina*; Taylor et al. 1995). However, this fruit body morphology is found in several other groups within the subphylum, making it difficult to rule out the possibility that this fossil belongs to a more basal *Ascomycota* lineage, such as *Taphrinomycotina* (typically members of this clade do not fruit, however some species have open apothecial fruit bodies), or even an earlier extinct

lineage (Taylor & Berbee, 2006). These doubts make the placement of this fossil into the *Ascomycota* phylogeny difficult and impair its use for calibrating phylogenetic trees.

2.2.2 The evolution of fungal symbioses

Some fungi live in symbiotic associations with photosynthetic partners, obtaining carbohydrates from their symbionts and providing water, nutrients, or protection in return. The two most remarkable types of symbioses involving fungi are lichens and mycorrhizal associations. In lichens fungi associate with green algae or cyanobacteria (or both) to form a vegetative structure called thallus. Lichens are remarkably successful, colonizing all kinds of habitats and regions (Nash, 1996). Lutzoni et al. (2000) used a phylogenetic framework to study the evolution of lichenization in the *Ascomycota* (including most of the lichenized fungi) and found that this life-style arose early in the evolutionary history of the phylum and that it has been easier for lineages to lose the ability to be lichenized than it is to become lichens. These results led them to conclude that many non-lichenized *Ascomycota* lineages (including important well-known fungi such as *Penicillium* and *Aspergillus*) descend from lichenized ancestors.

Mycorrhizal associations are symbioses involving fungi and plant roots. The mycorrhizal condition is the natural state for most plants under most ecological conditions. Mycorrhizas (the structure constituted by the root and the fungus) are the main organs of nutrient uptake in land plants (Smith & Read, 2008). The evolution of mycorrhizal associations had a tremendous impact on terrestrial ecosystems and is thought to have facilitated the initial colonization of land by plants (Pirozynski & Malloch, 1975). Ectomycorrhizal (ECM) fungi are one of the major groups of mycorrhizal fungi. They are mainly basidiomycetes and associate with about 30 plant families, including oaks, pines, poplars, and dipterocarps (Smith & Read, 2008). A broad phylogenetic analysis of mycorrhizal and free-living mushroom-forming fungi (*Homobasidiomycetes*, within *Agaricomycotina*) revealed that the ancestor of this group was free-living, and that ectomycorrhizal symbioses were lost and gained a number of times within the clade (Hibbett et al., 2001). This means that ectomycorrhizal fungal symbionts have evolved repeatedly from decomposer precursors and that there have been several reversals to this latter stage, with half of all *Homobasidiomycetes* potentially deriving from ectomycorrhizal ancestors. Such findings suggest that although ECM symbioses are widespread and play relevant ecological roles in nature, they are an evolutionary unstable mutualism.

3. Fungal diversity

3.1 Documenting fungal diversity

Fungi are cryptic and hyperdiverse organisms that assemble in complex and dynamic communities. For the most part, fungi grow as a network of thin filaments on the substrate (soil, wood, insect guts, living plant parts, etc.) making them difficult to detect. Species that produce spore-bearing structures can be easier to discover, although fruiting periods can be short and fructifications ephemeral. Some species can be cultured *in vitro*, however the vast majority are not amenable to culturing, often leaving mycologists with little to work with experimentally.

Traditionally, taxonomists have been responsible for undertaking the task of uncovering new fungi. Morphological, anatomical, and sometimes chemical characters are the basis for the description of fungal species. Interestingly for new species of fungi to be formally

accepted, a Latin diagnosis is still required, as recommended by the International Code of Botanical Nomenclature (McNeil et al., 2006), the code followed by mycologists to name fungi. Describing new species also requires the deposition of voucher specimens in official collections.

3.2 The rise of fungal molecular ecological studies

The last decade witnessed a substantial increase in studies focused on fungal community ecology. Conducting fungal surveys can be a tedious long-term undertaking and for a long time mycologists relied on fruit body occurrence or culturing of fungal isolates to document species occurrence and site-specific fungal diversity. Although such methods can provide important information, they tend to supply incomplete community descriptions for the reasons described in preceding sections.

The development of molecular tools to describe diversity allowed a much more straightforward, practical and rapid approach to the study of cryptic organisms such as fungi. These tools permit unveiling the communities colonizing soil (or other rich and dynamic substrates). Not only do they provide DNA-based information for identifying taxa, they also facilitate testing of ecological hypotheses, contributing for a better understanding of the structure and functioning of ecosystems. The vast majority of recent studies targeting the description of fungal communities are based on sequence data (Taylor, 2008).

In general, these molecular microbial studies target one specific short DNA region and rely on the identification of operational taxonomic units (OTUs): sequence similarity based surrogates for taxa (Sharpton et al., 2011). Although OTUs are difficult to define, they are the foundation for estimates of richness, frequency, abundance, and distributions. Most fungal environmental DNA-based diversity studies make use of the internal transcribed spacer (ITS), a nuclear ribosomal repeat unit composed of three parts, the rapidly evolving ITS1, the very conserved 5.8S, and the moderately rapid ITS2 (Horton & Bruns, 2001, Bridge et al, 2005; fig. 4).

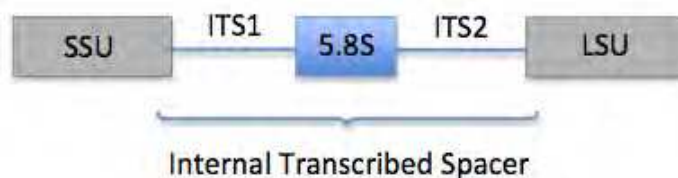


Fig. 4. Structure of the internal transcribed spacer (ITS), the nuclear ribosomal repetitive unit used to describe fungi to the species level. It is composed by the ITS1, 5.8S, and ITS2 regions, and flanked by SSU (ribosomal small subunit) and LSU (ribosomal large subunit).

ITS is used for identifying fungi at the species level. While it is far from being perfect, it offers several advantages that make it a popular that will likely be used for a long time. Genomes include numerous ribosomal DNA encoding genes distributed in tandem arrays along the same or different chromosomes (Rooney & Ward, 2005) and these copies are assumed to be extremely similar (Li, 1997). These coupled with the fact that ITS is easily amplified from low-quality samples (as opposed to single- or low-copy regions) makes it a fast and easy way to describe fungal diversity (Nilsson et al., 2008). However, there are several problems associated with using ITS to define fungal species. On the one hand, there are inherent biases associated with the use of DNA to document diversity, in particular problems with DNA extraction and amplification steps that might lead to distorted

community descriptions (Avis et al., 2009). On the other hand, it is known that there is within species variability in ITS, as the different copies within a genome are not exactly identical. Furthermore, intraspecific variation differs considerably across fungal groups (Karen et al., 1997, O'Donnell & Cigelnik, 1997, Glen et al., 2001, Horton, 2002, Rooney & Ward, 2005, Pawlowska & Taylor, 2004, Avis et al., 2006, Nilsson et al., 2008). These pose challenges in determining meaningful sequence similarity cut-offs (O'Brien et al., 2005). For the most part, OTUs are defined using a 95-97% similarity cut-off with the underlying assumption that resulting units are somewhat equivalent to fungal species. However, different fungal species have been reported to have ITS similarity as high as 99% (Dettman et al., 2001, Johannesson & Stenlid, 2003), while interspecific similarity of 90% or less has been found in other species (Kuniaga et al., 1997, O'Donnell, 2000). Despite these limitations and as mentioned above, ITS is the marker of choice for fungal diversity studies and is likely to remain so in the near future.

3.2.1 Ectomycorrhizal (ECM) fungal diversity

As mentioned above, ECM fungi are one of the major functional groups of mycorrhizal fungi. They associate with plant roots by creating a sheath of fungal tissue enclosing short root tips and a net with inward hyphal growth between plant root cells (called a Hartig net). Such anatomy allows for an extensive surface area of plant-fungal contact where fungi exchange soil nutrients for plant-produced carbohydrates. For the most part, ECM fungi belong to the phylum *Basidiomycota* and associate with about 30 plant families, mainly woody perennials (Smith & Read, 2008). These fungi assemble in hyperdiverse, complex and dynamic communities and play a crucial ecological role in most temperate and some tropical habitats.

Unraveling the diversity of ECM fungi is not trivial. Although fruit body inventories provide valuable information, they by no means offer accurate estimates of ectomycorrhizal fungal diversity. In a pioneer study Gardes & Bruns (1996) surveyed the fungi from pine forests both based on both fruitbody identification and molecular analyses of root samples. They discovered a profound disconnect between the results provided by these different types of data. In fact, the two species producing the majority of fruit bodies were not dominant at the root level, indicating fungal fruiting patterns do not reflect below ground dominances.

Root morphotyping is another approach to study ECM fungal diversity. It has been extensively developed by Agerer (1987-2002) and consists on distinguishing the different fungi based on the morphology and anatomy of ECM root tips. This is a difficult, slow and laborious method that requires extensive training.

As with other areas of mycology, molecular studies have recently revolutionized the study of ECM fungal diversity. In addition to clarifying the discrepancy between above and belowground fungal diversity, molecular surveys also revealed ECM communities as hyperdiverse (particularly when compared to plant host diversity) and composed mostly of rare species (Gehring et al., 1998, Taylor, 2002, Avis et al., 2003, Horton & Bruns, 2005, Walker et al., 2005, Avis et al., 2008, Morris et al., 2008, Branco & Ree, 2010). Figure 5 shows the typical patterns underlying ECM fungal communities: unsaturated species accumulation curves reveal the difficulty in obtaining complete community descriptions and a rank-frequency diagrams illustrate the rarity of most species. These patterns raise interesting questions, particularly from a functional perspective. The most striking question in ectomycorrhizal

ecology has been why are there so many fungal species in a given forest? What are they doing and how do they co-exist? Several explanations have been suggested, such as niche differentiation (Bruns, 1995). These could include vertical niche partitioning, where species have distinct microhabitat preferences that are distributed across a soil vertical gradient (Dickie et al., 2002), or temporal partitioning of ECM fungal communities, where species are active at different times of the year, promoting coexistence by reducing intraspecific competition (Koide et al., 2007). Although the majority of ECM fungal diversity studies are based on root tip data, fungal mycelia also live freely in soil and the community descriptions based on roots and mycelia provide different results (Koide et al., 2005), which adds another layer of complexity to the matter. Host-specificity, where different plant species associate with distinct assemblages of mycorrhizal fungi, has also been suggested as an explanation for the high ECM fungal diversity levels. In general, ECM fungi are known for not having high fidelity to their plant partners and tend to associate with a wide array of plant species. However, there is host preference, which seems to be an important factor in shaping local diversity (Dickie, 2007, Ishida et al., 2007). Inter-specific competition has been another topic of particular interest, given the high numbers of co-existing species. ECM fungi compete for access to the host, more specifically for carbon, as well as soil nutrients, and competition has recently been documented as a major player in ECM community structure (Kennedy, 2010).

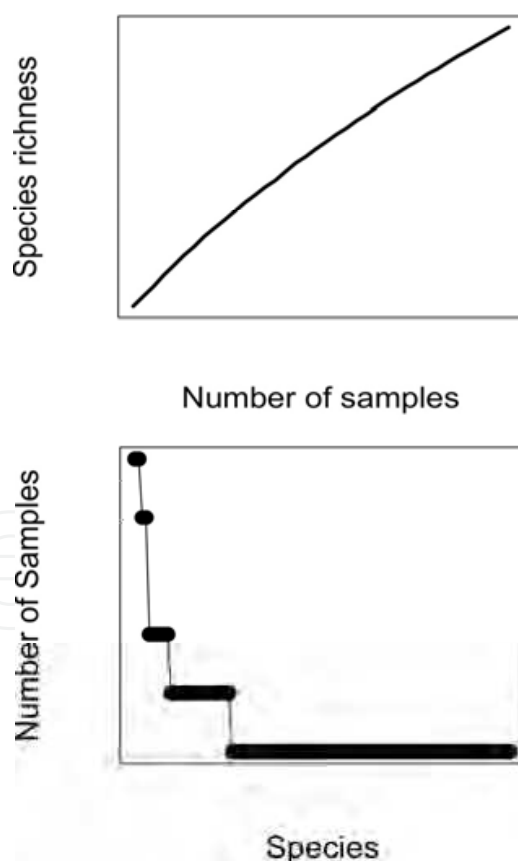


Fig. 5. Typical ECM fungal species accumulation curve (top) and species rank-frequency plot (bottom). (Adapted from Branco & Ree, 2010). As more samples are described, new species are discovered at a consistently rate. This indicates that the vast majority of species in the community are rare (see text for details).

3.3 The genomic revolution

The recent development of massively parallel DNA sequencing platforms, the so called next-generation sequencing (NGS), allowed for the democratization of genomic and metagenomic approaches due to cost reduction and wide availability (Shendure & Ji, 2008). Such technological development has been welcomed by the fungal research community, as it permits rapid studies of deeper scope than have been possible to date. Fungal communities can now be described based on millions of sequences in a very short time frame and at relatively reduced cost. Furthermore, NGS is also enabling an increase in the number of sequenced fungal genomes, providing valuable information crucial for a better understanding of fungal biology and evolution (Brockhurst et al., 2011).

Metagenomic fungal community studies have been based on massively parallel (454) pyrosequencing, a technology able to generate over a million ~500 base-pair sequences in a day (Margulis et al., 2005). 454 pyrosequencing has been preferred to other available technologies precisely because of the long sequence reads it generates, which is crucial for the OTU identification step. 454 has been used to study a wide array of fungal communities, including phyllosphere fungi (Jumpponen & Jones, 2009, 2010), ECM fungi (Jumpponen et al., 2010, Wallender et al., 2010), AMF (Öpik et al., 2009, Lumini et al., 2010), soil fungi (Buée et al., 2009, Rousk et al., 2010), and indoor fungi (Amend et al., 2010). These studies are important contributions representing the first steps in using metagenomics to study fungal diversity. Interestingly, the 454 results published so far confirm the trends of hyperdiversity and rarity described by traditional sequencing methods (Buée et al., 2009, Jumpponen & Jones, 2009, Tedersoo et al., 2010).

As with any new technology, pyrosequencing approaches introduce many biases that are still not completely understood, such as artefactual singletons due to sequencing errors and the formation of chimeric sequences, unintentionally formed during the polymerase chain reaction step (Bellemain et al., 2009, Quince et al., 2010, Tedersoo et al., 2010). Several attempts are being made to overcome these biases, such as the development of tools like a chimera checker (Nilsson et al., 2010) and a method for extracting the variable and informative regions of the NGS generated sequences (Nilsson et al., 2010b). There have also been some discussions on the usefulness of pyrosequencing data for determining fungal abundances, with some studies advising caution when using 454 data to quantify fungal communities (Amend et al., 2010b, Unterseher et al., 2011). Undoubtedly, the technological improvements on high-throughput sequencing coupled with refinement of analytic tools will significantly increase the quality of metagenomic results in the near future, making NGS an even more powerful and informative approach.

The massive amounts of information provided by metagenomic studies are by far the most substantial source of fungal diversity data today. As mentioned above, only a small fraction of the planet's fungal diversity has been documented and it has been suggested that the sequences generated in environmental studies should be the base for describing and naming new fungal species (Hibbett et al., 2011). The authors suggest a protocol to describe fungi based on molecular sequence similarity, but stress that sequence data should be used alone only when no other sources of information are available. Although sequences from environmental sampling offer limitations for taxonomy and phylogenetics (particularly analysis of single markers), they are practical and easy to obtain, accessible through databases, good for automated approaches, and used in phylogenetic studies. Formally naming fungal species from sequence data would imply some radical changes in the procedure for species descriptions (see section 3.1 above), however it would be a very effective way to rapidly accelerate the rate of fungal discovery.

The accessibility of genomics has also enabled the possibility of a dramatic increase in the number of fungal sequenced genomes. Sequencing the genomes of ecologically and taxonomically relevant fungi is and will continue to provide information not only on those specific species, but will also permit the study of genome structure, gene evolution, metabolic and regulatory pathways and life histories (Martin et al., 2011). The sequencing and analysis of fungal genomes is ongoing, mainly through the Fungal Genomics Program (FGP; <http://genome.jgi-psf.org/programs/fungi/about-program.jsf>), launched by the US Department of Energy Joint Genome Institute (JGI). This program will sequence the genomes of many species, including decomposer and mycorrhizal species enabling comparative studies focused on the pathways and mechanisms involved in being a symbiont or a decomposer across the fungal tree of life. The genomes of species from lineages with no genomic information will also be sequenced, allowing further studies on fungal evolution (Martin, 2011).

4. Fungal conservation

Although fungi are cryptic and understudied organisms, there has been increasing concern regarding their conservation. As with many other organisms, fungi are affected by habitat loss, pollution, climate change, and other environmental factors. Overall fungi have no legal protection and the potential decline in fungal diversity, affecting both known and unknown species, has been a major concern among mycologists. The main reason underlying the lack of fungal conservation protocols is the challenge in gathering data on fungal populations and geographic distributions. For the most part, conservation bodies, such as the International Union for the Conservation of Nature (IUCN), rely on data describing distributions, population size and population trends to assign threat categories to species (IUCN Standards and Petitions Subcommittee, 2010). These criteria make it very difficult to apply such categories to fungi.

Nevertheless, there have been efforts to gather fungal checklists and flag species of concern with red lists, particularly among European countries. One of the most relevant initiatives has been the European Council for the Conservation of Fungi (ECCF, currently the conservation group at the European Mycological Association), created in 1985 and aimed at promoting awareness about conservation of fungi, stimulating studies and publications on fungal distributions and fungal red lists, as well as promoting international collaborations towards the compilation of a European red list of threatened fungi (<http://www.wsl.ch/eccf/>). In the early 2000s, ECCF submitted a list of 33 threatened fungi in Europe to be included in the Bern Convention (Dahlberg & Croneborg, 2003). This report referred to rare European macrofungal species and, for the first time, aspired to obtain continental-level legal protection for fungi. This attempt was however unsuccessful, with the Bern Convention rejecting the proposal.

More recently, the International Society for the Conservation of Fungi was established specifically with the goal of protecting fungi worldwide (Minter, 2010, Williams, 2010; <http://www.fungal-conservation.org>). This is the first society devoted exclusively to the conservation of fungi and aims at developing actions on four fronts: infrastructure, science, education, and politics. The political aspect is regarded as a particularly important target, as the society plans to develop and lobby for fungal conservation policies worldwide.

Hopefully the recent genomic and metagenomic developments and all the multitude of new possibilities they open for fungal research, will contribute for the development of specific

protocols to describe fungal populations and distributions that can be the baseline for effective conservation strategies.

5. Conclusion

The last decades brought significant advancements to the understanding and appreciation of the kingdom *Fungi*. We have a much clearer picture of how fungi evolved, assemble and interact with each other and the environment. We also learned, however, how much we still do not know. With all the recent technological advancements, we are better poised to tackle this uncharted frontier than ever before. The use of genomic tools will enable mycology to flourish in the near future, making this a very exciting time to be a mycologist.

6. Acknowledgments

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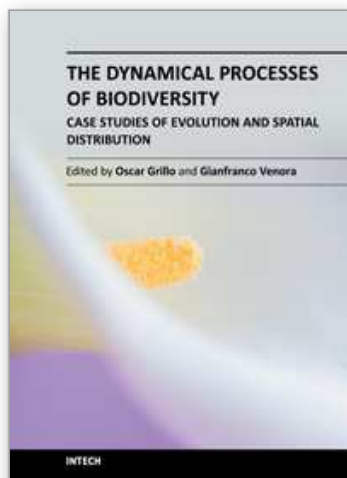
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Phone: +385 (51) 770 447
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中国上海市延安西路65号上海国际贵都大饭店办公楼405单元
Phone: +86-21-62489820
Fax: +86-21-62489821

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