



Commentary

Lives within lives: Hidden fungal biodiversity and the importance of conservation

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ABSTRACT

Nothing is sterile. Insects, plants, and fungi, highly speciose groups of organisms, conceal a vast fungal biodiversity. An approximation of the total number of fungal species on Earth remains an elusive goal, but estimates should include fungal species hidden in associations with other organisms. Some specific roles have been discovered for the fungi hidden within other life forms, including contributions to nutrition, detoxification of foodstuffs, and production of volatile organic compounds. Fungi rely on associates for dispersal to fresh habitats and, under some conditions, provide them with competitive advantages. New methods are available to discover microscopic fungi that previously have been overlooked. In fungal conservation efforts, it is essential not only to discover hidden fungi but also to determine if they are rare or actually endangered.

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1. Introduction

As heterotrophs, fungi are essential to the functioning of terrestrial ecosystems (Taylor and Osborn, 1996; Moore et al., 2011), and they form symbiotic associations with all groups of organisms (de Bary, 1879). For example, almost all plants contain fungal endophytes within their leaves, stems, and roots (Redlin and Carris, 1996; Schulz et al., 2006; Pirttilä and Frank, 2011; Mayerhofer et al., 2013), and fungal endophytes may arm their associates with competitive advantages (Cheplick and Faeth, 2009; White and Torres, 2009; Vega, 2018). Also, all groups of fungi have evolved mycoparasitic life histories independently on many occasions (see below), and 80% of land plant species are presumably associated with mycorrhizal fungi (van der Heijden et al., 2015; Watkinson et al., 2016). Some insects rely on gut microbes to enrich the nutrient content and digestibility of their often-limited diets, and

many fungi rely on insects for dispersal (Buchner, 1953, 1965; Vega and Dowd, 2005; Urubschurov and Jancyk, 2011; Douglas, 2015). Bacteria are increasingly being linked to associations with fungi and other organisms, and they may provide hosts with advantages over fungi because they fix nitrogen; fungi, however, synthesize sterols that are required by some of their associates, something that bacteria cannot do (Gibson and Hunter, 2010; Douglas, 2015; Blackwell, 2017a; 2017b).

The purpose of this commentary is to call attention to the fungal diversity hidden in associations with the highly speciose groups, plants, other fungi and insects (Vega and Blackwell, 2005; Watkinson et al., 2016), and to highlight the need to consider fungal genetic diversity when conservation decisions are made.

Despite multi-trophic level associations of fungi with bacteria and viruses in nature, they are mentioned only occasionally in this review. Because many fungal associates are microscopic, they are difficult to detect, and special effort is required to discover their presence.

2. Fungal diversity

Estimates of microbial diversity on Earth vary greatly, but since the development of methods to survey environmental DNA, the

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anticipated numbers have increased greatly, sometimes by several orders of magnitude. There are estimates of microbial numbers of all organisms on Earth that run as high as one trillion species, including bacteria, Archaea, and microscopic fungi (Locey and Lennon, 2016). Mycologists have been interested in fungal species diversity for several decades (Hawksworth, 1991, 2001; Hawksworth and Rossman, 1997; Blackwell, 2011; Hawksworth and Lücking, 2017) in order to understand better the effect of fungi on Earth and to justify their study (Hibbett et al., 2016). The number of fungi currently described is about 120,000 species (Hawksworth and Lücking, 2017). An estimate of the total number of undescribed fungi is more difficult to assess, and these estimates have varied depending on the methods used to collect and analyze them. Several estimates of total fungal numbers based on DNA data and extrapolation of fungi to plant ratios range from 3.5 to 5.1 (O'Brien et al., 2005) and 6 million species (Taylor et al., 2014). A more recent evaluation by Hawksworth and Lücking (2017) gave a lowered conservative estimate of 2.2–3.8 million species, based in part on new information of numbers of plant and insect species and their potential fungal associates. Based on these estimates, it is clear that “over 90% of fungal diversity on Earth remains to be discovered” (Tripp et al., 2017). See below (2.2. *Hidden fungal biodiversity: Fungi associated with insects*) for a continuation of this topic in connection with numbers of insects.

Studies involving fungal biodiversity and genes responsible for a distinctive biological process should be as important as research focused on functional-trait ecology (Crowther et al., 2014), which tries to elucidate the role of an organism on the community: “Looking at species number alone ... is like listing the parts of a car without saying what they do” (Cernansky, 2017). Not only are there a plethora of fungi to be discovered, but their role in ecological processes remains to be determined. Gradually, information on the contributions of fungi is being discovered. For example, Taylor et al. (2014) tied ecological observations to their accompanying phylogenetic analyses showing a pattern interpreted as niche partitioning in community assembly based on the divergent niches of closely related taxa in the ecologically and taxonomically diverse soil fungi they identified.

2.1. Hidden fungal biodiversity: Fungi associated with plants

Symptomless leaf, stem, and root endophytes are numerous (Petrini, 1991; Hyde and Soyong, 2008). Smith et al. (2008) referred to fungal and bacterial endophytes when they stated, “Each of the nearly 300,000 species of land plant on Earth is likely to host one or more endophyte species.” Based on the large majority of existing fungal endophyte surveys, this is very likely an underestimation of fungal endophyte presence. Several studies have reported on the large fungal biodiversity in plants: 418 morphospecies, equivalent to ca. 347 distinct taxa, in *Heisteria concinna* and *Ouratea lucens* (Arnold et al., 2000); 344 morphotaxa in *Theobroma cacao* (Arnold et al., 2003); 257 unique ITS genotypes in *Coffea arabica* (Vega et al., 2010); 196 species in *Arctostaphylos uva-ursi* (Widler and Müller, 1984); 155 species in *Carpinus caroliniana* (Bills and Polishook, 1991); and 149 species in *Quercus ilex* (Collado et al., 1999).

Surveys of fungal stem and leaf endophytes have resulted in the discovery of new ascomycete species: *Penicillium coffeae* in *C. arabica* (Peterson et al., 2005); *Muscodora yucatanensis* in *Bursaria simaruba* (González et al., 2009); *Mycosphaerella* sp. nov. in *Psychotria horizontalis*, with metabolites that include cercosporins with high potency against the causal agents of malaria, leishmaniasis, and Chagas disease (Moreno et al., 2011); and *Kabatiella bupleuri* in *Bupleurum gibraltarium* (Bills et al., 2012a). In a few cases, fungi in lineages known previously as insect associates and

wood decayers, are also being discovered to have a life as endophytes (Martin et al., 2015; Baral et al., 2018).

Even though many papers have reported the presence of fungal endophytes in roots (Stone et al., 2004; Schulz et al., 2006), these are not as well characterized as other root-associated fungi such as facultative biotrophic ectomycorrhiza or obligate biotrophic arbuscular mycorrhiza. According to Zuccaro et al. (2014), “There is growing evidence that root endophytic associations, which due to their inconspicuous nature have been often overlooked, can be of mutualistic nature and represent important players in natural and managed environments.” Fungal endophytism in the root is therefore an area that should be studied in earnest, not only because it might reveal new fungal species among other hidden fungal diversity, but also because these fungi might play an important role in the biology of the plant.

As was noted above, 80% of land plant species are presumably associated with mycorrhizal fungi. Ectomycorrhizas constitute the most species diverse group with 20,000 species including representatives of 16 orders of Basidiomycota, Ascomycota, and Endogonales (Tedersoo et al., 2010; van der Heijden et al., 2015). Arbuscular mycorrhizas (AM fungi) may be associated with up to 74% of all plant species (200,000 species) but are probably underestimated as numbering fewer than 2000 species (Wang and Qiu, 2006; van der Heijden et al., 2015). Accumulating evidence indicates that AM fungi are more species diverse than previously acknowledged, due in part to species delimitation based on few morphological characters (Bruns and Taylor, 2016; Savary et al., 2018). Other specialized mycorrhizal groups include those associated with ericoids and orchids. DNA studies indicate that the species rich Orchidaceae have rather specific fungal associates (Otero et al., 2002; Taylor et al., 2002).

2.1.1. Examples of interactions among fungi and plants

Numerous potentially useful compounds have been discovered in endophytes and endolichenic fungi and have encouraged searches for new medicines in the pharmaceutical industry. The fungi often play a protective or other interactive role in their hosts, and the associations between endophyte and host can be important in searches for bioactive compounds (Paranagama et al., 2007; Kannangara et al., 2009; Kusari et al., 2012; Card et al., 2016). The identification of *Neotyphodium coenophialum*, the agent of “fescue toxicosis” in cattle consuming infected strains of *Festuca arundinacea* 50 y ago was a landmark in the study of fungal secondary metabolites (Bacon et al., 1977).

Fungi produce secondary metabolites that can have adverse effects on other fungi, mammals, including humans (e.g., mycotoxins), and plants (e.g., phytotoxins). Some of the metabolites have important medicinal properties (e.g., cephalosporins, cyclosporins, penicillins), and others are used in the agrochemical (e.g., fumagillin, gibberellic acid) and cosmetics industry (e.g., kojic acid) (Bills and Gloer, 2016). One of the first examples of drug discovery involving a fungal endophyte was the antifungal drugs produced by various strains of *Cryptosporiopsis* isolated from plant roots (Tscherter and Dreyfuss, 1982). Some endophytes are known to have antiviral activity, e.g., inhibitors of human cytomegalovirus protease and HIV-1 division replication, activity against cancer, tuberculosis, fungi, and malaria; and control of cholesterol levels (Prakash, 2015).

In some cases, the search for potential drugs, including from endophytes, has been guided by traditional uses suspected long ago (Gordien et al., 2010). Not all researchers are convinced that the use of endophytes has paid off for commercial production of pharmaceuticals, although they offer suggestions to promote greater success (Kusari et al., 2014; Card et al., 2016). Newly developed techniques, including use of genomics, could be useful in making

bioprospecting more profitable (Collado et al., 2007; Bills et al., 2008; Chen et al., 2013).

In addition to pharmaceuticals, fungal endophytes offer potential new sources of compounds for agricultural and industrial uses (Strobel and Daisy, 2003; Bills et al., 2012b; Card et al., 2016; Macías-Rubalcava and Sánchez-Fernández, 2016). An analysis examining the effects of several ascomycete root endophytes on plant growth revealed a neutral to negative effect (Mayerhofer et al., 2013). In contrast, other studies have demonstrated a positive effect of endophytic fungal entomopathogens on plant growth, which has led to an interest in these fungi as biofertilizers (Vega et al., 2009; Vega, 2018). Fungal entomopathogens (*Metarhizium* spp. and *Beauveria bassiana*) and 18 fungal endophytes of saffron (*Crocus sativus*), and other plant-associated fungi have been reported to produce indole-3-acetic acid (IAA), an auxin phytohormone (Chanclud and Morel, 2016; Wani et al., 2016; Liao et al., 2017), although it is not known if they have biofertilizer effects in plants. The fungal endophyte *Pestalotiopsis* sp. also produces IAA, and production is significantly enhanced when a bacterium (*Luteibacter* sp.) colonizes the hyphae (Hoffman et al., 2013).

Above ground endophytes of plants can have important roles. A heat tolerant grass (*Dichanthelium lanuginosum*) growing near hot springs in Yellowstone National Park, USA, harbors the fungal endophyte *Curvularia* sp. and plants lacking the endophyte failed to grow at temperatures over 40 °C (Redman et al., 2002). The symbiosis, however, has since been discovered to involve virus-infected fungi, forming a three-way symbiosis (Márquez et al., 2007). Other examples of endophytes that protect their plant hosts from stress include cases of protection from plant pathogenic fungi and insects (Arnold et al., 2003; Cheplick and Faeth, 2009; Vega, 2018), and other plant-associated fungi that produce volatile organic compounds to lure a motile animal to join in an association or perhaps repel it (Johnstone and Bshary, 2002; Douglas, 2009, 2015; Davis et al., 2013; Spiteller, 2015).

2.2. Hidden fungal biodiversity: Fungi associated with other fungi

Mycoparasites are fungi that rely on other fungi for their nutrition at some time during their life history (Jeffries and Young, 1994; Jeffries, 1995; Boddy, 2016). A broader term, fungivorous, includes parasites, as well as other close fungus to fungus associations, such as commensals (Kirk et al., 2008). Mycoparasitism arose independently among all major groups of fungi (Alexopoulos et al., 1996; Boddy, 2016; Spatafora et al., 2016), and a report that three kinds of fungi were associated with arbuscular mycorrhizal fungi from the well-studied Rhynie chert suggests a diverse group of mycoparasites was present 410,000 million years ago (Krings et al., 2017), and many of the earliest diverging fungi were mycoparasites (Berbee et al., 2017). The host relations of several parasitic fungi, *Pyxidiophora* and rust fungi, played an important role in evolutionary thinking about the Laboulbeniales (Blackwell, 1994; Shattock and Preece, 2000).

Mycoparasites and the terminology to describe their parasitic approaches for obtaining nutrition from other fungi were described most recently by Boddy (2016). The conservatively estimated 1100 mycoparasites include necrotrophs that kill a broad range of hosts, and biotrophs that require living hosts, often with a narrower range of obligate coevolved hosts (Kirk et al., 2008; Boddy, 2016). Necrotrophic contact mycoparasites (e.g., *Cladosporium* sp., *Phlebiopsis gigantea*) do not penetrate the host, but interact with volatile or diffusible compounds that cause lysis of at least a part of the host. Invasive mycoparasites (e.g., *Rozella* spp., *Syncephalis* spp. *Nectria inventa*, *Cladosporium uredinicola*, *Trichoderma* spp., *Rhizoctonia solani*) penetrate the host, the host tissues disintegrate, and the host may die. For this reason, mycoparasitic fungi have been of interest for

biological control of other fungi for many years (Heydari and Pessaraki, 2010) and interest continues to increase (Druzhinina et al., 2018).

Three types of biotrophs have been described, each generally with increasing host specificity: (1) fusion (e.g., *Pyxidiophora* sp., *Gonadobotrys simplex*) in which the parasite fuses with the host and microdesmata-like connections are formed for passage of host nutrients to the parasite; (2) haustorial (e.g., *Piptocephalis* spp., *Cryptococcus depauperata*), in which the parasite produces a peg to a branched haustorial structure that usually penetrates the host wall but not the cell membrane; and (3) intracellular (e.g., *Ampelomyces* spp.), in which the entire thallus of the parasite enters the host. Fungal parasitism is a continuum, and depending on the conditions (time, host species, etc.) biotrophs may sometimes convert to necrotrophs. In certain cases, a fungus may be a temporary parasite, perhaps to overcome a competitor, before reverting back to life as a saprotroph (Boddy, 2016).

A specialized group of more than 1800 fungal species parasitize lichens (Lawrey and Diederich, 2003; Grube and Wedin, 2015). The specialized lichenicolous fungi (e.g., Leotiales, Tremellales, Cystobasidiomycetes, *Athelia arachnoidea*) withstand the harsh environment created by the lichens they infect. These fungi are often specialists of the fungal component of the lichen thallus, but some fungi use the algal cells (Grube and Wedin, 2015; Millanes et al., 2016; Hawksworth and Lucking, 2017). Other lichen-associated fungi are asymptomatic and are known as endolichenic fungi (Paranagama et al., 2007; Arnold et al., 2009); some of these produce metabolites with potential use in medicine (Paranagama et al., 2007) or against plant pathogens (Kannangara et al., 2009).

Early diverging fungal lineages include zoosporic fungi (e.g., *Rozella*), obligate endoparasites that “dress up as their hosts” because they insert their entire protoplast into other zoosporic fungi; the invasion is not revealed until their zoosporangia are produced (Held, 1981). Zygosporic fungi provide classic examples of all three kinds of biotrophs as well as necrotrophs. In the past, mycoparasitic mode was used to delimit taxonomic groups, some at order level and this character corresponds with current DNA phylogeny (Spatafora et al., 2016).

A number of genera and species of ascomycetes and basidiomycetes are necrotrophic and biotrophic mycoparasites (Jeffries, 1995; Boddy, 2016). Many mycoparasitic ascomycetes in the order Hypocreales (e.g., *Acremonium* spp., *Escovopsis* spp., *Emmonsia parva*, *Hortaea werneckii*, *Trichoderma* spp., and *Verticillium* spp.) are necrotrophs, some of which have been considered as potential biocontrol agents. Mycoparasitic ascomycetes such as *Verticillium fungicola* and *Mycogone perniciosa* cause disease in mushroom farms, and *Tolyposcladium* (as *Elaphocordyces*) *ophioglossoides* infects species of the false truffle, *Elaphomyces* spp., an ectomycorrhizal fungus, hidden beneath the soil (Sung et al., 2007). In addition to mushroom-forming basidiomycetes, some ascomycetes in Hypocreales (e.g., the necrotroph, *Cladosporium* sp.) parasitize the plant parasite *Exobasidium camelliae* (Mims et al., 2007), and additional species continue to be discovered as rust fungus mycoparasites (Torres et al., 2017). Mycoparasites such as *Ampelomyces quisqualis*, an intracellular biotroph of powdery mildews, have been suggested for biocontrol of other fungi for many years (Yarwood, 1932). Although experimental studies have shown that other fungal antagonists were more effective and faster at containing powdery mildew hosts, *Ampelomyces* had the effect of suppressing sporulation (Kiss et al., 2004).

Necrotrophic mycoparasitic interactions occur when hyphae come into contact, but the parasite does not penetrate the host. These contact parasitic or hyphal interference interactions involve potential biological control agents such as *P. gigantea* and *Cladosporium* spp. In other necrotrophic interactions the host

compartment is penetrated by the parasite before lysis occurs (*Trichoderma harzianum*). Many interactions do not fit the extremes of the mycoparasitic continuum and some may not kill a host but merely reduce its vigor so that it is less competitive (Boddy, 2016).

2.3. Hidden fungal biodiversity: Fungi associated with insects

Insect-fungal associations are quite common, and some of the associations are textbook examples of mutualistic (e.g., ambrosia beetles) or antagonistic associations (e.g., fungal entomopathogens) (Alexopoulos et al., 1996; Vega and Blackwell, 2005; Watkinson et al., 2016). Many new examples of insect-associated fungi are being discovered as new habitats are studied. For example, Suh and Blackwell (2004) identified more than 150 new yeast species in the alimentary canal of mushroom-feeding beetles in a diverse group of families on Barro Colorado Island (Panama) and several sites in the southern USA in fewer than 30 d of collecting, dissecting, and culturing. Vega et al. (2000) and Pérez et al. (2003) isolated over 55 different fungal species from the coffee berry borer (*Hypothenemus hampei*), including a new *Penicillium* species (*Penicillium brocae*; Peterson et al., 2003). Morais et al. (1992) identified 72 fungal species in the alimentary canal and cuticle of various *Drosophila* species, while Gillian et al. (1974a, 1974b) isolated 61 fungal species in the alimentary canal of honeybees (*Apis mellifera*). Zoberi and Grace (1990) identified 20 fungal species on the cuticle of the termite *Reticulitermes flavipes*. Malacrino et al. (2015, 2016) used Sanger sequencing and 454 pyrosequencing to identify about 15 fungal species associated with olive fruit flies (*Bactrocera oleae*), the most important insect pest of olives (*Olea europea*). The gut community of a dragonfly contained 12 fungal genera, some of which were associated with three bacterial endosymbionts, thus revealing further hidden bacterial biodiversity within the fungi (Shao et al., 2015). Harrison et al. (2016) examined fungi associated with egg, larvae, pupae and adults of the butterfly *Lycaeides melissa* and, after using next-generation sequencing, concluded that the insect fungi were also associated with host plant leaves (*Astragalus canadensis*).

Several highly specialized groups of fungi are associated with arthropods. Many species of microsporidia, previously thought to be protozoa, go undetected in the tissues of insects (Cali et al., 2016). Studies of fungal gut associations (e.g., zygospor-producing obligate commensals, Harpellales and Asellariales, and yeasts) have barely scratched the surface (and the gut) of the potential arthropod diversity (Lichtwardt et al., 2001; Blackwell, 2017a). The Laboulbeniomyces, are asymptomatic external ascomycete biotrophs of arthropods (Weir and Blackwell, 2005). Many entomologists and even mycologists have never seen these organisms, although they occur worldwide and comprise thousands of described species with more to be described, especially when cryptic species are discovered in what were considered widely distributed species (Haelewaters et al., 2015). Many of the more than a thousand known ascomycete yeasts (Saccharomycotina) are associated with insects (Kurtzman et al., 2011), especially when dispersal is considered. Xylose-assimilating and xylose-fermenting species in *Schefferosmyces* are usually associated with wood-ingesting beetles, and as mentioned above, fungus-feeding beetles and drosophilids usually contain gut-inhabiting species of *Suhomyces* that sometimes occupy specialized regions of the midgut. Collecting in similar habitats in outlying geographical regions indicates that species discovery has barely begun to reveal yeast biodiversity (Boekhout, 2005; Lachance et al., 2005; Blackwell, 2017a; 2017b).

Estimates of insect species numbers have been reduced over the last 20 years from of 5–10 million species (Ødegaard, 2000) to, more recently, 2.6–7.8 million with a mean of 5.5 million species

(Stork et al., 2015). Estimates (not “guesstimates” as the authors emphasize) are based on several methods including extrapolations from plant to beetle ratios and a new method based on beetle “body size and year of description” to account for their apparency in the landscape (Stork et al., 2015).

Estimates of total numbers of fungal species have varied, and these are somewhat lower than previously suggested (O'Brien et al., 2005; Blackwell, 2011; Taylor et al., 2014). More recent estimates of 2.2–3.8 million species include additional data on which to base extrapolations of plant to fungus ratios, more environmental sequences and recognition of cryptic species; environmental hot spots and under-collected habitats remain poorly known (Hawksworth and Lücking 2017). Based on their newest estimates, Hawksworth and Lücking (2017) suggested that only 3–8% of the Earth's fungi have been named. It still is not clear how many insect-associated fungi are included in the figures, but based on the known associations with insects, the total fungal numbers should increase considerably and approach or even surpass numbers of insects.

3. Conservation of fungal species

The important contribution of fungi is their continuing billion-year plus impact on our environment. Price (1988) recognized eons-old microbial efforts to shape the Earth, and his paraphrase of Haeckel (1899) to describe the process, “ecology recapitulates phylogeny,” should be cited widely. In conservation efforts we need not only to discover hidden fungi but also determine if they are rare or actually endangered. Microfungi are in need of study because they are overlooked in the environment. Microfungi added to local conservation lists (e.g., *Taphrina*) may have been detected because they cause distinctive host symptoms.

3.1. Distinguishing rare fungi from endangered species

Is a fungus rare or is it endangered? The answer requires knowledge of geographical range and degree of substrate and host specificity. High-throughput sequencing offers advantages of speed and lower cost over Sanger sequencing to collect huge amounts of data to answer these essential questions. Environmental genomics (metagenomics) is continually improving in order to distinguish species with targeted amplicon or shotgun sequencing. With the use of molecular and bioinformatics tools along with global collaborations, the “rare or endangered question” can be addressed by evaluating niches and global ranges. Evaluation of communities can also help to estimate species numbers with needed improvements to increase accuracy. Taylor et al. (2016) improved the accuracy of their sequencing methods to overcome past concerns with artifacts. The improvements were validated by use of low diversity “mock communities” of known composition.

Similar methods have been used to track specific taxa under different biotic and abiotic conditions and to assess communities, including fungal-plant interactions, across continents and soil gradients (Peay et al., 2010, 2016). The “staggering volume of data” from ecological sequencing (Hibbett et al., 2016) calls for a solution to all the unnamed sequences by integrating formal classification of environmental sequences and specimen-based sequences. Hibbett et al. (2011) estimated that this measure would speed the estimated 4000 y effort to complete inventorying the remaining fungi. Hibbett et al. (2016) once again called for improvements in the way that fungal systematics is practiced. The question of overwhelming numbers of fungi in databases, nameless and identified only by a barcode sequence (Schoch et al., 2012), was also addressed by acknowledging the need for more named sequences in curated databases such as GenBank and UNITE (Yahr et al., 2016). Yahr et al.

(2016) addressed the growing recognition of widespread fungal endemism and the need to use herbaria to match vouchers and sequences, a suggestion made earlier by Brock et al. (2009), and a disheartening solution with currently available methods.

Certain groups of symbiotic fungi are in special need of attention because species delimitation has been problematic. A combination of too few morphological characters and previous reliance on SSU rDNA of Glomeromycota and Laboulbeniomycetes has resulted in underestimation of species based on accumulating evidence of geographical and host specificity (Haelewaters et al., 2015; Bruns and Taylor, 2016).

3.2. Targeting the endangered species

As discussed above, arguing a place for fungi in the world is easy (Griffith, 2012; Watkinson et al., 2016), but we must speak in a way that rivets listeners. Previously, fungi had a problem of recognition, but phylogenetics has established them as a distinct major life form important for preservation, with high species diversity and intricate, often obligate, interactions with other organisms. The improvement of conservation efforts in some parts of the world has improved as perceptions of fungi have changed (Allen and Lendemer, 2015).

In the last decade, interest in the possibility of fungal loss has increased dramatically, including at regional levels, because of widespread habitat destruction or loss of a specific plant host. European mycologists were among the early proponents of fungal conservation (Scheidegger et al., 1995; Arnolds, 2001; Moore et al., 2001), followed soon after by others. Mycologists became involved on behalf of fungi in the International Union for Conservation of Nature (IUCN) Red List of Threatened Species (Red List). Conservation efforts were marked by the creation of the International Society for Fungal Conservation in 2010, publication of local lists and circulation of the newsletter *Fungal Conservation*. The goals of the newsletter were to provide parents for the “orphans of Rio,” as Minter (2010) called them, and the establishment of The Global Fungal Red List Initiative (<http://iucn.ekoo.se/en/iucn/welcome>) to provide information on the status of fungal biodiversity and to promote conservation. As fungal conservation efforts gained momentum, Mueller (2017) gave an optimistic review of the last decade of progress. The work continues with specific larger-scale projects such as the monitoring of ectomycorrhizas within the European Forest Monitoring Network (Suz et al., 2015). Today many projects use IUCN red-listing criteria for assessment and reporting the status of the fungi (Mueller and Schmit, 2007; Dahlberg and Mueller, 2011; Mueller and Dahlberg, 2013) and suggesting implementation of conservation policy (Suz et al., 2015).

In addition to education and lobbying activities, the Society for Fungal Conservation evaluates action plans of signers of the Rio Convention on Biological Diversity, which came into effect in 1994, and the evaluations by country are published on The Micheli Guide to Fungal Conservation website (<http://www.fungal-conservation.org/micheli.htm>). Most conservation efforts were reported around 2010, but few reports have been added since then. Scores are based on criteria from mere mention of fungi in a conservation context to explicit consideration of fungal conservation measures. Unfortunately, many countries earned the worst score, “totally deficient.”

Since 2009, the Fungi have received more attention from the IUCN Species Survival Commission. A Global Fungal Red List Initiative with five specialist groups evaluates the condition of fungi (Mueller, 2017; <http://iucn.ekoo.se/iucn/plans/>). Of the species categorized for conservation status on the IUCN Fungal Red List, most are basidiomycetes (including mycorrhizal species) and ascomycetes (mostly lichens). Fungi already assessed and published include 51 species threatened by loss of a specific habitat from

climate change or competition from invasive species (e.g., *Hypotrachyna virginica*, *Lecanora masana*, *Otidea platyspora*); loss of a symbiotic associate (e.g., *Hymenochaete ulmicola*, *Gyalecta ulmi*); pollution (*Anzia colpodes*); and over exploitation, perhaps by lichen collectors (*Ramalina confertula*). These fungi can be categorized as having conspicuous long-lived sporocarps or lichen thalli; inconspicuous fungi are more difficult to evaluate. As of May 2018, 505 fungi from 155 countries have been proposed for assessment. In addition to agarics, boletes, polypores, cup fungi, and lichens, some less conspicuous fungi, especially host specific symbiotic species of smuts and rusts (e.g., *Mundkurella heptapleuri*, *Nyssopsora echinata*), and several slime molds, such as *Barbeyella minutissima* with minute sporangia are listed. Thus far, few fungi that could be considered as insect symbionts or stem, leaf, or root endophytes are on the global list. Some regional lists (i.e., Japan), however, contain insect parasites (e.g., *Ophiocordyceps* spp.) and mycorrhizal basidiomycetes. Microscopic plant associated fungi cause symptoms on the host (e.g., *Taphrina* spp.), otherwise they are difficult to discover. Others have rare or endangered hosts.

Reports of presumably endangered foliicolous fungi (*Byssogene wilsonii*, *Janetia wilsonii* and *Ramichloridiopsis wilsonii*) are based on their critically endangered host, *Dimorphandra wilsonii*, in the Brazilian Cerrado (da Silva et al., 2016). A similar situation has been reported for six new fungal species recovered from another endangered host, *Coussapoa floccosa*, also in Brazil (Rocha et al., 2010). Fungal endophytes with rare or endangered hosts need attention.

The fungal lists are expected to grow longer as widely distributed taxa are more narrowly delimited by molecular methods (Hibbett and Donoghue, 1996). Also, fungi that seldom produce sporocarps, lichen thalli, or disease symptoms, will be discovered by molecular methods; mycorrhizal fungi that occupy the seldom-sporulating category might be most important to their symbiotic hosts and need evaluation (Horton and Bruns, 2001).

So, which fungi are missing from conservation discussions (Pereira-Carvalho et al., 2009)? Price (1988) observed that scientists interested in “Noah’s Ark ecology” have failed to recognize that the greatest transfer of energy occurs by microbes, not by organisms the size of “bird food” or larger. Of all microbes, mostly “charismatic megamycota,” including ectomycorrhizal fungi or parasites of larger flora and fauna are included in lists of threatened species. As mentioned above, a huge gap in our knowledge is of the generally non-parasitic fungi associated with arthropods and plants, and the absence of data affects estimates of insect-associated fungi and endophyte numbers, and draws attention to our inadequate knowledge of them in attempts to estimate total fungal numbers (Hawksworth, 1991; Hawksworth and Rossman, 1997; Hawksworth and Lücking, 2017). Our inability to estimate numbers of arthropod-associated fungi and fungal endophytes also precludes their inclusion in conservation discussions at the present time (see below). Saccharomycotina, Laboulbeniomycetes, Asellariales, and Harpellales with endangered insect hosts in Great Britain listed as may be locally extinct or critically endangered based on IUCN standards, and these include species likely as associates of gut fungi (Lane and Mann, 2016; Joint Nature Conservation Committee <http://jncc.defra.gov.uk/page-2132>).

Although the traits of few of the hidden fungi associated with insects, other fungi and plants are understood well enough to support conservation proposals, when centers of rich biodiversity (hot spots) such as plant hybrid zones and forests with structural complexity (Morrow et al., 1994; Whitham et al., 1994; Forzza et al., 2012; Dove and Keeton, 2015) are under consideration, insect-associated fungi and fungal endophytes may provide additional genetic diversity, and their likely presence could support arguments for conservation.

4. Conclusions

In an editorial in the *Journal of Insect Conservation*, New (2000) described invertebrates as “the meek inheritors,” and although recognition of fungi as a crown group improved conservation efforts, mycologists must continue to overcome a similar “invertebrate” perception by the public: “Many non-tangible values for invertebrates are indeed difficult to promote, and the common stance that, ‘they (Group *x*) may be important but we cannot really quantify this,’ is commonly viewed (correctly) with skepticism” (New, 2000). It is significant that publications on insect conservation outnumber those on fungal conservation. There is, however, increasing interest in fungal conservation judged by the increasing numbers of book chapters and refereed papers (Hawksworth, 1991; Mueller et al., 2004; Watling, 2005; Heilmann-Clausen et al., 2015) and the digital reprint (Moore et al., 2008) of the edited volume *Fungal Conservation: Issues and Solutions* (Moore et al., 2001). As Mueller (2017) has written, times are changing, not only because of better appreciation of fungi, but also because herbaria have been databased, often with information on associated organisms, and molecular methods make it easier to identify fungi in general and especially when they are hidden within other organisms.

Fungi are important for their symbiotic activities with all groups of organisms. Under certain conditions fungi support their associates to outcompete community members as they produce metabolites that might be important in commerce. Some fungi indicate environmental conditions and provide nourishment, all in aid of the associates in their symbiotic life styles (Dahlberg et al., 2010; Heilmann-Clausen et al., 2015). As the realization of the importance of fungal conservation increases, the efforts to ensure their preservation should include the fungi hidden within plants, other fungi and insects, and other groups of organisms not considered here.

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