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MINI-REVIEW

Current state and perspectives of fungal DNA barcoding and rapid identification procedures

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Abstract Fungal research is experiencing a new wave of methodological improvements that most probably will boost mycology as profoundly as molecular phylogeny has done during the last 15 years. Especially the next generation sequencing technologies can be expected to have a tremendous effect on fungal biodiversity and ecology research. In order to realise the full potential of these exciting techniques by accelerating biodiversity assessments, identification procedures of fungi need to be adapted to the emerging demands of modern large-scale ecological studies. But how should fungal species be identified in the near future? While the answer might seem trivial to most microbiologists, taxonomists working with fungi may have other views. In the present review, we will analyse the state of the art of the so-called barcoding initiatives in the light of fungi, and we will seek to evaluate emerging trends in the field. We will furthermore demonstrate that the usability of DNA barcoding as a major tool for identification of fungi largely depends on the development of high-quality sequence databases that are thoroughly curated by taxonomists and systematists.

Keywords Fungi · Barcode · ITS rDNA · Taxonomy · Next generation sequencing

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Introduction

Identification of fungi to species level is a fundamental component of many research efforts in life sciences. This is true in applied as well as in basic research fields. For example, correct species identification of plant pathogens is elementary for all aspects relating to plant diseases both in natural and agricultural ecosystems, and might even result in quarantine measures influencing international trade of plants and plant products (Wingfield et al. 2001; McNeil et al. 2004). Appropriate treatment of the increasingly frequent fungal diseases in humans is equally dependent on proper determination of the causal agents (Bialek et al. 2005; Rickerts et al. 2006). Furthermore, reliable species identification plays a central role in all studies relating to conservation biology and ecology, because all biological aspects of any given individual in an ecosystem can only be attributed meaningfully via an unambiguous identifier like a species name. Last but not least, the concept of biodiversity is fundamentally based on the species unit (i.e. alpha diversity) from which the higher levels of biodiversity (i.e. beta and gamma diversity) are derived (Whittaker 1970).

Since the times of the great explorations and fostered by Linnaeus' binomial system of naming all organisms, biologists were mainly occupied with describing and cataloguing species. As a result, a vast body of literature describing the fauna and flora of many countries and regions has accumulated over the past (Godfray 2002). As a consequence, the taxonomical information is scattered across this literature and difficult to access even for professional scientists, let alone for non-experts. Paradoxically however, while the number of expert taxonomists is dwindling, the need for taxonomic information is greater than ever before, largely due to the rapid growth of the life sciences and the biotechnology industry that produced a high demand for taxonomic expertise in many distinct research and production fields (Godfray 2002).

Nowadays, an ever-increasing rate of species extinction is resulting in destructive consequences for ecosystem functions and will also limit the potential economical benefits of biodiversity (Rockström et al. 2009). On top of that, global climate change is expected to cause far-reaching repercussions on many ecosystems and their biodiversity (Colwell et al. 2008). Changes in biodiversity (i.e. changes in species abundances and community composition of a given habitat) can only be measured and quantified if the underlying species richness is comprehensively assessed. Therefore, extensive species inventories of vulnerable habitats are urgently needed to monitor these changes in the future (Raxworthy et al. 2008). In addition, bioprospecting—the exploration of biological material for industrially valuable properties—has become an important topic, as many aspects of our daily life are linked to the discovery of new substances derived from microorganisms such as innovative healthcare products, drugs to cure serious diseases, food additives and biofuels (Alho 2008; Strobel et al. 2004, 2008). Due to the large amount of secondary metabolites found in already known fungi and the extensive biological diversity of the fungal kingdom, which is largely uncharted (Mueller et al. 2004; Zhang et al. 2006), fungi form one of the most promising resources in many efforts of systematic bioprospecting (Bills 1995; Wynberg and Laird 2007; King et al. 2009).

Fortunately, the last 20 years have witnessed much methodological advancement that make the often cumbersome process of species identification in fungi both faster and more reliable. Taxonomy and phylogeny-based systematics now rely to a large extent on phylogenetic trees derived from molecular data (e.g. Bruns et al. 1991; Hibbett et al. 2007; Shenoy et al. 2007; Kemler et al. 2009). Because of the numerous advantages of molecular data in systematic research, DNA-based taxonomy and DNA barcoding (i.e. the use of 500–800-bp long DNA sequences to delineate species) were explicitly proposed as tools for species identification in animals (Tautz et al. 2002, Hebert et al. 2003). In the meanwhile, the idea has been extended to other groups of organisms and has become a global enterprise (www.ibolproject.org). Here we explore the concept of biological barcoding of fungi and the ramifications it is likely to have on mycology as a discipline. After giving a short account of recent developments in marker selection, potential problems of barcoding that need to be overcome will be discussed, and eventually an outlook will be given on how next generation sequencing methods might transform the field in the near future.

Identification of fungi—the role of molecules

Even though molecular data is now widely used in fungal systematics and phylogeny, the valid description of a species still requires morphological characterization according to the Botanical Code of Nomenclature (McNeill et al. 2006). These morphological descriptions, together with further observations on the described species, represent a valuable and comprehensive source of information, which is still extensively used today. Nevertheless, relying solely on morphological characters in the identification process can be problematic. This is true because of the scarcity and plasticity of discriminatory yet easily accessible morphological characters in many fungi (Slepecky and Starmer 2009). Furthermore, the fungi's potentially di- and pleomorphic life cycles such as in yeast-mycelial transitions often hamper correct morphological identification to species level (Bemmann 1981; Begerow et al. 2000; Seifert and Samuels 2000; Klein and Tebbets 2007). Therefore, molecular tools were readily embraced by the mycological community when they became available. This is exemplified by the fact that nearly 6,000 fungal sequences were ready to be published when the US-based National Institute of Health initiated GenBank in 1993, and the yearly sequence submissions increased rapidly to a total number of more than 2.4 million fungal sequences in the core nucleotide set today (Fig. 1).

In retrospect, molecular information has proven highly useful to mycological endeavours such as taxonomic classification, phylogenetic inference and species delimitation and identification (e.g. Begerow et al. 1997; Kõljalg et al. 2005; James et al. 2006; Hibbett et al. 2007). In the light of molecular data, many morphological characters previously thought to be indicative of relatedness have been shown to be homoplasious or otherwise uninformative (Begerow et al. 2007; Lumbsch and Huhndorf 2007), and convergent morphological evolution as well as plesiomorphic character states appear to be widespread throughout the fungi (Blackwell et al. 2006; Hibbett et al. 2007; Shenoy et al. 2007). This has contributed to several radical taxonomic revisions in the past and an average synonymy rate of 2.5:1 for each accepted species (Hawksworth 2001).

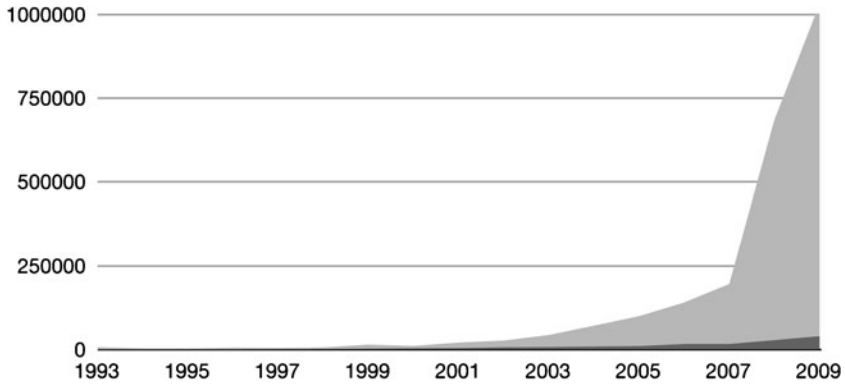

Fig. 1 Number of yearly submitted fungal sequences to GenBank/INSD. Since the establishment of GenBank in 1993 the amount of fungal DNA sequences has been growing continuously. *Light grey* all fungal sequences; *dark grey* ITS rDNA sequences. Thus, at present there are about 2.4 million fungal sequences and about 147,000 fungal ITS rDNA sequences deposited in the INSD

We believe that one of the most pressing current challenges is the linking of molecular identification with the existing species descriptions, thus tapping into the wealth of morphological and ecological information that has been accumulated over the long history of fungal taxonomy.

In spite of these recent progresses in fungal systematics and taxonomy (Stajich et al. 2009), we are still far from knowing the full extent of fungal diversity, particularly at a global scale. Only about 5% of the estimated 1.5 million species of extant fungi have been described (Hawksworth 2001), and sequence data are available for about 1% of the hypothesised number of fungal species (Nilsson et al. 2009b). Indeed, most phylogenetic studies of fungi reveal a much higher diversity than would be expected from the underlying morphological characters. Cryptic species are common throughout the fungi, and without the use of molecular phylogenetic methods the tremendous but inconspicuous and until recently overlooked diversity of the plant associated Glomeromycota (Wubet et al. 2004), Sebacinales (Weiß et al. 2004) or the fungus-like Peronosporomycetes (Spring et al. 2006) would be nearly unknown. These examples as well as environmental sampling in deep sea or in forest soil that revealed a plethora of unknown phylogenetic lineages make it clear that the fungal species known to us may well represent but the tip of the iceberg (Le Calvez et al. 2009; Porter et al. 2008). DNA-based approaches to the assessment of fungi thus open a window to a realm of diversity we presently know very little about.

In principal, molecular methods are comparatively objective and tend to be straightforward to apply when compared to morphological, anatomical, ultrastructural or chemical procedures (Brasier 1996, Crous et al. 2007). The use of sequence data to detect species in substrates where there may be no visible traces of the species present—such as soil, decaying wood, leaves or air—was quickly subsumed among the standard procedures in mycology (Gardes and Bruns 1993; Arnold et al. 2007; Taylor 2008, Fröhlich-Nowoisky et al. 2009). At the same time these studies clearly demonstrate that one of the dangerous bottlenecks is the lack of well-curated reference sequences, an issue that has been thoroughly analysed and discussed by Seifert (2009).

Barcoding as a tool for identification

Identification by molecular characters has some tradition, particularly for organisms that are very small or where species otherwise are difficult to tell apart. For example, it is now commonly accepted that a new bacterial species has to be published together with a sequence of the nuclear small subunit (SSU/16S) rDNA that distinguishes it from other known species (Sneath 1992). In a similar approach, Paul Hebert and co-workers proposed the use of a universally recognised gene—a DNA barcode—for biological identification some years ago (Hebert et al. 2003; Hajibabaei et al. 2007). The DNA barcoding initiative was founded by insect systematicians, and it has grown into an international collaboration providing the platform and facilities needed to establish databases with DNA barcodes of all organisms. Within insects the choice of an appropriate DNA region seemed straightforward, as the COI (COX1) gene was already in use for phylogenetic inference of insects and had been shown to give high resolution on various taxonomic levels (Hebert et al. 2003, Janzen et al. 2009). This gene has additionally proven useful in studies on birds and fish (Kerr et al. 2007; Hubert et al. 2008) and there were high hopes that it would prove equally useful in other groups of organisms as well.

In the meantime, it has been established that COI is unsuitable for barcoding several other groups of eukaryotes (e.g. Chase and Fay 2009). Among plants, the COI coding region often contains group I introns derived from various horizontal gene transfers (Cho et al. 1998). Furthermore, the overall slow substitution rate results in highly similar

sequences of COI even in distantly related plant families (Cho et al. 2004). In the meanwhile, several marker genes have been suggested for barcoding plants. Chase et al. (2005) proposed two sets of barcoding markers for plants; while species identification in a community context might be possible with the widely used internal transcribed spacer (ITS) rDNA or rbcL sequences, there is a need for more variable genes such as some other plastid regions for identification of closely related species on a global scale (Kress et al. 2005). Several studies have investigated the usability of different gene regions and concluded that even the combination of up to seven regions of the plastid genome cannot fully discriminate among all species (Borsch and Quandt 2009, CBOL Plant Working Group 2009). Recently, it was pragmatically proposed to use two or three plastid regions (rbcL, matK or rbcL, matK together with *trnH-psbA*, respectively), which can discriminate among most species—at least when additional information (region or habitat) is taken into consideration (CBOL Plant Working Group 2009; Kress et al. 2009).

Beside animal and plants, fungi are in focus of the barcoding initiative. The difficulties of finding the appropriate marker(s) have not been completely resolved so far and will be discussed later on. However, there are already several projects directly addressing fungi and their specific roles in the environment by using approaches similar to barcoding (Groenewald 2009). One project is focussing on indoor fungi, which are a common problem all over the world and identification of the involved mould species is often difficult and time consuming by traditional culturing methods. A second project is funded by the European Commission Framework Program 7 and is dedicated to develop barcoding for quarantine organisms. These examples show that applied aspects play a very important role for further methodological developments. Interestingly, there are already studies investigating high-throughput identification based on shorter sequences (less than 25 bp) that may prove sufficient to identify fungi from a set of 100–200 species (Summerbell et al. 2005).

Search for appropriate fungal marker genes

Although COI serves the purpose of barcoding well for a number of animal groups, the first reports on its usefulness in fungal barcoding were inconclusive (Seifert et al. 2007; Vialle et al. 2009). While COI seems to be appropriate to distinguish intraspecific and interspecific variability in *Penicillium* species (Seifert et al. 2007), a thorough analysis of the same marker in *Fusarium* revealed a significant number of multiple copies and various numbers of introns and highlighted the problems pertaining to primer design and the need for nested primers to amplify the entire region (Gilmore et al. 2009). Looking at other mitochondrial genes as candidates for barcode regions, Santamaria et al. (2009) demonstrated that introns are very common in the mitochondrial genome of Ascomycota and suggested NADH dehydrogenase 6 as a possible barcode marker. Based on these studies it is clear that COI will never be as useful for fungal barcoding as it is for many other groups of organisms.

The nuclear ribosomal RNA genes have been continuously discussed as promising candidates for fungal barcoding, because they have been the most widely used genes for phylogenetic studies for almost two decades. While SSU rDNA sequences are widely used for identification of glomeromycetes (Beck et al. 2007), they are too conserved for most other groups of fungi. However, it could be shown that species discrimination with SSU rDNA sequences is possible for a limited number of fungal species within a small habitat or to track species in manipulation experiments (Poll et al. 2009). The tripartite and highly variable internal transcribed spacer region of the ribosomal repeat unit (Fig. 2) has been in long use for species identification in fungi, and though not an officially recognised barcode, it has in reality played that role for many years (Kõljalg et al. 2005; Seifert 2009).

The ITS region is the most frequently sequenced genetic marker of fungi (Table 1) and it is routinely used to address research questions relating to systematics, phylogeny and identification of strains and specimens at and below the species level. Despite its universal usage it is not a DNA region without potential complications as a universal barcode of fungi. Intraspecific as well as intra-individual variability is known to occur (Smith et al. 2007; Simon and Weiß 2008), which may complicate automated attempts at species identification. Furthermore, the variability of the multicopy ITS region among species of different taxonomic groups does not appear to be uniform (Nilsson et al. 2008). The issue of when a query sequence is close enough to a reference sequence to be considered conspecific lies at the

Fig. 2 Barcode region proposed for fungi. The nuclear multicopy rDNA repeat of fungi codes for various parts of ribosomal RNA (small subunit (*SSU*), 5.8S, large subunit (*LSU*), and in some groups 5S as well). The tandem repeat includes two-spacer regions, the intergenetic spacer (*IGS*) and the internal transcribed spacer (*ITS*). The whole region spanning ITS1, 5.8S and ITS2 can easily be amplified by universal primers in the conserved flanking regions of SSU and LSU and is proposed as a DNA barcode for fungi (*indicated by black line*).

Table 1 Numbers of fungal sequences of several genes in GenBank (19 November 2009)

ITS including 5.8S	147,042
SSU	136,588
LSU	115,228
Beta-tubulin	14,921
Actin	4,124
RPB2	8,316
EF1a	7,235
COI	581

heart of molecular identification. The 3% threshold value, originally developed for full-length bacterial SSU sequences, has become something of a standard practice for fungi as well: if two ITS sequences differ by less than 3%, they are typically considered conspecific. And, perhaps surprisingly, there are indications to the effect that a 3% threshold value for ITS sequences may not be so bad (Hughes et al. 2009). It is nevertheless clear that no single threshold value will capture the boundary between interspecific and intraspecific variability for all fungi at the same time (Nilsson et al. 2008): a 3% threshold value will be too high in some cases (e.g. *Aspergillus*, *Penicillium*; Nilsson et al. 2009a) and too low in others (e.g. *Cantharellus* and *Craterellus*; Feibelman et al. 1994). In a way this argues for the abandonment of similarity-based identification to species level in favour of a phylogeny-based approach to the taxonomic assignment of query sequences. This was already supported by the comparison of phylogenetic resolution and accuracy of identification of mitochondrial sequences of various lengths (Min and Hickey 2007). The staggering size of many contemporary datasets of environmental sequences does however suggest that case-by-case solutions to identify sequence conspecificity may be a thing of the past. Indeed, automated approaches to species identification are resorted to already at this stage, and it seems clear that this trend is here to stay. Thus, we must come to terms with the fact that many of the taxonomic affiliations presented as a result of environmental sequencing of fungi will be approximate rather than exact. This will be one of the major tasks to be solved by the barcode consortium in the next years.

Another concern with the ITS region—as indeed with other regions—is the extent to which the most commonly used general primers discriminate against certain taxa. Because several groups of fungi can only be amplified using tailored ITS primers (Feibelman et al. 1994; Taylor and McCormick 2008), these taxa will typically not be picked up by merely molecular surveys of fungal communities in spite of potentially being very common and ecologically important. Their example serves as a disturbing reminder that there might be numerous groups of fungi of which we currently know little or nothing about because of primer mismatches.

Promises and challenges of fungal barcoding

It is trivial but nevertheless true that the reference sequences lie at the very core of DNA barcoding; any species identification effort employing DNA barcoding will be only as good as the available reference sequences permit. This understanding leads to guiding principles concerning the acquisition, handling and deposition of reference sequences. These sequences must stem from well-identified specimens including full voucher information and geo-reference data (Consortium for the barcode of life 2009). Furthermore, it will be very important to include type material and other specimens with a similar status, and several successful attempts to isolate and amplify DNA from old herbarium specimens have addressed this challenge (Telle and Thines 2008; Brock et al. 2009). In cases where the type material is too old or too scanty to allow DNA sequencing, epitypification based on sequenced specimens, conforming as closely as possible with the original type, may prove the most advantageous way of combining traditional knowledge with molecular information (Hyde and Zhang 2008; Pleijel et al. 2008).

The ideal situation would then be to have sequences of ten or more specimens per species to cover intraspecific variability of the barcode. This may be a somewhat optimistic goal since only the type material is known for many species. In addition, there will probably be a great artificial variability in the data, because often especially macrofungi like the mushrooms and toadstools had been assumed to be ubiquitous and therefore European names have been applied to specimens from all over the world due to similarities in morphology (Zhang et al. 2004). Several studies have shown that many such specimens may instead belong to species complexes that comprise several distinct, but morphologically very similar, cryptic species (Hallenberg and Larsson 1991; Hawksworth 2006; Kemler et al. 2006, 2009; Paulus et al. 2007). As a result we should expect a rapid increase of taxonomic novelties in the near future and we urgently need pipelines for fast publication of new taxa. The restrictions on electronic publication of names might, however, impede such efforts significantly. Journals like Fungal Planet (www.fungalplanet.org) or Mycosphere (www.mycospere.org) have however led the way in electronic publication of names with concomitant hardcopy releases. These are promising initiatives that hopefully will be picked up by the broad taxonomic community. Ultimately, we believe that de novo descriptions of species should be accompanied by sequence data, whenever possible and this should be regulated by the ICBN. All these actions will rely on extensive

support of conventionally trained and open-minded fungal taxonomists.

All barcoding efforts should not only provide raw sequences but also the underlying electropherograms and sequence quality information (Consortium for the barcode of life 2009). This is currently not the case for most of the sequences in the public sequence databases. At present, the database of the Canadian Centre for Barcoding (BOLD) is not fully prepared to use other barcodes than COI in a satisfying way, such that anyone seeking to make use of the ITS region for species identification of fungi will have to use other resources and databases instead. The reliability of the data in the public sequence databases such as the International Nucleotide Sequence Databases (INSD, Benson et al. 2009) is known to be moderate at best (Bidartondo 2008; Nilsson et al. 2006; Ryberg et al. 2009), which has prompted the development of independent databases, such as UNITE (http://unite.ut.ee), targeted at reliable identification of fungi to species level through high-quality reference sequences (Kõljalg et al. 2005). Needless to say, such tailored databases tend to sacrifice quantity for quality. Even the largest nucleotide sequence database, the INSD, features ITS sequences from less than 1% of the hypothesised number of fungal species (regardless of their suitability as reference sequences), which suggests that so far we are nowhere near a satisfactory sampling of fungal ITS sequences. It has been shown that the fungal herbaria worldwide are likely to form a key element in the procurement of reference ITS sequences (Brock et al. 2009), and yet the act of sequencing herbarium specimens is unlikely to attract any significant amount of funding. Other groups of fungi are likely to be poorly represented in the herbaria to begin with (Porter et al. 2008), which leaves us with two major impeding factors to DNA barcoding of fungi: lack of taxonomic knowledge and lack of funding for elementary DNA sequencing. It must be feared that none of these are likely to resolve in the positive in the foreseeable future (Hopkins and Freckleton 2002; Agnarsson and Kuntner 2007).

Prime field of application: environmental sampling

In addition to the use of DNA barcoding for the identification of organisms using reference sequences, there is a great potential to use barcodes in metagenomic approaches (Vandenkoornhuyse et al. 2002; Buée et al. 2009; Ghannoum et al. 2010). At present, the large amount of sequence data obtained with high-throughput sequencing techniques contrasts with the lack of high-quality reference sequences with sufficient taxonomic information. However, on the basis of such projects searchable sequence databases could be established much faster than using traditional taxonomy. This data will allow a census and provide status quo information of genotypic diversity (somehow reflecting the species diversity), which might serve as reference data for re-identification of genotypic lineages in the future. Using this approach, it will be possible to follow changes in species richness and species composition of a given area in the context of global climate change as well as for applied studies and conservation. While current biodiversity assessments are essentially based on data of vascular plants, birds and some other macroorganisms (cf. Heywood and Gardener 1995), high-throughput tools applied to determine microbial biodiversity should allow to describe biodiversity patterns more comprehensively and in much greater temporal and spatial resolution (Medinger et al. 2010). By combining current knowledge on functional biodiversity with additional information of genetic diversity of as yet poorly understood but functionally essential taxonomic groups like, for example, soil fungi, the biodiversity hot spots of the world may be newly pinpointed or redefined, because it is still unclear whether there is a general correlation of microbial diversity patterns with those reported for the abovementioned organisms (Bryant et al. 2008).

Biological barcoding relies heavily on improvements in DNA sequencing technology to maximise its full potential. Fortunately, the fields of DNA amplification and sequencing, as well as that of bioinformatics, presently undergo exceedingly large advancements to the effect that barcoding is not likely to be held back by technological or computational tardiness. Emerging sequencing technologies such as massively parallel ("454") pyrosequencing (Margulies et al. 2005) have the capacity to sequence hundreds of thousands of sequences from any given site or substrate overnight (Shendure and Ji 2008). As a consequence one can expect the discipline of ecology, rather than taxonomy, to be the one to discover the highest number of new species of fungi in the near future (Hibbett et al. 2009). Indeed, the three first 454-based studies of fungi (Buée et al. 2009; Jumpponen and Jones 2009; Öpik et al. 2009) jointly found more unidentifiable taxa than the number of new species described by the entire mycological community during all of 2008. Nevertheless, even though ecology is likely to be the discipline finding the highest number of undescribed species, one should not expect ecologists to describe many of these species formally. We are thus left with an ever-increasing array of fungal species, known only from sequence data, for which no Latin names exist. The International Code of Botanical Nomenclature (McNeill et al. 2006) prohibits description of species from sequence data alone, so these species will lack a Latin name for the foreseeable future. One possible approach of dealing with that problem is given by the UNITE database (http://unite.ut.ee) that will seek to provide informal, operational names of the accession number type for such taxa until the data is there to warrant formal

description of the species. Such operational names would provide an unambiguous way of referring to such taxa across study sites and publications to ensure that properties reported for any such taxon will be connected to a taxonomic lineage rather than to an arbitrary name (e.g. "*Cortinarius* clade 2"). Taken together, a much-needed emendation to the Botanical Code would be to mandate the provision of one relevant DNA sequence with each new species description. Unfortunately, this may well be deemed controversial enough not to happen in the near future (cf. Hibbett et al. 2009). Thus, we must be prepared to deal with very large sets of fungal sequences that simply cannot be identified to species level at present—either because a reference sequence or a taxonomist doing the isolation and formal description is lacking.

Concluding remarks

Molecular identification has enormous potential to further our understanding of fungal biodiversity, the ecological roles of fungi and their geographical distribution. It has also great potential to accelerate bioprospecting and other applied research fields. The ITS region has been used as the de facto standard 'barcoding' marker by mycologists for many years, and there is every reason to believe that it will remain at least one of the main fungal barcoding markers for many years to come. Even though the ITS region is the most sequenced genetic marker for fungi, there are currently ITS sequences from a moderate 14,000 fully identified fungal species available in the public sequence databases—a number that contrasts sharply with the estimated 1.5 million extant species of fungi. Therefore, it is critical that measures are taken to expand the reference dataset of ITS sequences from expertly identified fungi. The vouchers deposited in the herbaria worldwide are likely to form a key element in this pursuit, although it might probably prove difficult to secure funding for DNA sequencing of herbarium specimens. Similarly, the mycological community should seek to provide an ITS sequence along with each new species description. Other efforts, such as agreeing on informal names for taxa presently known only from sequence data, may also aid in the struggle to keep abreast of the rapidly accumulating environmental samples of fungi. Given the great potential molecular identification of fungi holds it would compare to culpable negligence if the mycological community could not agree on a joint effort to ensure as good a set of reference sequences as possible.

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