Supplementary Information

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1. Fungal Barcoding Resources Supplement

Extraction methods for improving yields and efficiency (Dentinger et al. 2009; Osmundson et al. 2013a) have been suggested, and to ensure high quality and representativeness, methodological improvements like using proofreading polymerase (Oliver et al. 2015), testing primers for bias against certain taxonomic groups (Bellemain et al. 2010; De Beeck et al. 2014; Tedersoo et al. 2015), and even user’s guides to 96-well specimen-based (Eberhardt 2012) and high-throughput fungal amplicon sequencing have been published, with step by step suggestions and cautions from sampling and lab methods to analysis and interpretation (Lindahl et al. 2013). Perhaps the most discussion has been paid to choice of primers, since so-called universal primers for rDNA have known mismatches for several groups of Fungi, some of which are abundant and presumably ecologically important (e.g for ITS; Rosling et al. 2011).

Fungal metabarcoding is almost always carried out using part or all of the ITS region, but there is some controversy about which portion and which primers to use to offer the best resolution with the best representativeness (Blaalid et al. 2013). The choice has important implications for species identification and any downstream application of those names (Nilsson et al. 2008). Some workers have suggested co-amplification with SSU in order to allow for phylogenetic studies and to anchor so-called orphan ITS sequences with no close analogues in databases (O’Brien et al. 2005; Richards et al. 2012), but as metabarcodes are necessarily limited in length by current sequencing technologies, most arguments are focussed on which part of the ITS should be targeted. If sequence read length is set to continue to increase with new technologies, ITS2 would benefit from the higher resolving power of the downstream LSU compared with ITS1 and its highly-conserved downstream 5.8S (Nilsson et al. 2008). On the other hand, ITS1 is reported to be more variable than ITS2 for a majority of basidiomycetes from dried collections tested.
(Osmundson et al. 2013b) and offers slightly better resolving power across a wide range of ascomycetes from sequence databases (Wang et al. 2015). The latter study and others also reported similar species identification success of ITS1 and ITS2 across a wide range of Basidiomycota (Blaalid et al. 2013; Wang et al. 2015). In tests in the EM fungal genus *Inocybe* (Ryberg et al. 2008), and across lichen fungi (Kelly et al. 2011), ITS1 and ITS2 performed more or less equally well at species discrimination, which is not surprising, since ITS1 and ITS variation tend to be correlated (Nilsson et al. 2008; Blaalid et al. 2013).

The choice of PCR primers has important consequences for what sequences are recovered, with some groups severely underrepresented (e.g. Bellemain et al. 2010; Schadt & Rosling 2015), and in some cases yield remarkably low species-level resolution, with only 45% (Pitkäranta et al. 2008) or even fewer than 25% of OTUs identified (Korpelainen et al. 2015) for indoor air fungi. Group I introns in the SSU can also result in non-amplification or overly long amplicons for some lichens (Kelly et al. 2011). Several new sets of primers have been proposed and tested (Toju et al. 2012; De Beeck et al. 2014; Tedersoo et al. 2015). Despite eliminating some taxon bias, there are disadvantages to using newly designed primers, notably the loss of comparability with other studies, and particularly with the difficulty in relative quantification of OTUs. However, to assay the bias in primers, a test in soil fungi found that shotgun sequencing versus amplicon sequencing revealed little to no bias (Tedersoo et al. 2015). Further similar tests should be completed in other fungal target groups and habitats.

Several Fungal-specific bioinformatics pipelines have also been developed, the best-known of which is UNITE, which includes the PlutoF workbench (Abarenkov et al. 2010) and modules for ITS extraction, chimera checking (including UCHIME(Edgar et al. 2011)) and identification, by matching query sequences with species hypotheses (including varying similarity cut-offs) and reference sequences determined by expert users. The integrated pipeline PIPITS takes advantage of many of the features of UNITE (Gweon et al. 2015), and another expressly for Illumina data was created to be both flexible and straightforward, having been used successfully by inexperienced students with only a few hours tuition (Seifert et al. 2007). The bioinformatics tools outlined here are based on MOTU discrimination and similarity thresholds, whereas evolutionary-aware approaches such as phylogenetic and coalescent-based criteria remain marginal and largely restricted to fungal taxonomists. The available fungal metabarcoding workflows are also designed for amplicon sequencing.
studies, and to our knowledge there are no optimised approaches for phylogenetic profiling of fungi in shotgun metagenomics datasets like there are for prokaryotes (Segata et al. 2012).

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2. Data for Figures.

Figure 1.

| Year | Species with unspecified names | Species with binomials |
|------|--------------------------------|------------------------|
| 1993 | 12                             | 438                    |
| 1994 | 8                              | 209                    |
| 1995 | 31                             | 338                    |
| 1996 | 22                             | 450                    |
| 1997 | 92                             | 712                    |
| 1998 | 266                            | 895                    |
| 1999 | 361                            | 1250                   |
| 2000 | 624                            | 1427                   |
| 2001 | 1106                           | 1422                   |
| 2002 | 1586                           | 1724                   |
| 2003 | 1640                           | 1463                   |
| 2004 | 1656                           | 1589                   |
| 2005 | 2184                           | 1316                   |
| 2006 | 3430                           | 1809                   |
| 2007 | 3797                           | 1436                   |
| 2008 | 5978                           | 1968                   |
| 2009 | 5460                           | 1537                   |
| 2010 | 7132                           | 1908                   |
| 2011 | 8287                           | 2086                   |
| 2012 | 8928                           | 2272                   |
| 2013 | 9689                           | 1910                   |
| 2014 | 11307                          | 2289                   |

In order to compare the annual set of binomials and unspecified names the following two sets of queries were done in Entrez Direct for each year since the inception of NCBI Taxonomy in 1993 e.g.:
esearch -db taxonomy -query "Fungi[organism] AND species[rank] AND specified[prop] AND 1993[edat]"
esearch -db taxonomy -query "Fungi[organism] AND species[rank] NOT specified[prop] AND 1993[edat]"
The yearly species names released in Index Fungorum and MycoBank was obtained. Each year’s combined set of names was then searched against the NCBI Taxonomy using the Taxonomy name/id Status Report tool (http://www.ncbi.nlm.nih.gov/Taxonomy/TaxIdentifier/tax_identifier.cgi) at NCBI. The output indicated names which were present in the NCBI Taxonomy and each year was subsequently tabulated. The validly published species names in Index Fungorum and MycoBank were obtained for each year. The combined non-redundant set of names was then searched against the NCBI Taxonomy database using the Taxonomy name/id Status Report tool (http://www.ncbi.nlm.nih.gov/Taxonomy/TaxIdentifier/tax_identifier.cgi) at NCBI. The output indicated names which were present/absent in the NCBI Taxonomy. In order to verify how many of these names were associated with ITS records present at the UNITE database we did an Entrez query (species name[orgn] AND loprovunite [filter]) in the Nucleotide Database at NCBI. Subsequently the presences of names in each database were counted and tabulated by the year a new name became published. Note, link out providers such as UNITE maintain their own LinkOut files and the search term loprovunite [filter] is dependent on that. The data for 2014 from UNITE were not up to date at of the date of data retrieval, and therefore not included in the final figure.

| Year of Publication | New Species in Index Fungorum / MycoBank | Total Binomials in GenBank | % | ITS record in UNITE | % |
|---------------------|------------------------------------------|----------------------------|---|---------------------|---|
| 1990                | 1210                                     | 198                        | 16.364 | 130                  | 10.744 |
| 1991                | 974                                      | 144                        | 14.784 | 88                   | 9.035  |
| 1992                | 889                                      | 198                        | 22.272 | 125                  | 14.061 |
| 1993                | 1286                                     | 193                        | 15.008 | 128                  | 9.953  |
| 1994                | 1197                                     | 217                        | 18.129 | 115                  | 9.607  |
| 1995                | 1221                                     | 226                        | 18.509 | 147                  | 12.039 |
| 1996                | 1327                                     | 268                        | 20.196 | 179                  | 13.489 |
| 1997                | 1435                                     | 245                        | 17.073 | 162                  | 11.289 |
| 1998                | 1164                                     | 292                        | 25.086 | 181                  | 15.550 |
| 1999                | 1200                                     | 278                        | 23.167 | 167                  | 13.917 |
| 2000                | 1180                                     | 286                        | 24.237 | 189                  | 16.017 |
| 2001                | 1290                                     | 375                        | 29.07  | 278                  | 21.550 |
| 2002                | 1133                                     | 251                        | 22.154 | 166                  | 14.651 |
| 2003                | 1311                                     | 332                        | 25.324 | 229                  | 17.468 |
| 2004                | 1425                                     | 504                        | 35.368 | 362                  | 25.404 |
| 2005                | 968                                      | 367                        | 37.913 | 230                  | 23.760 |
| 2006                | 1161                                     | 422                        | 36.348 | 308                  | 26.529 |
| 2007                | 1424                                     | 481                        | 33.778 | 356                  | 25.000 |
| 2008                | 1423                                     | 470                        | 33.029 | 344                  | 24.174 |
| 2009                | 1599                                     | 545                        | 34.084 | 401                  | 25.078 |
| 2010                | 1270                                     | 547                        | 43.071 | 386                  | 30.394 |
| 2011                | 1516                                     | 723                        | 47.691 | 525                  | 34.631 |
| 2012                | 1772                                     | 842                        | 47.517 | 648                  | 36.569 |
| 2013                | 1802                                     | 888                        | 49.279 | 715                  | 39.678 |
| 2014                | 1981                                     | 1080                       | 54.518 |                      |      |