HHS Public Access

Author manuscript

Fungal Genet Biol. Author manuscript; available in PMC 2020 October 01.

Published in final edited form as:

Fungal Genet Biol. 2019 October ; 131: 103249. doi:10.1016/j.fgb.2019.103249.

Fungal species boundaries in the genomics era

Daniel R. Matute[1], **Victoria E. Sepúlveda**[2]

[1]Biology Department, University of North Carolina, Chapel Hill

[2]Department of Microbiology and Immunology, School of Medicine, University of North Carolina, Chapel Hill

Abstract

Genomic data has opened new possibilities to understand how organisms change over time, and could enable the discovery of previously undescribed species. Although taxonomy used to be based on phenotypes, molecular data has frequently revealed that morphological traits are insufficient to describe biodiversity. Genomics holds the promise of revealing even more genetic discontinuities, but the parameters on how to describe species from genomic data remain unclear. Fungi have been a successful case in which the use of molecular markers has uncovered the existence of genetic boundaries where no crosses are possible. In this minireview, we highlight recent advances, propose a set of standards to use genomic sequences to uncover species boundaries, point out potential pitfalls, and present possible future research directions.

Keywords

Speciation; gene flow; fungi

Introduction

Species are defined as genetic clusters of organisms that are isolated from other clusters (Coyne and Orr, 2004; Nosil, 2012). This isolation can be assessed with phenotypic and genetic data alike. In spite of the simplicity of this idea, recognizing species is anything but simple (Figure 1). Gene exchange, phenotypic plasticity, and the infeasibility of culturing (let alone performing experiments) might impede species identification. Nonetheless identifying species boundaries is crucial to understanding how and why genetic variation is portioned in nature.

At least ten distinct conceptual frameworks and associated metrics for species delimitation have been proposed (listed and reviewed in(Coyne and Orr, 2004; De Queiroz, 2007; Taylor et al., 2000)). Arguably, the most influential concept for recognizing species boundaries is

Correspondence: Biology Department, University of North Carolina, Chapel Hill, North Carolina, 250 Bell Tower Drive, Genome Sciences Building, Chapel Hill, NC 27514, USA, dmatute@email.unc.edu.

the biological species concept (BSC; (Huxley, 1943; Dobzhansky, 1937)). Under this framework, groups of individuals that are reproductively isolated from each other are considered species. This approach constitutes the basis of diversity studies across domains of life (Bobay and Ochman, 2017). Nevertheless, in spite of its wide application, the BSC is impractical to apply for many types of organisms (De Queiroz, 2007; Sites and Marshall, 2003; Sokal and Crovello, 1970). A nontrivial proportion of species are either asexual or cannot be crossed in an experimental setting and thus are not amenable to the BSC. In other cases, there is evidence that sexual reproduction takes place in nature, but it is infrequent enough or is triggered by such rare conditions that the sexual stages of many species remain unknown. This is a particularly notorious issue for fungi (Cai et al., 2011; Crespo and Lumbsch 2010, Dettman et al., 2003a; Giraud et al., 2010; Grube and Kroken 2000, Harrington and Rizzo 1999, Taylor et al., 2000). Clearly, an alternative that is not based on experimental crosses would be advantageous for organisms where the BSC cannot be implemented.

Genetic data also reveal the signature of speciation (Bobay and Ochman, 2017; Taylor, 2006; Taylor et al., 2000). Several species concepts have been proposed based on population cohesion. All these concepts (henceforth referred to as ‘discontinuity concepts’) and the BSC have a common ground. According to the BSC, speciation has occurred in instances where reproductive isolation (RI) exists. According to the discontinuity concepts, speciation has occurred in instances where there are major genetic gaps between groups (i.e., discontinuities). If RI exists and speciation has proceeded, then there should be genetic differentiation between the putative species which should leave a signature of genetic discontinuity across the whole genome (Avise and Wollenberg, 1997; Bobay and Ochman, 2017; Coyne and Orr, 2004; Sobel et al., 2010). To date, there are few proposals on how to implement species concepts using genome-wide genetic data, and this is particularly evident for fungi. We aim to bridge this gap. In this piece, we review the main approaches to delimiting species, present an overview of the precedents on species boundaries delimitation in fungi to date, and propose four criteria to identify species boundaries using genome-wide data in fungi. We argue that a unified approach to study species boundaries is crucial for our understanding of fungal diversity but also has practical applications for plant pathology and medical mycology.

Current approaches to detect species boundaries in fungi

All discontinuity concepts are based on the detection of genetic clusters that are sufficiently differentiated from one another. Of all the discontinuity concepts, the phylogenetic species concept (PSC) is by far the most widely applied in fungi (Taylor et al., 2000). According to the PSC, speciation has occurred in instances where there are major genetic discontinuities. Under the PSC, species are diagnosed as a cluster of individuals that are sufficiently differentiated from other clusters as revealed by DNA sequences. Gene genealogies are frequently used to determine the clusters used in the PSC, although other approaches with a similar premise have been proposed (reviewed in (Coyne and Orr, 2004; Nixon and Wheeler, 1990; Renard et al., 2003; Wheeler and Platnick, 1989)). The PSC was originally proposed in the 1980s (Baum and Donoghue, 1995; Donoghue, 1985) but did not gain momentum in fungi until multilocus sequence typing was coopted from bacterial studies (Taylor and

Fisher, 2003). The PSC in fungi has two broadly-defined forms, the strict genealogic concordance (SGC) and the coalescent-based species delimitation (CBD) approaches (revised in (Taylor et al., 2000)). These approaches are not mutually exclusive as they both use phylogenetic trees but their scope is different. We summarize each of these groups as follows.

SGC approaches assess the extent of genetic concordance across loci in a semi-quantitative framework. Two seminal pieces, Dettman et al. (Dettman et al., 2003a, 2003b) and Liti et al. (Liti et al., 2006), studied what tree metrics might signal discontinuities in genetic variation that likely resulted from reproductive isolation and could thus differentiate between structured populations and true species. First, if all gene genealogies from unlinked loci in the genome show genealogies that are congruent with each other, then speciation might have occurred (i.e., genealogical concordance, or 'concordance rule'). Second, if a clade or group of putative species forms reciprocally monophyletic groups with Bayesian support of at least 90% and of bootstrap above 70%, then they are likely to be reproductively isolated (i.e., 'support rule'—based on (Hillis and Bull, 1993)). The SGC approach as proposed by Dettman et al. (Dettman et al., 2003a) is one of the unified approaches for species delimitation in fungi.

The mycology community has extensively used the two rules of the SGC to detect the signature of speciation, especially to identify cryptic species. The focus on this instance of divergence is due to the fact that this is the stage at which species boundaries are the most challenging to detect (reviewed in (Bickford et al., 2007; Cai et al., 2011; Restrepo et al., 2014; Roux et al., 2016)). At least 55 clades (64 studies) used SGC criteria to study potential cryptic speciation in fungi. The application of these two rules has led to the report of over 200 previously-unknown cryptic species, leading to an increase of almost three times in fungal diversity in the studied groups (from 116 to 343 species). Even though this list is certainly not complete, and should be taken as a sample from the complete literature, a pattern emerges from this body of work; most studies used either the support rule (described above; 24 out of 55) or both the support and the concordance rule (30/55; Figure 2A). Only one study used the concordance rule exclusively (1/55)—a study on species boundaries in *Histoplasma* using whole genome sequences ((Sepúlveda et al., 2017); discussed below). The reason for why the support rule is more commonly used than the concordance rule might be technical. Once a topology is inferred, assessing the level of support of the branches is almost automatic, while assessing the level of concordance requires different approaches.

The application of the SGC rules is not devoid of limitations. First, the 'rules' were proposed for a set of clades (*Neurospora* (Dettman et al., 2003b); *Saccharomyces* (Liti et al., 2006)), but it is unclear to what extent they can be generalized to other taxa. The evolution of reproductive isolation is particular to each clade and metrics to detect species boundaries might be required for each individual clade. A second caveat stems from the way that alignments are regularly analyzed. Notably, 31 out of the 5 5 studies that used the support rule used concatenated loci instead of individual genealogies. Gene concatenation has been shown to fail to recover true tree topologies when there are high levels of incomplete lineage sorting (as occurs in recently diverged species) and might thus obscure the relationships

between sibling species (Edwards et al., 2007; Kubatko and Degnan, 2007; Liu and Edwards, 2009; Mendes and Hahn, 2018).

A second set of approaches, the coalescent-based delimitation (CBD) involves understanding how several species are related by modeling the genealogical history of individuals back to a common ancestor (i.e., coalescent theory; reviewed in (Fujita et al., 2012)). Similar to the SGC approaches, the original coalescent-based methods to detect species boundaries leverage the fact that speciation might be inferred in cases where multiple loci show coincident splits for a sample of individuals. CBD can be divided into at least three families of methods. The first family of methods estimates the likelihood of obtaining a given set of gene genealogies given a species tree. This in turn allows for the estimation of the probability of a species tree and the probability of a particular species delimitation given multilocus data (e.g., BP&P, (Yang, 2015a, 2015b; Yang and Rannala, 2010)). These approaches sequentially collapse nodes to identify the potential species until all descendants are assigned to one species using a prespecified topology. A second family explores the full space of possible species tree topologies which allows them to delimit species and infer the species tree simultaneously (e.g., STEM (Kubatko et al., 2009), SpedeSTEM (Ence and Carstens, 2011), DISSECT (Jones et al., 2015), STACEY (Jones, 2017), and recent iterations of BP&P (Yang, 2015a)). A third family uses the distinct branching patterns between divergence (using either Yule or Poisson models) and intraspecific diversification (using coalescent models) to distinguish between species and populations. The transition between these different branching patterns is inferred to predict species boundaries. The main assumption of these methods is that within species branching events will be substantially more frequent than between species. GMYC (Generalized Mixed Yule Coalescent; (Fontaneto et al., 2007; Pons et al., 2006)) and PTP (Poisson Tree Processes; (Zhang et al., 2013)) are examples of this framework.

Each of these method families has been used in studies of fungal species boundaries. In a similar way to the compilation we did for SGC criteria, we focused on obtaining a sample from cases of cryptic speciation. We compiled a sample of 41 cases which studied potential cryptic speciation using coalescent-based species delimitation in fungi. Three general patterns emerged. First, the most common approach to delimit species boundaries in fungi under the CBD approach is modeling branching patterns across species following a Yule model, i.e., GMYC (Figure 2B, Table S2). A second pattern suggests that studies that delimit cryptic species in fungi using the CBD often use methods from more than one family. Ten (out of 41) studies used a combination of methods from different families, while two used more than one method from the same family (GMYC and PTP in both cases, Table S3). The species boundaries inferred by different approaches are usually similar but not always identical (see below). This discordance usually leads to authors choosing the most conservative number of species (e.g., (Pino-Bodas et al., 2018; Singh et al., 2015)) and highlights the subjectivity of current species boundaries delimitation.

It is worth noting that the effectiveness of the multispecies coalescent to detect species boundaries has been called into consideration as it cannot disentangle strong population structure from incipient speciation, especially in cases where the distribution of potential species is allopatric (Leaché et al., 2019; Sukumaran and Knowles, 2017). This is an

important distinction as not all populations become species, and most species show population structure usually associated with geography. The putative species identified using the multispecies coalescent (and the concordance-based PSC) must be considered as tentative hypotheses and must be confirmed through the collection of other sources of information.

Some general patterns also emerge for both the use of the SGC and CBD. First, a large proportion of cases used a set of common markers and most of them were nuclear loci (e.g., *ITS, TEF1-α, β-TUB, RPB2*; Figure 3A, B). This is certainly not a coincidence as these loci were originally chosen for being single copy genes, harboring polymorphism (suggestive of not being completely constrained by purifying selection), and being amenable to PCR-amplification using universal primers. Some of these markers (e.g., *ITS*) have come under scrutiny as they might underestimate the magnitude of differentiation (Gazis et al., 2011). Second, the mean number of loci studied to define species boundaries is 4.178 for SGC and 3.897 for CBD (with medians of 3 and 4, respectively). The ranges are broad and goes from one locus to fifteen unlinked loci (not including studies using whole genome sequencing, Figure 3C, D). This pattern can be concerning as individual loci (or a small group of loci) are more likely to be affected by selection, recombination, or even drift than large sets of unlinked loci (Dupuis et al., 2012; Edwards et al., 2007; Rokas et al., 2003). Simulations indicate that the accuracy of species delimitation is much higher when five or more loci are sampled for divergences larger than 2N generations (Avise and Ball, 1990; Baum and Shaw, 1995; Hudson and Coyne, 2002).

The range of reported cryptic species is large when using either the SGC or the CBD delimitation (Figure 3E, F), and in some notable cases a single species has been proposed to be split into dozens of species (e.g., (Lucking et al., 2014)). In the majority of cases, the number of proposed cryptic species is much lower (Figure 3E, F) and is remarkably similar for both approaches (mean ratio of resulting species to previously known species = 2.96 and 3.00 for SGC and CBD, respectively). Notably, the vast majority of studies that contemplated the possibility of cryptic speciation found a signature of it (Figure 3E, F). A handful of studies found no support for previously proposed species boundaries (Boluda et al., 2018; Chen et al., 2011; Douanla-Meli et al., 2018; Liu et al., 2016; Pino-Bodas et al., 2018; Saag et al., 2014; Zhao et al., 2015, 2017). This apparent prevalence of cryptic speciation begs the question whether the relative abundance of unidentified species is real or whether it is driven by the ascertainment bias of the published studies (i.e., ‘the drawer effect’—negative results do not get published). Few systematic studies have been published on the matter. The most influential, perhaps, studied the possibility of cryptic speciation in *Coccidioides* and its closest relatives, *Auxarthron zuffianum* and *Uncinocarpus reesii* (Koufopanou et al., 2001) . Using common criteria (i.e., the concordance and support rules) and genetic markers, the study found that the three genera harbored cryptic species, lending support to the hypothesis that unidentified species are common. Similar studies across all fungal groups are sorely needed.

A set of studies used both the SGC and CBD and the results were largely concordant (Table 1). This is not surprising given that the two lines of eviden ce aim to detect similar patterns across the genome which in turn signal deep splits. Yet, there are some exceptions. Studies

within the lichen-forming fungal genus *Protoparmellia* are representative of this situation (Singh et al., 2015). Using the concordance and support rules (SGC), *Protoparmellia* seemed to be composed by 25 species. CBD approaches did not fully agree: spedeSTEM suggested 19 species, while BP&P suggested 23. Not all species supported by BPP were supported by spedeSTEM; 16 received high support from all methods. This reflects the difficulties of applying cutoffs to species boundaries even when the premise of approaches is similar. There is no guarantee that the application of any PSC-related approach will give an unambiguous delimitation of species boundaries in any given case.

Some caveats apply to both the SGC and CBD. Both approaches work under the assumption that speciation is predicted by sequence divergence (Dettman et al., 2003b; Liti et al., 2006); nevertheless, this assumption is often violated. While the number of hybrid incompatibilities increase as genetic distance accrues (Matute et al., 2010; Matute and Gavin-Smyth, 2014; Moyle and Nakazato, 2010; Wang et al., 2015), reproductive isolation might be achieved with just one single gene (Orr, 2006; Richards et al., 2017) which in turn reduces the predictability of the signal of speciation given molecular divergence. For example, a single epistatic interaction can cause complete reproductive isolation. In interspecific hybrids between *Saccharomyces bayanus* and *S. cerevisiae,* the *S. bayanus Aep2* protein cannot regulate the translation of the *S. cerevisiae OLI1* mRNA (Chou et al., 2010; Lee et al., 2008). This interaction is sufficient to cause sterility in hybrids. On the other hand, some fungal species show an exceptionally high level of genetic diversity; individuals from the same species might be more differentiated than species from different genera, without being isolated from each other. Haploid genotypes of a split-gill fungus, *Schizophyllum commune,* show a staggering diversity at synonymous sites of 20%, the highest of any Eukaryote, without apparent incompatibility (Baranova et al., 2015; Leffler et al., 2012).

The need for revised standards for species delimitation in fungi

Dettman et al.'s (Dettman et al., 2003b, 2003a) criteria (SGC) and the CBD of species are milestones in the quest to identify species boundaries. Nonetheless, these rules were proposed for species delimitation using a set of unlinked loci. However, the application of these rules can be misinformative (Phillips et al., 2004; Rokas et al.,2003). First, bootstrap measures the sampling effects in an alignment but not if a tree topology is actually correct (Hillis and Bull, 1993; Holmes, 2003; Phillips et al., 2004; Sanderson, 1995; Susko, 2009). In large datasets (i.e., genomic data) of closely related species, sampling error should disappear and bootstrap values will almost invariably approach 100%. In cases of truly deep divergence, bootstrap might underestimate the support of branches because difficulties in detecting homology will increase the sampling error (Lemoine et al., 2018). New methods to assess branch support incorporating the challenges of genomic data have been proposed (Carstens and Knowles, 2007; Mirarab et al., 2014; Sayyari and Mirarab, 2016), but thus far no method has tried to determine to what extent different magnitudes of support (if any) signal reproductive isolation in different taxa.

Second, genome congruence cannot be assessed in the same way as multilocus gene genealogies. As the number of studied loci increases, the likelihood of complete reciprocal monophyly decreases precipitously (Knowles and Carstens, 2007). The genome of fungi

contains on average 5,000 to 10,000 genes ((Desjardins et al., 2011; Martinez et al., 2008; Seixas et al., 2019) reviewed in (Mohanta and Bae, 2015)) and some of these genes might show gene genealogies that are different from the species tree (i.e., are incongruent) just by chance (Knowles and Carstens, 2007). Sister species might retain ancestral alleles (Gao et al., 2015; Hudson and Coyne, 2002) that precede speciation or might exchange genes through hybridization (reviewed in (Schardl and Craven, 2003)). These two causes of shared genetic ancestry might lead to gene genealogies that could contradict the species tree. The increasing ease of obtaining genome-wide data uncovers the need to redefine the approaches to establish species boundaries. In light of these limitations, we propose a unified dataset and four criteria which incorporate the uncertainty associated with large datasets generated by genome sequencing but also leverage the power of such datasets.

Proposed standards for using genome sequences to delimit species boundaries

We propose that the use of whole genome sequences will allow for a systematic and comparable metric of species differentiation in fungi. This level of data not only reveals diagnostic markers, but allows for direct comparisons across studies (a major issue in reduced representation sequencing, such as RAD-Seq; (Burns et al., 2017)). We expect that whole genome sequences will become more ubiquitous over time and will end up largely replacing alternative markers. Here we propose four criteria that we consider sufficient and necessary to leverage genome sequences in identifying bona fide species boundaries in fungi.

1. Mostly reciprocal monophyly.

Reciprocal monophyly along the genome is one of the advanced stages of the speciation continuum. Groups that are reciprocally monophyletic are more likely to represent species. The proportion of loci across the genome that are reciprocally monophyletic is proportional to the the time since species divergence and represents an advanced stage in the speciation continuum (Figure 1; Hudson and Coyne 2002, Rosenberg et al. 2005, Mehta et al. 2016).

One way to identify reciprocally monophyletic groups is to generate a rooted phylogeny using the whole genome as a concatenated dataset. This approach is discouraged because when the gene trees of loci are discordant (as usually occurs in recently diverged species), concatenating data across loci can result in misleading inferences about the history of divergence (Kubatko and Degnan, 2007; Liu and Edwards, 2009). In particular, concatenated markers regularly fail at revealing the true species tree, especially in instances where there is incomplete lineage sorting ((Mendes and Hahn, 2018; Roch and Steel, 2015); discussed above). A different possibility is to find blocks of SNPs (single nucleotide polymorphisms) that are under linkage equilibrium and generate phylogenies for each block. In fungi with high levels of clonality, this genetic unit can be a chromosome (or a supercontig in unfinished genome assemblies). This procedure then produces several phylogenies that can be inspected for genome-wide support and concordance (see immediately below). Reciprocal monophyly is expected to occur after 4×Ne generations ((average number of generations until fixation where Ne is the effective population size) (Hudson and Coyne,

2002)) . Enforcing reciprocal monophyly will also ensure that all individuals in the population are placed within a phylogenetic species (exhaustive subdivision, (Dettman et al. 2003a)). Note that enforcing this criterion will prevent identification of species that are early in the speciation continuum as not all species are reciprocally monophyletic and some of them are nested within each other (Knowles and Carstens, 2007). Early stages of divergence should be treated with care as genetic data evidence might be insufficient to determine whether these groups are species or structured populations.

2. High concordance among genomic partitions.

The use of genome-wide markers will allow for species trees based on thousands of markers. In cases where speciation has truly occurred, the vast majority of the genome should show the signal that reflects the proposed species boundary. In essence, this is an extension of the genealogical concordance criterion (Dettman et al., 2003a). There are several methods to infer species trees and estimate the magnitude of genome-wide concordance (Chung and Ane, 2011; Jackson et al., 2017; Larget et al., 2010; Liu, 2008; Mirarab et al., 2014; Roch and Steel, 2015; Yang, 2015a; Zhang et al., 2018) and all of them have common elements. The main premise of concordance analyses is to generate species trees from individual gene trees and calculate the magnitude of discordance. Concordance trees and Bayesian Concordance Factors (CFs; range: 0-1) allow for this test. Each locus is assumed to have a unique genealogy, and different loci might have different genealogies. The estimated concordance tree (i.e., the dominant phylogenetic history for a group of organisms) is then built from the signal revealed from individual gene genealogies only for clades with the highest estimated CFs. The concordance tree reveals what is the most likely species tree while the CFs reveal the level of discordance among loci and reveal what proportion of genes are discordant with the rest of the genome. Formal concordance analyses account for biological processes like hybridization, incomplete lineage sorting, and lateral gene transfer, which may result in different loci having different genealogies (Ané et al., 2006; Baum, 2007).

What magnitude of CFs might be sufficient to detect species boundaries? This question was preemptively responded since the proposal of CFs: *'the search for a particular CF threshold that denotes the boundary between reticulation and divergence is doomed'* (Baum, 2007). One of the difficulties with the question is that, although the CF increases until it reaches 1 as speciation proceeds, truly isolated species might show CFs lower than 1 because of shared ancestry (see below). Similarly, the value of the metric depends on the number of loci included in a study. We propose that the solution to this difficulty is to implement CFs not in isolation but with all the other guidelines here proposed and to report the associated CF of any proposed species.

3. Lower interspecies differentiation than intraspecific differentiation.

Most species concepts use genetic discontinuities to identify species boundaries. This general premise is common to all the species concepts. Under this framework, individuals from the same species should be more closely related to each other than to individuals from other species. In other words, in cases where speciation has really occurred, the mean distance between individuals from the two species (Dxy) should be larger than the mean

distance between individuals within each of the species (π). Dxy and π can then be compared using permutation tests. This test reveals whether genetic variation in a putative species pair is partitioned across species or not, either at the individual locus level or genome wide (Hughes et al., 2009; Matute et al., 2006; Sepúlveda et al., 2017). It is worth noting that these comparisons require extensive sampling across the whole geographic range of a species to avoid missing clinal variation which with incomplete sampling might look like genetic discontinuities (Piedra-Malagón et al., 2011).

An alternative possibility is to use the magnitude of neutral differentiation to detect well-formed species. A seminal study found that the intermediate "gray zone" of speciation (i.e., cases in which population structure and incipient speciation cannot be differentiated thus making taxonomy controversial, Figure 1), usually happens in animals when synonymous divergence between the diverging species occurs between 0.5% to 2% (Roux et al., 2016). (A similar rule and rationale was proposed for single loci (Fujisawa and Barraclough, 2013)). Diverging populations in which divergence is lower than 0.5% are likely to have pervasive gene exchange and merge into a single lineage (Roux et al., 2016). The magnitude of gene exchange does fall precipitously after 2%. This allows us to formulate a tentative metric; if the magnitude of genome-wide synonymous divergence is above 2% and is significantly lower than the magnitude of polymorphism within either species, then the two lineages can be described as different species. Notably, no studies of this type have been done in fungi and the application of metrics devised for animals can be misinformative. As mentioned before, there is no perfect metric neither for Dxy/π comparisons nor for the magnitude of synonymous divergence to detect species boundaries (but see the 4× rule; (Barraclough et al., 2003; Birky, 2013)).

4. Low shared polymorphism.

As speciation progresses, genomes differentiate, and the likelihood of shared ancestry decreases between species (Figure 1). This occurs because the two events that lead to shared ancestry, retention of ancestral polymorphism (that pre-dates speciation) and gene exchange, are less likely as genetic divergence accrues. In the case of ancestral polymorphism, the magnitude of neutral polymorphism that is shared in sister species (i.e., variants that come from the ancestral species) decays precipitously as divergence increases (Gao et al., 2015; Hudson and Coyne, 2002). As the number of incompatible alleles increases rapidly with divergence time (Matute et al., 2010; Matute and Gavin-Smyth, 2014; Moyle and Nakazato, 2010; Wang et al., 2015), the likelihood of a neutral (or even advantageous) introgression being linked to a hybrid incompatibility increases and the permeability of the genome to introgression decreases (Carneiro et al., 2014, 2010; Muirhead and Presgraves, 2016; Payseur et al., 2004; Turissini and Matute, 2017). Several methods have been proposed to quantify shared ancestry and differentiate introgression and incomplete lineage sorting (Guerrero and Hahn, 2018), some of which have been specifically applied to fungi. These approaches fall into two types: the use of summary statistics, such as D (i.e., ABBA/BABA tests, (Green et al., 2010)) and fD (Martin et al., 2013), and the identification of shared haplotypes (e.g., (Guan, 2014; Hellenthal et al., 2014; Price et al., 2009; Turissini and Matute, 2017)). The former is amenable to all genomic analysis (including reduced representation approaches, reviewed in (Davey et al., 2011)), while the latter is better suited

for whole genome analyses but can be applied to reduced representation data in biological systems with extensive linkage disequilibrium and a reference genome (as is the case of humans; e.g., (Price et al., 2009)). Both approaches have been used in fungi, but summary statistics seem to be slightly more common than assessments of local ancestry (Table S3). A formal test of the magnitude of introgression across multiple species of fungi is sorely needed, but analyses of shared ancestry suggest that hybridization and introgression are of common occurrence in fungi. Similar to observations in animals, introgression seems to be less prevalent between diverged species (Maxwell et al., 2018b) than between recently diverged ones (Desjardins et al., 2017; Gladieux et al., 2015; Maxwell et al., 2018a) which follows expectations regarding the evolution of hybrid incompatibilities (Muirhead and Presgraves, 2016).

Why is it important to define species?

The ultimate goal of identifying species boundaries is not merely describing species by itself. Properly defining species boundaries is just the beginning of a robust research program in evolutionary biology. To understand diversification patterns in fungi, we first need to quantify the magnitude of extant diversity in the group. If cryptic species are particularly prevalent in fungi, then our understanding of macroevolutionary patterns—in fungi or any other taxon— will be warped by faulty species definitions. Similarly, in order to understand how fungal genomes evolve and how divergence unfolds, we need to have reliable species definitions that can be applied across taxa. Even our understanding of how genetic diversity is portioned across populations and species will be misinformed if species boundaries are not properly defined.

Properly defining species boundaries also has strong implications for other areas of biology. Cryptic species tend to be phenotypically similar, but this is not always the case. Moreover, since it is impossible to exhaustively measure the phenotype of an organism, it is difficult to rule out the possibility that they may differ in some important aspect. In fact, in hindsight some previously cryptic species differ in phenotypes that are medically or economically significant. Fungal pathogens provide an example of the correct delimitation of species boundaries providing insight into pathogenesis. For example, the application of the SGC approach in *Magnaporthe grisea* revealed the existence of two species, *M. oryzae* and *M. grisea sensu stricto* (Couch and Kohn, 2002). While the former infects rice cultivars, the latter is restricted to crabgrass *(Digitaria).* Notably, *M. oryzae* might contain multiple lineages, each of which is preferentially associated with different hosts, which in turns suggests incipient speciation following host shifts (Gladieux et al., 2018). Similarly, species of *Histoplasma* differ in their virulence, resistance to antifungals, and in some instances genome size (Kasuga et al., 2002; Goughenour et al., 2015; Sepúlveda et al., 2017, 2014). In contrast, the sibling species of the genus *Coccidioides, C. immitis* and *C. posadasii,* are deeply genetically differentiated but few phenotypic differences have been identified (Fisher et al., 2002; Neafsey et al., 2010; Ramani and Chaturvedi, 2007; Sharpton et al., 2009). Despite overlapping geographic ranges, which could facilitate introgression, these species share few alleles, indicating rare hybridization (Maxwell et al., 2018b; Neafsey et al., 2010).

Taxonomy is a hypothesis and if proposed species cannot be verified or are not used they can simply be archived. This is perhaps the ultimate 'species concept': if a species classification is evidence-based and turns out to be useful to researchers and others, then it will persist and spread. By infusing taxonomy with genetics and genomics, we can make the concept of 'species' in fungi more useful by making it more robust, testable, comparable to other taxa, and devoid of the personal attachments that researchers might feel to particular species names.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

ACKNOWLEDGEMENTS

We would like to thank Colin S. Maxwell, Juan G. McEwen and William Goldman for their comments on the manuscript. Three anonymous reviewers helped tremendously improve every aspect of this manuscript. This work was funded by grant R01GM121750 to DRM.

REFERENCES

Alamouti SM, Wang V, Diguistini S, Six DL, Bohlmann J, Hamelin RC, Feau N, Breuil C, 2011 Gene genealogies reveal cryptic species and host preferences for the pine fungal pathogen Grosmannia clavigera. Mol. Ecol 20(12): 2581–2602. 10.1111/j.1365-294X.2011.05109.x [PubMed: 21557782]

Alcoba-Flórez J, Méndez-Álvarez S, Cano J, Guarro J, Pérez-Roth E, Del Pilar Arévalo M, 2005 Phenotypic and molecular characterization of *Candida nivariensis* sp. nov., a possible new opportunistic fungus. J. Clin. Microbiol 43(8): 4107–4111. 10.1128/JCM.43.8.4107-4111.2005 [PubMed: 16081957]

Almeida P, Barbosa R, Bensasson D, Gonçalves P, Sampaio JP, 2017 Adaptive divergence in wine yeasts and their wild relatives suggests a prominent role for introgressions and rapid evolution at noncoding sites. Mol. Ecol 26(7): 2167–2182. 10.1111/mec.14071. [PubMed: 28231394]

Alors D, Lumbsch HT, Divakar PK, Leavitt SD, Crespo A, 2016 An integrative approach for understanding diversity in the *Punctelia rudecta* species complex (Parmeliaceae, Ascomycota). PLoS One. 11(2): e0146537 10.1371/journal.pone.0146537 [PubMed: 26863231]

Alves A, Crous PW, Correia A, Phillips a J.L., 2008 Morphological and molecular data reveal cryptic speciation in *Lasiodiplodia theobromae*. Fungal Diversity. 28: 1–13.

Ané C, Larget B, Baum DA, Smith SD, Rokas A 2006 Bayesian estimation of concordance among gene trees. Mol. Biol. Evol 24(2):412–426. 2007 doi:10.1093/molbev/msl170 [PubMed: 17095535]

Avise J, Ball RM, 1990 Principles of genealogical concordance in species concepts and biological taxonomy. Oxford Surv. Evol. Biol 7: 45–67

Avise JC, Wollenberg K, 1997 Phylogenetics and the origin of species. Proc. Natl. Acad. Sci 94 (15) 7748–7755. 10.1073/pnas.94.15.7748 [PubMed: 9223259]

Baranova MA, Logacheva MD, Penin AA, Seplyarskiy VB, Safonova YY, Naumenko SA, Klepikova AV, Gerasimov ES, Bazykin GA, James TY, Kondrashov AS, 2015 Extraordinary genetic diversity in a wood decay mushroom. Mol. Biol. Evol 32(10): 2775–2783. 10.1093/molbev/msv153 [PubMed: 26163667]

Barraclough TG, Birky CW, Burt A, 2003 Diversification in sexual and asexual to organisms. Evolution. 57(9): 2166–2172 10.1111/j.0014-3820.2003.tb00394.x [PubMed: 14575336]

Baum DA, Shaw KL, 1995 Genealogical perspectives on the species problem. Experimental and Molecular Approaches to Plant Biosystematics. (Hoch PC and Stephenson AG, eds.) Monogr. Syst, Missouri Bot. Gard 53: 289–303.

Baum DA, 2007 Concordance trees, concordance factors, and the exploration of reticulate genealogy. Taxon. 56(2): 417–426. 10.1007/sl0869-007-9037-x

Baum DA, Donoghue MJ, 1995 Choosing among alternative “phylogenetic” species concepts. Syst. Bot 20(4): 560–573.

Bazzicalupo AL, Buyck B, Saar I, Vauras J, Carmean D, Berbee ML, 2017 Troubles with mycorrhizal mushroom identification where morphological differentiation lags behind barcode sequence divergence. Taxon. 66(4): 791–810. 10.12705/664.1

Bickford D, Lohman DJ, Sodhi NS, Ng PKL, Meier R, Winker K, Ingram KK, Das I, 2007 Cryptic species as a window on diversity and conservation. Trends Ecol. Evol 22(3): 148–155. 10.1016/j.tree.2006.11.004 [PubMed: 17129636]

Bidochka MJ, Kamp AM, Lavender TM, Dekoning J, De Croos JNA, 2001 Habitat association in two genetic groups of the insect-pathogenic fungus *Metarhizium anisopliae*: uncovering cryptic species? Appl. Environ. Microbiol 67(3): 1335–1342. 10.1128/AEM.67.3.1335-1342.2001 [PubMed: 11229929]

Bidochka MJ, Small CLN, Spironello M, 2005 Recombination within sympatric cryptic species of the insect pathogenic fungus Metarhizium anisopliae. Environ. Microbiol 7(9): 1361–1368. 10.1111/j.1462-5822.2005.00823.x [PubMed: 16104859]

Birky CW Jr, 2013 Species detection and identification in sexual organisms using population genetic theory and DNA sequences. PLoS One. 8(1): e52544 10.1371/journal.pone.0052544 [PubMed: 23308113]

Bischoff JF, Rehner SA, Humber RA, 2006 *Metarhizium frigidum* sp. nov.: a cryptic species of *M. anisopliae* and a member of the *M. flavoviride* complex. Mycologia. 98(5): 737–745. 10.3852/mycologia.98.5.737 [PubMed: 17256577]

Bobay L-M, Ochman H, 2017 Biological Species Are Universal across Life’s Domains. Genome Biol. Evol 9(3): 491–501. 10.1093/gbe/evx026

Boluda CG, Rico VJ, Divakar PK, Nadyeina O, Myllys L, McMullin RT, Zamora JC, Scheidegger C, Hawksworth DL, 2018 Evaluating methodologies for species delimitation: the mismatch between phenotypes and genotypes in lichenized fungi (*Bryoria* sect. *Implexae, Parmeliaceae*). Persoonia - Mol. Phylogeny Evol. Fungi. 42(2019): 75–100. 10.3767/persoonia.2019.42.04

Brown EM, McTaggart LR, Zhang SX, Low DE, Stevens DA, Richardson SE, 2013 Phylogenetic analysis reveals a cryptic species *Blastomyces gilchristii*, sp. nov. within the human pathogenic fungus *Blastomyces dermatitidis*. PLoS One. 8(3): e59237 10.1371/journal.pone.0059237 [PubMed: 23533607]

Burns M, Starrett J, Derkarabetian S, Richart CH, Cabrero A, Hedin M, 2017 Comparative performance of double-digest RAD sequencing across divergent arachnid lineages. Mol. Ecol. Resour 17(3): 418–430. 10.1111/1755-0998.12575 [PubMed: 27454533]

Cai L, Giraud T, Zhang N, Begerow D, Cai G, Shivas RG, 2011 The evolution of species concepts and species recognition criteria in plant pathogenic fungi. Fungal Divers. 5: 121 10.1007/s13225-011-0127-8

Carneiro M, Albert FW, Afonso S, Pereira RJ, Burbano H, Campos R, Melo-Ferreira J, Blanco Aguiar JA, Villafuerte R, Nachman MW, Good JM, Ferrand N, 2014 The genomic architecture of population divergence between subspecies of the European rabbit. PLoS Genet. 10(8), e1003519. doi:10.1371/journal.pgen.1003519 [PubMed: 25166595]

Carneiro M, Blanco Aguiar JA, Villafuerte R, Ferrand N, Nachman MW, 2010 Speciation in the european rabbit (Oryctolagus cuniculus): islands of differentiation on the X chromosome and autosomes. Evolution. 64(12): 3443–3460. doi:10.1111/j.1558-5646.2010.01092.x [PubMed: 20666840]

Carriconde F, Gardes M, Jargeat P, Heilmann-Clausen J, Mouhamadou B, Gryta H, 2008 Population evidence of cryptic species and geographical structure in the cosmopolitan ectomycorrhizal fungus, Tricholoma scalpturatum. Microb. Ecol 56(3): 513–524. 10.1007/s00248-008-9370-2 [PubMed: 18305983]

Carstens BC, Knowles LL, 2007 Estimating species phylogeny from gene-tree probabilities despite incomplete lineage sorting: An example from Melanoplus grasshoppers. Syst. Biol 56(3): 400–411. 10.1080/10635150701405560 [PubMed: 17520504]

Chen J, Guo SX, Liu PG, 2011 Species recognition and cryptic species in the *Tuber indicum* complex. PLoS One. 6(1): e14625 10.1371/journal.pone.0014625 [PubMed: 21297969]

Chou J-Y, Hung Y-S, Lin K-H, Lee H-Y, Leu J-Y, 2010 Multiple molecular mechanisms cause reproductive isolation between three yeast species. PLoS Biol. 8(7): e1000432 10.1371/journal.pbio.1000432 [PubMed: 20652018]

Chung Y, Ané C, 2011 Comparing two bayesian methods for gene tree/species tree reconstruction: Simulations with incomplete lineage sorting and horizontal gene transfer. Syst. Biol 60(3): 261–275. 10.1093/sysbio/syr003 [PubMed: 21368324]

Corcoran P, Anderson JL, Jacobson DJ, Sun Y, Ni P, Lascoux M, Johannesson H, 2016 Introgression maintains the genetic integrity of the mating-type determining chromosome of the fungus Neurospora tetrasperma. Genome Res. 26(4): 486–98. doi: 10.1101/gr.197244.11. [PubMed: 26893460]

Correia A, Sampaio P, James S, Pais C, 2006 Candida bracarensis sp. nov., a novel anamorphic yeast species phenotypically similar to *Candida glabrata*. Int. J. Syst. Evol. Microbiol 56: 313–317. 10.1099/ijs.0.64076-0 [PubMed: 16403904]

Couch BC, Kohn LM, 2002 A multilocus gene genealogy concordant with host preference indicates segregation of a new species, *Magnaporthe oryzae*, from *M. grisea*. Mycologia. 94:4, 683–693. 10.1080/15572536.2003.11833196 [PubMed: 21156541]

Coyne JA, Orr HA, 2004 Speciation. Sunderland, MA 1–475.

Crous PW, Groenewald JZ, Pongpanich K, Himaman W, Arzanlou M, Wingfield MJ, 2004 Cryptic speciation and host specificity among *Mycosphaerella* spp. occurring on Australian *Acacia* species grown as exotics in the tropics. Studies in Mycology. 50: 457–469.

Crespo A and Lumbsch HT, 2010 Cryptic species in lichen-forming fungi. IMA fungus, 1(2), pp.167–170. [PubMed: 22679576]

Cruse M, Telerant R, Gallagher T, Lee T, Taylor JW, 2002 Cryptic species in *Stachybotrys chartarum*. Mycologia. 94(5): 814–822. 10.1080/15572536.2003.11833175 [PubMed: 21156555]

Davey JW, Hohenlohe PA, Etter PD, Boone JQ, Catchen JM, Blaxter ML, 2011 Genome-wide genetic marker discovery and genotyping using next-generation sequencing. Nat. Rev. Genet 12: 499–510. 10.1038/nrg3012. [PubMed: 21681211]

Del-Prado R, Divakar PK, Lumbsch HT, Crespo AM, 2016 Hidden genetic diversity in an asexually reproducing lichen forming fungal group. PLoS One. 11(8): e0161031 10.1371/journal.pone.0161031 [PubMed: 27513649]

De Queiroz K, 2007 Species concepts and species delimitation. Syst. Biol 56(6):879–886 10.1080/10635150701701083 [PubMed: 18027281]

Desjardins CA, Champion MD, Holder JW, Muszewska A, Goldberg J, Bailao AM, Brigido MM, da Silva Ferreira ME, Garcia AM, Grynberg M, Gujja S, Heiman DI, Henn MR, Kodira CD, Leon-Narvaez H, Longo LVG, Ma LJ, Malavazi I, Matsuo AL, Morais FV, Pereira M, Rodriguez-Brito S, Sakthikumar S, Salem-Izacc SM, Sykes SM, Teixeira MM, Vallejo MC, Walter MEMT, Yandava C, Young S, Zeng Q, Zucker J, Felipe MS, Goldman GH, Haas BJ, McEwen JG, Nino-Vega G, Puccia R, San-Blas G, de Soares CMA, Birren BW, Cuomo CA, 2011 Comparative genomic analysis of human fungal pathogens causing paracoccidioidomycosis. PLoS Genet. 7(10): e1002345 10.1371/journal.pgen.1002345 [PubMed: 22046142]

Desjardins CA, Giamberardino C, Sykes SM, Yu C-H, Tenor JL, Chen Y, Yang T, Jones AM, Sun S, Haverkamp MR, Heitman J, Litvintseva AP, Perfect JR, Cuomo CA, 2017 Population genomics and the evolution of virulence in the fungal pathogen Cryptococcus neoformans. Genome Res. 27, 1207–1219. 10.1101/gr.218727.116 [PubMed: 28611159]

Dettman JR, Jacobson DJ, Taylor JW, 2006 Multilocus sequence data reveal extensive phylogenetic species diversity within the Neurospora discreta complex. Mycologia. 98(3): 436–446. 10.1080/15572536.2006.11832678 [PubMed: 17040072]

Dettman JR, Jacobson DJ, Taylor JW, 2003a A multilocus genealogical approach to phylogenetic species recognition in the model eukaryote Neurospora. Evolution. 57(12): 2703–2720. 10.1111/j.0014-3820.2003.tb01514.x [PubMed: 14761051]

Dettman JR, Jacobson DJ, Turner E, Pringle A, Taylor JW, 2003b Reproductive isolation and phylogenetic divergence in Neurospora: Comparing methods of species recognition in a model eukaryote. Evolution. 57(12): 2721–2741. 10.1111/j.0014-3820.2003.tb01515. [PubMed: 14761052]

Dobzhansky T 1937 Genetics and the Origin of Species. Columbia University Press.

Donoghue MJ, 1985 A critique of the biological species concept and recommendations for a phylogenetic alternative. Bryologist. 88(3): 172–181. 10.2307/3243026

Dobzhansky T 1937 Genetics and the Origin of Species. Columbia University Press. Donoghue, M.J., 1985. A critique of the biological species concept and recommendations for a phylogenetic alternative. Bryologist. 88(3): 172–181. 10.2307/3243026

Douanla-Meli C, Unger JG, Langer E, 2018 Multi-approach analysis of the diversity in *Colletotrichum cliviae* sensu lato. Antonie van Leeuwenhoek, Int. J. Gen. Mol. Microbiol. 111(3): 423–435. 10.1007/s10482-017-0965-9

Dupuis JR, Roe AD, Sperling FAH, 2012 Multi-locus species delimitation in closely related animals and fungi: One marker is not enough. Mol. Ecol 21: 4422–4436. 10.1111/j.1365-294X.2012.05642.x [PubMed: 22891635]

Edwards SV, Liu L, Pearl DK, 2007 High-resolution species trees without concatenation. Proc. Natl. Acad. Sci 104(14): 5936–5941. 10.1073/pnas.0607004104 [PubMed: 17392434]

Ence DD, Carstens BC, 2011 SpedeSTEM: A rapid and accurate method for species delimitation. Mol. Ecol. Resour 11(3): 473–480. 10.1111/j.1755-0998.2010.02947.x [PubMed: 21481205]

Fisher MC, Koenig GL, White TJ, Taylor JW, 2002 Molecular and phenotypic description of Coccidioides posadasii sp. nov., previously recognized as the non-California population of *Coccidioides immitis*. Mycologia. 94(1): 73–84. 10.1080/15572536.2003.11833250 [PubMed: 21156479]

Fontaneto D, Herniou EA, Boschetti C, Caprioli M, Melone G, Ricci C, Barraclough TG, 2007 Independently evolving species in asexual bdelloid rotifers. PLoS Biol. 5(4): e87 10.1371/journal.pbio.0050087 [PubMed: 17373857]

Fujisawa T, Barraclough TG, 2013 Delimiting species using single-locus data and the generalized mixed yule coalescent approach: A revised method and evaluation on simulated data sets. Syst. Biol 62(5): 707–724. 10.1093/sysbio/syt033 [PubMed: 23681854]

Fujita MK, Leaché AD, Burbrink FT, McGuire JA, Moritz C, 2012 Coalescent-based species delimitation in an integrative taxonomy. Trends Ecol. Evol 27(9): 480–488. 10.1016/j.tree.2012.04.012 [PubMed: 22633974]

Gao Z, Przeworski M, Sella G, 2015 Footprints of ancient-balanced polymorphisms in genetic variation data from closely related species. Evolution 69, 431–446. doi:10.1111/evo.12567 [PubMed: 25403856]

Gazis R, Rehner S, Chaverri P, 2011 Species delimitation in fungal endophyte diversity studies and its implications in ecological and biogeographic inferences. Mol. Ecol 20(14): 3001–3013. 10.1111/j.1365-294X.2011.05110.x [PubMed: 21557783]

Geiser DM, Pitt JI, Taylor JW, 1998 Cryptic speciation and recombination in the aflatoxin-producing fungus *Aspergillus flavus*. Proc. Natl. Acad. Sci 95(1): 388–393. 10.1073/pnas.95.1388 [PubMed: 9419385]

Gerlach A da CL, Toprak Z, Naciri Y, Caviro EA., da Silveira RMB, Clerc P, 2019 New insights into the *Usnea cornuta* aggregate (Parmeliaceae, lichenized Ascomycota): Molecular analysis reveals high genetic diversity correlated with chemistry. Mol. Phylogenet. Evol 131: 125–137. 10.1016/j.ympev.2018.10.035 [PubMed: 30385309]

Gilgado F, Cano J, Gene J, Guarro J, 2005 Molecular phylogeny of the *Pseudallescheria boydii* species complex: Proposal of two new species. J. Clin. Microbiol 43(10): 4930–4942. doi:10.1128/JCM.43.10.4930-4942.2005 [PubMed: 16207945]

Giraud T, Gladieux P, Hood M, 2010 The origin of species in Fungi. Fungi. 3(4): 23–27.

Giraud T, Gladieux P, Hood M, 2010 The origin of species in Fungi. Fungi. 3(4): 23–27.

Gladieux P, Condon B, Ravel S, Soanes D, Maciel JLN, Nhani A, Chen L, Terauchi R, Lebrun MH, Tharreau D, Mitchell T, Pedley KF, Valent B, Talbot NJ, Farman M, Fournier E, 2018 Gene flow between divergent cereal- and grass-specific lineages of the rice blast fungus *Magnaporthe oryzae*. mBio. 9:e01219–17. 10.1128/mBio.01219-17 [PubMed: 29487238]

Gladieux P, Wilson BA, Perraudeau F, Montoya LA, Kowbel D, Hann-Soden C, Fischer M, Sylvain I, Jacobson DJ, Taylor JW, 2015 Genomic sequencing reveals historical, demographic and selective

factors associated with the diversification of the fire-associated fungus Neurospora discreta. Mol. Ecol 24, 5657–5675. 10.1111/mec.13417 [PubMed: 26453896]

Goughenour KD, Balada-Llasat JM, Rappleye CA, 2015 Quantitative microplate-based growth assay for determination of antifungal susceptibility of *Histoplasma capsulatum* yeasts. J Clin Microbiol 53(10):3286–3295. doi:10.1128/JCM.00795-15. [PubMed: 26246483]

Green RE, Krause J, Briggs AW, Maricic T, Stenzel U, Kircher M, Patterson N, Li H, Zhai W, Fritz MH-Y, Hansen NF, Durand EY, Malaspinas A-S, Jensen JD, Marques-Bonet T, Alkan C, Prüfer K, Meyer M, Burba no HA, Good JM, Schultz R, Aximu-Petri A, Butthof A, Hober B, Hoffner B, Siegemund M, Weihmann A, Nusbaum C, Lander ES, Russ C, Novod N, Affourtit J, Egholm M, Verna C, Rudan P, Brajkovic D, Kucan Z, Gusic I, Doronichev VB, Golovanova LV, Lalueza-Fox C, de la Rasilla M, Fortea J, Rosas A, Schmitz RW, Johnson PLF, Eichler EE, Falush D, Birney E, Mullikin JC, Slatkin M, Nielsen R, Kelso J, Lachmann M, Reich D, Paabo S, 2010 A Draft Sequence of the Neandertal Genome. Science. 328(5979): 710–722. doi: 10.1126/science.1188021 [PubMed: 20448178]

Groenewald M, Groenewald JZ, Crous PW, 2005 Distinct species exist within the *Cercospora apii* morphotype. Phytopathology. 95(8):951–959. 10.1094/PHYTO-95-0951 [PubMed: 18944418]

Grube M and Kroken S, 2000 Molecular approaches and the concept of species and species complexes in lichenized fungi. Mycological Research, 104(11), pp.1284–1294.

Grünig CR, Duò A, Sieber TN, 2006 Population genetic analysis of Phialocephala fortinii s.l. and Acephala applanata in two undisturbed forests in Switzerland and evidence for new cryptic species. Fungal Genet. Biol 43(6): 410–421. 10.1016/j.fgb.2006.01.007 [PubMed: 16631398]

Grünig CR, McDonald BA, Sieber TN, Rogers SO, Holdenrieder O, 2004 Evidence for subdivision of the root-endophyte Phialocephala fortinii into cryptic species and recombination within species. Fungal Genet. Biol 41(7): 676–687. 10.1016/j.fgb.2004.03.004 [PubMed: 15275663]

Guan Y, 2014 Detecting structure of haplotypes and local ancestry. Genetics. 196(3): 625–642. doi: 10.1534/genetics.113.160697 [PubMed: 24388880]

Guerrero RF, Hahn MW, 2018 Quantifying the risk of hemiplasy in phylogenetic inference. Proc. Natl. Acad. Sci. 115(50): 12787–12792. 10.1073/pnas.1811268115 [PubMed: 30482861]

Guillin EA, Grijalba PE, Oliveira L.O. de, Gottlieb AM, 2014 Specific boundaries between the causal agents of the soybean stem canker. Trop. Plant Pathol 39(4):316–325. 10.1590/s1982-56762014000400006

Hagen F, Khayhan K, Theelen B, Kolecka A, Polacheck I, Sionov E, Falk R, Parnmen S, Lumbsch HT, Boekhout T, 2015 Recognition of seven species in the *Cryptococcus gattii/Cryptococcus neoformans* species complex. Fungal Genet. Biol 75: 16–48 10.1016/j.fgb.2015.02.009

Harrington TC and Rizzo DM, 1999 Defining species in the fungi In Structure and dynamics of fungal populations (pp. 43–71). Springer, Dordrecht.

Hellenthal G, Busby GBJ, Band G, Wilson JF, Capelli C, Falush D, Myers S, 2014 A genetic atlas of human admixture history. Science. 343(6172): 747–751. doi: 10.1126/science.1243518. [PubMed: 24531965]

Hillis DM, Bull JJ, 1993 An empirical test of bootstrapping as a method for assessing confidence in phylogenetic analysis. Syst. Biol 42(2): 182–192. 10.1093/sysbio/42.2.182

Holmes S, 2003 Bootstrapping phylogenetic trees: theory and methods. Stat. Sci 18(2): 241–255. 10.1214/ss/1063994979

Hubka V, Nováková A, Jurjević Z, Sklenář F, Frisvad JC, Houbraken J, Arendrup MC, Jørgensen KM, Siqueira JPZ, Gené J, Kolařík M, 2018 Polyphasic data support the splitting of Aspergillus candidus into two species; proposal of *Aspergillus dobrogensis* sp. nov. Int. J. Syst. Evol. Microbiol 68: 995–1011. 10.1099/ijsem.0.002583 [PubMed: 29458472]

Hudson RR, Coyne JA, 2002 Mathematical consequences of the genealogical species concept. Evolution. 56(8): 1557–1565. 10.1111/j.0014-3820.2002.tb01467.x. [PubMed: 12353748]

Hughes KW, Petersen RH, Lickey EB, 2009 Using heterozygosity to estimate a percentage DNA sequence similarity for environmental species' delimitation across basidiomycete fungi. New Phytol. 10.1111/j.1469-8137.2009.02802.x

Huxley J, 1943 Systematics and the origin of Species: from the Viewpoint of a Zoologist. Nature. 151, 347–348. 10.1038/151347a0

Jackson ND, Carstens BC, Morales AE, O'Meara BC, 2017 Species delimitation with gene flow. Syst. Biol 66(5): 799–812. 10.1093/sysbio/syw117 [PubMed: 28003535]

Jones G, 2017 Algorithmic improvements to species delimitation and phylogeny estimation under the multispecies coalescent. J. Math. Biol 74: 447–467. 10.1007/S00285-016-1034-0 [PubMed: 27287395]

Jones G, Aydin Z, Oxelman B, 2015 DISSECT: An assignment-free Bayesian discovery method for species delimitation under the multispecies coalescent. Bioinformatics. 31(7): 991–998. 10.1093/bioinformatics/btu770 [PubMed: 25422051]

Kasuga T, White TJ, Koenig G, Mcewen J, Restrepo A, Castaneda E, Da Silva Lacaz CDA, Heins-Vaccari EM, De Freitas RS, Zancope-Oliveira RM, Qin Z, Negroni R, Carter DA, Mikami Y, Tamura M, Taylor ML, Miller GF, Poonwan N, Taylor JW, 2003 Phylogeography of the fungal pathogen Histoplasma capsulatum. Mol. Ecol 12: 3383–3401. 10.1046/j.1365-294X.2003.01995.x [PubMed: 14629354]

Kasuga T, White TJ, Taylor JW, 2002 Estimation of nucleotide substitution rates in Eurotiomycete fungi. Mol. Biol. Evol 19(12): 2318–2324. 10.1093/oxfordjournals.molbev.a004056 [PubMed: 12446823]

Knowles LL, Carstens BC, 2007 Delimiting species without monophyletic gene trees. Syst. Biol 56(6): 887–895. 10.1080/10635150701701091 [PubMed: 18027282]

Koufopanou V, Burt A, Szaro T, Taylor JW, 2001 Gene genealogies, cryptic species, and molecular evolution in the human pathogen *Coccidioides immitis* and relatives (Ascomycota, Onygenales). Mol. Biol. Evol 18(7): 1246–1258. 10.1093/oxfordjournals.molbev.a003910 [PubMed: 11420364]

Kubatko LS, Carstens BC, Knowles LL, 2009 STEM: Species tree estimation using maximum likelihood for gene trees under coalescence. Bioinformatics. 25(7): 971–973. 10.1093/bioinformatics/btp079 [PubMed: 19211573]

Kubatko LS, Degnan JH, 2007 Inconsistency of phylogenetic estimates from concatenated data under coalescence. Syst. Biol 56(1): 17–24. 10.1080/10635150601146041 [PubMed: 17366134]

Larget BR, Kotha SK, Dewey CN, Ane C, 2010 BUCKy: Gene tree/species tree reconciliation with Bayesian concordance analysis. Bioinformatics. 26(22): 2910–2911. 10.1093/bioinformatics/btq539 [PubMed: 20861028]

Laurence MH, Summerell BA, Burgess LW, Liew ECY, 2014 Genealogical concordance phylogenetic species recognition in the *Fusarium oxysporum* species complex. Fungal Biol. 118(4): 374–384. 10.1016/j.funbio.2014.02.002 [PubMed: 24742832]

Le Gac M, Hood ME, Giraud T, 2007a Evolution of reproductive isolation within a parasitic fungal species complex. Evolution. 61(7): 1781–1787. 10.1111/j.1558-5646.2007.00144.x [PubMed: 17598756]

Le Gac M, Hood MEME, Fournier E, Giraud T, 2007b Phylogenetic evidence of host-specific cryptic species in the anther smut fungus. Evolution. 61(1): 15–26. 10.1111/j.1558-5646.2007.00002.x [PubMed: 17300424]

Leaché AD, Zhu T, Rannala B, Yang Z, 2019 The Spectre of too many species. Syst. Biol 68(1): 168–181. 10.1093/sysbio/syy051 [PubMed: 29982825]

Leavitt S, Fernández-Mendoza F, Pérez-Ortega S, Sohrabi M, Divakar P, Lumbsch T, St. Clair L, 2013a DNA barcode identification of lichen-forming fungal species in the Rhizoplaca melanophthalma species-complex (Lecanorales, Lecanoraceae), including five new species. MycoKeys. 7: 1–22. 10.3897/mycokeys.7.4508

Leavitt SD, Esslinger TL, Divakar PK, Crespo A, Lumbsch HT, 2016 Hidden diversity before our eyes: Delimiting and describing cryptic lichen-forming fungal species in camouflage lichens (Parmeliaceae, Ascomycota). Fungal Biol 120(11): 1374–1391. 10.1016/j.funbio.2016.06.001 [PubMed: 27742095]

Leavitt SD, Fankhauser JD, Leavitt DH, Porter LD, Johnson LA, St. Clair LL, 2011 Complex patterns of speciation in cosmopolitan "rock posy" lichens - Discovering and delimiting cryptic fungal species in the lichen-forming *Rhizoplaca melanophthalma* species-complex (Lecanoraceae, Ascomycota). Mol. Phylogenet. Evol 59(3): 587–602. 10.1016/j.ympev.2011.03.020 [PubMed: 21443956]

Leavitt SD, Fernández-Mendoza F, Pérez-Ortega S, Sohrabi M, Divakar PK, Vondrak J, Thorsten Lumbsch H, Clair LLS, 2013b Local representation of global diversity in a cosmopolitan lichen-forming fungal species complex (Rhizoplaca, Ascomycota). J. Biogeogr 40(9): 1792–1806. 10.1111/jbi.12118

Leavitt SD, Westberg M, Nelsen MP, Elix JA, Timdal E, Sohrabi M, St. Clair LL, Williams L, Wedin M, Lumbsch HT, 2018 Multiple, distinct intercontinental lineages but isolation of Australian populations in a cosmopolitan lichen-forming Fungal Taxon, *Psora decipiens* (Psoraceae, Ascomycota). Front. Microbiol 9: 283 10.3389/fmicb.2018.00283 [PubMed: 29527197]

Leducq JB, Nielly-Thibault L, Charron G, Eberlein C, Verta JP, Samani P, Sylvester K, Hittinger CT, Bell G, Landry CR, 2016 Speciation driven by hybridization and chromosomal plasticity in a wild yeast. Nat. Microbiol 1:1–10. 10.1038/nmicrobiol.2015.3

Lee H-Y, Chou J-Y, Cheong L, Chang N-H, Yang S-Y, Leu J-Y, 2008 Incompatibility of nuclear and mitochondrial genomes causes hybrid sterility between two yeast species. Cell. 135(6): 1065–1073. 10.1016/j.cell.2008.10.047. [PubMed: 19070577]

Leffler EM, Bullaughey K, Matute DR, Meyer WK, Segurel L, Venkat A, Andolfatto P, Przeworski M, 2012 Revisiting an old riddle: what determines genetic diversity levels within species? PLoS Biol. 10(9): e1001388 10.1371/journal.pbio.1001388 [PubMed: 22984349]

Lemoine F, Domelevo Entfellner JB, Wilkinson E, Correia D, Davila Felipe M, De Oliveira T, Gascuel O, 2018 Renewing Felsenstein's phylogenetic bootstrap in the era of big data. Nature. 556: 452–456. 10.1038/s41586-018-0043-0 [PubMed: 29670290]

Li YM, Shivas RG, Cai L, 2017 Cryptic diversity in *Tranzscheliella* spp. (Ustilaginales) is driven by host switches. Sci. Rep 7: 43549 10.1038/srep43549 [PubMed: 28256543]

Liti G, Barton DBH, Louis EJ, 2006 Sequence diversity, reproductive isolation and species concepts in *Saccharomyces*. Genetics. 174(2): 839–850. 10.1534/genetics.106.062166 [PubMed: 16951060]

Liu F, Wang M, Damm U, Crous PW, Cai L, 2016 Species boundaries in plant pathogenic fungi: A *Colletotrichum* case study. BMC Evol. Biol 16(81). 10.1186/s12862-016-0649-5

Liu L, 2008 BEST: Bayesian estimation of species trees under the coalescent model. Bioinformatics. 24(21): 2542–2543.10.1093/bioinformatics/btn484 [PubMed: 18799483]

Liu L, Edwards SV, 2009 Phylogenetic analysis in the anomaly zone. Syst. Biol 58(4): 452–460. 10.1093/sysbio/syp034 [PubMed: 20525599]

Lombard L, Crous PW, Wingfield BD, Wingfield MJ, 2010 Multigene phylogeny and mating tests reveal three cryptic species related to Calonectria pauciramosa. Stud. Mycol 66: 15–30. 10.3114/sim.2010.66.02 [PubMed: 20806004]

Lucking R, Dal-Forno M, Sikaroodi M, Gillevet PM, Bungartz F, Moncada B, Yanez-Ayabaca A, Chaves JL, Coca LF, Lawrey JD, 2014 A single macrolichen constitutes hundreds of unrecognized species. Proc. Natl. Acad. Sci 111(30). 11091–11096. 10.1073/pnas.1403517111 [PubMed: 24982168]

Ludlow CL, Cromie GA, Garmendia-Torres C, Sirr A, Hays M, Field C, Jeffery EW, Fay JC, Dudley AM, 2016 Independent origins of yeast associated with coffee and cacao fermentation. Curr. Biol 26(7): 965–971. 10.1016/j.cub.2016.02.012 [PubMed: 27020745]

Marimon R, Cano J, Gene J, Sutton DA, Kawasaki M, Guarro J, 2007 *Sporothrix brasiliensis, S. globosa*, and *S. mexicana*, three new Sporothrix species of clinical interest. J. Clin. Microbiol 45(10): 3198–3206. 10.1128/JCM.00808-07 [PubMed: 17687013]

Martin SH, Dasmahapatra KK, Nadeau NJ, Salazar C, Walters JR, Simpson F, , Blaxter M, Manica A, Mallet J, Jiggins CD, 2013 Genome-wide evidence for speciation with gene flow in *Heliconius* butterflies. Genome Res. 23(11): 1817–1828. doi: 10.1101/gr.159426.113 [PubMed: 24045163]

Martinez D, Berka RM, Henrissat B, Saloheimo M, Arvas M, Baker SE, Chapman J, Chertkov O, Coutinho PM, Cullen D, Danchin EGJ, Grigoriev IV, Harris P, Jackson M, Kubicek CP, Han CS, Ho I, Larrondo LF, De Leon AL, Magnuson JK, Merino S, Misra M, Nelson B, Putnam N, Robbertse B, Salamov AA, Schmoll M, Terry A, Thayer N, Westerholm-Parvinen A, Schoch CL, Yao J, Barbote R, Nelson MA, Detter C, Bruce D, Kuske CR, Xie G, Richardson P, Rokhsar DS, Lucas SM, Rubin EM, Dunn-Coleman N, Ward M, Brettin TS, 2008 Genome sequencing and analysis of the biomass-degrading fungus *Trichoderma reesei* (syn. *Hypocrea jecorina*). Nat. Biotechnol 26(5): 553–560. 10.1038/nbt1403 [PubMed: 18454138]

Matute DR, Butler IA, Turissini DA, Coyne JA, 2010 A test of the snowball theory for the rate of evolution of hybrid incompatibilities. Science. 329(5998): 1518–1521. 10.1126/science.1193440. [PubMed: 20847270]

Matute DR, Gavin-Smyth J, 2014 Fine mapping of dominant X-linked incompatibility alleles in Drosophila hybrids. PLoS Genet. 10(4): e1004270. doi:10.1371/journal.pgen.1004270 . [PubMed: 24743238]

Matute DR, McEwen JG, Puccia R, Montes BA, San-Blas G, Bagagli E, Rauscher JT, Restrepo A, Morais F, Nino-Vega G, Taylor JW, 2006 Cryptic speciation and recombination in the fungus Paracoccidioides brasiliensis as revealed by gene genealogies. Mol. Biol. Evol 23(1): 65–73. 10.1093/molbev/msj008 [PubMed: 16151188]

Maxwell CS, Mattox K, Turissini DA, Teixeira MM, Barker BM, Matute DR, 2018a Gene exchange between two divergent species of the fungal human pathogen, *Coccidioides*. Evolution. 73(1): 42–58. 10.1111/evo.13643 [PubMed: 30414183]

Maxwell CS, Sepúlveda VE, Turissini DA, Goldman WE, Matute DR, 2018b Recent admixture between species of the fungal pathogen Histoplasma. Evol. Lett 2(3): 210–220. 10.1002/evl3.59 [PubMed: 30283677]

Mehta RS, Bryant D, Rosenberg NA. 2016 The probability of monophyly of a sample of gene lineages on a species tree. Proc. Natl. Acad. Sci 7 19;113(29):8002–9. [PubMed: 27432988]

Mendes FK, Hahn MW, 2018 Why concatenation fails near the anomaly zone. Syst. Biol 67(1): 158–169. 10.1093/sysbio/syx063 [PubMed: 28973673]

Millanes AM, Truong C, Westberg M, Diederich P, Wedin M, 2014 Host switching promotes diversity in host-specialized mycoparasitic fungi: Uncoupled evolution in the biatoropsis-usnea system. Evolution. 68(6): 1576–1593. 10.1111/evo.12374 [PubMed: 24495034]

Mirarab S, Reaz R, Bayzid MS, Zimmermann TSSwenson M, Warnow T., 2014 ASTRAL: Genome-scale coalescent-based species tree estimation. Bioinformatics. 30(17): i541–i548. 10.1093/bioinformatics/btu462 [PubMed: 25161245]

Mishra B, Choi YJ, Thines M, 2018 Phylogenomics of Bartheletia paradoxa reveals its basal position in Agaricomycotina and that the early evolutionary history of basidiomycetes was rapid and probably not strictly bifurcating. Mycol. Prog 17(3): 333–341. 10.1007/s11557-017-1349-2

Mohanta TK, Bae H, 2015 The diversity of fungal genome. Biol. Proced. Online. 17:8 10.1186/s12575-015-0020-z [PubMed: 25866485]

Molina MC, Del-Prado R, Divakar PK, Sanchez-Mata D, Crespo A, 2011 Another example of cryptic diversity in lichen-forming fungi: The new species Parmelia mayi (Ascomycota: Parmeliaceae). Org. Divers. Evol 11(5): 331–342. 10.1007/s13127-011-0060-4

Moyle LC, Nakazato T, 2010 Hybrid incompatibility “Snowballs” between Solanum species. Science. 329(5998): 1521–1523. 10.1126/science.1193063 [PubMed: 20847271]

Muirhead CA, Presgraves DC, 2016 Hybrid incompatibilities, local adaptation, and the genomic distribution of natural introgression between species. Am. Nat 187(2): 249–261. 10.1086/684583 [PubMed: 26807751]

Neafsey DE, Barker BM, Sharpton TJ, Stajich JE, Park DJ, Whiston E, Hung C-Y, McMahan C, White J, Sykes S, Heiman D, Young S, Zeng Q, Abouelleil A, Aftuck L, Bessette D, Brown A, FitzGerald M, Lui A, Macdonald JP, Priest M, Orbach MJ, Galgiani JN, Kirkland TN, Cole GT, Birren BW, Henn MR, Taylor JW, Rounsley SD, 2010 Populatio n genomic sequencing of Coccidioides fungi reveals recent hybridization and transposon control. Genome Res. 20(7): 938–946. 10.1101/gr.103911.109 [PubMed: 20516208]

Nguyen NH, Landeros F, Garibay-Orijel R, Hansen K, Vellinga EC, 2013 The *Helvella lacunosa* species complex in western North America: cryptic species, misapplied names and parasites. Mycologia. 105(5): 1275–1286. 10.3852/12-391 [PubMed: 23709487]

Nixon KC, Wheeler QD, 1990 An amplification of the phylogenetic species concept. Cladistics. 6(3): 221–223. 10.1111/j.1096-0031.1990.tb00541.x

Nosil P, 2012 Ecological speciation, Ecological Speciation. 10.1093/acprof:osobl/9780199587100.001.0001

O’Donnell K, Kistler HC, Tacke BK, Casper HH, 2000 Gene genealogies reveal global phylogeographic structure and reproductive isolation among lineages of Fusarium graminearum,

the fungus causing wheat scab. Proc. Natl. Acad. Sci 97(14): 7905–7910. 10.1073/pnas.130193297 [PubMed: 10869425]

O'Donnell K, Sutton DA, Fothergill A, McCarthy D, Rinaldi MG, Brandt ME, Zhang N, Geiser DM, 2008 Molecular phylogenetic diversity, multilocus haplotype nomenclature, and in vitro antifungal resistance within the Fusarium solani Species complex. J. Clin. Microbiol 46(8): 2477–2490. 10.1128/JCM.02371-07 [PubMed: 18524963]

Oono R, Lutzoni F, Arnold AE, Kaye L, U'Ren JM, May G, Carbone I, 2014 Genetic variation in horizontally transmitted fungal endophytes of pine needles reveals population structure in cryptic species. Am. J. Bot 101(8): 1362–1374. 10.3732/ajb.1400141 [PubMed: 25156984]

Orr HA, 2006 Is single-eene speciation possible? Evolution. 45(3): 764–769. 10.2307/2409927

Parnmen S, Rangsiruji A, Mongkolsuk P, Boonpragob K, Nutakki A, Lumbsch HT, 2012 Using phylogenetic and coalescent methods to understand the species diversity in the Cladia aggregata complex (Ascomycota, Lecanorales). PLoS One. 7(12): e52245 10.1371/journal.pone.0052245 [PubMed: 23272229]

Payseur BA, Krenz JG, Nachman MW, 2004 Differential patterns of introgression across the X chromosome in a hybrid zone between two species of house mice. Evolution 58(9): 2064–2078. 10.1554/03-738 [PubMed: 15521462]

Pelin A, Selman M, Aris-Brosou S, Farinelli L, Corradi N, 2015 Genome analyses suggest the presence of polyploidy and recent human-driven expansions in eight global populations of the honeybee pathogen Nosema ceranae. Environ. Microbiol 15(11): 4443–4458. 10.1111/1462-2920.12883

Peris D, Langdon QK, Moriarty RV, Sylvester K, Bontrager M, Charron G, Leducq JB, Landry CR, Libkind D, Hittinger CT, 2016 complex ancestries of lager-brewing hybrids were shaped by standing variation in the wild yeast *Saccharomyces eubayanus*. PLoS Genet. 12(7): e1006155 10.1371/journal.pgen.1006155 [PubMed: 27385107]

Perrone G, Stea G, Epifani F, Varga J, Frisvad JC, Samson RA, 2011 *Aspergillus niger* contains the cryptic phylogenetic species *A. awamori*. Fungal Biol. 115(11): 1138–1150. 10.1016/j.funbio.2011.07.008 [PubMed: 22036292]

Phillips MJ, Delsuc F, Penny D, 2004 Genome-scale phylogeny and the detection of systematic biases. Mol. Biol. Evol 21(7): 1455–1458. 10.1093/molbev/msh137 [PubMed: 15084674]

Piedra-Malagón EM, Sosa V, Ibarra-Manríquez G, 2011 Clinal variation and species boundaries in the Ficus petiolaris Complex (Moraceae). Syst. Bot 31(1): 80–87. 10.1600/036364411X553153

Pino-Bodas R, Burgaz AR, Ahti T, Stenroos S, 2018 Taxonomy of *Cladonia angustiloba* and related species. Lichenologist. 50(3): 267–282. 10.1017/S002428291800018X

Pons J, Barraclough TG, Gomez-Zurita J, Cardoso A, Duran DP, Hazell S, Kamoun S, Sumlin WD, Vogler AP, 2006 Sequence-based species delimitation for the DNA taxonomy of undescribed insects. Syst. Biol 55(4): 595–609. 10.1080/10635150600852011 [PubMed: 16967577]

Powell JR, Monaghan MT, Opik M, Rillig MC, 2011 Evolutionary criteria outperform operational approaches in producing ecologically relevant fungal species inventories. Mol. Ecol 10.1111/j.1365-294X.2010.04964.x

Price AL, Tandon A, Patterson N, Barnes KC, Rafaels N, Ruczinski I, Beaty TH, Mathias R, Reich D, Myers S, 2009 Sensitive detection of chromosomal segments of distinct ancestry in admixed populations. PLoS Genet. 5(6): e1000519. doi: 10.1371/journal.pgen.1000519 [PubMed: 19543370]

Pringle A, Baker DM, Platt JL, Wares JP, Latge JP, Taylor JW, 2005 Cryptic speciation in the cosmopolitan and clonal human pathogenic fungus Aspergillus fumigatus. Evolution. 59(9): 1886–1899. 10.1111/j.0014-3820.2005.tb01059.x [PubMed: 16261727]

Ramani R, Chaturvedi V, 2007 Antifungal susceptibility profiles of Coccidioides immitis and Coccidioides posadasii from endemic and non-endemic areas. Mycopathologia 163: 315–319. 10.1007/s11046-007-9018-7. [PubMed: 17484074]

Rehner SA, Buckley E, 2005 A Beauveria phylogeny inferred from nuclear ITS and EF1-a sequences: evidence for cryptic diversification and links to Cordyceps teleomorphs. Mycologia. 97(1): 84–98. 10.1080/15572536.2006.11832842 [PubMed: 16389960]

Renard D, Valle G Della, Eds, Y P., Royal T., 2003 Species concepts and phylogenetic theory: a debate. Edited by Wheeler Q, and Meier R Columbia University Press.

Restrepo S, Tabima JF, Mideros MF, GrOnwald NJ, Matute DR, 2014 Speciation in fungal and Oomycete plant pathogens. Annu. Rev. Phytopathol. 52(1): 289–316. 10.1146/annurev-phyto-102313-050056 [PubMed: 24906125]

Richards PM, Morii Y, Kimura K, Hirano T, Chiba S, Davison A, 2017 Single-gene speciation: Mating and gene flow between mirror-image snails. Evol. Lett1(6): 282–291. 10.1002/evl3.31 [PubMed: 30283656]

Roch S, Steel M, 2015 Likelihood-based tree reconstruction on a concatenation of aligned sequence data sets can be statistically inconsistent. Theor. Popul. Biol100: 56–62. 10.1016/j.tpb.2014.12.005

Rokas A, Williams BI, King N, Carroll SB, 2003 Genome-scale approaches to resolving incongruence in molecular phylogenies. Nature. 425: 798–804. 10.1038/nature02053 [PubMed: 14574403]

Rosenberg NA 2003 The shapes of neutral gene genealogies in two species: Probabilities of monophyly, paraphyly, and polyphyly in a coalescent model. Evolution 57(7): 1465–1477. [PubMed: 12940352]

Roux C, Fra'isse C, Romiguier J, Anciaux Y, Galtier N, Bierne N, 2016 Shedding light on the grey zone of speciation along a continuum of genomic divergence. PLoS Biol. 14(12): e2000234 10.1371/journal.pbio.2000234 [PubMed: 28027292]

Saag L, Mark K, Saa A, Randlane T, 2014 Species delimitation in the lichenized fungal genus Vulpicida (Parmeliaceae, Ascomycota) using gene concatenation and coalescent-based species tree approaches. Am. J. Bot 101(12): 2169–2182. 10.3732/ajb.1400439 [PubMed: 25480713]

Sadowska-Deś AD, Dal Grande F, Lumbsch HT, Beck A, Otte J, Hur JS, Kim JA, Schmitt I, 2014 Integrating coalescent and phylogenetic approaches to delimit species in the lichen photobiont Trebouxia. Mol. Phylogenet. Evol 76: 202–210. 10.1016/j.ympev.2014.03.020 [PubMed: 24685499]

Salgado-Salazar C, Rossman AY, Chaverri P, 2013 Not as ubiquitous as we thought: taxonomic crypsis, hidden diversity and cryptic speciation in the cosmopolitan fungus *Thelonectria discophora* (Nectriaceae, Hypocreales, Ascomycota). PLoS One. 8(10): e76737 10.1371/journal.pone.0076737 [PubMed: 24204665]

Samuels GJ, Ismaiel A, Bon M-C, De Respinis S, Petrini O, 2010 Trichoderma asperellum sensu lato consists of two cryptic species. Mycologia. 102(4): 944–966. 10.3852/09-243 [PubMed: 20648760]

Sanchez-Ramírez S, Tulloss RE, Guzmán-Dávalos L, Cifuentes-Blanco J, Valenzuela R, Estrada-Torres A, Ruan-Soto F, Diaz-Moreno R, Hernandez-Rico N, Torres-Gómez M, León H, Moncalvo JM, 2015 In and out of refugia: Historical patterns of diversity and demography in the North American Caesar's mushroom species complex. Mol. Ecol 24(23): 5938–5956. 10.1111/mec.13413 [PubMed: 26465233]

Sanderson MJ, 1995 Objections to bootstrapping phylogenies: A critique. Syst. Biol 10.1093/sysbio/44.3.299

Sato H, Murakami N, 2008 Reproductive isolation among cryptic species in the ectomycorrhizal genus Strobilomyces: Population-level CAPS marker-based genetic analysis. Mol. Phylogenet. Evol 48: 326–334. 10.1016/j.ympev.2008.01.033 [PubMed: 18331802]

Sato H, Yumoto T, Murakami N, 2007 Cryptic species and host specificity in the ectomycorrhizal genus Strobilomyces (Strobilomycetaceae). Am. J. Bot 94(10): 1630–1641. 10.3732/ajb.94.10.1630. [PubMed: 21636360]

Savary R, Masclaux FG, Wyss T, Droh G, Cruz Corella J, Machado AP,Morton JB, Sanders IR, 2017 A population genomics approach shows widespread geographical distribution of cryptic genomic forms of the symbiotic fungus Rhizophagus irregularis. ISME J. 12(1): 17–30. doi:10.1038/ismej.2017.153 [PubMed: 29027999]

Sayyari E, Mirarab S, 2016 Fast coalescent-based computation of local branch support from quartet frequencies. Mol. Biol. Evol 33(7): 1654–1668. 10.1093/molbev/msw079 [PubMed: 27189547]

Schardl CL, Craven KD, 2003 Interspecific hybridization in plant-associated fungi and oomycetes: A review. Mol. Ecol 12(11): 2861–2873. 10.1046/j.1365-294X.2003.01965.x [PubMed: 14629368]

Seixas I, Barbosa C, Mendes-Faia A, GQldener U, Tenreiro R, Mendes-Ferreira A, Mira NP, 2019 Genome sequence of the non-conventional wine yeast *Hanseniaspora guilliermondii* UTAD222 unveils relevant traits of this species and of the Hanseniaspora genus in the context of wine fermentation. DNA Res. 26(1): 67–83. 10.1093/dnares/dsy039 [PubMed: 30462193]

Sepúlveda VE, Marquez R, Turissini DA, Goldman WE, Matute DR, 2017 Genome sequences reveal cryptic speciation in the human pathogen Histoplasma capsulatum. mBio. 8:e01339–17. 10.1128/mBio.01339-17. [PubMed: 29208741]

Sepúlveda VE, Williams CL, Goldman WE, 2014 Comparison of phylogenetically distinct Histoplasma strains reveals evolutionarily divergent virulence strategies. mBio. 5(4):e01376–14. doi:10.1128/mBio.01376-14. [PubMed: 24987093]

Sharpton TJ, Stajich JE, Rounsley SD, Gardner MJ, Wortman JR, Jordar VS, Maiti R, Kodira CD, Neafsey DE, Zeng Q, Hung C-Y, McMahan C, Muszewska A, Grynberg M, Mandel MA, Kellner EM, Barker BM, Galgiani JN, Orbach MJ, Kirkland TN, Cole GT, Henn MR, Birren BW, Taylor JW, 2009 Comparative genomic analyses of the human fungal pathogens Coccidioides and their relatives. Genome Res. 19(10): 1722–1731. doi: 10.1101/gr.087551.108. [PubMed: 19717792]

Silva DN, Varzea V, Paulo OS, Batista D, 2018 Population genomic footprints of host adaptation, introgression and recombination in coffee leaf rust. Mol. Plant Pathol19(7): 1742–1753. 10.1111/mpp.12657 [PubMed: 29328532]

Singh G, Aptroot A, Rico VJ, Otte J, Divakar PK, Crespo A, Cáceres ME da 5, Lumbsch HT, Schmitt I, 2018 Neoprotoparmelia gen. nov. and Maronina (Lecanorales, Protoparmelioideae): species description and generic delimitation using DNA barcodes and phenotypical characters. MycoKeys. 10.3897/mycokeys.44.29904

Singh G, Dal Grande F, Divakar PK, Otte J, Crespo A, Schmitt I, 2017 Fungal-algal association patterns in lichen symbiosis linked to macroclimate. New Phytol.214(1): 317–329. 10.1111/nph.14366. [PubMed: 27918622]

Singh G, Dal Grande F, Divakar PK, Otte J, Leavitt SD, Szczepanska K,Crespo A, Rico VJ, Aptroot A, Da Silva Cáceres ME, Lumbsch HT, Schmitt I., 2015 Coalescent-based species delimitation approach uncovers high cryptic diversity in the cosmopolitan lichen-forming fungal genus Protoparmelia (Lecanorales, Ascomycota). PLoS One. 10(5): e012462510.1371/journal.pone.0124625 [PubMed: 25932996]

Sites JW, Marshall JC, 2003 Delimiting species: a Renaissance issue in systematic biology. Trends Ecol. Evol 18(9): 462–470. 10.1016/S0169-5347(03)00184-8

Sklenář F, Jurjević, Zalar P, Frisvad JC, Visagie CM, Kolařík M, Floubraken J, Chen AJ, Yilmaz N, Seifert KA, Coton M, Déniel F, Gunde-Cimerman N, Samson RA, Peterson SW, Flubka V, 2017 Phylogeny of Xerophilic aspergilli (subgenus *Aspergillus*) and taxonomic revision of section Restrict!. Stud. Mycol 88: 161–236. 10.1016/j.simyco.2017.09.002 [PubMed: 29158611]

Sobel JM, Chen GF, Watt LR, Schemske DW, 2010 The biology of speciation. Evolution.64(2): 295–315. 10.1111/j.1558-5646.2009.00877.X [PubMed: 19891628]

Sokal RR, Crovello TJ, 1970 The biological species concept: a critical evaluation. Am. Nat 104(936). 10.1086/282646

Soto Medina E, Prieto M, Wedin M, 2018 A new Bunodophoron species (*Sphaerophoraceae*, *Lecanorales*) from the Neotropics. Lichenologist. 50(3): 255–266. 10.1017/S0024282917000743

Starkey DE, Ward TJ, Aoki T, Gale LR, Kistler HC, Geiser DM, Suga H, Toth B, Varga J, O'Donnell K, 2007 Global molecular surveillance reveals novel *Fusarium* head blight species and trichothecene toxin diversity. Fungal Genet. Biol 44(11): 1191–1204. 10.1016/j.fgb.2007.03.001 [PubMed: 17451976]

Steenkamp ET, Wingfield BD, Desjardins AE, Marasas WFO, Wingfield MJ, 2002 Cryptic speciation in Fusarium subglutinans. Mycologia. 94(6): 1032–1043. 10.1080/15572536.2003.11833158. [PubMed: 21156574]

Sukumaran J, Knowles LL, 2017 Multispecies coalescent delimits structure, not species. Proc. Natl. Acad. Sci. U. S. A 114(17): 1607–1612. 10.1073/pnas.1607921114. [PubMed: 28137871]

Sun Y, Corcoran P, Menkis A, Whittle CA, Andersson SGE, Johannesson H, 2012 Large-scale introgression shapes the evolution of the mating-type chromosomes of the filamentous

Ascomycete *Neurospora tetrasperma*. PLoS Genet. 8(7): e1002820. doi:10.1371/journal.pgen.1002820 [PubMed: 22844246]

Susko E, 2009 Bootstrap support is not first-order correct. Syst. Biol 58(2): 211–223. 10.1093/sysbio/syp016 [PubMed: 20525579]

Svantesson S, Larsson K-H, Kõljalg U, May TW, Cangren P, Nilsson RH, Larsson E, 2019 Solving the taxonomic identity of *Pseudotomentella tristis* s.l. (Thelephorales, Basidiomycota) - a multi-gene phylogeny and taxonomic review, integrating ecological and geographical data. MycoKeys. 50: 1–77. 10.3897/mycokeys.50.32432 [PubMed: 31043855]

Taylor JW, 2006 Evolution of human-pathogenic fungi: phylogenies and species, in: molecular principles of fungal pathogenesis. American Society of Microbiology, pp. 113–132.

Taylor JW, Fisher MC, 2003 Fungal multilocus sequence typing - It's not just for bacteria. Curr. Opin. Microbiol 6(4): 351–356. 10.1016/S1369-5274(03)00088-2 [PubMed: 12941403]

Taylor JW, Jacobson DJ, Kroken S, Kasuga T, Geiser DM, Hibbett DS, Fisher MC, 2000 Phylogenetic species recognition and species concepts in fungi. Fungal Genet. Biol, 10.1006/fgbi.2000.1228

Teixeira MM, Theodoro RC, De Oliveira FFM, Machado GC, Hahn RC, Bagagli E, San-Blas G, Felipe MSS, 2015 *Paracoccidioides lutzii* sp. nov.: biological and clinical implications. J. Music Ther 52(1): 19–28. 10.3109/13693786.2013.794311.

Teixeira MM, Theodoro RC, Nino-Vega G, Bagagli E, Felipe MSS, 2014 Paracoccidioides species complex: ecology, phylogeny, sexual reproduction, and virulence. PLoS Pathog. 10(10): e1004397 10.1371/journal.ppat.1004397 [PubMed: 25357210]

Turissini DA, Gomez OM, Teixeira MM, McEwen JG, Matute DR, 2017 Species boundaries in the human pathogen *Paracoccidioides*. Fungal Genet. Biol 106: 925 10.1016/j.fgb.2017.05.007.

Turissini DA, Matute DR, 2017 Fine scale mapping of genomic introgres sions within the Drosophila yakuba clade. PLoS Genet. 13(9): e1006971 10.1371/journal.pgen.1006971. [PubMed: 28873409]

Udayanga D, Castlebury LA, Rossman AY, Chukeatirote E, Hyde KD, 2014 Insights into the genus *Diaporthe*: phylogenetic species delimitation in the *D. eres* species complex. Fungal Divers. 67(1): 203–229. 10.1007/s13225-014-0297-2

Vaghefi N, Kikkert JR, Hay FS, Carver GD, Koenick LB, Bolton MD, Hanson LE, Secor GA, Pethybridge SJ, 2018 Cryptic diversity, pathogenicity, and evolutionary species boundaries in *Cercospora* populations associated with Cercospora leaf spot of *Beta vulgaris*. Fungal Biol. 122(4): 264–282. 10.1016/j.funbio.2018.01.008 [PubMed: 29551200]

Van Niekerk JM, Crous PW, Groenewald JZ, Fourie PH, Halleen F, 2004 DNA phylogeny, morphology and pathogenicity of *Botryosphaeria* species on grapevines. Mycologia. 96(4): 781–298. 10.1080/15572536.2005.11832926 [PubMed: 21148899]

Vieira A, Silva DN, Varzea V, Paulo OS, Batista D, 2018 Novel insights on colonization routes and evolutionary potential of *Colletotrichum kahawae*, a severe pathogen of Coffea arabica. Mol. Plant Pathol 19(11): 2488–2501. 10.1111/mpp.12726 [PubMed: 30073748]

Walker A-S, Gautier A, Confais J, Martinho D, Viaud M, Le Pecheur P, Dupont J, Fournier E, 2011 *Botrytis pseudocinerea*, a new cryptic species causing gray mold in french vineyards in sympatry with *Botrytis cinerea*. Phytopathology. 101(12): 1433–1445. 10.1094/PHYT0-04-11-0104 [PubMed: 21830954]

Wang RJ, White MA, Payseur BA, 2015 The Pace of hybrid incompatibility evolution in house mice. Genetics 201,229-242 201(1): 229–242. 10.1534/genetics.115.179499 [PubMed: 26199234]

Wang X-H, Huhtinen S, Hansen K, 2016 Multilocus phylogenetic and coalescent-based methods reveal dilemma in generic limits, cryptic species, and a prevalent intercontinental disjunct distribution in Geopyxis (Pyronemataceae s. l., Pezizomycetes). Mycologia. 108(6): 1189–1215. 10.3852/16-100 [PubMed: 27760850]

Wheeler QD, Platnick NI, 1989 The phylogenetic species concept and phylogenetic theory: a debate. Columbia University Press.

Whitehead MR, Catullo RA, Ruibal M, Dixon KW, Peakall R, Linde CC, 2017 Evaluating multilocus Bayesian species delimitation for discovery of cryptic mycorrhizal diversity. Fungal Ecol. 26: 74–84. 10.1016/j.funeco.2016.11.009

Yang Z, 2015a The BPP program for species tree estimation and species delimitation. Curr. Zool 61(5): 854–865. 10.1093/czoolo/61.5.854

Yang Z, 2015b A tutorial of BPP for species tree estimation and species delimitation. Curr. Zool 10.1093/czoolo/61.5.854

Yang Z, Rannala B, 2010 Bayesian species delimitation using multilocus sequence data. Proc. Natl. Acad. Sci 10.1073/pnas.0913022107

Zhang C, Ogilvie HA, Drummond AJ, Stadler T, 2018 Bayesian inference of species networks from multilocus sequence data. Mol. Biol. Evol 35(2): 504–517. 10.1093/molbev/msx307 [PubMed: 29220490]

Zhang J, Kapli P, Pavlidis P, Stamatakis A, 2013 A general species de limitation method with applications to phylogenetic placements. Bioinformatics. 29(22): 2869–2876. 10.1093/bioinformatics/btt499 [PubMed: 23990417]

Zhang W, Zhang X, Li K, Wang C, Cai L, Zhuang W, Xiang M, Liu X, 2018 Introgression and gene family contraction drive the evolution of lifestyle and host shifts of hypocrealean fungi. Mycology. 9(3): 176–188. 10.1080/21501203.2018.1478333 [PubMed: 30181924]

Zhao P, Liu F, Li YM, Cai L, 2016 Inferring phylogeny and speciation of *Gymnosporangium* species, and their coevolution with host plants. Sci. Rep 6: 29339 10.1038/srep29339 [PubMed: 27385413]

Zhao P, Wang QH, Tian CM, Kakishima M, 2015 Integrating a numerical taxonomic method and molecular phylogeny for species delimitation of *Melampsora* species (Melampsoraceae, Pucciniales) on willows in China. PLoS One. 10(12): e0144883 10.1371/journal.pone.0144883 [PubMed: 26680416]

Zhao X, Fernández-Brime S, Wedin M, Locke M, Leavitt SD, Lumbsch HT, 2017 Using multi-locus sequence data for addressing species boundaries in commonly accepted lichen-forming fungal species. Org. Divers. Evol 17(2): 351–363. 10.1007/s13127-016-0320-4

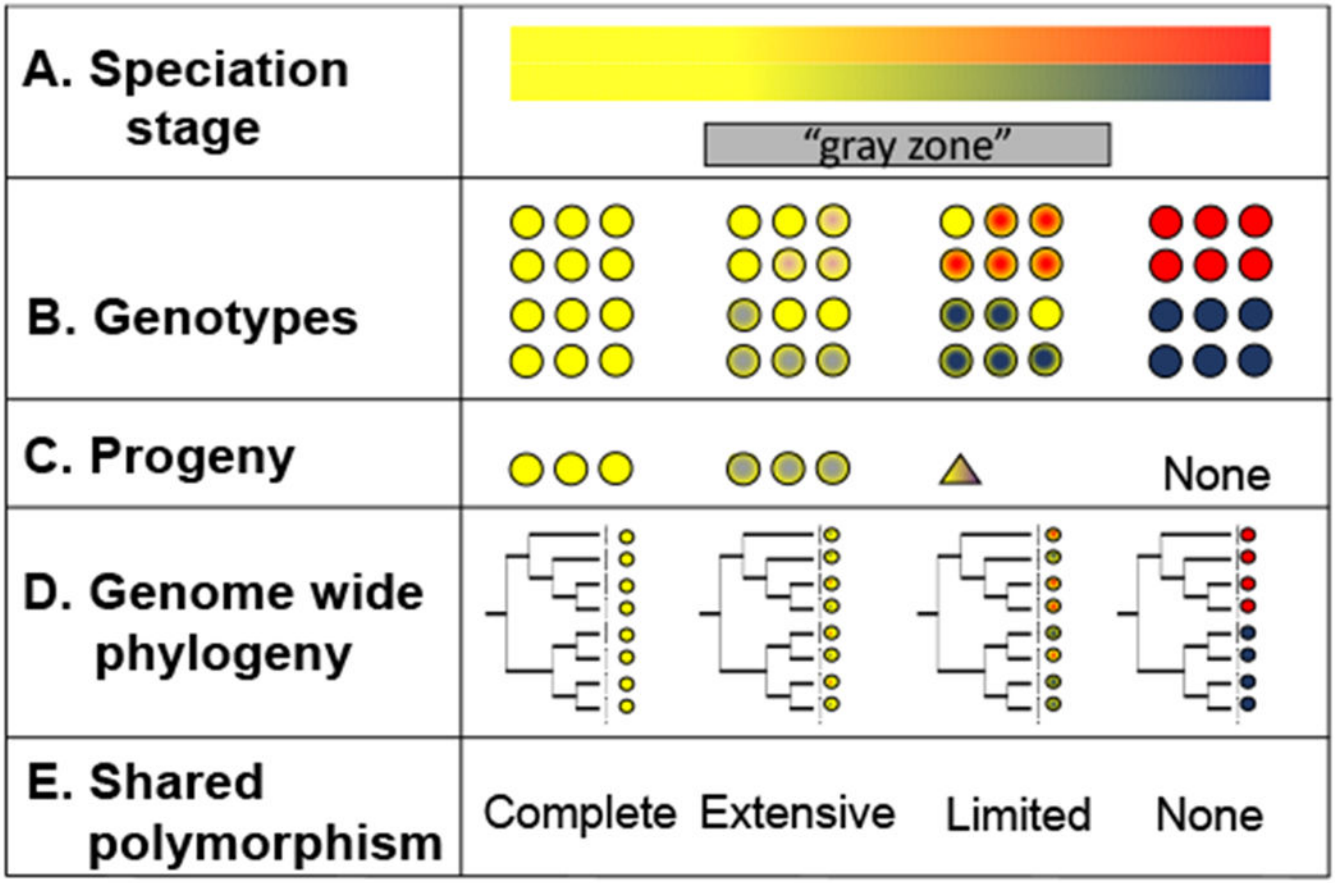

FIGURE 1. Simplified representation of the process of speciation continuum and the different signatures of speciation.

A. Speciation stage: genetic differences accrue in the nascent species to the extent that they become fully differentiated. Detecting speciation can be difficult in cases where divergence is recent (i.e., the gray zone). **B.** Genotypes: genetic differentiation will manifest in differences in the allele frequencies of the resulting populations (middle panel). Genetic differentiation might also lead to phenotypic differences but that is not always the case. **C.** Progeny: as divergence accumulates, the resulting species are less likely to interbreed and produce fit progeny to the extent that interbreeding will cease completely (bottom panel). **D.** Genome wide phylogeny: before speciation occurs (left), populations will exchange genes and polymorphic sites across the genome will be present in both species. As divergence accrues, the likelihood of reciprocal monophyly across the genome increases, and the likelihood of shared polymorphism decreases. Speciation is complete when genomes become reciprocally monophyletic, the magnitude of shared polymorphism is negligible, and reproductive isolation precludes the possibility of admixture. **E.** Level of shared polymorphism.

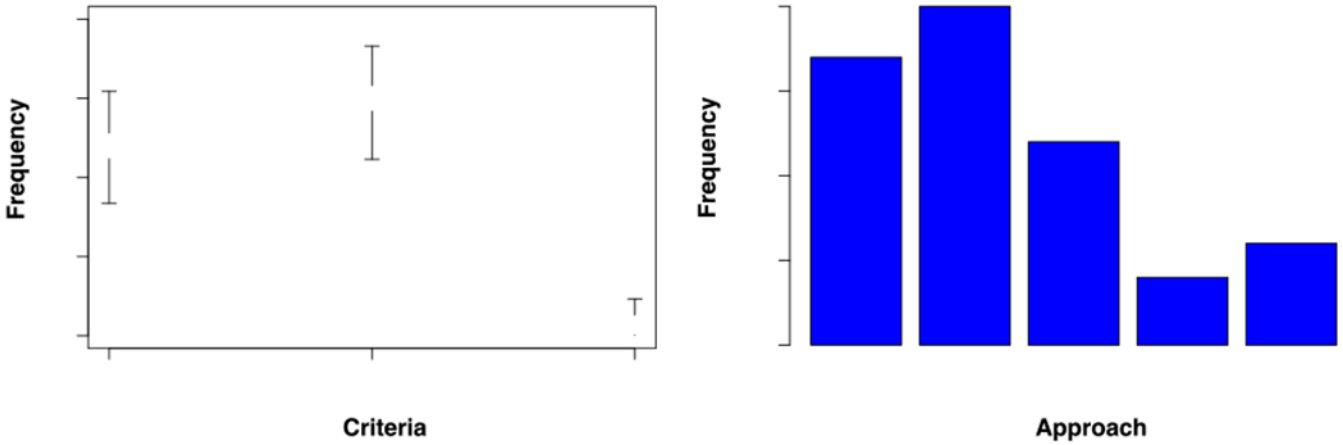

FIGURE 2. Summary of the approaches used to delimit species in fungi.

A. Proportion of studies that have used either one or both rules of the SGC to detect species boundaries in fungi. Error bars show the Bayesian 95% confidence intervals of the proportions. **B.** Proportion of studies that have used different CBD approaches to detect species boundaries in fungi. Please note that we reviewed 41 studies but the total for this panel is 60 as 12 studies used more than one approach.

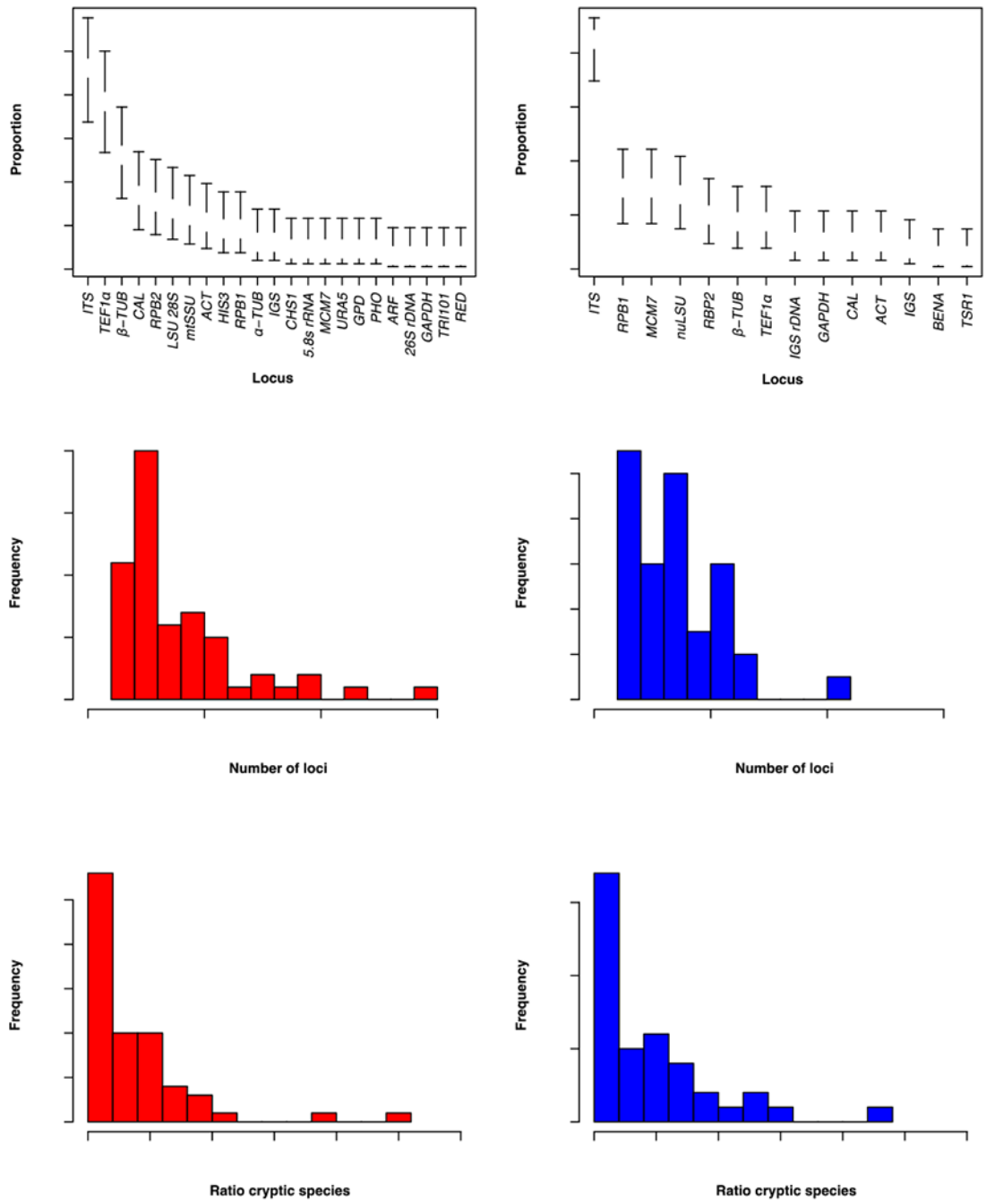

FIGURE 3. Characteristics of the SGC and CBD approaches in fungi.

A. Histogram showing the most used loci in SGC studies in fungi. **B.** Histogram showing the most used loci in CBD studies in fungi. **C.** Distribution of the number of loci used in SGC studies in fungi. Error bars show the Bayesian 95% confidence intervals of the proportions. **D.** Distribution of the number of loci used in CBD studies in fungi. **E.** Ratio of resulting species to initially reported species for studies using the SGC criteria. When this index is higher than one, the study found cryptic species; when it is lower, the study collapsed species (i.e., found no support for previously names species). **F.** Ratio of resulting species to

initially reported species for studies using the CBD criteria. Note that we excluded a study (Lucking et al. 2014), that found 126 potentially cryptic species from the histogram but not from the mean calculations.

TABLE 1.

A sample of studies that have used both the SGC and the CBD criteria in fungi.

Table S2 shows a complete list of studies using SGC; Table S3 shows a complete list of studies using CBD.

Taxon	SGC	Species reported with SGC	CBD	Species reported with CBD
Protoparmelia s. str.	Concordance + Support	25	BP&P, spedeSTEM	19,23
Trebouxia	Concordance + Support	6	GMYC	4,6
Geopyxis	Concordance + Support	6	BP&P	8
Diploschistes	Support	8	BP&P	*8*
Serendipita vermifera	Support	8	BP&P	8
Colletotrichum cliviae	Support	3	PTP	3
Cryptococcus gattii/ Cryptococcus neoformans	Support	7	GMYC	6-8
Cladonia mediterranea	Concordance + Support	2	spedeSTEM	2
Peltigera	Support	5	bGMYC, bPTP, spedeSTEM, BP&P	14 *
Montanelia	Support	at least 8	GMYC, PTP	6-8
Oropogon	Support	at least 10	GMYC	at least 10
Collemopsidium	Support	26	GMYC	26-28
Hesperomyces virescens	Concordance + Support	3	GMYC, PTP	3
Macalpinomyces	Support	1	GMYC, PTP	10,11
Graphis scripta	Support	at least 7	GMYC	6,7
Fomitopis pinicola	Support	3	BP&P	4

* authors state that this result might be intraspecific population structure.