Dissertation Title Exploring Fungal Species Boundaries Using Morphology, Divergence Times, Coalescent and Distance Based Methods

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Degree Doctor of Philosophy (Biological Science)

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ABSTRACT

Fungi are an essential component of any ecosystem, but they can also cause mild and severe plant diseases. Plant diseases are caused by a wide array of fungal groups that affect a diverse range of hosts with different tissue specificities. Species delimitation is the process by which species boundaries are determined and new species are discovered. This is a major topic in modern systematics with increasing amount of molecular data available on public databases and the development of new methods for species delimitation. Fungi were previously named based only on morphology and, in many cases, host association, which has led to superfluous species names and synonyms. Morphology-based identification represents an important method for genus level identification and molecular data are important to accurately identify species. Accurate identification of fungal pathogens is vital as the scientific name links the knowledge concerning a species including the biology, host range, distribution, and potential risk of the pathogen, which are vital for effective control measures. Thus, in the modern era, a polyphasic approach is recommended when identifying fungal pathogens. It is also important to determine if the organism is capable of causing host damage, which usually relies on the application of Koch's postulates for fungal plant pathogens. The importance and the challenges of applying Koch's postulates are discussed. Bradford Hill criteria, which are generally used in

establishing the cause of human disease, are briefly introduced. We provided guidelines for pathogenicity testing based on the implementation of modified Koch's postulates incorporating biological gradient, consistency, and plausibility criteria from Bradford Hill. We provided a set of protocols for fungal pathogenicity testing along with a severity score guide, which takes into consideration the depth of lesions. The application of a standard protocol for fungal pathogenicity testing and disease assessment in plants will enable inter-studies comparison, thus improving accuracy. When introducing novel plant pathogenic fungal species without proving the taxon is the causal agent using Koch's postulates, we advise the use of the term *associated with the* "*disease symptoms*" *of* "*the host plant*".

This study also emphasizes on the application of different molecular-based methods for species delineation in two important pathogenic genera, *Bipolaris* and *Colletotrichum*. *Bipolaris* species are important plant pathogens with a worldwide distribution in tropical and temperate environments. Species recognition in *Bipolaris* has been problematic due to a lack of molecular data from ex-type cultures, the use of few gene regions for species resolution and overlapping morphological characters. In this study, we evaluated the efficiency of different DNA barcodes in species delimitation in *Bipolaris* by phylogenetic analyses, Automatic Barcode Gap Discovery and Objective Clustering. GAPDH is determined to be the best single marker for the genus. These approaches are used to clarify the taxonomic placement of all sequences currently named as *Bipolaris* in GenBank based on ITS and GAPDH gene sequence data. In checking various publications, we found that the majority of new host records published in the Plant Disease journal between 2010 and 2019 were based on blast searches of the ITS sequences and up to 82% of those records could be erroneous. Therefore, ITS Blast searches of GenBank to name species is not recommended. Editorial boards of journals and reviewers of new record papers should be aware of this problem. In naming *Bipolaris* species, whether new or known, it is recommended to perform phylogenetic analyses based on GAPDH using the correct

taxon sampling for accurate results and the clade should have reliable statistical support. At least two additional species are represented by molecular data in GenBank and we provide an updated taxonomic revision of *Bipolaris*. We accepted 45 species in *Bipolaris* and notes are provided for all the species including hosts and geographic distribution.

Colletotrichum is one of the most important plant pathogenic genera that is responsible for numerous diseases which can have a profound impact on the agricultural sector. Species delineation is difficult due to a lack of distinctive phenotypic variation. Therefore, in this study three different genomic approaches based on phylogenetic, evolutionary and coalescent-based methods were applied to establish robust species boundaries. The efficiency of five different DNA barcodes was determined for species delineation. The ITS region can resolve the generic placement of taxa up to the species complex level. The GAPDH and TUB2 markers are determined to be the most informative for most complexes. However, no single marker could discriminate between species in all complexes, therefore different molecular approaches based on multi-locus datasets is recommended. This is the first study to provide an estimated divergence time for all species complexes in *Colletotrichum*. The estimated divergence time for species complexes ranged between 4.8 to 32.2 MYA. Based on congruent results among different molecular approaches, a new species complex, the *Colletotrichum-agaves* complex was introduced. This complex consists of five taxa which are characterised by the presence of straight or slightly curved conidia with obtuse apices. This study shows that coalescent approaches and multi-locus phylogeny are crucial to establish species boundaries in *Colletotrichum*. The taxonomic placement of three singleton taxa *Colletotrichum axonopodi, C. cariniferi* and *C. parallelophorum* is revised. We accepted 248 species and provided recommendations regarding species boundaries in the graminicola-caudatum complex.

This study also resulted in the introduction of several novel taxa from several hosts. A new species, *Colletotrichum artocarpicola,* collected on *Artocarpus heterophyllus* from Chiang Rai, Thailand, was introduced using both morphological and molecular approaches. Combined phylogenetic analysis of ITS, GAPDH, CHS-1, ACT and TUB2 sequence data demonstrated that *Colletotrichum artocarpicola* is a distinct species within the gloeosporioides species complex. The new species is illustrated and compared with related taxa, and evidence of its pathogenicity was provided. A novel genus, *Anastomitrabeculia,* was also introduced for a distinct species, *Anastomitrabeculia didymospora*, collected as a saprobe on dead bamboo culms from a freshwater stream in Thailand. *Anastomitrabeculia* is distinct in its trabeculate pseudoparaphyses and ascospores with longitudinally striate wall ornamentation. A new family, *Anastomitrabeculiaceae,* is introduced to accommodate *Anastomitrabeculia*. *Anastomitrabeculiaceae* formed an independent lineage basal to *Halojulellaceae* in *Pleosporales* and it is closely related to *Neohendersoniaceae* based on phylogenetic analyses of a combined LSU, SSU and TEF1α dataset. In addition, divergence time estimates provided further support for the establishment of *Anastomitrabeculiaceae.* The family diverged around 84 million years ago (MYA) during the Cretaceous period, which supports the establishment of the new family. The crown and stem age of *Anastomitrabeculiaceae* was also compared to morphologically similar pleosporalean families. This study also introduced a new species from leaf litter samples. Leaf litter is an important component of the ecosystem as it is a major source of organic material. *Phyllosticta doitungensis* was introduced from leaf litter samples of *Dasymaschalon obtusipetalum.* The host, *Dasymaschalon obtusipetalum* is widely distributed in Asia and has been used as traditional medicine. Morphological descriptions and illustrations of the novel microfungi was also provided.

Fungi play vital roles in the ecosystems as endophytes, pathogens and saprobes. The current estimate of fungal diversity is highly uncertain, ranging from

1.5 to 12 million, but only around 150,000 species have been named and classified to date. Since the introduction of DNA based methods, the number of newly described fungal taxa has increased from approximately 1,000 to around 2,000 yearly. This demonstrates the importance of DNA based methods to identify and distinguish species, especially morphologically similar taxa. Many novel species from recent studies have been found in historically understudied regions and habitats, but these still represent only a small percentage of the estimated species. In this study, we estimate the number of taxa in the top 30 most speciose genera as listed in Species Fungorum. The genera that are treated herein are *Cercospora, Diaporthe, Meliola, Passalora, Phyllachora*, *Phyllosticta, Pseudocercospora, Ramularia* (ascomycetes) and *Cortinarius*, *Entoloma*, *Inocybe*, *Marasmius*, *Psathyrella*, *Puccinia, Russula, Uromyces* (basidiomycetes). We discuss why these genera have some of the largest number of species.

Keywords: BEAST, Disease Severity, DNA Barcoding, Dothideomycetes, Fungal Diversity, General Mixed Yule Coalescent Method, Host-specificity, Image Analysis, Integrative Taxonomy, Multi-rate Poisson Tree Process, Pathogenicity, Plant Disease Assessment