Chapter

Diagnosis of Fungal Plant Pathogens Using Conventional and Molecular Approaches

Monika C. Dayarathne, Amin U. Mridha and Yong Wang

Abstract

Fungi are a large group of eukaryotes found as saprophytes, pathogens or endophytes, which distribute in every corner of our planet. As the main pathogens, fungi can cause 70–80% of total plant diseases, leading to huge crop yield reduction and economic loss. For identification of fungal plant pathogens, mycologists and plant pathologists have mainly gone through two stages, viz. morphological observation and morphology/phylogeny, and the next era might be utilizing DNA barcodes as the tool for rapid identification. This chapter accounts i) the brief history of development for fungal identification tools and main concepts, ii) the importance and confusion of "One fungus, one name" for pathogen identification, iii) more or fewer species that we need in agricultural practice, and iv) the foreground of fungal plant pathogen identification. These will help to solve the practical problems of identification of fungal pathogens in agricultural production.

Keywords: DNA barcode, morphology, phylogeny, plant diseases, rapid identification

1. Introduction

Plant parasitic fungi are a large group of eukaryotic living organisms lack of photosynthetic pigment and chitinous cell wall. It has been estimated around 15,000 species of them cause diseases in plants [1, 2], and annual crop losses exceed 200 billion euros [3–5]. **Figure 1** shows differently infected plants by various fungal pathogens. During occurrence of plant diseases, they produce various types of essential elements to complete their life cycle [6]. Most of the plants are attacked by one species or several phytopathogenic fungi but also the individual species of fungi can parasitize one or many different kinds of plants [7, 8].

In the pre-molecular era, the detection of fungal pathogens was mostly depending on microscopic, morphological and cultural approaches [9]. The culture-based diagnosis is time consuming and impractical when rapid results are required. With the advancement of molecular methods, detection and identification of phytopathogenic fungi have sped up and become more reliable [4], because of its high degree of specificity to distinguish closely related organisms at different taxonomic levels [10]. Polymerase Chain Reaction (PCR) technologies include multiplex PCR, nested PCR, real-time PCR and reverse transcription (RT)-PCR and DNA barcoding have been recently used as a molecular tool for detection and identification of fungal pathogens [11].

Figure 1.
A. Leaf spot disease of Houttuynia cordata, B. Downy mildew of Cucumis sativus, C. Peach brown rot of Amygdalus persica, D. Rust disease of Prunus salicina, E-F. Brown rot of Cerasus pseudocerasus leaves and fruit.

The rapid identification of fungal disease is an effective management practice and may help control and prevent their spread and progress successfully. Phylogenetic analyses have been employed for rapid identification of different kinds of fungi. However, the accuracy and reliability of DNA based methods depended largely on the experience and skill of the person making the diagnosis.

Besides that, few plant pathogenic fungi were sometimes also detected and identified using different types of proteomics approaches [6, 11]. In this chapter, we also discussed the importance and confusion of "One fungus, one name", and its impacts on identification of fungal plant pathogens. Finally, some suggestions were referred to the foreground of molecular identification.

2. The brief history of development for fungal identification tools and main concepts

[12] provided a chronological and systematic assessment of conventional methods of plant pathogen identification [13]. The application of light microscopy in the 1840s, the first evidence of plant disease was reported which was caused by *Phytophthora infestans* [14]. In the mid-nineteenth century, spore characters were accepted widely in classification [15]. In the middle of the twentieth century, different fungal structures were given emphasis in taxonomic systems, and separate scientific names (e.g., *Cercospora* were given for more or less similar fungi growing on different plant genera [16]. The observations of ornamentals of spores through scanning electron microscope (SEM) in the mid-1960s helped in separation of very similar plant pathogens and it also aided in clarifying patterns of conidiogenesis [17]. Then when came to the era of Transmission Electron Microscopy (TEM) which led to the discovery of fundamental differences in the major groups [18]. **Figures 2** and **3** represent the ultrastructural morphology of spongy tissue cells of tea leaves infected by fungal pathogens and control leaves by TEM [19].

During 1960s and 1970s thin-layer chromatography (TLC) and isozyme profiles were used to find out the chromosome numbers [20]. Vegetative compatibility groups (VCGs) were developed and it was found importance in many research studies on pathogenic *Fusarium* spp. [21]. The cluster analysis was performed

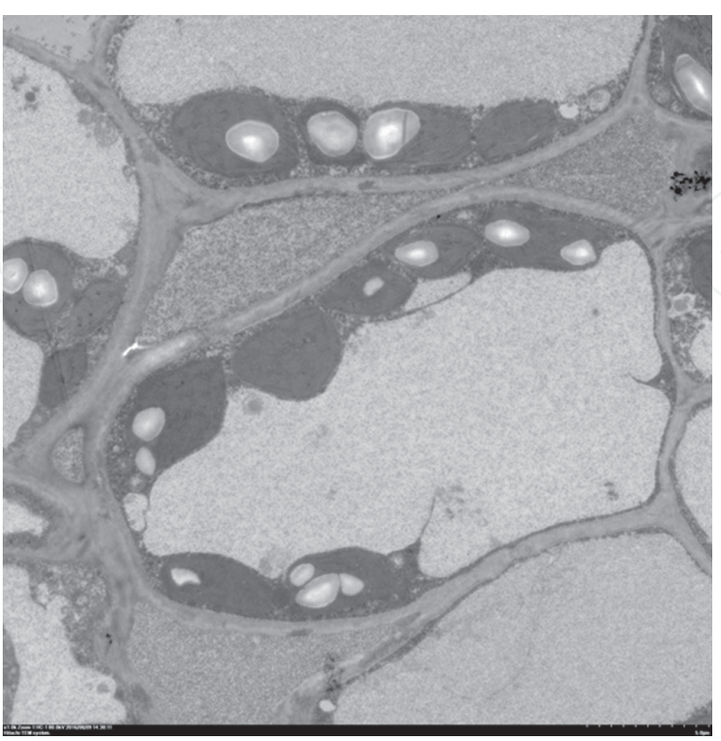

Figure 2.
The healthy spongy tissue of tea leaves, observed by TEM.

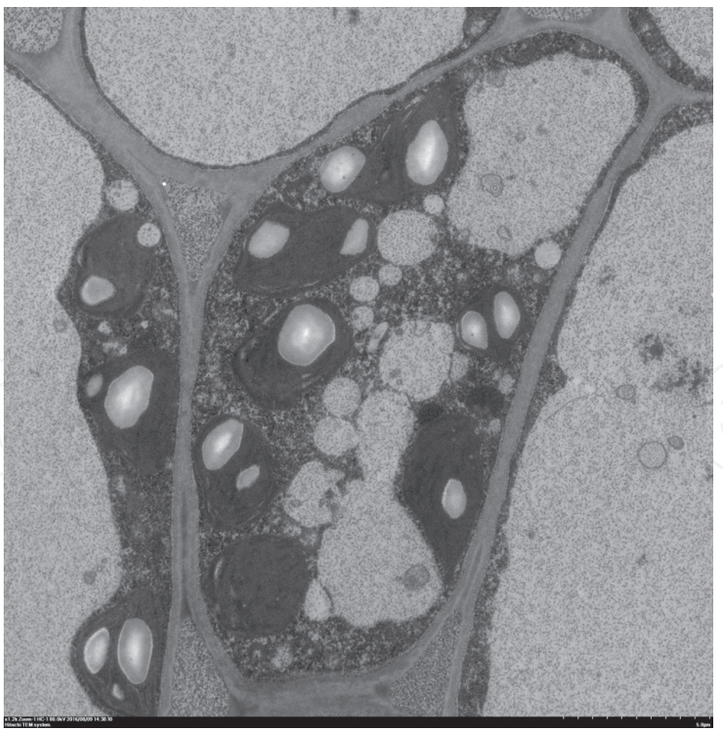

Figure 3.
Exobasidium vexans infects spongy tissue cells of tea leaves, observed by TEM.

after having powerful computers in the 1970s which revealed large numbers of morphological, cultural, and physiological characteristics should be computed and analyzed together. With that situation, DNA-based methodologies moved from occasional to common use [22].

[23] mentioned that the identification of fungi up to generic/species level or the *formae speciales* strains depended on their morphological characteristics and various kinds of reproductive organs. However, varieties or biotypes have to be identified by following pathogenicity, biochemical and immunological properties or nucleotide sequences of the genomic DNA, isozyme analysis, vegetative compatibility group (VCG) analysis and electrophoretic mobility of cell wall proteins etc. The development of enzyme-linked immunosorbent assay (ELISA) and monoclonal antibodies exhibit greater sensitivity and specificity in identifying fungi [23].

The molecular technologies are widely used in identifying plant pathogenic fungi and have been studied by mycologist and plant pathologists throughout the world. Many different types of diagnostic techniques may be used for detection, identification and quantification of fungal pathogens present in the infected above ground and below ground parts of plants and propagating and reproductive organs of different types of plants [23]. The nucleotide sequences of the pathogen DNA have become the preferred ones, because of their greater speed, specificity, sensitivity, reliability, and reproducibility of the results obtained, following the development of PCR [23]. [24] mentioned that the researchers over the last few years devoted their affords to develop the methods for detecting and identifying plant pathogens based on DNA/RNA probe technologies and PCR amplification such as [25] developed techniques for the rapid detection of plant pathogens; [26] used PCR for identifying plant pathogens; [27] used the modern assays for identification, detection and quantification of plant pathogenic fungi: and impacts of molecular diagnostic technologies on plant disease management was evaluated by [28]. The RT-PCR advances are helping the accurate detection and quantification of plant pathogens quickly and now being used routinely in most of the aspects of plant pathology.

In all molecular technology, DNA technology is most important in recovering from living cultures but is also useful to revise major groups of obligate fungi that cannot be cultivated, such as the powdery mildews [29], rusts and smuts [30]. Whole-genome sequence analyses indicated that the millions of dried fungal specimens preserved in different collection centers could hold great promise for understanding the evolution of many major fungal pathogens and their associated diseases and epidemics over time [31, 32]. [33] described a large number of various important common leaf diseases (from 2004 to 2019) caused by fungal plant pathogens with their symptoms and references of publications. [34] mentioned that the PCR and flow cytometry may be used in the genetic recognition of existing pathogens and the identification of emergent ones. The minute quantities of DNA in plant pathogens may be detected because of sensitiveness of DNA-based PCR technologies [35]. Further, genetic investigations could detect sources of pathogen and host resistance in diseases such as powdery mildew. [4] mentioned the different molecular diagnostics techniques (**Table 1**) used by many researchers throughout the world for the identification of phytopathogenic fungi with their advances and disadvantages.

Accurate identification and diagnosis of plant pathogens with reliable technologies and methods are needed to control them for sustainable plant diseases management [84] as well as prevention of the spread of invasive pathogens [85]. [86] published their works on fungal protocols and the primers for the ITS were first introduced, and it is still valid and widely used [32] in identification of plant pathogens. [9] reported that species identification was frequently difficult because fungi are a large and diverse assemblage of eukaryotes and have complex and

Molecular method	**Reference**
Conventional PCR	[36, 37]
Nested PCR	[38–40]
Multiplex PCR	[41]
Reverse transcriptase (RT) PCR	[10, 42]
Real-time PCR (Q PCR)	[43–45]
Serial analysis of gene expression (SAGE)	[46, 47]
DNA barcoding	[32, 48–51]
DNA/RNA probe-based methods	[24]
Northern blotting	[52–54]
In situ hybridization	[55–57]
FISH	[58, 59]
Post amplification techniques	[60–62]
Macroarray	[62–64]
The isothermal amplification-based methods	[58, 65, 66]
Loop-mediated isothermal amplification (LAMP)	[66–71]
Nucleic acid sequence-based amplification (NASBA):	[68, 72–75]
RNA interference methods (RNAi)	[76–79]
RNA-Seq-based next-generation sequencing methods	[46, 80–83]

Table 1.
PCR-based molecular methods for the detection of fungi.

poorly understood life cycles [87]. They have mentioned that molecular (DNA sequence) data as an essential tool for the identification of plant pathogenic fungi by the nuclear ribosomal internal transcribed spacer (ITS) region. The barcode gene for the fungi could be used to identify a wide range of plant-pathogenic fungi [9]. Protein-coding genes [glyceraldehyde-3-phosphate dehydrogenase (GAPDH), beta-tubulin (*tub2*) gene, translation elongation factor 1-alpha (tef1), actin (act), and histone H3 (his3)], generally prove a valuable supplement to ribosomal genes at the species level. More conserved gene regions such as large subunit (LSU), small subunit (SSU), and RNA polymerase II (RPB2) gene provide a better discrimination at the generic and/or family level [88–90]. The Q-bank fungal database contains DNA barcodes supplemented by morphological, phenotypical, and ecological data for more than 725 species of relevance to phytopathology. The database continues to be actively expanded, and parties interested in participating or contributing can contact its curators (http://www.q-bank.eu). The molecular identification of fungi (http://unite.ut.ee), is available on the basis of the results from a total of 31,954 changes incorporated and made available through the UNITE database, standalone FASTA files of sequence data for local BLAST searches and also use in the next-generation sequencing analysis platforms QIIME and mother [9]. The results were incorporated in UNITE made available publicly (http://unite.ut.ee/repository.php) e.g., local sequence similarity searches and sequence processing pipelines such as QIIME [91, 92], mothur [93], SCATA (http://scata.mykopat.slu.se/), CREST [94], and other downstream applications. UNITE also serves as one of the data providers for BLAST [95] searches in the EUBOLD fungal barcoding database (http://www.cbs.knaw.nl/eubold/). The maximum parsimony, maximum likelihood, and/or Bayesian inference are currently practiced to identify in many genera of phytopathogenic fungi [12]. Interestingly, improvement in molecular techniques has begun to allow a rapid alternative rDNA sequencing to whole genome sequencing [96]. LAMP of DNA is a newer molecular technology for affordable, specific, highly sensitive, and rapid diagnostic testing of pathogens in both laboratory and field conditions [97], and subsequently been optimized for portable instruments in field. Recently several protocols for a rapid detection of woody pathogens, such as *Ceratocystis platani, Fusarium circinatum, F. euwallaceae, Xylella fraxineus,* and *Phytophthora ramorum,* have been established [98–100].

Additionally, the application of proteomics such as two-dimensional gel electrophoresis (2-DE) and mass spectrometry (MS) is used to characterize cellular and extracellular virulence and pathogenicity factors produced by pathogens as well as to identify changes in protein levels in plant hosts upon infection by pathogenic organisms and symbiotic counterparts [101]. Many of the techniques used in proteomics, in particular the 2-DE method was developed two decades before the term proteomics was coined [102, 103]. Two-dimensional gel electrophoresis (2-DE) have been carried out to study the proteome of phytopathogenic fungi, mainly due to the difficulty of obtaining fungal protein extracts and/or the lack of available fungal protein databases [6]. In the last few years proteomic, in conjunction with genomic, has become one of the most relevant techniques for studying phytopathogenic fungi. Currently, the complete genome of over four hundred different species has been sequenced and this number is still increasing. With the availability of genome information for more and more species and the advancement in mass spectrometry technologies, proteomics has come into true since 1990s [104]. The advent of proteomics has allowed researchers to identify a broad spectrum of proteins in living systems.

Almost a little earlier, [86, 105–107] used immunological techniques with fungal plant pathogens-aspects of antigens, antibodies and assays for diagnosis. To speed up the identification of plant pathogens and allow their identification in

field, a number of serological methods have been developed, mainly based upon the enzyme-linked immunosorbent assay (ELISA). These methods are used to detect pathogens using a monoclonal antibody labeled with fluorescent compounds [108, 109]. Lateral flow devices (LFDs) are a simple paper-based dipstick assay able to detect and identify the causal agents of disease [110, 111]. [112] also described the methods using allozyme and isozyme markers to rapidly differentiate inter-sterility groups of *Heterobasidion annosum* [113], *Phytophthora cinnamomi,* and *Seiridium* sp. isolates [114].

3. The importance and confusions of "One fungus, one name" concept in plant pathogen identification

3.1 "One fungus, one name" concept

Scientific names (Latin binomials) are an integral part for communicating details about fungi causing plant diseases. Assembled knowledge on fungal pathogens *viz* biology, distribution, ecology, host range, control measures and the risks are accessible through these names [12]. In naming new fungal species mycologists are governed by the ICN and, more specifically by the *International Code for Nomenclature of plants, algae, and fungi* [115]. The Code provides a platform to abolish any bias or taxonomic confusion where multiple names are used for the same species [116]. '1 Fungus = 1 Name' was a meeting organized by CBS in the Netherlands that resulted in the 'Amsterdam Declaration' signed by some 80 participants [117], that strictly proposed the move to a unified nomenclature. Each fungal species should have one accurate name which is nomenclaturally accepted in a particular classification. Any of the previously used names for a particular species should be considered as a synonym with the oldest epithet taking priority over any younger name. If there is a wish/desire/movement to use a widely known younger name, then such usage must be in accordance with Art. 57.2 of the Code and its adoption should be accepted by the Nomenclature Committee for Fungi (NCF). Nevertheless, application of "one fungus one name" (1F1N) is in its infancy in mycology because most of the fungi are commonly known in only their sexual or asexual morph [116].

3.2 Significance of 1F1N concept in plant pathogen identification

Pleomorphism (having diverse fungal propagules) can be seen in many pathogenic fungi especially in Ascomycetes and in basidiomycetous rust fungi [118, 119]. Until the early 2000s, fungi were primarily classified on the basis of their sporing structures, and separate names were given to the sexual structures (formerly called the teleomorph) and asexual structures (formerly called the anamorph or if there are several asexual morphs, synanamorphs) even where the relationship between different morphs was proved by the culture of single spores [119]. For example, *Calonectria* with *Cylindrocladium* asexual morphs [120], *Chaetosphaeria* with *Menispora* asexual morphs [121], *Cladosporium* with *Venturia* asexual morphs [122], *Gibberella* with *Fusarium* asexual morphs [122], *Ceratocystis* with *Thielaviopsis* asexual morphs [123] and *Grosmannia* with *Leptographium* asexual morphs [124].

However, the concept of dual name became controversial to mycology in 21st century especially when a single DNA sequence could be attached to two names; one being the sexual morph name and the other under the asexual morph name (e.g. species of *Diaporthe* and *Phomopsis*) [125]. Many people working in fields related to agriculture/horticulture and plant pathology are confused by having to

deal with two names for a single pathogen [119]. This can be very important when dealing with fungi of quarantine significance and quarantine regulations linked to import and export requirements. Some countries may list the asexual morph name for an organism, whereas others list the sexual morph name. It is true that the two names refer to the one genetically identical organism, but quarantine officers are not necessarily aware of these details when dealing with constantly changing asexual–sexual morph taxonomy. For instance, identification of an invasive new rust (*Uredo rangelii*) on Myrtaceae in Australia [126]. This raised confusion as to whether or not the much-feared Eucalyptus rust (*Puccinia psidii*), a serious quarantine organism, and a restricted fungus on quarantine lists in countries in which eucalypts are cultivated [127, 128], was identical and had been introduced into Australia. Genetically, these names represent the same fungus or, at least, very closely related fungi causing the same disease, which suggests that they should be treated in a similar fashion when it comes to quarantine decisions. However, the names have not been treated equally and this has caused substantial complications relating to the treatment of the new *P. psidii sensu lato* invasion in Australia [126].

Dual nomenclature also conflicts with biological philosophy; a type is the type of a single organism that can have only one legitimate name [129]. The concept of permitting separate names for asexual morph of fungi with a pleomorphic life-cycle has been also an issue for mycologists to collect and describe new fungal species, mostly with one morph [116]. Therefore, depending on the accepted recommendations of 1F1N concept, all legitimate fungal names are now treated equally for the purposes of establishing priority. Asexual morph genera compete with sexual morph genera based on priority. For example, the asexual genera names *Alternaria* (1817) takes precedence over the sexual genus name *Lewia* (1986), *Cladosporium* (1816) over *Davidiella* (2003), *Fusarium* (1809) over *Gibberella* (1877), *Phyllosticta* (1818) over *Guignardia* (1892), *Sphaceloma* (1874) over *Elsinoë* (1900), *Trichoderma* (1794) over *Hypocrea* (1825). However, the reverse can also happen where an older sexual genus name takes priority over a younger asexual genus name, e.g., *Diaporthe* (1870) over *Phomopsis* (1905). However, there are exceptions where younger, widely used names get priority over an older name, for example *Hypomyces* (1860) over *Cladobotryum* (1816).

[130] documented five alternatives which can be followed when deciding on a single name for a fungus with a pleomorphic life cycle. These are: 1) strict priority, ignoring names originally typified by asexual morph or sexual morph by considering the priority of both generic names and species epithets [131, 132]; 2) sexual morph priority, with asexual morph species epithets [133]; 3) sexual morph priority without considering earlier asexual morph species epithets [134–136]; 4) teleotypification and 5) single species names but allowing two genera per clade (*Hypomyces*/*Cladobotryum*) [137, 138]. A number of sexual and asexual morph fungal genera have been linked by applying the oldest available name for the lineage (strict priority) in various studies. For example, *Neofusicoccum* was assigned for the clade with unnamed *Botryosphaeria*-like sexual morphs [139] included asexual *Phialophora*-like fungi in the sexual morph genus *Jattaea* [120, 140–142]; *Cylindrocladium* species were included under the older generic name *Calonectria*, and *Phomopsi* species in the older, sexual genus *Diaporthe* [139]. Importantly, 1F1N is important to link asexual morphs of pathogenic fungi to sexual morph-typified generic names, even without ever having seen the sexual morphs (e.g *Teratosphaeria toledana* and *Phaeophleospora toledana*) [119]. Further, this approach is also crucial for the widely emerging whole genome sequencing projects specially to compare species representing single entities with their closest relatives [143]. Such as comparing *Mycosphaerella tritici* (now *Zymoseptoria*) with *Mycosphaerella fijiensis* (now *Pseudocercospora*), is not instructive, as they are just two genera within a family, but not two species of one genus [119].

4. Controversies associated with one fungus one name concept in fungal plant pathogen identification

Although the application of 1F1N has become a reality, determination of which name to use for certain fungal species is somewhat more complex. Also, it is doubtful when accepting the other morph if it has been described elsewhere with a different name especially when lacking molecular data [116]. According to 1F1N mycologists must now select a genus name formerly applied to taxa with either asexual or sexual reproductive modes, that decision often influences the scope of genotypic and phenotypic diversity of a genus, and even its monophyly. [144] showed that many pairs of legitimate asexual-sexual morph names are not homotypic synonyms and merging them may not be justified. Therefore, dual names continue to be available for use following [145] e.g. the name pairs *Aspergillus niveus – Fennellia nivea* and *Aspergillus flavipes – Fennellia flavipes*, were not conspecific in a molecular study by [146].

Another problem arises when pathogenic species have one or more generic names for sexual morph associated with one or more asexual states. The best example is *Aspergillus* species which are mostly opportunistic pathogens. There are 11 sexual generic names associated with this genus; phenotypic variation and genetic divergence within the asexual genera are low but between sexual genera they are high [147]. Applying the asexual name *Aspergillus* to the many sexual genera masks information now conveyed by the sexual genus names. This would lead to taxonomic inconsistency in the Eurotiales because the large *Aspergillus sensu lato* would embrace more genetic divergence than neighboring clades comprised of two or more genera. However, [148] proposed a phylogeny combined aspect to apply one name to one fungal genus in a scientific manner in such a case.

Establishment and full use of the single name concept may take a long time as it is difficult to discard fungal names in publications before 2013, and these materials are still in use. The old name of some species whose name has been changed is still used in many publications [148]. When identifying fungi that cause diseases in humans, animals or plants, it may be difficult to determine which is the correct name because there are different names for these fungi in the literature. It is unlikely that all researchers and workers in agricultural industries, or border protection officers will have a good knowledge and understanding of fungal taxonomy. Acceptance and widespread use of the fungal names that change due to 1F1N will take time. Therefore, in some ways we are trapped in the past and there is difficulty in applying recent knowledge, due to long-standing and traditional rules that define how we name fungi.

5. More or fewer species that we need in agricultural practice

With the advent of "One fungus, one name" times from 2010s, many important fungal genera and species, for example *Gibberella*, *Hypocrea*, *Phomopsis* and *Magnaporthe grisea* causing worldwide rice blast towards the end became the synonyms of *Fusarium*, *Trichoderma*, *Diaporthe* and *Pyricularia grisea* respectively approved by the Nomenclature Committee of the Fungi and the General Committee (Art. 14.13). [149] listed nearly 7,000 generic names for eventual adoption, which made up just less than 50% of the total [24, 38] legitimate generic names) from Index Fungorum/ MycoBank database. For these changes, molecular techniques play an important role in the emergence of this great change, although for the species concept of fungi, we do still not get rid of the cruse of pragmatism. Thus, this has led to a very puzzling phenomenon, viz. on one side oceans of known species walk towards death, but on the other side mycologists spare no effort to 'create'

many new species and even many higher-level taxa (genus, family, and order, etc.). *Trichoderma harzianum* as an ubiquitous species in the environment and also effective bio-control agents against the devastating plant diseases, became an aggregate species recognized by [150], using genealogical concordance and recombination analyses confirmed there were two genetically isolated agamospecies and two hypothetical holomorphic species related to *T. harzinanum* species-complex [151], but surprisingly split into at least 14 species based on morphological, ecological, biogeographical and phylogenetic data [152, 153]. For *Alternaria* and allied genera, even the whole Kingdom Fungi, 2013 was destined to go down in history because of "*Alternaria* redefined", up to eighteen old generic name, for example, *Embellisia*, *Nimbya*, *Ulocladium* and *Lewia* turned into the synonyms of *Alternaria*, but in the meantime, 16 new *Alternaria* section were born [154].

Immediately, Hyde and Crous as well as their research groups open the dazzling "re-" doors published in Fungal Diversity, Studies in Mycology, Persoonia, IMA Fungus, Mycosphere. They provided a series of "backbone" trees of fungal genera, family, order or even higher taxonomic level based on DNA sequences from ex-type, epitype and authoritative strains. From 2014 to 2020, "One stop shop: backbones trees for important phytopathogenic genera: I-IV", were published in Fungal Diversity and led by [155] and [156–158] with international co-operations, which provided phylogenetic frameworks of 100 groups or genera of plant pathogenic fungi in the Ascomycota, Basidiomycota, Mucormycotina (Fungi), and Oomycota. Almost at the same time, in Studies in Mycology, a series of "Genera of phytopathogenic fungi: GOPHY1-3", which introduced stable platforms for the taxonomy of 62 phytopathogenic genera, including 5 new genera, 88 new species, 38 new combinations, four new names and 13 typifications of older names [159–161]. For these publications, the important disease information, viz. distribution, hosts and disease symptoms were referred, but without the key pathogenicity test (Koch's postulates) to clarify whether they were real pathogens or not. In spite of this, these contributions still make us get rid of the embarrassment of using morphology as the only approach of pathogen identification and provide primary and secondary DNA barcodes for rapid and accurate recognition. After census of new pathogens report in the international mainstream journals of plant pathology, we discovered that in the latest three years, more than 200 new pathogens and first reports were recorded per year in our planet.

Now more and more mycologists and plant pathologists accepted that fungi causing plant or post-harvest diseases should be identified on the basis of morphology and phylogeny or at least ITS-blast on NCBI database (for example, https://www.apsnet.org/publications/plantdisease). Especially, [162] solemnly declared that the optimal identity thresholds to discriminate filamentous fungi on the species level were 99.6% for ITS and 99.8% for LSU regions using more than 24,000 DNA barcode sequences originated from 12,000 ex-type strains. Even so, for important plant pathogenic fungal group (*Alternaria*, *Botrysphaeria*, *Colletotrichum* and *Diaporthe*), if only sequences of ITS or LSU region, the result will be considered rash and superficial. We have to admit that for identification of fungal pathogens, the agricultural practitioners welcomed fewer and simpler, but mycologists always looked ahead into the future and back into the past to creation or elimination.

6. The foreground of fungal plant pathogen identification

Accurate identification of pathogens must be the first step of plant pathology. Linnaeus published "Species Plantarum" in 1753 and then "Systema Naturae" (10th edition) in 1758 for planting naming with binomial nomenclature, which were

continued in Kingdom Fungi. A dual system of fungal nomenclature for asexual fungi was promulgated by [15], at one time, which played an important role in the identification of plant pathogenic fungi but came to the end in 2013. [147] compared the distinction between theoretical and operational species concepts, and pointed that PSR (Phylogenetic Species Recognition) by genealogical concordance was well suited to fungi and developed and adopted at an increasing rate [163]. DNA barcode, as a relative short specific DNA sequence was able to utilize in taxonomic practice referring to OTUs (Operational Taxonomic Units), which was comprehensively discussed by [164]. Urgently [165, 166] even attempted to propose DNA sequences without vouchered specimens to serve as types for fungal taxon names, but was unfortunately rejected by Nomenclature Committee for Fungi and International Mycological Congress (IMC 11) [167]. Almost at the same time, [168] further pointed out ASVs (Amplicon Sequence Variants) could replace OTUs as the standard units by high-throughput marker-gene sequencing data analysis.

The rapid development about identification approach of fungi has entered a dazzling but seemingly at a loss stage in plant pathology and other related practical or applied scientific fields. Although this, we have to admit the reality or the status quo is existing mycological research networks, especially e-books or publications do really facilitate the rapid development of DNA identification and information sharing. We can even update our knowledge in almost days and more comprehensive. It can also be understood in this way, viz. easier to make mistakes but also correct them. Although [167] fully expounded the deficiencies of Hawksworth's proposals, for identification of plant pathogenic fungi, we believe that accuracy sometimes gives way to quickness. Thus, DNA identification is competent to become a core or sole approach for fungal pathogens.

For plant pathologists in consideration of this method, we can quickly start the following two works, i) to make full use of the achievements of taxonomists to all-round confirm or correct the scientific name of old fungal pathogens, like "one fungus, one name" and "backbone trees" of fungal groups, which needs to be simultaneously done by pathologists in different countries of the world, or at least one continent, and 2) to standard the identification parameters of plant pathogenic fungi, for example the barcoding gene markers (only ITS or ITS plus a secondary generic marker) for PCR amplification (including forward/reverse primers), sequences threshold (99.6% for ITS or 99.8% for LSU is OK, or adopt the new standard?) and international specialized open database for rapid alignment. Of course, we also should keep pace with mycologists, and update our identification system on time.

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References

[1] Hawksworth DL, Kirk PM, Sutton BC. Pegler DN. Ainsworth and Bisby's Dictionary of the Fungi, International Mycological Institute, Surrey, UK, 2001.

[2] Hawksworth DL. The fungal dimension of biodiversity: magnitude, significance, and conservation, Mycol. Res. 1991;95(6):641-655.

[3] Arora DK, Bridge PD, Bhatnagar D. Fungal Biotechnology in Agricultural, Food and Environmental Applications, Marcel Dekker, New York, NY, USA, 2004.

[4] Aslam S, Tahir A, Aslam MF, Alam MW, Shedayi AA, Sadia S. Recent advances in molecular techniques for the identification of phytopathogenic fungi – a mini review. J. Plant Interact. 2017;12:493-504, DOI: 10.1080/17429145.2017.1397205.

[5] Ristaino JB. Tracking historic migrations of the Irish potato famine pathogen, *Phytophthora* infestans. Microbes and Infection 2002;4:1369-1377. DOI:10.1016/S1286-4579(02)00010-2.

[6] Fernández-Acero FJ, Carbú M, Garrido C, Vallejo I. Cantoral JM. Proteomic Advances in Phytopathogenic Fungi. Current Proteomics, 2007;4:79-88.

[7] Agrios. 5th ed., Plant Pathology WSU, OSU, U of I, 2005, Pacific Northwest Plant Disease. Handbook Moore, Randy1998, Botany, 2005. http:/www.mycolog.com/fifthloc.html.

[8] Tao WC, Zhang W, Yan JY, Hyde KD, McKenzie EH, Li XH, Wang Y. A new Alternaria species from grapevine in China. Mycol. Prog. 2014;13(4):999.

[9] Nilsson RH, Hyde KD, Pawłowska J, Ryberg M, Tedersoo L, Aas AB, Alias SA, Alves A, Anderson CL, Antonelli A, Arnold AE. Improving ITS sequence data for identification of plant pathogenic fungi. Fungal Divers. 2014;67:11-19. DOI 10.1007/s13225-014-0291-8.

[10] Capote N, Pastrana AM, Aguado A, Sánchez-Torres P. Molecular tools for detection of plant pathogenic fungi and fungicide resistance. In: Cumagun CJ (Ed.), Plant Pathology, InTech, Rijeka, Croatia; 2012. 362 p.

[11] González FR, Jorrín Novo JV. Proteomics of fungal plant pathogens: the case of *Botrytis cinerea*. Current Research, Technology and Education Topics in Applied Microbiology and Microbial Biotechnology, A. Méndez-Vilas (Ed.), 2010;205-216.

[12] Crous PW, Hawksworth DL, Wingfield MJ. Identifying and Naming Plant pathogenic Fungi: Past, present and Future. Annu. Rev. Phytopathol. 2015;53:247-267

[13] Crous PW, Slippers B, Wingfield MJ, Rheeder J, Marasas WF, Philips AJ, Alves A, Burgess T, Barber P, Groenewald JZ. Phylogenetic lineages in the Botryosphaeriaceae. Studies in mycology. 2006;55:235-253. DOI:10.3114/sim.55.1.235.

[14] Berkeley MJ. Observations, botanical and physiological, on the potato murrain. J. R. Hortic. Soc. 1846;1:9-34

[15] Saccardo PA. De Diagnostica et nomenclatura mycologica, Admonita quaedam. Ann. Mycol. 1904;2:195-198. [English translation by Clements, F.E. J. Mycology. 1904;109-112.

[16] Chupp C. A Monograph of the Fungus Genus *Cercospora*. Ithaca, NY, 1954.

[17] Cole GT, Samson RA. *Patterns of Development in Conidial Fungi*. London: Pitman Publ. 1979.

[18] von Arx JA, van der Walt JP, Liebenberg NVDM. The classification of *Taphrina* and other fungi with yeast-like cultural states. Mycologia, 1982;74:285-296.

[19] Zhao XZ, Wang Y, Ren YF, Li DX, Chen Z. The morphology observation of infection process for the pathogen *Exobasidium vexans* of tea blister blight against tea leaf. Chinese Agri Sci Bull. 2020;34:117-122

[20] Wieloch W. Chromosome visualisation in filamentous fungi. J. Microbiol. Meth. 2006;67:1-8

[21] Leslie JF, Summerell BA, eds. The *Fusarium* Laboratory Manual. Ames, IA: Blackwell Publishers 2006.

[22] Schloss PD, Westcott SL, Ryabin T, et al. Introducing mothur: open-source, platform-independent, community-supported software for describing and comparing microbial communities. Appl. Environ. Microbiol. 2009;75:7537-7541.

[23] Narayanasamy, P. Detection of Fungal Pathogens in Plants. Microbial Plant Pathogens-Detection and Disease Diagnosis. 2010;5-199.

[24] McCartney HA, Foster S J, Fraaije BA, Ward E. Molecular diagnostics for fungal plant Pathogens. Pest Manag Sci. 2003;59:129-142 DOI: 10.1002/ps.575.

[25] Duncan JM, Torrance L. Techniques for the rapid detection of plant pathogens, Blackwell Scientific Publications, Oxford .1992.

[26] Ward E. Use of the Polymerase Chain Reaction for identifying plant pathogens, in Ecology of plant pathogens, In: Blakeman JP, Williamson B. (editors) CAB International, Wallingford, UK, 1994; pp. 143-160

[27] Schots A, Dewey FM, Oliver R. (eds), Modern assays for plant pathogenic fungi: Identification, detection and quantification, CAB International, Wallingford, UK. 1994.

[28] Martin RR, James D, Le'vesque CA. Impacts of molecular diagnostic technologies on plant disease management. Annu. Rev. Phytopathol. 2000;38:207-239.

[29] Takamatsu S, Kano Y. PCR primers useful for nucleotide sequencing of rDNA of the powdery mildew fungi. Mycoscience 2001;42:135-139.

[30] Shivas RG, Beasley DR, McTaggart AR. Online identification guides for Australian smut fungi (*Ustilaginomycotina*) and rust fungi (*Pucciniales*). IMA Fungus, 2014;5:195-202.

[31] Gibbons A. On the trail of ancient killers. Science. 2013;340:1278-1282

[32] Schoch CL, Seifert KA, Huhndorf S, Robert V, Spouge JL, et al. Nuclear ribosomal internal transcribed spacer (ITS) region as a universal DNA barcode marker for *Fungi*. Proceedings of the Nat. Acad. Sci. 2012;109:6241-6246.

[33] Jaina A, Sarsaiya S, Wua Q, Lua Y, Shi J. A review of plant leaf fungal diseases and its environment speciation. Bioengineered. 2019;10(1):409-424. https://doi.org/10.1080/21655979.2019.1649520

[34] Sarsaiya S, Jia Q, Fan X, et al. First report of leaf black circular spots on Dendrobium nobile caused by *Trichoderma longibrachiatum* in Guizhou Province, China. Plant Dis. 2019. DOI:10.1094/pdis-03-19-0672-pdn

[35] Lee SB, Taylor JW. Isolation of DNA from fungal mycelia and single

spores, in PCR protocols. A guide to methods and applications, In: Innis MA, Gelfand DH, Sninsky JJ and White TJ, (editors) Academic Press, San Diego, USA; 1990. pp. 282-287

[36] Fang Y, Ramasamy RP. Current and prospective methods for plant disease detection. Biosensors. 2015;5(3):537-561.

[37] Mancini V, Murolo S, Romanazzi G. Diagnostic methods for detecting fungal pathogens on vegetable seeds. Plant Pathol. 2016;65:691-703.

[38] Bhat R, Browne G. Specific detection of Phytophthora cactorum in diseased strawberry plants using nested polymerase chain reaction. Plant Pathol. 2010;59(1):121-129.

[39] Tsai HL, Huang LC, Ann PJ, Liou RF. Detection of orchid Phytophthora disease by nested PCR. Bot Stud. 2006;47(4).

[40] Yeo SF, Wong B. Current status of nonculture methods for diagnosis of invasive fungal infections. Clin. Microbiol. Rev. 2002;35(3):465-484

[41] Cho HJ, Hong SW, Kim HJ, Kwak YS. Development of a multiplex PCR method to detect fungal pathogens for quarantine on exported cacti. Plant Pathol. J. 2016;32(1):53-54.

[42] Brown NA, Bass C, Baldwin TK, Chen H, Massot F, Carion PW, Urban M, Van De Meene AM, Hammond-Kosack KE. Characterisation of the *Fusarium graminearum*-wheat floral interaction. J. Pathogens, 2011;9. DOI:10.4061/2011/626345

[43] Badali H, Nabili M. Molecular tools in medical mycology; where we are! Jundishapur J. Microbiol. 2012;6(1):1-3.

[44] Black J. Quantitative real-time polymerase chain reaction (qPCR) of filamentous fungi in carpet. Research Triangle Park, NC: RTI Press; 2009.

[45] Dasmahapatra K, Mallet J. DNA barcodes: recent successes and future prospects. Heredity. 2006;97(4):254-255.

[46] Dawei W, Peng Y. Assessing the impact of dominant sequencing base gene expression profiling techniques (SGEPTs) on phytopathogenic fungi. Chiang Mai J. Sci. 2014; 41:922-9444.

[47] Velculescu VE, Zhang L, Vogelstein B, Kinzler KW. Serial analysis of gene expression. Science. 1995;270(5235):484-487.

[48] Krishnamurthy PK, Francis RA. A critical review on the utility of DNA barcoding in biodiversity conservation. Biodiversity and Conservation. 2012;21 (8):1901-1919.

[49] Li DZ, Gao LM, Li HT, Wang H, Ge XJ, Liu JQ, Chen ZD, Zhou SL, Chen SL, Yang JB. Comparative analysis of a large dataset indicates that internal transcribed spacer (ITS) should be incorporated into the core barcode for seed plants. Proc. Natl. Acad. Sci. 2011;108 (49):19641-19646..

[50] Monteiro F, Romeiras MM, Figueiredo A, Sebastiana M, Baldé A, Catarino L, Batista D. Tracking cashew economically important diseases in the West African region using metagenomics. Front. Plant Sci. 2015;6:482-483.

[51] Roe AD, Rice AV, Bromilow SE, Cooke JE, Sperling FA. Multilocus species identification and fungal DNA barcoding: insights from blue stain fungal symbionts of the mountain pine beetle. Mol Ecol. Resour. 2010;10(6):946-959.

[52] Berg Jeremy M. Biochemistry. 6th ed. New York: W. H. Freeman and Company; 2007.

[53] Kim SW, Li Z, Moore PS, Monaghan AP, Chang Y, Nichols M, John B. A sensitive non-radioactive northern blot method to detect small RNAs. Nucleic Acids Res.2010;38:e98..

[54] Qi M, Yang Y. Quantification of *Magnaporthe grisea* during infection of rice plants using real-time polymerase chain reaction and northern blot/phosphoimaging analyses. Phytopath. 2002;92 (8):870-876.

[55] Ellison MA, McMahon MB, Bonde MR, Palmer CL, Luster DG. In situ hybridization for the detection of rust fungi in paraffin embedded plant tissue sections. Plant Methods. 2016;12(1):37

[56] Jensen E. Technical review: in situ hybridization. Anatomical Records 2014; 297(8):1349-1353.

[57] Qian X, Lloyd RV. Recent developments in signal amplification methods for in situ hybridization. Diagn. Mol. Pathol.2003;12(1):1-13.

[58] Tsui NB, Kadir RA, Chan KC, Chi C, Mellars G, Tuddenham EG, Leung TY, Lau TK, Chiu RW, Lo YM. Noninvasive prenatal diagnosis of hemophilia by microfluidics digital PCR analysis of maternal plasma DNA. Blood. 2011;117(13):3684-3691. DOI:10.1210/jc.2014-1118

[59] Volpi EV, Bridger JM. Fish glossary: an overview of the fluorescence in situ hybridization technique. Biotechniques. 2008;45(4):385-386.

[60] Eshaque B, Dixon B. Technology platforms for molecular diagnosis of cystic fibrosis. Biotechnological Adv. 2006;24(1):86-93.

[61] Robinson B, Erle D, Jones D, Shapiro S, Metzger W, Albelda S, Parks W, Boylan A. Recent advances in molecular biological techniques and their relevance to pulmonary research. Thorax. 2000;55(4):329-339.

[62] Singh A, Kumar N. A review on DNA microarray technology. Int J Curr. Res. Rev. 2013;5(22):01-05.

[63] Sato T, Takayanagi A, Nagao K, Tomatsu N, Fukui T, Kawaguchi M, Kudoh J, Amagai M, Yamamoto N, Shimizu N. Simple PCR based DNA microarray system to identify human pathogenic fungi in skin. J. Clinical Microbiol. 2010;48(7):2357-2364.

[64] Zhang N, McCarthy ML, Smart CD. A macroarray system for the detection of fungal and oomycete pathogens of solanaceous crops. Plant Dis. 2008;92(6):953-960

[65] Ahmed OB, Asghar AH, Elhassan MM. Comparison of three DNA extraction methods for polymerase chain reaction (PCR) analysis of bacterial genomic DNA. African J. Microbiol. Res. 2014;8(6):598-602. DOI:10.5897/AJMR2013.6459.

[66] Kuhn J, Binder S. RT–PCR analysis of 5′ to 3′-end-ligated mRNAs identifies the extremities of cox2 transcripts in pea mitochondria. Nucleic Acids Res. 2002;30(2):439-446. Doi:10.1093/nar/30.2.439.

[67] Das A, Babiuk S, McIntosh MT. Development of a loop-mediated isothermal amplification assay for rapid detection of capripox viruses. J. Clinical Microbiol. 2012;50(5):1613-1620.

[68] Fakruddin MD. Loop mediated isothermal amplification (LAMP)–an alternative to polymerase chain reaction (PCR). Bangladesh Res. Publications J. 2011;5(4): 425-439 .

[69] Liu W, Huang S, Liu N, Dong D, Yang Z, Tang Y, Ma W, He X, Ao D, Xu Y. Establishment of an accurate and fast detection method using molecular beacons in loop-mediated isothermal

amplification assay. Sci. Rep. 2017; 7:40125. doi:10.1038/srep40125 (2017).

[70] Ren CH, Hu CQ, Luo P, Wang QB. Sensitive and rapid identification of Vibrio vulnificus by loop-mediated isothermal amplification. Microbiol. Res. 2009;164(5):514-521.

[71] Villari C, Tomlinson JA, Battisti A, Boonham N, Capretti P, Faccoli M. Use of loop-mediated isothermal amplification for detection of Ophiostoma clavatum, the primary blue stain fungus associated with Ips acuminatus. Appl. Environ. Microbiol. 2013;79(8):2527-2533.

[72] Chang SS, Hsieh WH, Liu TS, Lee SH, Wang CH, Chou HC, Yeo YH, Tseng CP, Lee CC. Multiplex PCR system for rapid detection of pathogens in patients with presumed sepsis–a systemic review and meta-analysis. PloS one. 2013;8(5):e62323. DOI:10.1371/journal.pone.0062323

[73] Lee D, La Mura M, Allnutt TR, Powell W. Detection of genetically modified organisms (GMOs) using isothermal amplification of target DNA sequences. BMC Biotechnol. 2009;9(1):7.

[74] Lizardi PM, Huang X, Zhu Z, Bray-Ward P, Thomas DC, Ward DC. Mutation detection and single-molecule counting using isothermal rolling-circle amplification. Nature Genetics. 1998;19(3):225-232.

[75] Sergentet-Thevenot D, Montet MP, Vernozy-Rozand C. Challenges to developing nucleic acid sequence-based amplification technology for the detection of microbial pathogens in food. Rev. Med. Vet. 2008;159:514-527.

[76] Chen X, Steed A, Harden C, Nicholson P. Characterisation of *Arabidopsis thaliana–Fusarium graminearum* interactions and identification of variation in resistance among ecotypes. Mol. Plant Pathol. 2006;7:391-403.

[77] Ishii H, Holloman DW. Fungicide resistance in plant pathogens. Tokyo: Springer; 2015.

[78] Nakayashiki T, Kurtzman CP, Edskes HK, Wickner RB. Yeast prions [URE3] and [PSI+] are diseases. Proc. Natl. Acad. Sci. 2005;102(30):10575-10580.

[79] Panwar V, McCallum B, Bakkeren G. Endogenous silencing of Puccinia triticina pathogenicity genes through in planta-expressed sequences leads to the suppression of rust diseases on wheat. Plant J. 2012;73:521-532. doi:10.1111/tpj.12047.

[80] de Jonge R, van Esse HP, Maruthachalam K, Bolton MD, Santhanam P, Saber MK, Zhang Z, Usami T, Lievens B, Subbarao KV. Tomato immune receptor Ve1 recognizes effector of multiple fungal pathogens uncovered by genome and RNA sequencing. Proc. Natl. Acad. Sci. 2012;109:5110-5115

[81] Grabherr MG, Haas BJ, Yassour M, Levin JZ, Thompson DA, Amit I, Adiconis X, Fan L, Raychowdhury R, Zeng Q. Full-length transcriptome assembly from RNA-Seq data without a reference genome. National Biotech. 2011;29:644-652.

[82] Mortazavi A, Williams BA, McCue K, Schaeffer L, Wold B. Mapping and quantifying mammalian transcriptomes by RNA-Seq. Nat Methods. 2008;5:621-628.

[83] Metzker ML. Sequencing technologies – the next generation. Nat. Rev. Genet. 2009;11:31-46.

[84] Belete T. Boyraz N. Biotechnological tools for detection, identification and management of plant diseases. African J. Biotech. 2019;18(29):797-807. DOI: 10.5897/AJB2018.16591

[85] Balodi R, Bisht S, Ghatak A, Rao KH. Plant disease diagnosis: technological advancements and challenges. Indian Phytopath. 2017;70(3):275-281.

[86] White TJ, Bruns TD, Lee SB, Taylor JW. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Sninsky JJ, White TJ (eds). PCR protocols: a guide to methods and applications. Academic Press, New York; 1990. 315-322 p. DOI: 10.1016/B978-0-12-372180-8.50042-1.

[87] Werres S, Steffens C. Immunological techniques used with fungal plant pathogens—aspects of antigens, antibodies and assays for diagnosis. Ann. Appl. Biol. 1994;125:615-643.

[88] Hansen K, Lobuglio KF, Pfister DH. Evolutionary relationships of the cup-fungus genus *Peziza* and *Pezizaceae* inferred from multiple nuclear genes: RPB2, beta-tubulin, and LSU rDNA. Mol. Phylogenetics Evo. 2005;36:1-23.

[89] Hyde KD, Jones EBG, Lui J-K, Ariyawansa H, Boehm E, et al. Families of *Dothideomycetes*. Fungal Divers. 2013;63:1-313.

[90] Wang HK, Aptroot A, Crous PW, Hyde KD, Jeewon R. The polyphyletic nature of *Pleosporales*: an example from *Massariosphaeria* based on rDNA and RPB2 gene phylogenies. Mycol. Res. 2007;111:1268-1276

[91] Caporaso JG, Kuczynski J, Stombaugh J et al. QIIME allows analysis of high-throughput community sequencing data. Nat Methods. 2010;7:335-336.

[92] Bates ST, Ahrendt S, Bik HM, Bruns TD, Caporaso JG, Cole J, Dwan M, et al. Meeting report: fungal ITS workshop (October 2012). Stand Genomic Sci. 2013;8:118-123.

[93] Schoch CL, Robbertse B, Robert V, Vu D, Cardinali G, Irinyi L, Meyer W, Nilsson RH, Hughes K, Miller N, Kirk PM. Finding needles in haystacks: linking scientific names, reference specimens and molecular data for Fungi. Database. 2014. DOI:10.1093/database/bau061.

[94] Lanzén A, Jørgensen SL, Huson DH, GorferM, Grindhaug SH, Jonassen I, Øvreås L, Urich T. CREST – classification resources for environmental sequence tags. PLoS One. 2012;7:e49334.

[95] Altschul SF, Madden TL, Schäffer AA, Zhang J, Zhang Z, Miller W, Lipman DJ. Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucleic Acids Res. 1997;25:3389-3402.

[96] Olson A, Aerts A, Asiegbu F, Belbahri L, Bouzid O, Broberg A. et al. Insight into trade-off between wood decay and parasitism from the genome of a fungal forest pathogen. New Phycologist. 2012;194:1001-1013. DOI:10.1111/j.1469-8137. 2012.04128.x.

[97] Notomi T, Okayama H, Masubuchi H, Yonekawa T,Watanabe K, Amino N, Hase T. Loop-mediated isothermal amplification of DNA. Nucleic Acids Res. 2000;28:e63. DOI:10.1093/nar/28.12.e63.

[98] Aglietti C, Luchi N, Pepori AL, Bartolini P, Pecori F, Raio A, Capretti P, Santini A. Real-time loop-mediated isothermal amplification: an early-warning tool for quarantine plant pathogen detection. AMB Express. 2019a;9:50-14. DOI:10.1186/s13568-019-0774-9

[99] Aglietti C, Stehliková D, Paap T, Luchi N, Pecori F, Santini AA. new loop mediated isothermal amplification assay based on assimilating probe for early sequence-specific detection of *Fusarium circinatum* and *F. euwallaceae*. Joint

Meeting of the IUFRO, Working Parties "Shoot, foliage and stem diseases" and "Wilt diseases" (7.02.02 and 7.02.03) on Phyllosphere Diseases. 6-10 May 2019, Figline Valdarno, Florence, Italy, 2019b 49 p.

[100] Harrison C, Tomlinson J, Ostoja-Starzewska S, Boonham N. Evaluation and validation of a loop-mediated isothermal amplification test kit for detection of *Hymenoscyphus fraxineus*. Eur. J.Plant Pathol. 2017;149:253-259. DOI:1007/s10658-017-1179-8

[101] Kav NN, Srivastava S, Yajima W, Sharma N. Application of proteomics to investigate plant-microbe interactions. Current Proteomics. 2007;4(1):28-43.

[102] Klose J. Protein mapping by combined isoelectric focusing and electrophoresis of mouse tissues. Humangenetik. 1975;26(3):231-243.

[103] O'Farrell PH. High resolution two-dimensional electrophoresis of proteins. J. Biol. Chem. 1975;250(10):4007-4021.

[104] Komatsu S, Kamal AH, Hossain Z. Wheat proteomics: proteome modulation and abiotic stress acclimation. Frontiers in plant sci. 2014;5:684.

[105] Barker I. Serological methods in crop protection, in Diagnostics in crop production, BCPC Symposium Proceedings No 65, In: Marshall G, British Crop Protection Council, Farnham, UK; 1996. pp 13-22.

[106] Dewey FM, Thornton CR, Gilligan CA. The use of monoclonal antibodies to detect, quantify and visualise fungi in soils. Adv. Bot. Res. 1997;24:275-308

[107] Dewey FM, Thornton CR. Fungal immunodiagnosis in plant agriculture. 1995.

[108] Halk EL, De Boer SH. Monoclonal antibodies in plant disease research. Ann. Rev. Phytopathol. 1985;23:321-350. DOI:10. 1146/annurev.py.23.090185.001541.

[109] Torrance L. Use of monoclonal antibodies in plant pathology. Europion J. plant Pathol. 1995;101:351-363. DOI:10.1007/BF01874849.

[110] Boonham N, Glover R, Tomlinson J, Mumford R. Exploiting generic platform technologies for the detection and identification of plant pathogens. Eur. J. Plant Pathol. 2008;121:355-363. DOI:10.1007/s10658-008-9284-3.

[111] Tomlinson JA, Dickinson M, Hobden E, Robinson S, Giltrap PM, Boonham N. A five-minute DNA extraction method for expedited detection of *Phytophthora ramorum* following prescreening using *Phytophthora* spp. lateral flow devices. J.Microbiol. Methods. 2010;81:116-120. DOI:10.1016/j.mimet.

[112] Luchi N, Loos R, Santini A. Fast and reliable molecular methods to detect fungal pathogens in woody plants. Appl. Microbiol. Biotechnol. 2020;104:2453-2468. DOI:10.1007/s00253-020-10395-4.

[113] Otrosina WJ, Ghase TE, Gobb FW, Korhonen K. Allozyme differentiation of intersterility groups of *Heterobasidion annosum* isolated from conifers in the Western United States. Phytopathol. 1992;82:540-545. DOI:10.1094/Phyto-82-540.

[114] Raddi S, Santini A, Casini N. Comparison of enzymatic polymorphism in different *Seiridium* isolates. Proc Congr Medit Phytopathology Union, 9th, Kusadasi, Izmir: J. Turkish phytopath. 1994;281-285.

[115] McNeill, J. Barrie FR, Buck WR, Demoulin V, Greuter W, Hawksworth DL, Herendeen PS, Knapp S, Marhold K, Prado J,

Prud'homme Van Reine WF. International Code of Nomenclature for algae, fungi, and plants (Melbourne Code) adopted by the Eighteenth International Botanical Congress Melbourne, Australia, July 2011. [Regnum Vegetabile No. 154.] Königsten: Koeltz Scientific Books; 2012.

[116] Dayarathne MC, Boonmee S, Braun U, Crous PW, Daranagama DA, Dissanayake AJ, Ekanayaka H, Jayawardena R, Jones EBG, Maharachchikumbura SSN, Perera RH, Phillips AJL, Stadler M, Thambugala KM, Wanasinghe DN, Zhao Q, Hyde KD, Jeewon R. Taxonomic utility of old names in current fungal classification and nomenclature: Conflicts, confusion & clarifications. Mycosphere. 2016;7(11):1622-1648. DOI: 10.5943/mycosphere/7/11/2.

[117] Hawksworth DL. A new dawn for the naming of fungi: impacts of decisions made in Melbourne in July 2011 on the future publication and regulation of fungal names. MycoKeys. 2011,7-20. DOI:10.5598/imafungus.2011.02.02.06.

[118] Guarro J, Gené J, Stchigel AM. Developments in fungal taxonomy. Clinical Microbiology Reviews. 1999;12(3):454-500. DOI: 10.1128/CMR.12.3.454.

[119] Wingfield MJ, De Beer ZW, Slippers B, Wingfield BD, Groenewald JZ, Lombard L, Crous PW. One fungus, one name promotes progressive plant pathology. Mol. plant pathol. 2012;13(6):604-613. DOI:10.1111/j.1364-3703.2011.00768.x.

[120] Lombard L, Crous PW, Wingfield BD, Wingfield MJ. Multigene phylogeny and mating tests reveal three cryptic species related to *Calonectria pauciramosa*. Stud. Mycol. 2010a;66:15-30. DOI:10.3114/sim.2010.66.02.

[121] Gams W, Jaklitsch W. A critical response to the 'Amsterdam Declaration'. Mycotaxon. 2011;116:501-512. DOI:10.5598/imafungus.2013.04.01.06.

[122] Hawksworth DL, McNeill J, de Beer W, Wingfield MJ. Names of fungal species with the same epithet applied to different morphs: how to treat them. IMA fungus. 2013;4(1):53-56. DOI:10.5598/imafungus.2013.04.01.06.

[123] de Beer ZW, Duong TA, Barnes IBD, Wingfield BD, Wingfield MJ. Redefining *Ceratocystis* and allied genera. Studies in Mycology. 2014;79:187-219. DOI:10.1016/j.simyco.2014.10.001.

[124] Jankowiak R, Strzałka B, Bilański P, Linnakoski R, Aas T, Solheim H, Groszek M, de Beer ZW. Two new *Leptographium* spp. reveal an emerging complex of hardwood-infecting species in the Ophiostomatales. Antonie Van Leeuwenhoek. 2017;110:1537-1553. DOI:10.1007/s10482-017-0905-8.

[125] Taylor JW. One Fungus = One Name: DNA and fungal nomenclature twenty years after PCR. IMA Fungus. 2011;2:113-120. DOI:10.5598/imafungus.2011.02.02.01.

[126] Carnegie AJ, Cooper K. Emergency response to the incursion of an exotic myrtaceous rust in Australia. Australasian Plant Pathol. 2011;40:346-359. DOI:10.1007/s13313-011-0066-6.

[127] Coutinho TA, Wingfield MJ, Alfenas AC, Crous PW. *Eucalyptus* rust: a disease with the potential for serious international implications. Plant Dis. 1998;82:819-825. DOI:10.1094/PDIS.1998.82.7.819.

[128] Glen M, Alfenas AC, Zauza EA, Wingfield MJ, Mohammed C. *Puccinia psidii*: a threat to the Australian environment and economy–a review. Australasian Plant Pathol. 2007;36(1):1-6. DOI:10.1071/AP06088.

[129] Gams W. A new nomenclature for fungi. Mycologia Iranica. 2014;1(1):1-5. DOI:10.22043/MI.2014.2959.

[130] Rossman A, Seifert K. Phylogenetic revision of taxonomic concepts in the Hypocreales and other Ascomycota-a tribute to Gary J. Samuels. Stud. Mycol. 2011;68.

[131] Gräfenhan T, Schroers HJ, Nirenberg HI, Seifert KA. An overview of the taxonomy, phylogeny, and typification of nectriaceous fungi in *Cosmospora*, *Acremonium*, *Fusarium*, *Stilbella*, and *Volutella*. Studies in Mycology. 2011;68:79-113. DOI:10.3114/sim.2011.68.04.

[132] Summerbell RC, Gueidan C, Schroers HJ, De Hoog GS, Starink M, Rosete YA, Guarro J, Scott JA. *Acremonium* phylogenetic overview and revision of *Gliomastix*, *Sarocladium*, and *Trichothecium*. Stud. Mycol. 2011;68:139-162. DOI:10.3114/SIM.2011.68.06.

[133] Chaverri P, Salgado C, Hirooka Y, Rossman AY, Samuels GJ. Delimitation of *Neonectria* and *Cylindrocarpon* (Nectriaceae, Hypocreales, Ascomycota) and related genera with Cylindrocarpon-like anamorphs. Studies in Mycology. 2011;68:57-78. Doi:10.3114/sim.2011.68.03.

[134] Hirooka Y, Rossman AY, Chaverri P. A morphological and phylogenetic revision of the *Nectria cinnabarina* species complex. Studies in Mycol. 2011;68:35-56. Doi:10.3114/sim.2011.68.02.

[135] Mejía LC, Castlebury LA, Rossman AY, Sogonov MV, White Jr JF. A systematic account of the genus *Plagiostoma* (Gnomoniaceae, Diaporthales) based on morphology, host-associations, and a four-gene phylogeny. Stud. Mycol. 2011;68:211-235. Doi:10.3114/sim.2011.68.10.

[136] Sultan A, Johnston PR, Park D, Robertson AW. Two new pathogenic ascomycetes in *Guignardia* and *Rosenscheldiella* on New Zealand's pygmy mistletoes (*Korthalsella*: Viscaceae). Stud. Mycol. 2011;68:237-247. Doi:10.3114/sim.2011.68.11.

[137] Põldmaa K. Tropical species of *Cladobotryum* and *Hypomyces* producing red pigments. Stud. Mycol. 2011;68:1-34. DOI:10.3114/sim.2011.68.01.

[138] Réblová M, Seifert KA. Discovery of the teleomorph of the hyphomycete, *Sterigmatobotrys macrocarpa*, and epitypification of the genus to holomorphic status. Stud. Mycol. 2011;68:193-202. DOI:10.3114/sim.2011.68.08.

[139] Crous PW, Groenewald JZ, Shivas RG, Edwards J, Seifert KA, Alfenas AC, Alfenas RF, Burgess TI, Carnegie AJ, Hardy GS, Hiscock N. Fungal Planet description sheets: 69-91. Persoonia, 2011; 26:108-156. DOI: 10.3767/003158511x581723 PMID: 22025808 PMCID: PMC3160798

[140] Lombard L, Crous PW, Wingfield BD, Wingfield MJ. Phylogeny and systematics of the genus *Calonectria*. Stud. Mycol. 2010b;66:31-69. DOI:10.3114/sim.2010.66.03.

[141] Lombard L, Crous PW, Wingfield BD, Wingfield MJ. species concepts in *Calonectria* (*Cylindrocladium*). Stud. Mycol. 2010c;66:1-3. DOI:10.3114/sim.2010.66.01.

[142] Lombard L, Rodas CA, Crous PW, Wingfield BD, Wingfield MJ. *Calonectria* (*Cylindrocladium*) species associated with dying Pinus cuttings. Persoonia. 2009;23:41. DOI:10.3767/003158509X471052.

[143] Aylward J, Steenkamp ET, Dreyer LL, Roets F, Wingfield BD, Wingfield MJ. A plant pathology perspective of fungal genome

sequencing. IMA fungus. 2017;8(1):1-5 DOI:10.5598/imafungus.2017.08.01.01.

[144] Braun U, Cook RTA. Taxonomic Manual of the Erysiphales (Powdery Mildews). CBS Biodiversity Series. 2012; 11:1-707.

[145] Hawksworth DL. Global species numbers of fungi: Are tropical studies and molecular approaches contributing to a more robust estimate? Biodivers. Conserv. 2012;21:2425-2433.

[146] Peterson SW. Phylogenetic relationships in *Aspergillus* based on rDNA sequence analysis. In: Integration of Modern Taxonomic Methods for *Penicillium* and *Aspergillus* Classification, Samson RA, Pitt JI, eds. Amsterdam: Harwood Academic Publishers; 2000. 323-356 p.

[147] Taylor JW, Jacobson DJ, Kroken S, Kasuga T, Geiser DM, Hibbett DS, Fisher MC. Phylogenetic species recognition and species concepts in fungi. Fungal Genet. Biology. 2000;31(1):21-32. DOI:10.1006/fgbi.2000.1228.

[148] Taylor JW, Göker M, Pitt JI. Choosing one name for pleomorphic fungi: the example of *Aspergillus* versus *Eurotium*, *Neosartorya* and *Emericella*. Taxon. 2016;65(3):593-601. DOI:10.12705/653.10.

[149] Kirk PM, Stalpers JA, Braun U, Crous PW, Hansen K, Hawksworth DL, Hyde KD, Lücking R, Lumbsch TH, Rossman AY, Seifert KA. A without-prejudice list of generic names of fungi for protection under the International Code of Nomenclature for algae, fungi, and plants. IMA fungus. 2013;4(2):381-443. DOI:10.5598/imafungus.2013.04.02.17.

[150] Rifai MA. A Revision of the Genus *Trichoderma*. Mycological Papers. 1969;116:1-56.

[151] Druzhinina IS, Kubicek CP, Komoń-Zelazowska M, Mulaw TB, Bissett J. The *Trichoderma harzianum* demon: complex speciation history resulting in coexistence of hypothetical biological species, recent agamo species and numerous relict lineages. BMC Evolutionary Biology. 2010;10(1):1-4. DOI:10.1186/1471-2148-10-94.

[152] Chaverri P, Branco-Rocha F, Jaklitsch W, Gazis R, Degenkolb T, Samuels GJ. Systematics of the *Trichoderma harzianum* species complex and the re-identification of commercial biocontrol strains. Mycologia. 2015;107(3):558-590. DOI:10.3852/14-147.

[153] Li QR, Tan P, Jiang YL, Hyde KD, Mckenzie EH, Bahkali AH, Kang JC, Wang Y. A novel *Trichoderma* species isolated from soil in Guizhou, *T. guizhouense*. Mycol. Prog. 2013;12(2): 167-172. DOI:10.1007/s11557-012-0821-2.

[154] Woudenberg JH, Groenewald JZ, Binder M, Crous PW. *Alternaria* redefined. Stud. Mycol. 2013;75:171-212. DOI:10.3114/sim0015.

[155] Hyde KD, Nilsson RH, Alias SA, Ariyawansa HA, Blair JE, Cai L, de Cock AW, Dissanayake AJ, Glockling SL, Goonasekara ID, Gorczak M. One stop shop: backbones trees for important phytopathogenic genera: I (2014). Fungal Divers. 2014;67(1):21-125. DOI:10.1007/s13225-014-0298-1.

[156] Jayawardena RS, Hyde KD, Jeewon R, Ghobad-Nejhad M, Wanasinghe DN, Liu N, Phillips AJ, Oliveira-Filho JR, da Silva GA, Gibertoni TB, Abeywikrama P. One stop shop II: taxonomic update with molecular phylogeny for important phytopathogenic genera: 26-50 (2019a). Fungal Divers. 2019a;94(1):41-129. DOI:10.1007/s13225-019-00418-5.

[157] Jayawardena RS, Hyde KD, McKenzie EH, Jeewon R, Phillips AJ, Perera RH, de Silva NI, Maharachchikumburua SS, Samarakoon MC, Ekanayake AH, Tennakoon DS. One stop shop III: taxonomic update with molecular phylogeny for important phytopathogenic genera: 51-75 (2019). Fungal Divers. 2019b;98(1):77-160. DOI: 10.1007/s13225-019-00433-6(0123456789.

[158] Jayawardena RS, Hyde KD, McKenzie EHC. et al. One stop shop IV: taxonomic update with molecular phylogeny for important phytopathogenic genera: Fungal Diversity. 2020;99:76-100. DOI:10.1007/s13225-020-00460-8.

[159] Marin-Felix Y, Groenewald JZ, Cai L, Chen Q, Marincowitz S, Barnes I, Bensch K, Braun U, Camporesi E, Damm U, de Beer ZW, Dissanayake A, Edwards J, Giraldo A, Hernandez-Restrepo M, Hyde KD, Jayawardena RS, Lombard L, Luangsa-ard J, McTaggart AR, Rossman AY, Sandoval-Denis M, Shen M, Shivas RG, Tan YP, van der Linde EJ, Wingfield MJ, Wood AR, Zhang JQ, Zhang Y, Crous PW. Genera of phytopathogenic fungi: GOPHY 1. Stud. Mycol. 2017;86:99-216. Doi:10.1016/j.simyco.2017.04.002.

[160] Marin-Felix Y, Hernández-Restrepo M, Iturrieta-González I, García D, Gené J, Groenewald JZ, Cai L, Chen Q, Quaedvlieg W, Schumacher RK, Taylor PWJ, Ambers, C, Bonthond G, Edwards J, Krueger-Hadfield SA, Luangsa-ard JJ, Morton L, Moslemi A, Sandoval-Denis M, Tan YP, Thangavel R, Vaghefi N, Cheewangkoon R, Crous PW. Genera of phytopathogenic fungi: GOPHY 3. Stud. Mycol. 2019b;94:1-124. Doi:10.1016/j.simyco.2019.05.001.

[161] Marin-Felix Y, Hernández-Restrepo M, Wingfield MJ, Akulov A, Carnegie AJ, Cheewangkoon R, Gramaje D, Groenewald JZ, Guarnaccia V, Halleen F, Lombard L, Luangsa-ard J, Marincowitz S, Moslemi A, Mostert L, Quaedvlieg W, Schumacher RK, Spies CFJ, Thangavel R, Taylor PWJ, Wilson AM, Wingfield BD, Wood AR, Crous PW. Genera of phytopathogenic fungi: GOPHY 2. Stud. Mycol. 2019a;92:47-133. DOI:10.1016/j.simyco.2018.04.002

[162] Vu D, Groenewald M, De Vries M, Gehrmann T, Stielow B, Eberhardt U, Al-Hatmi A, Groenewald JZ, Cardinali G, Houbraken J, Boekhout T. Large-scale generation and analysis of filamentous fungal DNA barcodes boosts coverage for kingdom fungi and reveals thresholds for fungal species and higher taxon delimitation. Stud. Mycol. 2019;92:135-154. DOI:10.1016/j.simyco.2018.05.001.

[163] Seifert KA. Progress towards DNA barcoding of fungi. Mol. Ecol. Resources. 2009;9:83-89. DOI:10.1111/j.1755-0998.2009.02635.x

[164] Goldstein PZ, DeSalle R. Integrating DNA barcode data and taxonomic practice: determination, discovery, and description. Bioessays. 2011;33(2):135-147. DOI:10.1002/bies.201000036.

[165] Hawksworth DL, Hibbett DS, Kirk PM, Lücking R. (308-310) Proposals to permit DNA sequence data to serve as types of names of fungi. Taxon. 2016;65(4):899-900. DOI:10.12705/654.31.

[166] Hawksworth DL, Hibbett DS, Kirk PM, Lücking R. (F-005-006) Proposals to permit DNA sequence data to be used as types of names of fungi. IMA Fungus. 2018;9:v–vi.

[167] Zamora JC, Svensson M, Kirschner R, Olariaga I, Ryman S, Parra LA, Geml J, Rosling A, Adamčík S, Ahti T, Aime MC.

Considerations and consequences of allowing DNA sequence data as types of fungal taxa. IMA fungus. 2018;9(1):167-175. DOI:10.5598/imafungus.2018.09.01.10.

[168] Callahan BJ, McMurdie PJ, Holmes SP. Exact sequence variants should replace operational taxonomic units in marker-gene data analysis. The ISME J. 2017;11(12):2639-2643. DOI:10.1038/ismej.2017.119.