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REVIEW ARTICLE

Advances in Taxonomy of Genus *Phoma*: Polyphyletic Nature and Role of Phenotypic Traits and Molecular Systematics

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Abstract *Phoma* is a highly polyphyletic genus with its unclear species boundaries. The conventional system of identification is functional but it has its limitations. Besides morphological studies, chemotaxonomy, secondary metabolite and protein profiling have been assessed for the classification and identification of these fungi. Molecular datasets have provided a better outlook towards the phylogenetic and evolutionary trends of *Phoma*. Molecular markers such as ITS-rDNA, tubulin, actin, translation elongation factor have been widely used by the taxonomists to demarcate species. However, outcomes gained up till now represent preliminary step towards the study of *Phoma* systematics and a combined approach would be beneficial in the understanding of this polyphyletic group members. Lately, on the base of molecular phylogeny of the type species of the seven *Phoma* sections a new teleomorph family, Didymellaceae has been established, besides the Phaeosphaeriaceae related to sect. Paraphoma anamorphs, and the Leptosphaeriaceae to sect. Heterospora anamorphs. The estimated ratio is about 70 % of the recognized *Phoma*-like species can be associated with the Didymellaceae ascomycetous family.

Keywords Phoma · Systematics · Didymellaceae · ITS-rDNA · Translation elongation factor · *tef1* sequences · β-tubulin

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Introduction

Phoma species are geographically prevalent and are found in diverse ecological niches. In spite of having numerous harmless saprobic species, *Phoma* species have been well known as imperative plant pathogen on economically important plants [1, 2]. The identification of isolates based on morphological characters is often conceded beneath extreme time restraints. However, generation of vast morphological and anatomical data over the years had built up a strong base for the taxonomic studies of *Phoma*. But, this often lacked satisfactory resolution and thus should always be compared with the conclusions from molecular data sources. Moreover, there have been efforts to classify fungi based on secondary metabolite profiles [3]. Chemotaxonomy is the use of chemical diversity as a taxonomic tool which means classification and identification of filamentous fungi based on profiles of secondary metabolites.

The major objective of any taxonomic study includes systematic grouping of taxa of interest through generation of robust natural classification based on constant characteristics which reveal their factual evolutionary record and development of trustworthy identification key(s) for uncomplicated taxon determination. Molecular taxonomic methods are the most widely accepted tools for identification of the appropriate taxonomic level at which it would be most informative and its correlation with morphologically definable taxonomic groupings.

Identification on the Basis of Phenotypic Characteristics

Phoma is taxonomically a controversial genus. It consists of over 200 known species, found all over the world. The original genus concept of Saccardo [4] was emended by

Boerema and Bollen [5] and after more than 40 years of taxonomical research, the admitted *Phoma* species were arranged in nine sections [6, 7], which are mainly based on a single or just a few morphological characters and have not been confirmed as biologically realistic by molecular biological studies [2, 8].

Recently there is a convention of using a single generic name, based on priority but regardless of whether the genus is "anamorphic" or "teleomorphic". This classification is used for all unambiguous monophyletic phylogenetic lineages [2].

The nine *Phoma* sections have teleomorph relations described in the genera *Didymella*, *Mycosphaerella*, *Leptosphaeria* and *Pleospora* [6] indicating that *Phoma* anamorphs represent a polyphyletic group. For a long time the genera *Phoma* and *Ascochyta*, both classified in the Pleosporales of Ascomycota, have already been considered as closely related [9]. The species of genus *Phoma* fungi reproduce asexually and a typical colony of the genus has a velvety texture which can be slightly powdery, depending on the species. It may be white to gray with pink, yellow, and reddish purple colourations. Pycnidia contain single celled masses of spores which are known as pycn(idi)ospores or conidia. With the effect of different factors like pH, temperature, light, etc. the culture characteristics and morphology differs [10–12]. They are also known to produce variety of secondary metabolites in the form of dyes, antibiotics, etc. [13–29].

Phoma species are ubiquitous and are common inhabitants of soil [1, 30–35] and they may periodically infect plants by causing root infections and different spot/blotch diseases [36–41]. Moreover, *Phoma* species have also been reported as opportunistic invasive pathogen in humans [42–48].

A variety of significant agronomic crops are susceptible to the wide-ranging species of the genus *Phoma* [49–52].

Until the late seventies, majority of the Indian species of *Phoma* were erected on the basis of host alone [53–64], and thus the importance of host specificity for the taxonomy of *Phoma* has been much emphasized and overestimated. Up to eighties many mycologist in India did not consider morphological characters for identification and differentiation of *Phoma* species in culture. Usually, a morphological species may attack various host plants. For example, *P. exigua* was reported on different hosts [65]. The criterion of identification should be in such a way so that it should be possible to identify a *Phoma* species in case host identification is difficult particularly when floral parts are lacking, or when the fungus is grown on artificial media. These have created taxonomic dilemmas and misinterpretations with superfluous species descriptions.

The growth and colour of the colony helps in differentiating the species in the genus *Phoma* [66]. On the basis of cultural and morphological characteristics of *Phoma* was assembled in 20 broad groups [38, 67–73]. Furthermore, there were studied effects of different factors such as different colours of light, temperature and different media on the morphology and cultural characteristics of different *Phoma* species [10, 14, 36, 74, 75].

Previously the erection of new species of *Phoma* was mostly based on host and sometimes on shape and size of pycnidia and pycnospores. The key to identification of *Phoma* was summarized by Boerema et al. [7] on the basis of their morphological appearances. This key is enormously helpful in identifying strains up to species level but it has its limitations as several taxa exhibit characters that are representative of different sections [76].

The erection of *Phoma* species on the basis of traditional methods particularly on host was a criterion which dominated up to early seventies. Later, mycologists realized the importance of morphological characters for creating a new *Phoma* species. The most important morphological characteristics include the formation of pycnidia, conidia and chlamydospores. Although, size and septation of the conidia are also important criteria for species identification, at times the conidium dimensions may differ in the strains of the same species.

As numbers of fungi grow on wide range of culture media, fungal taxonomists use these physiological and biochemical tools for the identification and classification purpose [53]. Growth rates on distinct media can also be exploited for the differential studies of filamentous fungi.

As *Phoma* species are morphologically similar and hence discrimination of the species based on the production of secondary metabolites, cultural characteristics, mostly depends on the growth conditions, which are not reliable. Thus, it can be conferred that phenotypic characters cannot always be distinctive between taxa and the aid of molecular systematics will rather helpful in delineating the unclear species boundaries of this controversial genus.

Determining the Identity of *Phoma* by Multiple Approach

Secondary metabolite profiling has also been assessed as one of the marker for differentiation among filamentous fungi including *Phoma*-like species [3, 77]. Secondary metabolites are usually a mixture of closely related molecules with a peculiar and exceptional chemical structure such as steroids, terpenes, alkaloids, cyclopeptides, and coumarins. Some of these are mycotoxins. With the advent of modern chromatographic techniques the profiling is easy fast and reliable which has resulted in a vast array of secondary metabolite data. Conversely, this method has a drawback as the production of these secondary metabolites can be affected by different factors such as environmental conditions, temperature and pH [53].

Isozyme comparative studies were made to delineate of α-esterase isozyme profiles of some *Phoma* species and varieties [78]. Simple band-counting technique is used for differentiation, although phylogenetic information can be retrieved from allelic frequencies and other genetic interpretation data. Furthermore, this analysis can be used to identify unknown species or to identify a pathogen in a mixed infection.

Identification Based on Molecular Datasets

Recently, the use of molecular datasets in phylogenetic assessment has gained much popularity among the mycologists. Molecular data such as RAPD, RFLP analyses and the use of DNA sequences are very commonly used by mycologists [79]. ITS-rDNA sequences are divergent and vary between species within a genus hence it is the most popular choice in molecular systematics.

Generic Regions Responsible for Identification of *Phoma* sp.

The molecular based phylogenetic analyses within *Phoma* genus have only been used for defining phylogenetic relationships among isolates within one or closely related species, however recently serial publications came out with re-classification consequences of *Phoma*-like fungi on the base of molecular data [1, 2, 8, 9, 49, 76, 80–82].

Molecular taxonomy is an essential part for authentication of established species concept, identification and taxonomic revision of well-established species based on phenotypic and ecological characters and also desired for the detection of cryptic species (species where no morphological differences exist) [83–85]. With the advent of PCR wide range of molecular markers viz. nuclear ribosomal markers (ITS-rDNA), protein coding genes (tubulin genes, translation elongation factor, actin, histone gene, etc.) have been expedited by fungal taxonomists for the study of both phylogenetic and population structure studies [86]. Some of these markers which are used predominantly have been discussed briefly below.

Nuclear rDNA Sequences

Bi-parental, nuclear ITS regions are one of the most popular choices for phylogenetic inference for genus level or below due to higher rate of base substitution than most of the organellar genes [2, 49, 79, 80]. Hence, it has typically been most useful for molecular systematics at the species level, and even within species to identify geographic races.

ITS, refers to a piece of non-functional RNA situated between structural ribosomal RNAs (rRNA) on a common precursor transcript. This polycistronic rRNA precursor transcript contains the 5′-external transcribed sequence (5′-ETS), 18S rRNA, ITS1, 5.8S rRNA, ITS2, 26S rRNA and finally the 3′-ETS. During rRNA maturation, ETS and ITS pieces are excised and as non-functional maturation by-products rapidly degraded. Genes encoding ribosomal RNA and spacers occur in tandem repeats that are thousands of copies long, each separated by regions of non-transcribed DNA termed intergenic spacer (IGS) or non-transcribed spacer (NTS) [86]. Sequence comparison of the ITS region is widely used in taxonomy and molecular phylogeny.

Tubulin Gene

Tubulin is one of the members of globular heterodimeric proteins. The most common classes are alpha (α) and beta (β) tubulin having molecular weight of about 55 kDa [87–90]. These proteins are responsible for the production of microtubules. β-tubulin is encoded by highly conserved multigene families or in some cases single genes. On the basis of tubulin genes wide range of organisms are erected and characterized leading to the successful determination of inter- and intraspecific relationships [49, 80, 91].

Translation Elongation Factors (tef) Gene

Elongation factors are set of proteins used in protein synthesis. They facilitate translational elongation starting from the first to the last peptide bond in ribosome. The translation elongation factor 1 alpha gene (*tef1*) has been widely used for the phylogenetic and taxonomic evaluation study of *Phoma* [80]. Single-copy genes have the benefit that any sequence variation within a spore can be recognized explicitly to disparity among nuclei.

Actin Gene

The actin gene encodes actin, a multifunctional protein found in all eukaryotic cells and is one of the highly conserved proteins. These genes have been used to study evolutionary relationships among *Phoma* species [92]. Moreover, these genes assist in exploring the additional information about genetic structure in these fungi.

Histone Protein Gene

Eukaryotic genomic DNA is wrapped with histone proteins in nucleosomes the fundamental units of chromatin [93–95]. The nucleosome comprises of the DNA enfolded around an octamer of core histones consisting of two copies each of histones H2A, H2B, H3, and H4 assembled in one H3–H4 heterotetramer and two H2A–H2B heterodimers [96]. Histone protein gene helps in the comparative studies of intron insertion sites among the different organisms.

DNA Barcoding

With the advancement in sequencing and computational methodologies DNA sequences have become a major source of information for evolutionary and genetic relationships. Comparative sequence analysis has emerged in almost all fields of biological sciences. A short standardized sequence that can distinguish the individual from the species forms the basis of DNA barcoding [97]. Barcoding is usually carried out by the retrieval of a DNA sequence i.e. a barcode from a specific gene region. This unknown barcode is then compared with the other barcodes present in the library of reference barcode sequences.

The most preferred DNA barcode region for fungi is ITS but it not enough to delineate all the taxa [98, 99]. However, according to Aveskamp et al. [81] actin gene can also be considered for developing DNA barcodes for *Phoma* species.

Conclusions

Phoma with its unclear species boundaries still remains a taxonomically controversial genus. A vast array of morphological as well as molecular data have been generated still there are many questions that has to be addressed with numerous species yet to be discovered. Though the traditional method of identification lacks the specificity it still cannot be outlined in the presence of modern techniques of molecular systematics. Morphological studies still forms the basis of the preliminary level identification and thus is applicable and aid to morphological species recognition. Nevertheless, it can be concluded that a multiple approach, including molecular tools, would provide a better understanding for the constant and reliable identification of *Phoma* species. Lately, towards a reclassification of the *Phoma* complex, on the data of molecular phylogeny, a new ascomycetous teleomorph family, Didymellaceae has been established, besides to the Phaeosphaeriaceae and Leptosphaeriaceae [9]. Based on the sequence data, it is estimated that approximately 70 % of the species recognised by Boerema et al. [7] can be associated with the Didymellaceae [2].

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