STUDIES OF EVOLUTIONARY RELATIONSHIPS OF *PHOMA* SPECIES BASED ON PHYLOGENETIC MARKERS

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Introduction

Phoma is a cosmopolitan genus of coelomycetous fungi. Many species have been reported from wide range of hosts, substrates, particularly as pathogens from plants, as well as soil-borne but predominantly saprophytic and opportunistic species have also been isolated. Almost 2000 *Phoma* species have been reported throughout the world till now (Boerema et al., 2004).

There are several ways in the traditional and modern mycology to contribute to taxonomical studies of fungi including morphology, biochemistry, nucleic acid sequences and many others.

The three most commonly discussed species concepts are morphological, biological, and phylogenetic ones. Since the beginning of mycology, studies of species concept in fungi have been mainly based on morphological elements. The most of the species and other taxa of *Phoma* have so far been determined on the basis of morphology on standardized media, and gene sequence analysis was only used as a confirmative or distinctive complement. Thus, members of the genus are primarily defined by the application of the Morphological Species Recognition (MSR). The weakness of MSR is that species diagnosed by this often comprise more than one species when diagnosed by Biological Species Recognition (BSR) or Phylogenetic Species Recognition (PSR). Biological species concept defines species as groups of actually or potentially interbreeding natural populations which are reproductively isolated from other such groups (Mayr, 1942). BSR is acceptable for many fungi, where sexual reproduction occurs. But there are also fungal groups, where sexual reproduction has never been discovered. Approximately 20% of fungi are morphologically asexual and do not produce meiospores (Reynolds and Taylor, 1993). Since strains of *Phoma* spp. apparently can not be crossed, the application of the BSR concept is impracticable. Though, despite reproducing asexually, many anamorphic fungi including *Phoma* spp. are known to possess a surprisingly high level of genetic variation (Khon, 1995; Talhinhas et al., 2002).

The current advances in biochemical and molecular research have provided mycologists with powerful tools that can be used for delineation of fungal taxa. The PSR defines species as the smallest aggregation of populations with a common lineage that share unique, diagnosable phenotypic characters (Harrington and Rizzo, 1999). According to Taylor et al. (2000) seems to be well suited for fungi and likely to become very popular with mycologists, because it can be applied equally both to sexual and to asexual organisms. Taylor et al. (2000) proposed the Genealogical Concordance Phylogenetic Species Recognition (GCPSR) *for species defining,* which *could be* an attractive alternative or complement to the morphological species concept, but has not been widely applied to *Phoma* spp. yet. It requires the analysis of several unlinked genes and implies that the phylogenetic position of a true species is concordant in at least some of them and can not be contradicted in the others.

Up to now the characterization of *Phoma* species has been mostly applied on the basis of morphology, phenotype and physiology. Recently, Boerema et al. (2004) published *Phoma* Identification Manual, based on morphological studies which contains 223 cultural descriptions of specific and intraspecific taxa of *Phoma* Sacc.

In the middle of 90s, due to the advances in molecular and biochemical research of that time molecular markers were identified in *Phoma*. Some isozyme analyses were applied to distinct some morphologically identical *Phoma* species from each other (Kövics and Gruyter, 1995). Protein polymorphisms comparing to DNA polymorphisms is unfavorable, because protein electrophoresis assays the genotype indirectly, and a high proportion of the variation occurs at the DNA level may not be detectable as it does not alter the amino acid composition of the protein. Similarly, some changes in amino acid composition do not change the electrophoretic mobility of the protein, and remain undetected, leading to different genotypes being assigned to the same allozyme allele.

DNA polymorphisms are based on differences in DNA sequences and have three enormous advantages over protein polymorphisms. The first is, that the sequence differences are detected directly. The second advantage is that they occur in a genome at very high frequency, and finally, they are not subject to selection pressure, in case they do not affect the phenotype. But morphological characterization besides molecular tools will remain a basic and powerful key in the identification of *Phoma* species.

One of the most commonly used molecular techniques for assessing phylogenetic relationships is to evaluate the sequences of certain fungal DNA regions. Phylogenetic sequence comparisons concentrate on a comparison of the coding portions of the ribosomal genes and their RNA products, allowing discrimination at different taxonomic levels. Many phylogenetic works are based on the internally transcribed spacers (ITS),

which are one of the most widely used molecular markers due to their highly variability in nucleotide sequences.

According to Lutzoni et al. (2004) 83.9% of fungal phylogenies are based exclusively on sequences from the ribosomal RNA tandem repeats. Because of it, there is a consequent trend toward inclusion of other gene loci in the data sets, gathered for phylogenetic analysis. Among these genes, protein-coding genes like β-tubulin and translation elongation factor (*tef1*) can contribute greatly to resolving deep phylogenetic relationships with high support and/or increase support for topologies inferred using ribosomal RNA genes.

In this study we have obtained DNA sequences from ITS and translation elongation factor coding genes to resolve phylogenetic relationships among several *Phoma* species, since it has been shown that usage of multigene datasets can increase the resolution of molecular phylogenetic analyses.

Ribosomal DNA (rDNA) has long been used as a potential marker for phylogenetic studies (reviewed in Avise, 2004). rRNA genes are organized in clusters of tandemly repeated units, each of which consists of coding regions (18S, 5.8S, and 28S; Gerbi, 1985) and 2 internal transcribed spacers (ITS) and intergenic spacer (formerly called the „ Non-Transcibed Spacer, NTS) region. While the coding regions are evolutionarily conserved and have been utilized for phylogenetic inferences for major phyla (reviewed in Hills and Dixon 1990), the 2 ITS regions are appropriate for detecting differences between co-specific individuals and are hence potentially useful markers to study the relationships of populations and closely related species in fungal, plant, and animal taxa due to their relatively rapid evolutionary rates (Baldwin, 1992; Schlötterer et al., 1994; Mai and Coleman, 1997; Weekers et al., 2001; Oliverio et al., 2002; Chen et al., 2000, 2002). In this study we have obtained a region of nuclear rDNA, containing the internal transcribed spacer regions 1 and 2 and the 5.8S rDNA (Figure 1).

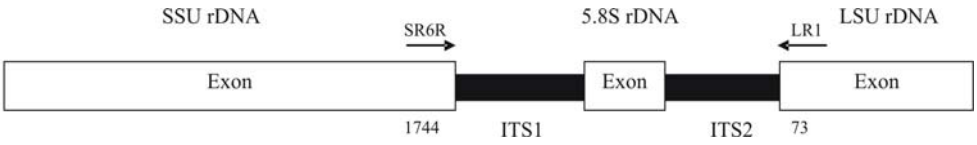

Figure 1. Schematic structure of ITS region in *Phoma* spp. and location of primers for phylogenetic analyses

Translation elongation factor 1 subunit alpha (EF1α=*tef*1) is part of the cytosolic EF1 complex, whose primary function is to promote the binding of aminoacyl-tRNA to the ribosome in a GTP-dependent process (Moldave, 1985). It is an essential component of the protein synthesis process in eukaryotes and archeabacteria. Complexed with GTP, it carries the aminoacyl-tRNA to the A site of the ribosome-mRNA–peptidyl-tRNA complex; upon hydrolysis of GTP it leaves the ribosome as EF-1α-GDP.

Simultaneously, elongation factor 1α (EF-1α) is a highly conserved ubiquitous protein that has been suggested to have desirable properties for phylogenetic inference (Roger et al., 1999). EF-1α is well suited for determining phylogenetic relationships, due to its universal occurrence and presence typically as a single copy within the genome (Baldauf and Doolittle, 1997). It has been proven to be a useful gene to resolve phylogenetic relationships at species level as well as in deeper divergences where amino acid substitutions provide phylogenetic resolution. Here we have used primer pair which facilitates the PCR amplification of the large intron of tef1 gene (Druzhinina and Kubicek, 2005, Figure 2).

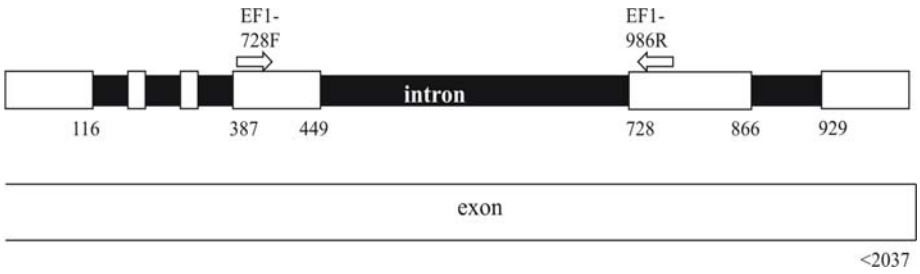

Figure 2. Schematic structure of *tef1* gene in *Phoma* spp. and location of primers for phylogenetic analyses

Materials and Methods

Twelve isolates of eleven *Phoma* species were tested for phylogenetic analyses in this present study (Table 1). All isolates were identified morphologically according to Boerema et al. (2004) based on physiological and morphological characteristics.

Mycelium from isolates were transferred to 100 ml Erlenmayer flasks containing 50 ml malt broth (2% malt exract). The cultures were grown at room temperature for 48 hours in the dark on a rotary shaker (125rpm). The mycelium was harvested by vacuum filtration. Total genomic DNA was extracted from freeze-dried mycelium and isolated using the E.Z.N.A.® TM

Fungal DNA Isolation Kit (Omega Bio-tek, Inc., USA) according to the protocol (as following the manufacture instructions).

Primers used to amplify the ITS region containing the internal transcribed spacer regions 1 and 2 and the 5.8S rDNA are based on published composite sequences, SR6R and LR1 (White et al., 1990) with the following amplification protocol: 3 min initial denaturing at 95°C, followed by 5 cycles of 1 min at 95°C, 1 min annealing at 50°C, 1 min at 72°C and 25 cycles of 1 min at 90°C, 1 min annealing at 50°C, 1min at 72°C and 15 min final extension at 72°C. The large intron of the *tef1* gene was amplified by the EF1-728F and EF1-986R primer pair (Druzhinina and Kubicek, 2005) according to the following program: 3 min initial denaturing at 95°C, followed by 5 cycles of 1 min at 95°C, 1 min annealing at 59°C, 1 min at 72°C and 25 cycles of 1 min at 90°C, 1 min annealing at 59°C, 1min at 72°C and 15 min final extension at 72°C. Purified amplification products were sequenced by MWG Biotech Company in Germany.

The obtained DNA sequences were aligned first with ClustalX (Thompson et al., 1997) and manually adjusted using Genedoc (Nicholas et al., 1997). Single gaps were treated either as missing data or as the fifth base and multistate characters were treated as uncertain.

Phylogenetic analyses were performed in PAUP*4.0b (Swofford, 2002). The following settings were used: heuristic search with tree bisection-reconnection (TBR), with random addition of sequences with 1000 replicates. Stability of clades was assessed with 1000 bootstrap replications.

Results

Twelve isolates of eleven *Phoma* species were compared in this study. The morphological identification of the isolates was done following the descriptions of Boerama et al. (2004). The obtained results indicated that the microscopical and cultural characteristics of the concerned *Phoma* isolates fit to the identity of *Phoma* species given in Table 1.

Table 1. Isolates of *Phoma* species

Species	Isolate number		Host of origin
	Our collection	**Original**	
Phoma eupyrena	D/058	CBS 375.91	*Phaseolus vulgaris*
Phoma destructiva	D/033	?	*Lycopersicon esculentum*
Phoma pinodella	D/035	D/035	*Glycine max*
Phoma foveata	D/048	PD 76/1021	*Chenopodium quinoa*
Phoma herbarum	D/143	MTCC 2319	?
Phoma exigua var. *exigua*	D/075	D/075	*Glycine max*
Phoma exigua var. *exigua*	D/077	D/077	*Glycine max*
Phoma exigua var. *linicola*	D/071	PD 86/73	*Linum usitatissimum*
Phoma glomerata	D/034	D/034	*Glycine max*
Phoma multirostrata	D/044	PD 77/508	*Phylodendron* sp.
Phoma plurivora	D/072	PD 75/907	*Medicago sativa*
Phyllosticta sojicola (=*Phoma exigua* var. *exigua* ?)	D/050	CBS 301.39	*Glycine max*

Translation elongation factor

We amplified and sequenced a 0.2 kb fragment of the large intron of the *tef1* gene from twelve isolates of eleven *Phoma* species and subjected it together with the ITS1-5.8Sr-DNA-ITS2 to a combined parsimony analysis with PAUP.

For phylogenetic analyses of *tef1* fragments we involved other *Phoma* and *Ascochyta* species as well as *Claviceps* and *Leptosphaeria* species as outgroup, all were downloaded from GenBank maintained by the NCBI (Table 2). *Didymella fabae* and *Didymella lentis* are the teleomorph of *Ascochyta fabae* and *Ascochyta lentis*, (Kaiser et al., 1997).

Table 2. Species involved in the phylogenetic analyses of *tef1* fragments

Species	**Isolation code**	**Accession number**
Leptosphaerulina trifolii	WAC 6693	AY831543.1
Ascochyta pisi	AP2	DQ386494.1
teleomorf: *Didymella lentis* anamorf: *Ascochyta lentis*	SAT AL	AY831546.1
Ascochyta fabae f. sp. *viciae* (= *Ascochyta fabae*)	AV11	DQ386498.1
teleomorf: *Didymella lentis* anamorf: *Ascochyta lentis*	AL1	DQ386493.1
teleomorf: *Didymella fabae* anamorf: *Ascochyta fabae*	AF1	DQ386492.1
Claviceps sorghi	?	AY960837.1
Claviceps sorghi	?	AY960836.1
Phoma pinodella	CBS 318.90	AY831542.1
Phoma pinodella	WAC 7978	AY831545.1

According to the phylogenetic tree based on *tef*1 sequences (Figure 3), the *Phoma* species are well separated from their closely related *Ascochyta* taxa.

As the identification of *Phoma* and *Ascochyta* genus based on morphological characteristics is often problematic, this new phylogenetic marker can be a useful tool for mycologists identifying an unknown species.

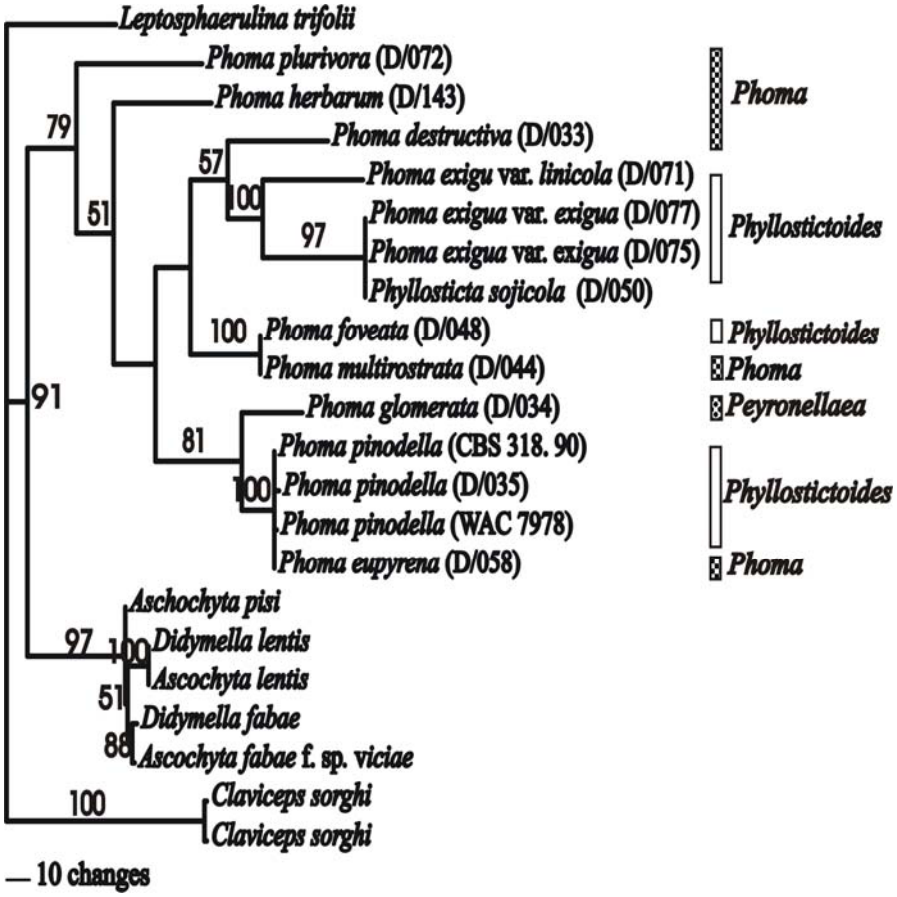

Figure 3. Phylogenetic relationships of *Phoma* strains inferred by the parsimony analysis of *tef1* sequences. The numbers above the lines represent the bootstrap (bootstrap=1000) values. The columns on the right side represent the *Phoma* sections based on morphological characterization

Most of *Phoma* species (*P. plurivora, P. herbarum, P. desrtuctiva, P. glomerata*) are well separated from the other tested *Phoma* species. Some *Phoma* species constitute clades but there are some species which can not be distinguished on the basis of *tef1* sequences (*Phoma pinodella* and *Phoma eupyrena* as well as *Phoma foveata* and *Phoma multirostrata*). The species represented by more than one isolate are classified in the same subgroup, which proves that the *tef1* sequences are well suited for delineating phylogenetic relationships within the *Phoma* genus.

The *Phyllosticta sojicola* associates with the *Phoma exigua* var. *exigua* subgroups which support the statement that the two species are identical (Kövics et al., 1999).

The phylogenetic tree based on *tef1* sequences does not support the traditional *Phoma* sections based on morphological characterization (Boerema et al., 2004).

On the basis of our investigation, carried out with *tef1* sequences all *Phoma* species form a well distinguishable group from the *Ascochyta* species, which proves the monophyletic origin of *Phoma* genus.

Up to now, phylogenetic analyses within *Phoma* genus have only been used for defining phylogenetic relationships among isolates within one species (Mendes et al., 2003 and Balmas et al., 2005).

Here we have used the translational elongation factor to resolve phylogenetic relationships within *Phoma* genus at higher taxonomic levels.

The present study has proved the *tef1* region to be phylogenetically useful tool for defining *Phoma* species but further investigations would be necessary to clarify whether the *tef1* gene sequence as phylogenetic molecular marker is well suited for the classification of *Phoma* species.

ITS sequences

In the PCR reaction 0.6kb fragment of the rDNA gene containing the internal transcribed spacer regions 1 and 2 and the 5.8S regions was amplified.

For phylogenetic analyses of ITS region we involved other *Phoma* and *Ascochyta* species as well as *Didymella* and *Leptosphaeria* species as outgroup, all were downloaded from GenBank maintained by the NCBI (Table 3).

The phylogenetic tree based on ITS sequences (Figure 4) is drawn by parsimony analyis.

The difference between the different *Phoma* and *Ascochyta*, *Leptosphaeria* and *Didymella* species was not significant, 23 sites were considered as informative for the parsimony analysis. Moreover only 3 clades were supported by the bootstrap analysis with more than 80% probability.

We can state from the parsimony tree that the *Phyllosticta sojicola* grouped with the *Phoma exigua*, as we were able to state from the analysis of *tef1* sequences. *Phoma foveata* and *Phoma multirostrata* also grouped together both in ITS and *tef1* analysis.

ITS sequences should analyzed with other methods (like MEGA or maximum likelihood), which may draw a much supported tree.

Table 3. Species involved in the phylogenetic analyses of ITS fragments

Species	**Isolation code**	**Accession number**
Phoma exigua var. *heteromorpha*	?	AY899262.1
Phoma exigua	CSL 20316964	AY550992.1
Phoma exigua var. *populi*	CBS 100167	AF268189.1
Phoma exigua var. *linicola*	CBS 113.28	AF268187.1
Phoma exigua	?	AY927784.1
Phoma herbarum	?	DQ132841.1
Phoma herbarum	ATCC 12569	AY293803.1
Phoma pinodella	VPRI 32177	DQ087402.1
Phoma pinodella	VPRI 32171	DQ087400.1
Phoma pinodella	CBS 318.90	AY831562.1
Phoma pinodella	WAC 7978	AY831556.1
Phoma glomerata	?	AF126816.1
Phoma glomerata	?	AY618248.1
Phoma glomerata	?	AY183371.1
Phoma eupyrena	Gr61	AJ890436.1
Leptosphaerulina trifolii	WAC 6693	AY831558.1
Ascochyta sp.	Georgia6	DQ383955.1
Ascochyta pisi	AP1	DQ383954.1
Ascochyta lentis	MU AL1	AY131201.1
Didymella lentis	AL1	DQ383953.1
Didymella fabae	AF1	DQ383952.1

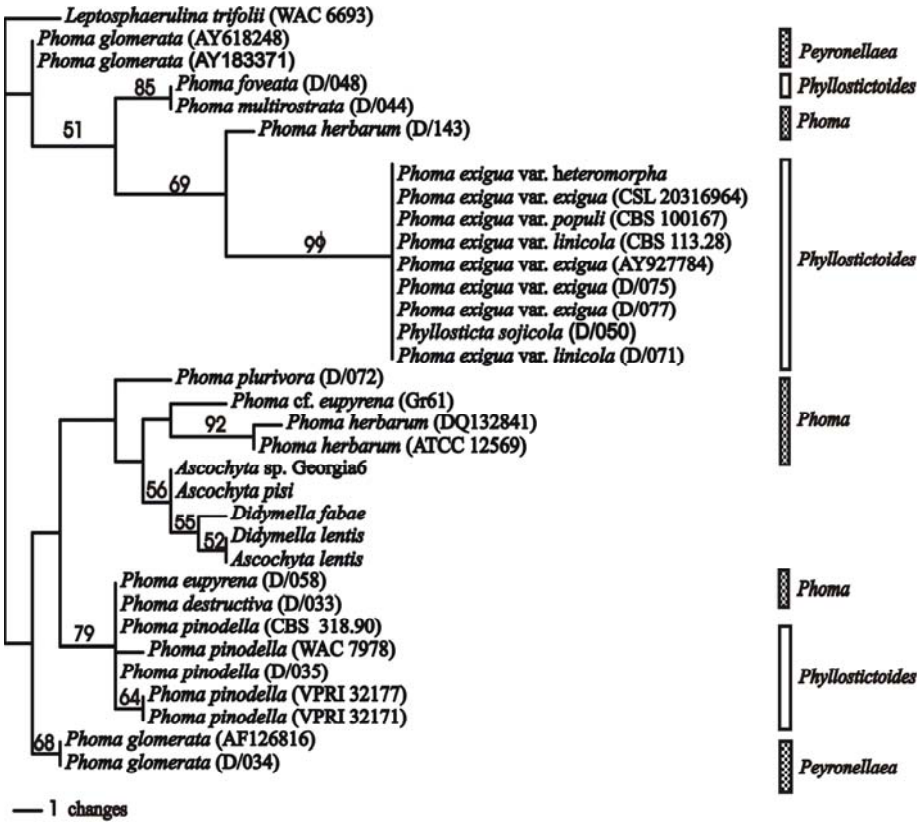

Figure 4. Phylogenetic relationships of *Phoma* strains inferred by the parsimony analysis of ITS sequences. The numbers above the lines represent the bootstrap (bootstrap=1000) values. The columns on the right side represent the *Phoma* section based on morphological characterization.

Discussion

tef1 sequences are well suitable for phylogenetic analysis of *Phoma* species similarly to other mycetous fungi (Druzhinina and Kubicek, 2005). The phylogenetic analysis of ITS sequences by parsimony method has not given a definite result. One possible reason for this can be that there were limited variable sites in ITS sequences. This can mean that the evolutionary distance by ITS sequences within *Phoma* species is too small to get well based consequences for the phylogenetic relationships of *Phoma* genus. So similarly to the *Trichoderma* genus we should involved other sequences in phylogenetic analysis like *tef1* or β-tubulin sequences.

Both sequence analyses confirmed that the *Phyllosticta sojicola* species is identical to the *Phoma exigua* var. *exigua* species as Kövics et al., 1999 claimed.

References

Avise, J.C. (2004): Molecular markers, natural history, and evolution. 2nd ed. Underland, MA: Sinauer Associates

Baldauf, S.L. and Doolittle, W.F. (1997): Origin and evolution of slime molds (Mycetozoa). Proc. Natl. Acad. Sci. USA 94: 12007-12012.

Baldwin, B.G. (1992): Phylogenetic utility of the internal transcribed spacers of nuclear ribosomal DNA in plants: an example from the Compositae. Mol. Phylogenet. Evol. 1: 3-16.

Balmas, V., Scherm, B., Ghignone, S., Salem, A.O.M., Cacciola, S.O., Migheli, Q. (2005): Characterisation of *Phoma tracheiphila* by RAPD-PCR, microsatellite-primed PCR and ITS rDNA sequencing and development of species primers for in planta PCR detection. European Journal of Plant Pathology 111: 235-247.

Boerema, G.H., Gruyter, J. de, Noordeloos, M.E., Hamers, M.E.C. (2004): *Phoma* identification manual. CABI Publishing. CAB International Wallingford, Oxfordshire, UK

Chen, C.A., Wallace, C.C., Wolstenholme, J. (2002): Analysis of mitochondrial 12S RNA gene supports a two-clade hypothesis of the evolutionary history of scleractinian corals. Mol. Phylogenet. Evol. 23: 137-149.

Chen, C.A., Yu, J.K., Wei, N.W. (2000): Strategies for amplification by polymerase chain reaction of the complete sequence of nuclear large subunit ribosomal RNA-encoding gene in corals. Mar. Biotechnol. 6: 558-570.

Druzhinina, I. and Kubicek, C.P. (2005): Species concepts and biodiversity in *Trichoderma* and *Hypocrea*: from aggregate species to species cluster? J. Zhejiang Univ. Sci. 6B (2): 100-112.

Gerbi, S.A. (1985): Evolution of ribosomal DNA. pp. 419-517. In: Molecular evolutionary genetics. Macintyre, R. J. (Ed.) Plenum, New York

Harrington, T.C., and Rizzo, D.M. (1999): Defining species in the fungi. pp. 43-70. In: Structure and Dynamics of Fungal Populations. Worral, J.J. (Ed.) Kluwer Academic, Dordrecht

Hillis, D.M., Dixon, M.T. (1991): Ribosomal DNA: molecular evolution and phylogenetic inference. Q. Rev. Biol. 66: 411-453.

Kaiser, W.J., Wang, B.-C., and Rogers, J.D. (1997): *Ascochyta fabae* and *A. lentis*: Host specificity, teleomorphs (*Didymella*), Hybrid analysis, and taxonomic status. Plant Dis. 81: 809-816.

Khon, L.M. (1995): The clonal dynamic in wild and agricultural plant-pathogen populations. Canadian Journal of Botany 73. (Suppl. 1): S 1231-1240.

Kövics, G.J. and Gruyter, J. de (1995): Comparable esterase isozyme analysis of some *Phoma* species occur on soybean. (A szóján előforduló néhány *Phoma* faj észteráz izoenzim mintázatainak összehasonlító vizsgálata.) Proceedings of Debrecen Agricultural University (DATE Tudományos Közleményei) 31: 191-207.

Kövics, G.J., Gruyter, J. de, Aa, H.A. van der (1999): *Phoma sojicola* comb. nov. and other hyaline-spored coelomycetes pathogenic on soybean. Mycol. Res. 103 (8): 1065-1070.

Lutzoni, F., Kauff, F., Cox, C.J., McLaughlin, D., Celio, G., Dentinger, B., Padamsee, M., Hibbett, D., James, T.Y., Baloch, E., Grube, M., Reeb, V., Hofstetter, V., Schoch, C., Arnold, A. E., Miadlikowska, J., Spatafora, J., Johnson, D., Hambleton, S., Crockett, M., Shoemaker, R., Sung, G.H., Lucking, R., Lumbsch, T., O'Donnell, K., Binder, M., Diederich, P., Ertz, D., Gueidan, C., Hansen, K., Harris, R.C., Hosaka, K., Lim, Y.W., Matheny, B., Nishida, H., Pfister, D., Rogers, J., Rossman, A., Schmitt, I., Sipman, H., Stone, J., Sugiyama, J., Yahr, R., Vilgalys, R. (2004): Assembling the fungal tree of life: progress classi.cation and evolution of subcellular traits. Am. J. Bot. 91: 1446-1480.

Mai, J.C. and Coleman, A.W. (1997): The internal transcribed spacer 2 exhibits a common secondary structure in green algae and flowering plants. J. Mol. Evol. 44: 258-271.

Mayr, E. (1942): Systematics and the origin of species from a viewpoint of a zoologist. Columbia University Press, New York

Mendes-Pereira, E., Balesdent, M.-H., Brun, H., Rouxel, T. (2003): Molecular phylogeny of the *Leptosphaeria maculans-L. biglobosa* species complex. Mycol. Res. 107 (11): 1287-1304.

Moldave, K. (1985): Eukaryotic protein synthesis. Annu. Rev. Biochem. 54: 1109-1149.

Nicholas, K.B., Nicholas, H.B.Jr., Deerfield, D.W. II. (1997): GeneDoc: Analysis and Visualization of Genetic Variation, Embnew. news 4: 14

Oliverio, M., Cervelli, M., Mariottini, P. (2002): ITS2 rRNA evolution and its congruence with the phylogeny of muricid neogastropods (Caenogastropoda, Muricoidea). Mol. Phylogenet. Evol. 25**:** 63-69.

Reynolds, D.R., Taylor, J.W. (1993): The fungal holomorph: An overview. pp. 15-25. In: The fungal holomorph: mitotic, meiotic and pleomorphic speciation in fungal systematics. Reynolds, D.R. and Taylor, J.W. (Eds.) CAB International, Wallingford, UK

Roger, A.J., Sandblom, O., Doolittle, W.F., Philippe, H. (1999): An evaluation of elongation factor 1α as a phylogenetic marker for eukaryots. Mol. Biol. Evol. 16: 218-233.

Schlötterer, C., Hauser, M., Haeseler, A. von, Tautz, D**.** (1994): Comparative evolutionary analysis of rDNA ITS regions in *Drosophila*. Mol. Biol. Evol. 11: 513-522.

Swofford, D.L. (2002): PAUP: Phylogenetic Analysis Using Parsimony (and other methods). Version 4b10. Sinauer Associates, Sunderland, MA

Talhinhas, P., Sreenivasaprasad, S., Neves-Martins, J. Oliveira, H. (2002): Genetic and morphological characterization of *Colletotrichum acutatum* causing anthracnose of lupins. Phytopathology 92: 986-996.

Taylor, J.W., Jacobson, D.J., Kroken, S., Kasuga, T., Geiser, D.M., Hibbett, D.S., Fisher, M.C. (2000): Phylogenetic species recognition and species concepts in fungi. Fungal Genet. Biol. 31: 21-32.

Thompson, J.D., Gibson, T.J., Plewniak, F., Jeanmougin, F., Higgins, D.G. (1997): The ClustalX windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic Acids Research, 24: 4876-4882.

Weekers, P.H.H., Jonckheere, F.J. de, Dumont, H.J. (2001): Phylogenetic relationships inferred from ribosomal ITS sequences and biogeographic patterns in representative of the genus *Calopteryx* (Insecta: Odonata) of the West Mediterranean and adjacent west European zone. Mol. Phylogenet. Evol. 20**:** 89-99.

White, T.J., Bruns, T., Lee, S., Taylor, J. (1990): Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. pp. 315-322. In: PCR protocols. A guide to methods and applications. Innis, M.A., Gelfand, D.H., Sninsky, J.J., White, T.J. (Eds.) Academic Press, New York

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Summary

The cosmopolitan *Phoma* genus contains mainly phytopathogenic, opportunistic parasite, and saprophyte fungal species. Up to now the characterization of *Phoma* species and other taxa of *Phoma* has so far been determined on the basis of morphology on standardized media, and gene sequence analysis was only used as a confirmative or distinctive complement.

In this study we have tried to find molecular markers which can be used as phylogenetic markers in the molecular based classification in the *Phoma* genus.

We employed a part of the translation elongation factor 1 subunit alpha (EF-1α=tef1) containing both introns and exons and ITS region containing the internal transcribed spacer regions 1 and 2 and the 5.8S rDNA, as a potential genetic markers to infer phylogenetic relationships among different *Phoma* taxa. Twelve different *Phoma* species sequences were analysed together with the closely related *Ascochyta* ones. The constructed phylogenetic trees based on *tef1* and ITS sequences, do not support the traditional *Phoma* sections based on morphological characterization. However we have managed to distinct the *Phoma* strains and *Ascochyta* species comparing their *tef1* sequences by parsimony analysis. We have proved that a *tef1* can be a useful phylogenetic marker to resolve phylogenetic relationships at species level in *Phoma* genus.

Both parsimony sequence analyses confirmed that the *Phyllosticta sojicola* species is identical to the *Phoma exigua* var. *exigua* species as Kövics et al. (1999) claimed. However the evolutionary distance by ITS sequences within *Phoma* species is too small to get well based consequences for the phylogenetic relationships of *Phoma* genus.

Further investigations would be necessary to clarify whether the *tef1* and ITS sequences as phylogenetic molecular markers are well suited for the classification of *Phoma* species.