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Recent Concepts in Fungal Taxonomy: A Review.

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Review Article

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ABSTRACT

Kingdom Fungi, which has been estimated at around 1.5 million species, with about 5% of these having been formally classified. Species identification based on conventional phenotypic methods is often time-consuming and laborious and is hindered by the unstable and subjective nature of phenotypic characteristics, which are readily influenced by culture conditions. Such phenotypic approaches has a clear limitation on fungi that do not grow in culture. Molecular systematics involving the use of genes to gain taxonomic status of organisms and their evolutionary relationships have influenced inducting significant changes in the traditional concepts of systematics. Techniques such as RFLP, RAPD, rDNA analysis, SSR and ISSR are are now gaining importance in fungal systematics and related studies. These techniques are much faster, more specific and more accurate. These developments have provided new information that has caused the biological-species concept to come under criticism in favor of the phylogenetic-species concept and have helped reshaping the classification of Kingdom Fungi.

INTRODUCION

The fungus kingdom encompasses an enormous diversity of taxa with varied ecologies, life cycle strategies, and morphologies ranging from single-celled aquatic chytrids to large mushrooms. However, little is known of the true biodiversity of Kingdom Fungi, which has been estimated at around 1.5 million species, with about 5% of these having been formally classified. Historically, classification is based on macroscopically and microscopically observable characteristics - the phenotypic approach. Morphological characteristics differ greatly depending on the species, and may not be reproduced under artificial conditions. Furthermore, the characteristics accepted as typical for fungi have arisen polyphyletically, and there seems to be a definite trend toward asexual reproduction, reducing the spectrum of morphological characteristics [1]. Species identification based on conventional phenotypic methods is often time-consuming and laborious and is hindered by the unstable and subjective nature of phenotypic characteristics, which are readily influenced by culture conditions [2].

The failure to adequately identify plant pathogens from culture-based morphological techniques has led to the development of culture-independent molecular approaches [3]. Two recent and important developments have greatly influenced and caused significant changes in the traditional concepts of systematics. These are the phylogenetic approaches and incorporation of molecular biological techniques, particularly the analysis of DNA sequences, into modern systematics. This new concept has been found particularly appropriate for fungal groups in which no sexual reproduction has been observed (deuteromycetes) [4]. The molecular revolution in fungal taxonomy commenced in the early 1990s, with analyses of PCR-amplified ribosomal RNA genes [5]. The advent of PCR has allowed the analysis of small numbers of fungal cells or even single spores, dried herbarium material [6]. The selection of universal oligonucleotide primers specific to fungi [7] has provided easy access to nucleotide sequences. The parts of the rDNA are highly conserved [8] and serve as reference points for evolutionary divergence studies.

Comparisons of the 18S rRNA sequences have been performed to assess the relationships of the major groups of living organisms [9]. For phylogeny of filamentous fungi, the 18S sequence is mostly used completely or in subunits of over 600 bp [10]. In the yeasts, the D1 and D2 variable regions of 25S rDNA regions are almost

exclusively used [11]. The GC content of nDNA has been established for many fungi, primarily yeasts. A difference of 2% in the GC content has been considered to indicate that two strains should be assigned to different species [12]. On the basis of molecular-genetic evidence, it is now clear that several groups traditionally treated as fungi belong to some other groups [13] eg- Oomycetes.

Why should we classify?

- Taxonomy is a language for communication. Taxonomists provide the basic vocabulary of biology if you like, defining each new species (individual words) and making sure there are accurate definitions of those species (like a dictionary).
- Ease of communication with other scientists. In many diverse situations communication further requires the recognition of higher-level units of classification. Communication also requires that plants have widely accepted and recognized scientific names that reflect their positions in the hierarchy of classification.
- If the taxonomy is poor, studies may be conducted on different taxa but reported by the same name – creates confusion.
- Taxa, with poor taxonomy, may not even be possible to do research on. If a researcher doesn't have a reliable name on her study object, it is impossible to communicate and publish on the results.

Taxonomy

Taxonomy is a branch of biology concerned with the naming and grouping of organisms. Taxonomy is the science of classifying, naming, and describing organisms. Taxonomy is a part of the scientific practice known as systematics, which entails the evolutionary relationships between organisms.

The varying degrees of relatedness in organism lead to the concept of a hierarchy of different levels of classification:

- Species – Consist of a group of individuals that can breed and produce fertile offspring.
- Genus – consists of a group of closely related species.
- Family – group of closely related genera.
- Order – group of related families
- Class – group of related orders
- Phylum – group of related classes
- Kingdom – group of related phyla.

Some fungi have more than one scientific name – Why?

The dual modality of fungal propagation, i.e., sexual and asexual, has meant that since the last century [14] there has been a dual nomenclature.

- **Teleomorph**: The sexual reproductive stage (morph), typically a fruiting body (e.g., *Morchella esculenta*, *Agaricus brunescens*).
- **Anamorph**: An asexual reproductive stage (morph), often mold-like (e.g. *Aspergillus flavus*, *Fusarium solani*). When a single fungus produces multiple morphologically distinct anamorphs, they are called synanamorphs.
- **Holomorph**: The fungus, as a whole, comprises a teleomorph (sexual state) and one or more anamorphs (asexual states) [15].

Concept of species in fungi

The basic rank in taxonomy is the species. Different concepts have been used by mycologists to define the fungal species [4].

- **The morphological (phenetic or phenotypic) concept** is the classic approach used by mycologists. In this approach, units are defined on the basis of morphological characteristics and ideally by the differences among them.
    - **Developmental studies**
      Some features, present in the young stages of a fruiting body, may disappear at maturity and, conversely, some features appear only late in the fruiting body development. So, studies of fruiting body development provide another insight into fungal classification. For example, basidia are present in immature puffballs but absent from the mature puffball. Without knowledge of the young stage we wouldn't know that puffballs are basidiomycetes. Developmental studies have proven very useful in all groups of fungi.

- **The polythetic concept** is based on a combination of characters, although each strain does not have to have the same combination.
- **The ecological concept**, which is based on adaptation to particular habitats rather than on reproductive isolation, is often used for plant-pathogenic fungi.
- **The biological concept**, which was developed before the advent of modern phylogenetic analysis, emphasizes gene exchange (i.e., sexual and parasexual reproduction) within species and the presence of barriers that prevent the cross-breeding of species [16]. A species is considered to be an actual or potential interbreeding population isolated by intrinsic reproductive barriers [17]. However application of the biological-species concept to fungi is complicated by the difficulties in mating and in assessing its outcome [18].

Classification of Fungi

According to Ainsworth (1966), the kingdom fungi (Myceteae) is divided into two divions [19]:

- Myxomycota
- Eumycota

MYXOMYCOTA: it is divided into four classes:

- Acrasiomycetes
- Hydromyxomycetes
- Myxomycetes
- Plasmodiophoromycetes

EUMYCOTA: it is divided into five subdivisions:

- **Mastigomycotina**
- Chytridomycetes
- Hyphochytridomycetes
- **Oomycetes**
- **Zygomycotina**
- Zygomycetes
- Trichomycetes
- **Ascomycotina**
- Hemiascomycetes
- Plectomycetes
- Pyrenomycetes
- Discomycetes
- Laboulbeniomycetes
- Loculoascomycetes
- **Basidiomycotina**
- Teliomycetes
- Hymenomycetes
- Gasteromycetes
- **Deuteromycotina**
- Blastomycetes
- Hyphomycetes
- Coelomycetes

Recent classification of fungi given by Ainsworth

The fungi and fungal-like organisms that causes diseases on plants are a diverse group. Some fungal like organisms, often reffered to as lower fungi, are now considered to belong to the kingdom Chromista [20].

Fungal-like organisms (Chromista)

- **Phylum Oomycota**
- Class: Oomycetes

True fungi

- **Phylum Chytridiomycota**
- Class: Chytridiomycetes

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- **Phylum Zygomycota**
- Class: Zygomyecetes
- **Phylum Ascomycota**
- Class: Archiascomycetes
- Class: Saccharomycetes
- Filamentous ascomycetes
- Plectomycetes
- Pyrenomycetes
- Loculoascomycetes
- Discomycetes
- Deuteromycetes
- **Phylum Basidiomycota**
- Class: Basidiomycetes

Why are Oomycota not true Fungi?

The Oomycota have long been considered fungi because they obtain their nutrients via absorption and many of them produce the filamentous threads known as mycelium characteristic of many fungi. The Oomycota now are classified as a distinct group based on a number of unique characteristics (Table). All members of the Oomycota undergo oogamous reproduction, meaning that diploid oospores are produced as zygotes following fertilization of haploid oospheres by haploid gametes. These oospores may be large and solitary or smaller and numerous inside the oogonium. None of the true Fungi produce oospores [21].

Character	Oomycota	True Fungi
Sexual reproduction	Fertilization of oospheres by nuclei from antheridia forming oospores.	Oospores not produced; sexual reproduction results in zygospores, ascospores or basidiospores.
Cell wall composition	Beta glucans, cellulose	Chitin. Cellulose rarely present
Type of flagella on zoospores, if produced	two types, one whiplash, directed posteriorly, the other tinsel, directed anteriorly	If flagellum produced, usually of only one type: posterior, whiplash
Mitochondria	With tubular cristae	With flattened cristae

Disadvantages of Phenotypic Approach

The phenotypic approach has been largely criticized for its lack of standardized and stable terminology and for its high subjectivity. Moreover, some phenotypic characteristics have been considered to be unstable and dependent on environmental conditions, as with growth in artificial culture. A clear limitation of phenotypic approaches is that they cannot be applied to fungi that do not grow in culture. Consequently, there are many fungi that will remain unclassified as long as taxonomists rely solely on phenotypic characteristics. Another notable problem of classification based on morphological criteria is the dual system of classification, with no consistent correlation between the taxonomies of the ascomycetes and deuteromycetes [22]. This is an important difficulty in establishing the taxonomic concept of the fungus as a whole. Identification based on culture characteristics requires experience and is time-consuming [2]. It is difficult to identify fungal isolates to species level by the traditional methods. e.g. The mycelial state of the fungus is very similar between the different species. The biological-species concept cannot be applied to organisms that do not undergo meiosis.

Phylogenetic Concept - DNA based

Molecular taxonomy, also known as molecular systematics, is the use of genes to gain information on an organisms evolutionary relationship. Two recent and important developments have greatly influenced and caused significant changes in the traditional concepts of systematics. These are the phylogenetic approaches and incorporation of molecular biological techniques, particularly the analysis of DNA nucleotide sequences, into modern systematics. Molecular techniques, which were previously used only in research laboratories, are now commonplace. These developments have provided new information that has caused the biological-species concept to come under criticism in favor of the phylogenetic-species concept [4].

Advantages of Molecular Taxonomy

Molecular methods are much faster, more specific, and more accurate, and can be performed and interpreted by personnel with no specialized taxonomical expertise. Molecular methods are universally applicable [4]. One of the most important advantages is the power to distinguish closely related organisms. This new concept has been found particularly appropriate for fungal groups in which no sexual reproduction has been observed

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(deuteromycetes) [4]. The increased use and availability of modern techniques have opened up many new areas within systematics and have enabled more traditional ones to be developed further [23].

Molecular Methods in Fungal Taxonomy

- DNA base composition
- DNA hybridization
  - RFLP (Restriction fragment length polymorphisms)
- PCR based
  - RAPD (Random amplified polymorphic DNAs)
  - rDNA analysis
  - PCR-RFLP
  - SSR (Simple Sequence Repeats)
  - ISSR (Inter-Simple Sequence Repeats)

DNA base composition: Determination of guanine-plus-cytocine content

Nuclear DNA base composition is the simplest of the molecular techniques that allow resolution of taxa. The base composition of a single species is theoretically a fixed property; thus, a comparison of the G+C content in different species can reveal the degree of relatedness among them. Among the classical DNA-based methods is the determination of the nuclear DNA (nDNA) guanine-plus-cytosine content. The GC content of nDNA has been established for many fungi, primarily yeasts. A difference of 2% in the GC content has been considered to indicate that two strains should be assigned to different species [12]. In some insufficiently resolved fungal groups, a difference of 8% has been allowed within species.

DNA base ratio is expressed as:

$$\frac{G + C}{A + T + G + C} \times 100$$

GC content of DNA prepatrations from *Rhizoctonia solani*

Anastomosis group	Isolate	Mole percent guanine plus cytosine
AG–1	R 71	49.3
	CS-Ka	48.8
	A–10	49.3
	OO 1–7	48.0
	K–18	49.1
	SFBV–1	48.5
AG–2–1	R 123	45.3
	SHM 1–2	42.8
	RPS–4	43.1
AG–2–2	B 52	44.9
	B 60	44.0
	C–112	44.6
	R 88	45.3
	BR–2	43.9
	48HDTN	44.6
AG–3	ST3–1	47.0
	ST4–1	46.6
	ST6–3	47.9
AG–4	RR5–2	47.1
	Chr–3	48.0
	R 101	46.9
AG–5	TE 1–5	45.5
	R3P	45.6
	K–7	44.4
AG–6	AT 2–1	42.2
	HAM 1–1	41.4
	SO 2–1	41.8
AG–BI	SH 1–2	41.0
	KA 1–1	42.3
	KO 1–1	40.9

Comparisons of DNA Base Compositions Among Anastomosis Groups of *Rhizoctonia solani* [24]

Base composition of DNAs of 30 isolates of *Rhizoctonia solani* was compared. The range of guanine plus cytocine content of *R. solani* was 40.9 to 49.3 mole %. AG-1 shows the highest GC content (avg. 48.8 mole %).The widest intra-group variation of GC content was observed in AG-2-1.Average GC content of AG-3 was very close to AG-4.Intra-group variation of AG-6 was lower than others.GC content of AG-B1 was lowest among all AG group. AG-1 Shows the highest GC content (avg. 48.8 mole %) among all the anastomosis groups, followed by AG-4(avg. 47.3

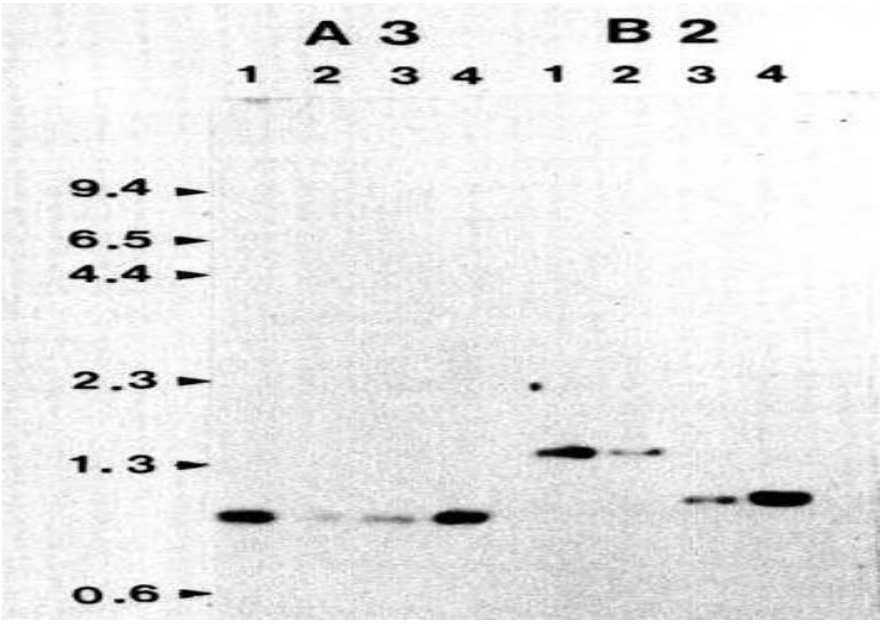
mole %), AG-3(avg. 47.1 mole %), AG-5(avg. 45.2 mole %), AG-2-2(avg. 44.6 mole %), AG-2-1(avg. 43.7 mole %), AG-6(avg. 41.8 mole %), and AG-BI(avg. 41.4 mole %). The GC contents of the isolates within each anastomosis group was almost same. Therefore, each anastomosis group must be regarded as a genetically independent unit.

DNA Hybridization Based

It is the hybridization between complementary strands of DNA. Genomic hybridization measures the degree of sequences similarity and is useful for differentiating very closely related organisms. DNA isolated from one organism is made radioactive with ^{32}P or ^{3}H, sheared to a relatively small size, heated to denature, and mixed with an excess of unlabeled DNA prepared in the same way from a second organism. The DNA mixture is than cooled to allow it to reanneal, and double-stranded duplex DNA is separated from any remaining unhybridized DNA.The amount of radioactivity in the hybridized DNA is determined and compared with the control, which is taken as 100%. A relative hybridization value of over 80% is generally regarded as indicating membership in the same species, whereas values of less than 20% are proof of nonidentity [4].

Genetic relatedness among anastomosis groups in *Rhizoctonia* measured by DNA-DNA hybridization [25]

Isolates of rhizoctonia are known to vary greatly in their morphology, pathogenecity, and physiology, making taxonomic study difficult. Isolates of r. solani (telomorph = thanatephorus cucumis) can be distinguished by their multinucleate hyphae, in contrast to isolates of rhizoctonia having binucleate cells. Genetic relationship of 44 mutinucleate and binucleate isolates of *Rhizoctonia* were investigated by the technique of DNA-DNA hybridizaton. Genomic DNA was isolated from representative isolates of eight different anastomosis groups(AG) in *Rhizoctonia solani*, five different anastomosis groups (CAG) of binucleate Rhizoctonia, and several different isolates of uncertain anastomosis affinity.Isolates belonging to different AG show little DNA sequence complementarity, usually less than 25%. DNA hybridization values within each AG range considerably (30-100%) and indicate that substantial genetic variation is present within some AG. Within AG, reduced DNA hybridization values (<60%) indicate the presence of genetically divergent subgroups, some of which had been recognized on the basis of colony morphology, pathogenecity, or anastomosis behavior. The lowest DNA hybridization values from this study were observed between the multinucleate *R. solani* reference isolates and isolates representing five CAG of binucleate *Rhizoctonia.* Highly reduced levels of DNA hybridization indicate that multinucleate and binucleate *Rhizoctonia spp*. are unrelated and provide genetic evidence to support the taxonomic separation of the telomorph genera *Thanatephorus and Ceratobasidium*



Autoradiogram of restriction fragment length polymorphisms of Southern blots of four isolates of Fusarium oxysporium [*1-Fusarium oxysporum f.sp. danthi* (isolate 1), *2- Fusarium oxysporum f.sp. dianthi (isolate 2), 3- Fusarium oxysporum f.sp. lycopersici* (race1) and 4- *Fusarium oxysporum f.sp. gladioli (isolate 2)]* digested with Hind III.

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Restriction fragment length polymorphism (RFLP)

Among the electrophoretic methods, restriction fragment length polymorphism (RFLP) is particularly significant for taxonomy [4]. This approach is to digest genomic DNA with a restriction enzyme and directly examine the resulting bands in agarose or polyacrylamide gels after electrophoresis. Depending upon the size of the genome, it is possible to directly compare digests of genomic DNAs from different species. The patterns can be tabulated and compared [26] or phenetic trees can be constructed [27].

Use of Random DNA probes and restriction Fragment length polymorphism in the taxonomy of *Fusarium* [28]

A range of *Fusarium* species was screened for DNA restriction fragment length polymorphisms in ethidium-stained agrose gel and by hybridization to random probes (A3 and B2) generated from total DNA of an isolate of *Fusarium oxysporum f.sp. dianthi.* DNA was digested with HIND III. The fragments obtained were identical over the four isolates for probe A3 (950bp) but polymorphic for probe B2 (1,550 bp), enabling a differentiation between *Fusarium oxysporum* f.sp. *danthi* and the other two forma specials

PCR-BASED

Random amplified polymorphic DNAs (RAPD)

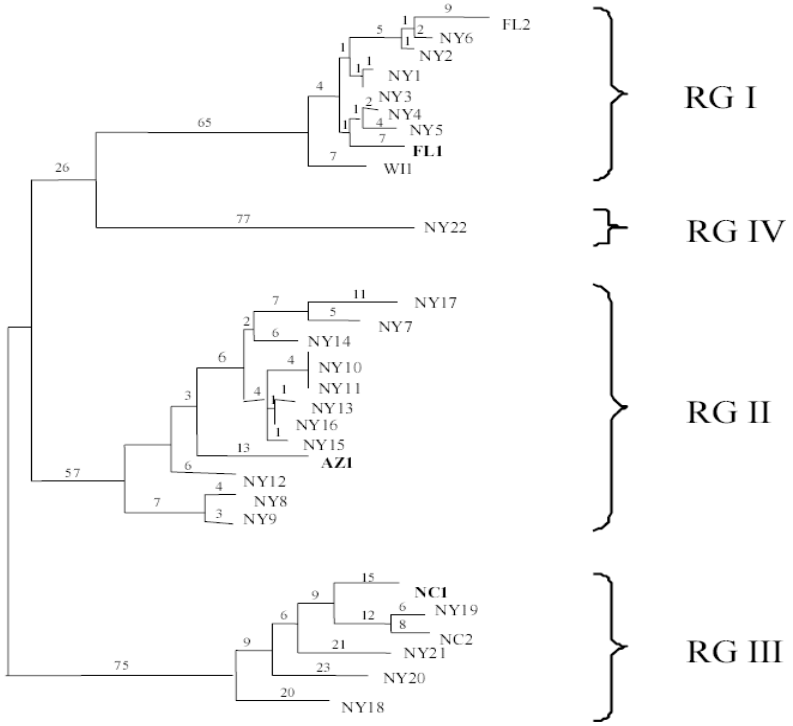
RAPD analysis is a PCR- based molecular technique. Here, single short oligonucleotide primers are arbitrarily selected to amplify a set of DNA segments distributed randomly throughout the genome. A particular fragment generated for one individual but not for other represents polymorphism [29]

RAPD analysis detects two types of genetic variations:

- In the length of DNA between the two primer binding sites
- In sequence variation at the priming regions.

Nucleotide substitutions in the region of PCR primer binding, particularly at the 3′ ends, can prevent binding of the primer to the DNA template. As a result, this band will be missing in a PCR reaction. Similarities in banding profiles among strains (i.e., the number and mobility) can be calculated and used to infer strain relationships. When multiple primers are screened, RAPD analysis can be very sensitive to detect variation among isolates that cannot be observed using other methods. RAPD analysis can detect minute variation among strains because even a single nucleotide mismatch in the priming region may prevent annealing and the absence of a characteristic band on gels.

RAPD Analysis of *Didymella bryoniae and Phoma* species Isolated from Cucurbits



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Didymella bryoniae (anamorph Phoma cucurbitacearum) is the causal agent of gummy stem blight of cucurbits.Isolates of *D. bryoniae and Phoma obtained* from cucurbits subjected to RAPD analysis to determine the molecular and phylogenetic relationships within and between these fungi. Using RAPD fingerprinting, 59 isolates placed into four phylogenetic groups, designated RAPD group (RG) I, RG II, RG III, and RG IV. Ten primers were used OPK-1, OPK-4, OPK-8, OPK-9, OPT-01, OPT-07, OPT-12, OPT-13, OPT-14 and OPT-18. *D. bryoniae* isolates clustered in either RG I (33 isolates), RG II (12 isolates), or RG IV (one isolate), whereas all 13 *Phoma* isolates clustered to RG III.

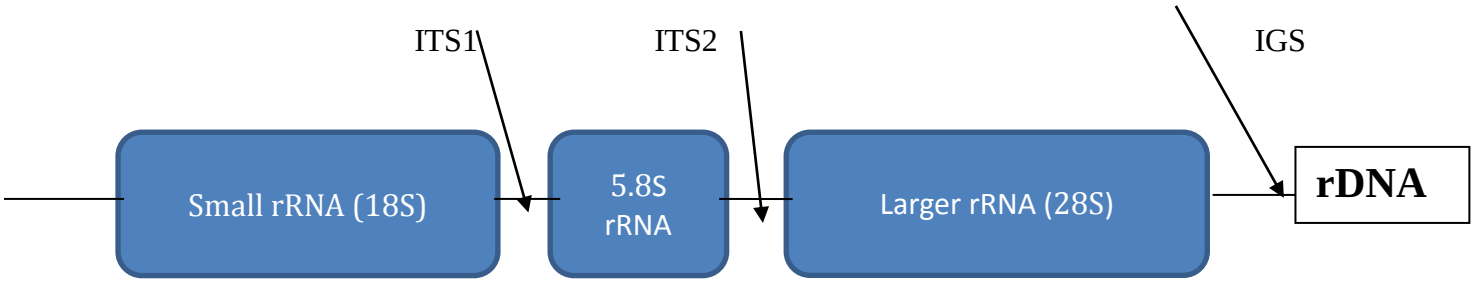
Results of Phylogenetic Analysis based on RAPD fingerprints of isolates of *Didymella bryoniae and Phoma*
rDNA ANALYSIS

rDNA region is found universally in living cells and corresponds to an important function in the cell, and so its evolution might reflect the evolution of the whole organism.This region contains some highly conserved sequences and some variable sequences, allowing the comparison of organisms at different taxonomic levels.

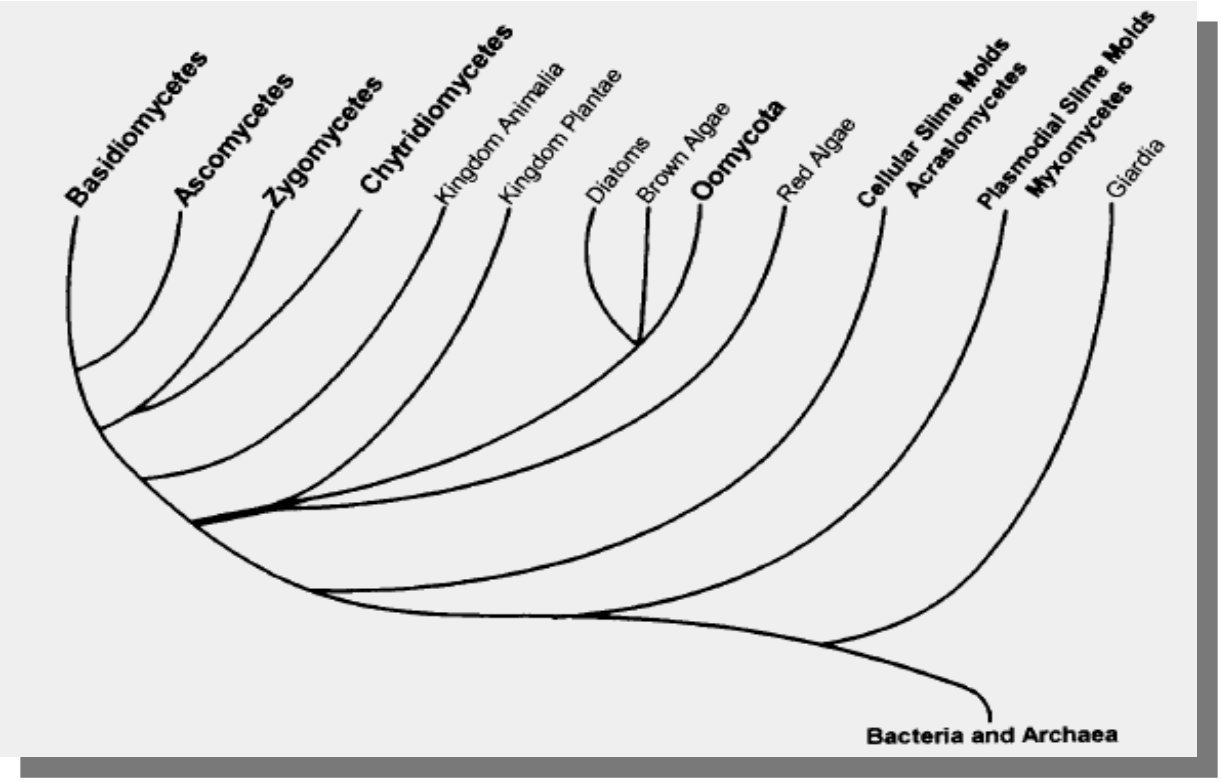
A rDNA unit includes three rRNA genes:

- The small nuclear (18S-like) rRNA,
- The 5.8S rRNA, and
- The large nuclear(28S-like) rRNA genes.

In one unit, the genes are separated by two internal transcribed spacers (ITS1 and ITS2). Two rDNA units are separated by the inter-genic spacer (IGS) [23].



Phylogenetic tree showing relationships of eukaryotes, based on the nucleotide sequence of 18S rDNA [4].



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Phylogenetic analysis has shown that the fungal kingdom is part of the terminal radiation of great eukaryotic groups, which occurred one billion years ago [30]. Surprisingly, although mycology has been classically considered a branch of botany, there is also evidence that the kingdom Fungi is more closely related to Animalia than to Plantae [31]. The analysis of amino acid sequences from numerous enzymes indicated that plants, animals, and fungi last had a common ancestor about a billion years ago and that plants diverged first [32].

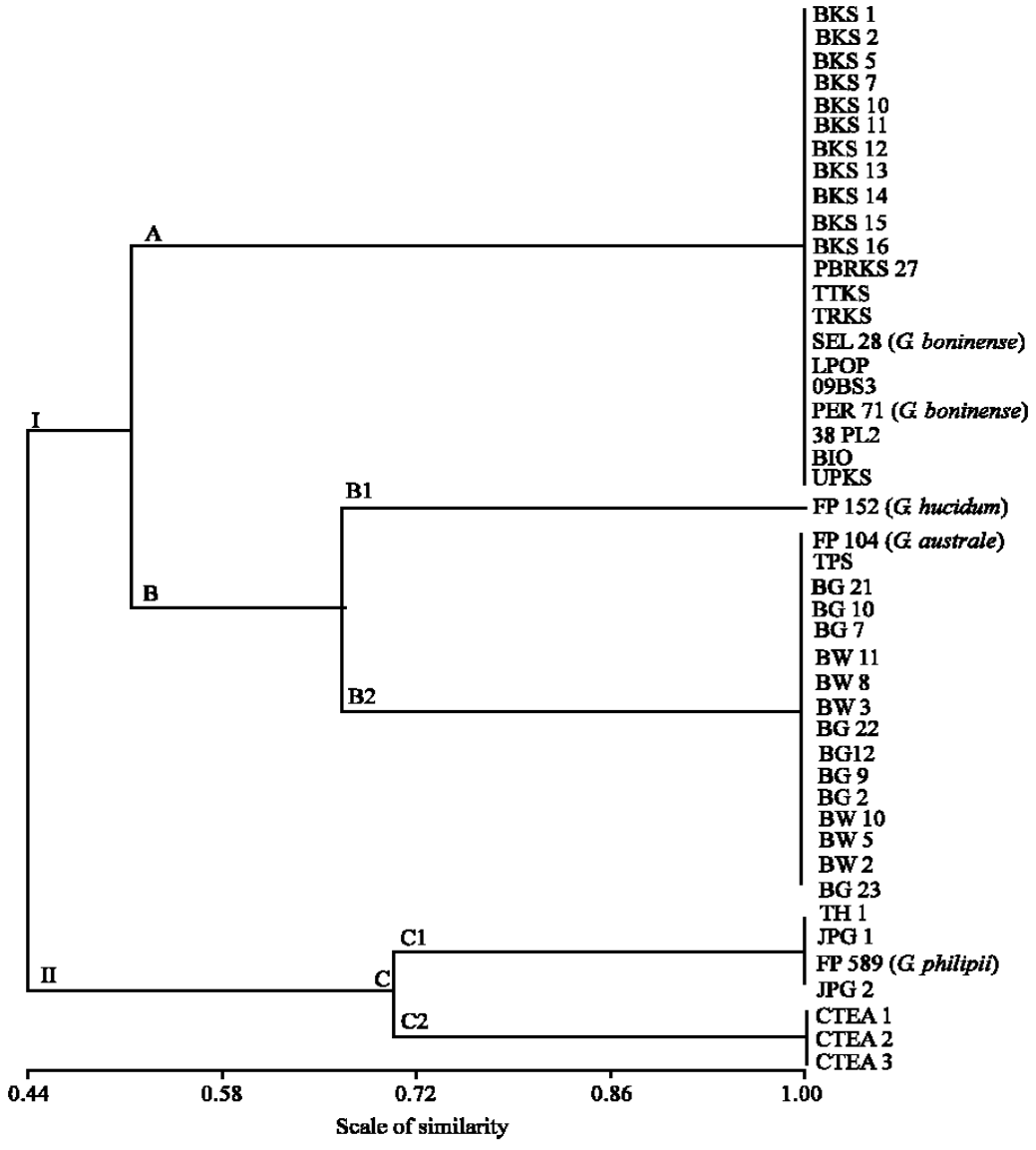
PCR-RFLP

Basis : PCR primers amplify a particular gene from representative strains followed by digestion of the amplified products with restriction enzymes to screen for variability.

Analysis of *Ganoderma* species from Different Hosts [33]

Ganoderma isolates from different hosts namely, *G. boninense* from oil palm, *G. philiplii* from rubber and *G. austral* from forest trees characterized using PCR-RFLP of ITS+5.8S regions.Restriction analysis of ITS+5.8S regions using six restriction enzymes (Mspl, Bsu151, Hin61, HindIII, Hinf1 and Taq1) shows the restriction patterns from the same species generates similar patterns. Cluster analysis of PCR-RFLP of ITS+5.8S regions clusters Ganoderma species from the same host together. Dendrogram of different *Ganoderma* isolates based on PCR-RFLP of ITS + 5.8S bands.

Dendrogram of different Ganoderma isolates based on PCR-RFLP of ITS + 5.8S bands



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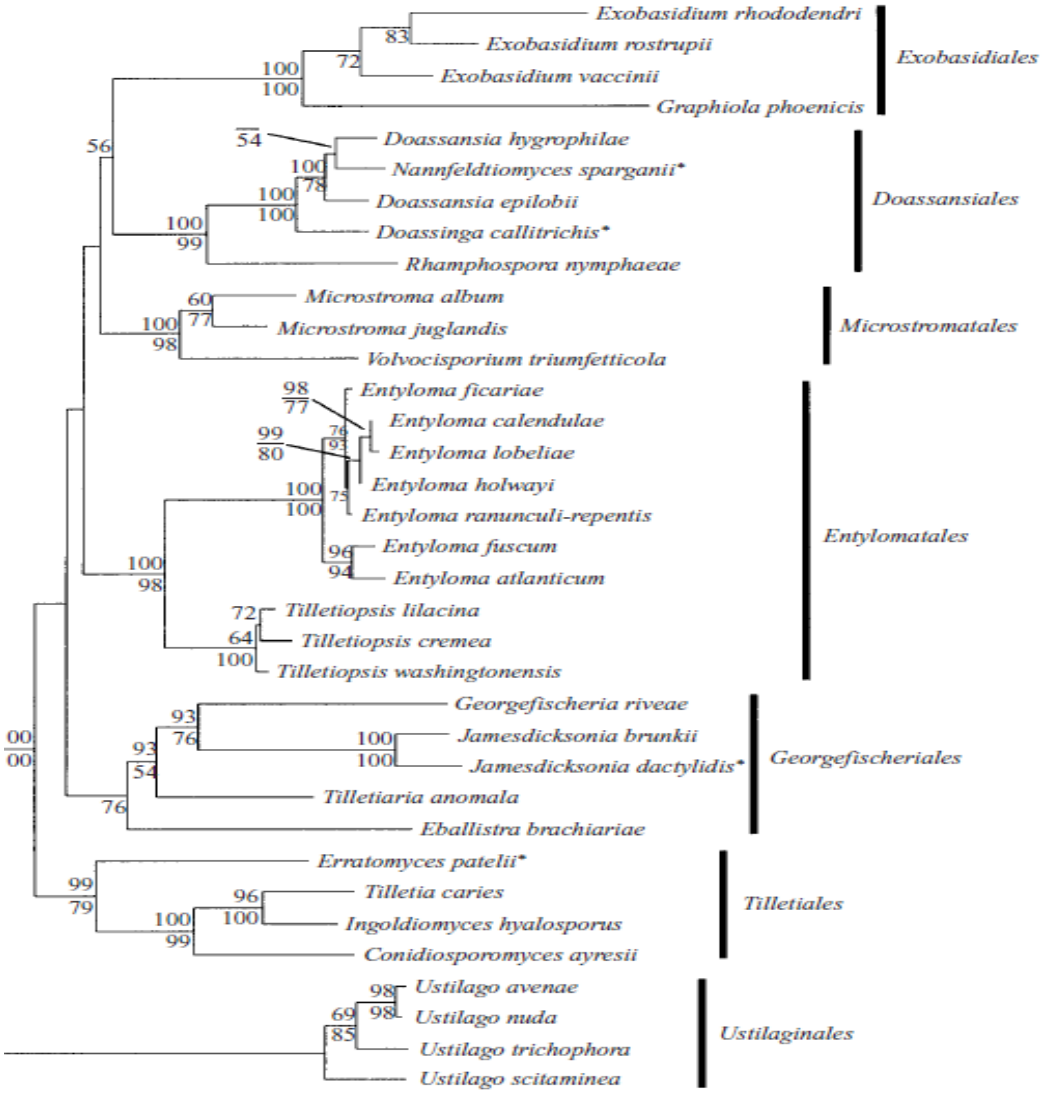
Implications rDNA for the phylogeny of the genus *Entyloma* [34]

The genus *Entyloma* was characterized by de Bary (1874) by the formation of teliospores, the germination of these spores with *Tilletia*-like basidia, and the characteristic white dense leaf spots. Because of the life cycle, he placed the genus in the smut fungi, although the species do not produce dark coloured, loose teliospores. The systemic position was confirmed by ultrastructural and molecular data. Many species formerly listed in *Entyloma* have been removed and now are placed in several orders. Analysis of LSU and ITS sequences of rDNA support the monophyly of the order *Entylomatales. Entyloma callitrichis* (now *Doassinga callitrichis*) was transferred to the Doassansiales. *Entyloma vignae* (now *Erratomyces patelii*) to the *Tilletiales. Entyloma fluitans* (now *Ustilentyloma fluitans*) to the microbotryales. *Entyloma sparganii* (now *Nannfeldtiomyces sparganii*) to the Doassansiales

Phylogram obtained by analysis of LSU region sequences of 35 members of the Ustilaginomycetes (The stars refer to species which were listed formerly in the genus Entyloma)



Simple Sequence Repeats

The use of simple sequence repeats or microsatellites as genetic markers has become very popular because of their abundance and length variation between different individuals. SSRs are tandem repeat units of 1 to 6 base pairs that are found abundantly in many prokaryotic and eukaryotic genomes. SSR length polymorphisms at individual loci are detected by PCR, using locus-specific flanking region primers where the sequence is known.

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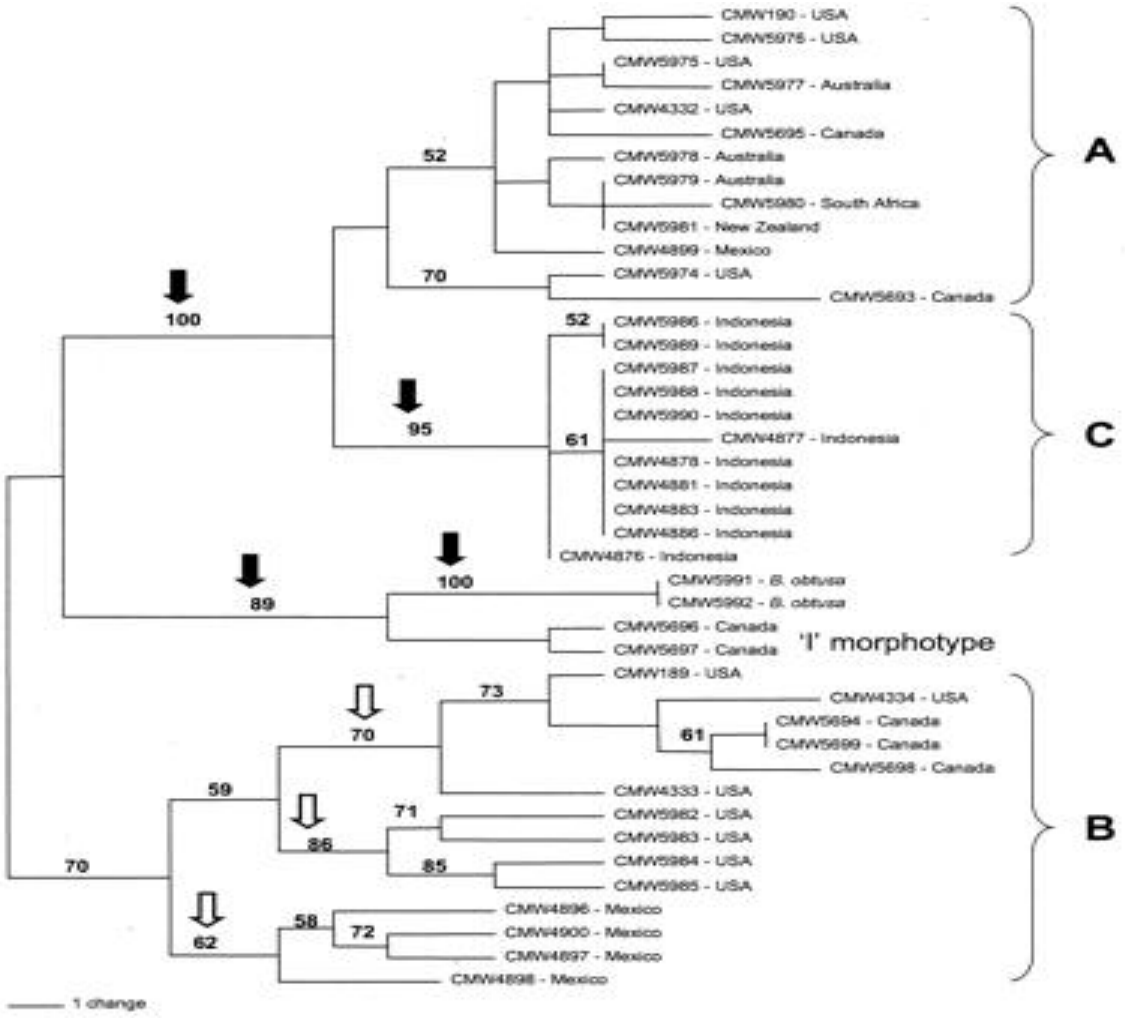
Simple Sequence Repeat Markers Distinguish among Morphotypes of *Sphaeropsis sapinea*[35]



Dendrogram of morphotypes of *Sphaeropsis sapinea* generated from SSR polymorphic data

Four morphotypes of *S. sapinea* have been described within the natural range of the fungus.Eleven polymorphic SSR markers were tested on 40 isolates of *S. sapinea* representing different morphotypes alongwith 2 isolates of the closely related species *Botryosphaeria obtusa*. The putative I morphotype was found to be identical to *B. obtusa*.The markers clearly distinguished the remaining three morphotypes and showed that the C morphotype was more closely related to the A than the B morphotype.

Inter-Simple Sequence Repeats

ISSR is a term for a genome region between microsatellite loci. The complementary sequences to two neighboring microsatellites are used as PCR primers; the variable region between them gets amplified.

Use of Inter-Simple Sequence Repeatst o Analyze Genetic Relationships Among Species of *Ustilago* [36]

In the smut fungi, DNA-based molecular techniques are useful in expanding the traits considered in determining relationships among these fungi. The phylogenetic relationships among seven species of *Ustilago* (*U. avenae, U. bullata, U. hordei, U. kolleri, U. nigra, U. nuda, and U. tritici)* using inter-simple sequence repeats (ISSRs) has been examined.Fifty-four isolates of different *Ustilago spp. analyzed using ISSR* primers.The variability among isolates within species was low for all species except *U. bullata. The isolates of U. bullata, U. nuda, and U. tritici were* well separated. *U. avenae and U.kolleri isolates did not separate from each other and there was little* variability between these species. *U. hordei and U. nigra isolates also* showed little variability between species, but the isolates from each species grouped together. *U. avenae and U. kolleri* are monophyletic and should be considered one species, as should *U.hordei and U. nigra.*

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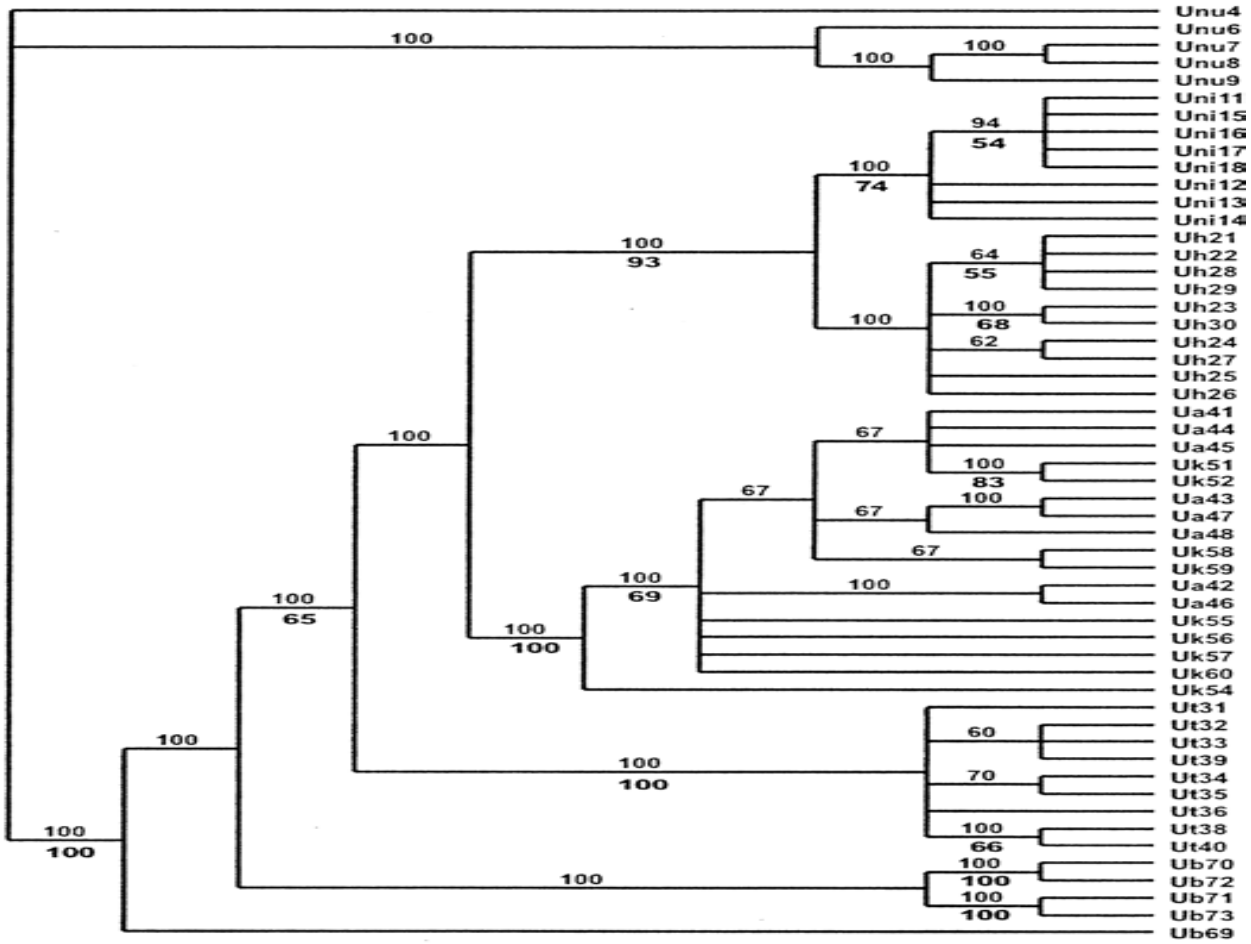
Phylogenetic relationships among 54 isolates of Ustilago spp.



CONCLUSIONS

- Phylogenetic studies have helped reshape the classification of Kingdom Fungi.
- Advances in molecular techniques have opened the way for DNA analysis to be incorporated into taxonomy such as RFLP, RAPD, rDNA analysis, SSR and ISSR.
- These techniques are much faster, more specific and more accurate.
- These techniques allow detection of non-culturable microorganisms.
- They have power to distinguish closely related organisms.
- Molecular techniques becoming an important tool to establish the taxonomic status of different fung.

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