Molecular Characterization of the Wild Edible Mushrooms of the *Pleurotus* species in Kenya

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DECLARATION

This thesis is my original work and has not been presented for a degree in any other University.

Signature…….…….. ……. Date…….

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This thesis has been submitted for examination with our approval as University Supervisors.

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DEDICATION

This Thesis is dedicated to, first and foremost, my loving mother Hilda Atieno and my wife Berine Awuor whose strength, patience and encouragement enabled me to overcome the many challenges throughout my studies.

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LIST OF ABBREVIATIONS

AMOVA	Analysis of Molecular Variance
BLAST	Basic Local Alignment Search Tool
EDTA	Ethylenediaminetetraacetic acid
FAO	Food and Agriculture Organization
ITS	Internal Transcribed Spacer
JKUAT	Jomo Kenyatta University of Agriculture and Technology
KARI	Kenya Agricultural Research Institute
NCBI	National Centre for Biotechnology Institute

ABSTRACT

Members of the genus *Pleurotus* are macro fungi belonging to the phylum Basiomycetes. They are important source of food and medicinal compounds among many local communities. Limited studies have been done to identify and characterize *Pleurotus* species based on genetic characteristics in different parts of the world. However, no previous studies have been undertaken to understand the genetic characteristics of the wild species in Kenya. A total of 71 samples of wild *Pleurotus* species were randomly collected from Kakamega Forest, Arabuko Sokoke Forest and Mount Kenya Forest. Thirteen samples of commonly cultivated *Pleurotus* species were obtained from Jomo Kenyatta University of Agriculture and Technology. Genetic variability and phylogenetic relationships were evaluated using amplified fragment length polymorphic markers and ITS sequences of the ribosomal DNA respectively. Five primer combinations used generated 330 polymorphic loci across 84 samples. The mean diversity estimate between the wild (0.27) and cultivated (0.24) species was small and is not statistically significant. However, diversity was great within (89%; P>0.001) than among populations. Phylogenetic analysis revealed *Pleurotus ostreatus*, *Pleurotus eryngii*, *Pleurotus* sp. 'Florida' and *Pleurotus* sp.YL005 as part of diversity of *Pleurotus* species in Kenya. The broad diversity within populations suggests the possibility of obtaining commercially suitable wild species for cultivation.

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background information

Pleurotus species commonly known as oyster mushrooms are distributed all over the world and usually grow on hardwood in terrestrial ecosystems (Vilgalys and Sun 1994). Similar to other white-rot fungi, they are important agents of biodeterioration, due to their ability to break down plant materials, especially cellulose and lignin (Carlile and Watkinson, 1994; Buswell *et al.*, 1996). They have high commercial value, and thus they have been widely cultivated (Cohen *et al.*, 2002; Stamets, 2000). Consumption of wild *Pleurotus* species is common among the diets of many rural communities during the rainy seasons whereas, urban dwellers have great preference for cultivated species. Consumption of *Pleurotus* species has increased due to their high nutritional composition, taste and aroma. A recently published FAO study recommends consumption of edible mushrooms to supplement carbohydrate rich diets common among many developing countries (FAO, 2006).

Many members of the genus *Pleurotus* are found distributed worldwide in nature. A few of them have been domesticated and are under commercial production. The commonly cultivated species include *Pleurotus sajor-caju, Pleurotus cystidiosus*, *Pleurotus eryngii* and *Pleurotus tuberregium* (Chang and Miles, 1989a). Production of *Pleurotus* species is increasing due to their ability to grow fast on a wide range of agro-

wastes. Production of *Pleurotus* species is gaining popularity and it is the second most produced mushroom in the world market after *Agaricus* species (Chang, 1999).

Growth of mushroom industry requires new strains with better characteristics. Farmers require mushroom varieties with fast maturity period, increased resistance to both pests and diseases and high yield. Kenya's rich mushroom biodiversity has great potential to provide new mushroom strains with desirable characteristics for commercial cultivation. The exact characterization and identification at the species level is thus an important step in systematically utilizing the full potential of fungi in specific applications (Lieckfeldt *et al.*, 2001). Many *Pleurotus* species have been identified and characterized in the past using morphological features. The previous studies on *Pleurotus* species in Kenya have also been based on morphological characteristics. However, morphological features in Basidiomycetes fungi are influenced by environmental factors and often fail to detect variations among species and strains that are closely related.

Molecular markers have been used to discriminate mushroom lineages at the species level. They are more stable, reproducible and are not affected by environmental factors hence provide more information on genetic characteristics of any species. Identification and characterization of local strains of *Pleurotus* species using molecular

tools is necessary in selecting new strains for commercial cultivation. Morphological characters alone used in the past are often inadequate for exact strain identification and in resolving the systematics and evolutionary relationships within Basidiomycetes fungi. Molecular genetic data is therefore useful for establishing a reliable taxonomic scheme for *Pleurotus* taxa. The aim of this study was to evaluate variability and relatedness of the wild *Pleurotus* species collected from different parts of Kenya.

1.2 Problem statement

In recent times, edible mushrooms have assumed greater importance in the diets of both rural and urban dwellers in Kenya, unlike previously when consumption was confined to rural communities (Wambua, 2004). Increase in demand for edible mushrooms has resulted in setting up of several mushroom units in different parts of the country. Currently the mushroom production stands at slightly over 500 tons per annum with the production of *Pleurotus* species being the second most produced after *Agaricus* (Concern/GTZ/MOA., 2005). The total annual mushroom production in Kenya is low and hardly enough to meet the local demand. Rural communities therefore, rely on the collection and consumption of wild species during the rainy seasons. Unfortunately, the seasonality of wild edible mushrooms makes them unreliable source of nutrition. Similarly, lack of clear-cut identification and limited information on their genetic diversity limit their exploitation for commercial production and breeding purposes.

1.3 Justification of the study

Increased productivity of mushroom industry in Kenya requires new mushroom species with improved characteristics such as high yields and increased resistance to pests and diseases. Characterization and identification of wild species is likely to provide strains with desired characteristics. Accurate taxonomic identification and phylogenetic classification is therefore necessary for selecting strains with potential for commercial production and breeding purposes. Molecular markers including rapid amplified polymorphic DNA (RAPD) markers, amplified fragment length polymorphic markers (AFLP), restriction fragment length polymorphic (RFLP) markers and microsatellite have all been employed to discriminate different kinds of organisms including mushrooms (Barroso *et al.,* 2000; Vos *et al.,* 1995). AFLP markers have proved to be more reliable compared to other molecular tools for genotyping mushroom lineages. AFLP technique has been successfully applied to discriminate the genomes of *Pleurotus ostreatus* (Meng *et al.,* 2003), *Tricholoma matsutake* (Chen *et al.,* 2003), *Lentinula edodes (*Zhuo *et al.,* 2006*), Agaricus bisporus (*Gu *et al.,* 2003) *G. lucidum (*Zheng *et al.,* 2007*).* Similarly, the internal transcribed spacer (ITS) region of the ribosomal DNA (rDNA) has also been widely used for the phylogenetic identification of mushrooms at both the species and genus level (Sanchez-Ballesteros *et al.,* 2000). Different regions of rDNA also evolve at variable rates and this makes them suitable for investigating fungal relationships at different taxonomic levels (Bruns *et al.,* 1991).

1.4 Research hypothesis

Wild *Pleurotus* species in Kenya have broad genetic diversity suitable for commercial cultivation.

1.5 Objectives

1.5.1 General objective

To determine the genetic potential of wild *Pleurotus* species in Kenya for commercial cultivation.

1.5.2 Specific objectives

To examine genetic variability and phylogenetic relationships of the wild *Pleurotus* species collected from different parts of Kenya using AFLP markers and ITS sequences of the ribosomal DNA.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Description and classical taxonomy of *Pleurotus* species

Pleurotus is a genus of gilled mushroom with wide cap shaped like an oyster shell. Members of the genus *Pleurotus* form basidia and they usually have a mycelial thallus (Hawksworth *et al.*, 1995). The systematic position of *Pleurotus* has been much debated with several species being placed in the former Polyporaceae, tribus Lentineae and the latter in the Tricholomataceae, tribus Resupinateae (Singer, 1951; Corner, 1981). Some mycologists have also placed *Pleurotus* in the family Pleurotaceae, irrespective of sporeprint color and other micro-morphological characters. Current taxonomic classification places *Pleurotus* species in the phylum Basiomycetes, order Agaricales and family Tricholomataceae (Bernardo, 2004; Hawksworth *et al.*, 1995).

Figure 1. *Pleurotus* species growing on dead trunk of wood

2.2 World distribution of *Pleurotus* species

Pleurotus species are distributed throughout the world as shown in Table I. To date, approximately 70 species of *Pleurotus* have been recorded and new species are discovered more or less frequently although some of these are considered identical to previously recognized species (Singer, 1986). *Pleurotus pulmonarius* and *Pleurotus cystidiosus* are known to be distributed in tropical and subtropical region, while *Pleurotus eryngii* are collected in Europe, Africa and most of Asia except Korea and Japan, where the mushroom is commercially cultivated (Walser *et al.*, 2003; Zervakis *et al.*, 1994; Zervakis and Balis, 1996; Vilgalys and Sun, 1994; Lindequist *et al.*, 2005; Lieckfeldt *et al.*, 2001; Kües, 2000; Kües and Liu, 2000; Kalac and Svobod, 2005; Cohen *et al.*, 2002; Borchers *et al.*, 1999). *Pleurotus ostreatus* is the most important commercial mushroom species within the genus *Pleurotus* and it is widespread in temperate areas (Chang, 1999). The species is quite adaptable to a range of climates and substrate materials, making it one of the most preferred edible mushrooms to many farmers.

Table 1. Worldwide distribution of some commonly cultivated *Pleurotus* species

	Europe	Asia	N.America	S.America	Africa	Australasia
P.ostreatus	+	+	+	+	+	+
P.pulmonarius	+	+	+	_	_	+
P.populinus	+	_	+	_	_	_
P.djamor	_	+	+	+	+	+
P.eryngii	+	+	_	_	+	_
P.tuber-regium	_	+	_	_	+	+

Source: Zervakis and Balis, (1996); +present;-absent

2.3 Nutrient composition

Pleurotus species have been used as human food for centuries due to the variety of flavours and textures they can provide. Nutritional composition of *Pleurotus* species compared to other commonly consumed mushroom species is illustrated in Table 2. *Pleurotus* species are rich in protein and low in fat, and carbohydrates. They also contain vitamins like riboflavin and thiamine that are necessary for good health.

Table 2. Nutritional composition of some commonly consumed mushroom species

Nutrient composition	*Auricularia* species	*Lentinus* species	*Volvariela* species	*Pleurotus* species
Crude protein (%w/w)	7.7	12.7	21.2	30.4
Fat (%w/w)	0.8	2	10.1	2.2
Carbohydrate (%w/w)	87.6	79.6	58.6	57.6
Thiamine (mg/100g of d.wb)	0.2	7.8	1.2	4.8
Riboflavin (mg/100g of d.wb)	0.9	4.9	3.3	4.7

Source: Crisan and Sands (1978)

2.4 Medicinal value

The consumption of *Pleurotus* species has several positive effects on the general human health because of a number of health promoting substances they possess (Kües and Liu, 2000). Many *Pleurotus* species have yielded potential biologically active compounds that exhibit anticancer activity *in vitro* or in animal models (Borchers *et al.*, 1999). These compounds include hemicellulose, polysaccharides, lipopolysaccharides, peptides, proteins, glycoproteins, nucleosides, triterpenoids, complex starches, lectins, lipid derivatives and other metabolites (Kalac and Svobod, 2005; Lindequist *et al.*, 2005).

2.5 Role in environmental management

Many basidiomycetes have the capability to produce simultaneously the hydrolytic and oxidative enzymes which are needed to degrade complex lignocellulosic substrates (Kirk *et al.*, 2008; Buswell *et al.*, 1996). Great diversity within *Pleurotus* species suggests variability in terms of yield and Biological Efficiency (BE) (Buswell *et al.*, 1996). *Pleurotus* species can therefore be used to profitably manage the agricultural waste materials left after harvesting and at the same time used as important source of food.

2.6 Life cycle and growth of *Pleurotus* species

The development of fruiting bodies is a highly organized process, which requires the coordination between genetic, environmental and physiological factors (Kües, 2000). Formation of various tissues within the developing primordium alternate between light and dark phases (Boulianne *et al.*, 2000; Walser *et al.*, 2003) This promotes the elongation of the stripe and the expansion of the cap, giving rise to a fully developed fruiting body (Moore *et al.*, 1979; Kües and Liu, 2000).

During fruit body formation, nuclear fusion and meiosis occur only in the specialized basidia. Haploid nuclei migrate into a tetrad of basidiospores, external to the basidium. Each Basidium has commonly four monokaryotic basidiospores. These spores germinate into homokaryotic hyphae (Stamets, 1993; Kang, 2004). A single basidiospore germinates to be a mass of homokaryotic mycelium, each cell of which contains a single haploid nucleus (Chang and Miles, 1989b). The homokaryotic mycelia

continue to grow until the hypha fuse with the other hyphae which have compatible mating type. After fusion between compatible homokaryotic hyphae, reciprocal nuclear migration occurs and a heterokaryotic mycelium is formed.

2.7 Mating system and gene flow potential

Members of the genus *Pleurotus* are heterothallic (self-sterile) and sexual reproduction is governed by the mating type genes (Eugenio and Anderson, 1968b). The spore gets off the gill and away from the mushroom cap. Once the spores have cleared the bottom of the cap, air currents carry them away. When the spores are a few millimetres away from the cap they can be picked up by the faster winds and carried considerable distances thus enabling them to cross with the same species (Perberdy *et al.*, 1993; Terakawa, 1957).

Mating type genes prevent mating between genetically identical cells. They have a bifactorial tetrapolar incompatibility mating systems which has two unlinked mating type factors designated as A and B (Eugenio and Anderson, 1968a). Factor A controls nuclear pairing, clamp cell formation, coordinate cell division and clamp cell septation whereas factor B is responsible for the control of nuclear migration, septa dissolution and clamp cell fusion. Two monokaryotic mycelia are compatible if they have different alleles at both loci. Multiple allelism for mating type genes was also reported by Terakawa (1957). Because of this multiple allelism of mating type, the out breeding potential is estimated close to 100% in nature and the inbreeding potential can be as low as 25% (Eugenio and Anderson, 1968a)

2.8 Molecular systematic

Most taxonomic and phylogenetic studies of Basidiomycota have been based on the analysis of morphological characters. Recently, relationships among species in several genera of Basidiomycota have often been established by amplification of nuclear sequences by Polymerase Change Reaction (Pringle *et al.*, 2000; Bos, 1996). Investigations have mainly focused on nucleotide sequences of the internal transcribed spacer (ITS) located between the nuclear rDNA 18S and 28S subunit genes, and made it possible to determine the relationships between fungal species from the genus *Pleurotus* (Molcalvo *et al.*, 1995). Ribosomal RNA genes exist in genomes as multiple copies arranged in tandem repeats along one or more chromosomes (Figure 2).

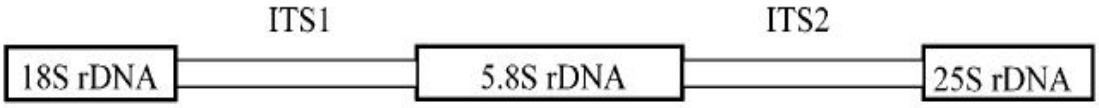

Figure 2. A schematic representation of the location of ITS region.

Several features of rDNA make it appropriate for systematic and phylogenetic studies. First, this region of the genome is well characterized and conserved. Many primers already are available to amplify regions of the rDNA repeat that would supply sequence data for a wide range of taxa (White *et al.*, 1990). Second, substantial research has been done on rDNA from many fungi, so ample datasets are available for reference. Additionally, different regions of rDNA evolve at variable rates, which can be used to investigate fungal relationships at different taxonomic levels (Bruns *et al.*, 1991).

2.9 Biogeography and speciation

The information on phylogeny and biogeography provides a framework for understanding the relationship among different components of evolution at the species level, including geographic variation, genetic isolation mechanisms, and morphological evolution (Avise, 1989). Speciation in many mushroom groups is often associated with tremendous levels of genetic divergence that suggest an ancient origin for some species. Because of their ephemeral fruiting patterns, the ranges and distributions of most mushroom species are poorly known (Vilgalys and Sun, 1994).

2.10 Characterization techniques

2.10.1 Morphological tools

Macrofungi have been traditionally characterized based on their micro and macroscopic features. Macroscopic descriptions are based on the size, shape, color and texture of the pileu. Other descriptors include the size of lamellae (height, thickness, breadth, and width), shape (attachment), color and texture; Stipe size including length, width, texture, color and cuticle (aculopellis) feel Color (Kirk *et al.*, 2008). Unfortunately, the phenotypic approach has been largely criticized for its lack of standardized and stable terminology and for its high subjectivity to environmental conditions (Brasier, 1997).

2.10.2 Molecular tools

Perspectives for fingerprinting the genomes of mushrooms have recently arisen from molecular markers based on the polymerase chain reaction. These procedures have provided novel and very powerful reproducible and reliable DNA fingerprinting methods, (Vos *et al.*, 1995). Molecular markers such as rapid amplified polymorphic DNA (RAPD) markers, restriction fragment length polymorphic (RFLP) markers, microsatellite and mitochondrial genotypes have all been used to discriminate mushroom species (Barroso *et al.*, 2000).

2.10.2.1 Random amplified polymorphic DNA markers

Random amplified polymorphic DNA (RAPD) has been successfully applied in the determination of genetic diversity in several mushroom breeding materials intended for crossing (Khush *et al.*, 1991). This is because RAPD technique is simple and efficiency, and it requires no prior sequence knowledge (Karp, 1997a). However, the RAPD technique has proved not to be reproducible especially between laboratories as it is highly influenced by experimental conditions (Jones *et al.*, 1997b; Virk *et al.*, 2000; Staub and Serquen, 1996). The preferential amplification of DNA fragments also masks relatedness between taxa or populations and limit reproducibility (Mueller and Wolfenbarger, 1999).

2.10.2.2 Restriction fragment length polymorphic markers

Restriction fragment length polymorphic markers (RFLPs) have been used for analysis of genetic diversity of fungal species because of their specificity and codominant nature (Chyi *et al.*, 1992). However, the RFLP analysis generates relatively small numbers of polymorphisms and is therefore not suitable for studying new or alternative crops such as wild mushrooms where little prior data is available (Pradhan *et al.*, 1992; Lanner *et al.*, 1997).

2.10.2.3 Amplified fragment length polymorphic markers

Amplified fragment length polymorphic (AFLP) is a highly accurate method to detect polymorphisms among individuals, populations, and independently evolving mushroom lineages (Mueller and Wolfenbarger, 1999). The visible polymorphism of AFLP fragments is primarily generated through variations in restriction enzymes sites, and the incorporation of PCR allows for rapid and efficient marker generation. AFLP technique has widely been used to study many mushroom lineages including *Pleurotus ostreatus (*Zhuo *et al.*, 2006 Zheng *et al.*, 2007*)*.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Sample collection

Samples of *Pleurotus* species growing on either tree barks or other substrates (wood, soil or leaf litters) were randomly collected from Arabuko Sokoke Forest, Kakamega Forest and Mt. Kenya Forest in Kenya (Table 3). Each collection site constituted a population. Individual sample in each population was collected 10-20 m apart to avoid sampling the same individual several times. Similarly, populations were over 300km apart. Thirteen samples were obtained from Jomo Kenyatta University of Agriculture and Technology (JKUAT).

Table 3. Wild and cultivated of *Pleurotus* species used in this study

Sample identification codes							
AS01	AS32	AS66	AS70	KK14	KK53	KK94	MK95
AS03	AS33	AS67	AS80	KK15	KK65	MK07	KK12
AS06	AS34	AS68	AS83	KK21	KK73	MK10	KK14
AS08	AS35	AS69	AS86	KK23	KK76	MK11	KK15
AS13	AS39	AS29	AS87	KK38	KK77	MK16	KK21
AS17	AS40	AS32	AS89	KK42	KK78	MK20	KK94
AS18	AS41	AS33	AS90	KK43	KK79	MK27	MK07
AS24	AS51	AS34	AS96	KK44	KK82	MK31	MK10
AS26	AS52	AS35	AS96	KK45	KK84	MK36	MK11
AS28	AS54	AS39	KK05	KK47	KK91	MK71	MK16
AS29	AS55	AS40	KK07	KK50	KK53	MK88	MK20
AS41	AS51	AS41	KK12	93JK	75JK	74JK	48JK
72JK	30JK	92JK	19JK	22JK	25JK	04JK	46JK

AS, MK, KK represent species from Arabuko Sokoke, Mt. Kenya and Kakamega Forests. JK represents cultivated species obtained from JKUAT

3.2 Preparation of tissue cultures

The young and healthy fruit bodies of *Pleurotus* species were prepared by breaking either the cap or stem to expose the interior tissue, followed by excising and inoculating small tissue fragments using a sterile scalpel in petri dishes containing potato dextrose agar as described by Stamets (2000). A total of 84 samples formed mycelium after incubation at 25-28^{o}C for 5 days. Mycelia were sub cultured after every 10 days until pure cultures were obtained. The cultures were then preserved at 4°C as stock cultures.

3.3 Isolation and visualization of genomic DNA

3.3.1 DNA isolation

Total genomic DNA was extracted following the cetyltrimethyl ammonium bromide method (Doyle and Doyle, 1988). Mycelium (0.1g) of each sample was collected using sterile scalpel from the agar medium and put into 1.2 ml tubes and then ground into fine powder for 5 min using a 2000 Geno/Grinder (Troemner, Inc., Beirut, Lebanon). The crushed mycelia were resuspended in 0.5 ml extraction buffer (100 mM Tris-HCl [pH 8], 2% [wt/vol] CTAB, 50 mM EDTA, 0.7 M Nacl, 1% [vol/vol] β-mercaptoethanol and 1% [w/v] PVP) and incubated for 1 hr at 65°C. Solution (0.5ml) of chloroform-isoamyl alcohol (24:1 vol/vol) was added into the mixture of extraction buffer and the two phases were mixed several times by inverting tubes gently. The resulting emulsion was centrifuged at 4500 x *g*, 20°C, for 5 min using Beckman

Coulter, Allegra™ 25R Centrifuge (Beckman Coulter, Inc., CA, USA). The upper aqueous phase was mixed with 50µL of NaAC and 400µL of isopropanol in 1.2 ml tubes. Samples of DNA were left to precipitate for 12h at 4^{o}C and centrifuged at 3500 x g, 20^{o}C for 5 min. The supernatant was discarded and pellets air- dried on a clean paper towel in the hood for 1 h before washing two times with an equal volume of 70% ethanol. Pellets of DNA were then resuspended in a low-salt TE buffer (Tris-HCl pH 8.0, EDTA 0.5M) and incubated at 37^{o}C for 30 min with 2µL of DNAse-free RNAseA (10 mg/ml). Purified DNA was then stored at 4°C.

3.3.2 Gel electrophoresis

Quality and quantity of DNA was confirmed using agarose gel electrophoresis. Solution of 1% agarose was prepared by melting 1.0 g agarose in 100 ml of 1x TBE (0.1M Tris-HCl pH 8.0; 0.1M Boric acid; 0.5M EDTA) buffer in a microwave for 2 min. The solution was allowed to cool for 5 min minutes then 1 µl of ethidium bromide was added and stirred to mix. The gel was cast using a supplied tray and comb and allowed to set for of 30 min at 25^{o}C on a flat surface. DNA sample was mixed with 2 µl 1x loading buffer and loaded alongside 5 µl of 1kb ladder into the separate wells. DNA samples were run in the gel for 1hr at 80V after which the gel was photographed using UVP Bioimaging Camera (SFC Inc., CA, USA). Purified DNA was diluted to 200mn/µL using NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific Inc., Wilmington, USA).

3.4 AFLP analysis

3.4.1 Template preparation and adaptor ligation

AFLP analysis was carried out following the standard procedure described by Vos *et al*. (1995) and adapted in the AFLP® Plant Mapping protocol of the Applied Biosystems Inc. (Forster City, CA, USA). The suitability of the restriction enzymes used to cut the genomic DNA was initially tested. The genomic DNA was digested with *Mse*I (frequent-4-base cutter-TAA) and *Eco*RI (rare-6-base cutter-AATTC) restriction enzymes supplied by Applied Biosystems (Forster City, CA, USA) separately and then in combination. A restriction-ligation enzyme master mix was prepared by combining 0.5 µl of T4 DNA ligase (1 U/µl in 10 mM Tris-HCl (pH 7.5), 1 mM DTT, 50 mM KCl, 50% (v/v) glycerol) with 4.5 µl adapter/ligation solution (*Eco*RI/*Mse*I adapters, 0.4 mM ATP, 10 mM Tris-HCl (pH 7.5), 10 Mm Mg-acetate, 50 mM K-acetate). The genomic DNA (5.5µl) was incubated for 2.5 hr at 37°C with 0.5 µl of *Eco*RI/*Mse*I (1.25 U/µl each in 10 mM Tris-HCl (pH 7.4), 50 mM NaCl, 0.1 mM EDTA, 1 mM DTT, 0.1 mg/mL BSA, 50% glycerol (v/v), 0.1% Triton® X-100), and 5 µl of 5× reaction buffer (50 mM Tris-HCl (pH 7.5), 50 mM Mg-acetate, 250 mM K-acetate). The adapter pairs were first annealed to make them double stranded by heating the tubes in a water bath at 95°C for 5 min. The tubes were then left to stand at 25ºC for 10 min. This reaction mixture was incubated at room temperature overnight. The restriction-ligation products were diluted by adding 189 µL of low TE buffer (0.1 mM EDTA, 15mM Tris-HCL) to 11 µL of the reaction mixture in a 1.5 ml micro-centrifuge tube to give the appropriate

concentration for subsequent PCR. An aliquot (5 µl) of each digested products was run on 1.5% agarose gel in 1× TBE buffer to check for complete digestion of DNA samples. A 1kb DNA size marker was used to check the size of the DNA.

3.4.2 Pre-selective amplification

Amplification of the adapter-ligation restriction products was performed for subsequent selective amplification using pre-selective primers provided by Applied Biosystem, USA. Diluted restriction-ligation reaction product (4.0 µl) was mixed with 1.0 µl AFLP pre-selective primer pairs and 15 µl core mix from AFLP ligation and preselective amplification module P/N 402004. PCR amplification was carried out at initial hold-time of 2 min at 72 ^{o}C followed by 20 cycles of 20 s at 94 ^{o}C, 20 cycles of 30 s at 56 ^{o}C and 20 cycles of 2 min at 72 ^{o}C and a further hold time of 30 min at 60^{o}C using Applied Biosystem GeneAmp 9700 thermo cycle machine (Applied Biosystem, CA, USA). The pre-selective amplification reaction products were verified by mixing 10 µl of each pre-selective amplification product with 2 µl of 1× loading dye and run on 1.5% agarose gel in 1× TBE buffer at 90 V/cm for 45 min. The gel was stained with 1 µg1/100ml ethidium bromide and photographed using UVP Bioimaging Camera (SFC Inc., Osaka, Japan).

3.4.3 Selective amplification

Selective AFLP amplification was performed following the method described by Vos *et al.* (1995). Pre-selective amplification product (10 µl) was diluted with 190 µl

low salt TE (Tris-EDTA) buffer. The selective PCR amplification was performed using various combinations of two AFLP primers specific for *Eco*RI and *Mse*I primer adapters. A total of 14 primer pairs; EcoR1-AAC/MseI-CTC, EcoR1-ACA/MseI-CAT, EcoR1-AT/MseI-CTG, EcoR1-AGG/MseI-CTG, EcoR1-AGG/MseI-CAT, EcoR1-AG/Mse-C, EcoR1-AT/Mse-CTA, EcoR1-AG/Mse-CAT, EcoR1-TA/Mse-C, EcoR1-ACA/Mse-CTC, EcoR1-ACA/Mse-CTG, EcoR1-ACA/Mse-CTA, EcoR1-AGG/Mse-CTC and EcoR1-AGG/Mse-CTA were screened on eight samples for protocol optimization and to identify the primer pairs that produced the most polymorphic fragments. The diluted pre-selective amplification product (3 µl) was mixed with 1 µl fluorescently labeled *Eco*RI primer, 1 µl of *Mse*I primer and 15 µl of AFLP core mix. Amplification of selective reaction product was performed on a Applied Biosystems GeneAmp 9700 thermocycler (ABI, Forster City, CA, USA) with the following parameters; an initial two minutes at 94 ^{o}C followed by one cycle of 94 ^{o}C for 20 s, 66 ^{o}C for 30 s and 72 ^{o}C for 2 min. This cycle was repeated eight times with a lowering of the annealing temperature of 1 ^{o}C per cycle. This was followed by 20 cycles of 94 ^{o}C for 20 s, 56 ^{o}C for 30 s and 72 ^{o}C for 2 min and a further hold time of 30 min at 60 ^{o}C. The selective PCR product was prepared by adding 12 µl of Gene Scan 500 LIZ internal size standard supplied by Applied Biosystems Inc. (Forster City, CA, USA) to 1 ml deionised formamide HiDi. Loading buffer (9 lµ) was added to 1 µl of the selective amplification products in a MicroAmp PCR Plate and resolved in ABI capillary electrophoresis system (ABI Inc., Forster City, CA, USA) and analyzed on ABI 3130 genetic analyzer (Forster City, CA, USA).

3.5 DNA amplification and sequencing

DNA (2 µl) isolated from 12 strains of *Pleurotus* species were mixed with 18 µl Accupower PCR Premix cat. #K-2016 (Bioneer Inc. Daejeon, South Korea) and amplified using forward ITS-1 (5'-TCCGTAGGTGAACCTGCGG-3') primers and reverse ITS-4 (5' –TCCTCCGCTTATTGATATGC-3') primers. PCR was performed using GeneAmp 9700 Eppendorf thermocycler (Applied Biosystem Inc., CA, USA) with the following program: 95°C for 1 min, 35 cycles at 94°C for 30 s, 60°C for 30 s and 72°C for 2 min, and a final elongation at 72°C for 10 min. PCR products were treated with ExoSAP-IT (USB Corporation, Cleveland, OH, USA) and examined on a 1.6% (w/v) agarose gel stained with ethidium bromide. For sequencing, an ABI PRISM 3.1 BigDye Terminator kit (Perkin Elmer, USA) was used and the electrophoresis was carried out on an ABI PRISM 3700 Genetic Analyzer. Sequencing was carried out for both strands using the forward and reverse primers used for initial amplification.

3.6 Sequence editing and alignment

Resulting sequences with readable chromatograms were assembled and edited using DNA Baser Version 3 (DNAStar, Inc. Madison, USA). However, unreadable chromatograms characterized by a more or less sudden overlapping of sequence peaks starting at certain given positions in the sequence could not be assembled together in the DNA Baser program. It was therefore not possible to edit these chromatograms manually and to reconstruct complete sequence hence they were removed from the

analyses. Each unique ITS sequences were first used as a query to retrieve closely related sequences from the GeneBank.

The unique ITS and the retrieved sequences were aligned using CLUSTALW multiple alignment program available at http://www.ebi.ac.uk/Tools/msa/clustalw2. The aligned sequences were visually checked, adjusted and then analyzed using MEGA v5.05. In this analysis, because of the inclusion of divergent sequences and the differences in length among the aligned DNA sequences, a large number of gaps (i.e. insertions and deletions) were introduced in the aligned dataset. As a result, the gaps were treated as missing data.

3.7 Data analysis

Genotypes were scored for presence (1) and absence (0) of AFLP bands using GeneMapper Software version 4.0 (Applied Biosystem Inc., Forster City, CA, USA). Only sharp and precise bands were scored to generate a data matrix. Category bins were created to group peaks based on the sizes of the allele. A threshold peak height was set at 50 -500 relative fluorescent units (Palsson *et al.*, 1999). Bands present in all accessions were not scored. Distance matrices for all pairs of genotypes were constructed from the AFLP data matrix using the Euclidean distance method (Kaufman and Rousseeuw, 1990).

The AFLP data set was subjected to Nei's gene diversity index (H) to quantify variability within the population and to investigate genetically close populations. Allele frequency-based Nei's genetic distance and unweighted pair group method of arithmetic

averages (UPGMA) clustering methods were employed using tools for population genetic analysis (TFPGA) software version 1.3 (North Arizona University, Arizona, USA) (Miller, 1997). Cluster analysis was performed using the genetic distance matrices generated by the Euclidean distance method to reveal the patterns of genetic relationships among genotypes. The results of cluster analysis were presented in the form of dendrograms to infer relationships among genotypes.

Neighbor-Joining (NJ) and Maximum Parsimony (MP) analyses were performed as described by Tamura *et al.* (2011). NJ and MP analyses were performed using MEGA software version. 5.05 developed by Arizona State University, USA (Tamura *et al.*, 2011). Support for phylogenetic groupings was assessed by bootstrap analysis (1,000 replicates) with random addition of sequences during each heuristic search (Felsenstein, 1985). Only significant bootstrap replication frequencies above 50% were indicated. Other indices for the generated topology, including tree length, a consistency index as well as retention index were calculated. The database search of sequences for a possible match to the ITS sequences was performed using the basic sequence alignment Basic Local Alignment Search Tool (BLAST) program run against the GenBank database (http://www. ncbi.nlm.nih.gov/blastn) on 5th October, 2011. Additional four sequences were included as reference ITS sequences. The four reference sequences were chosen based on their comparable sequence lengths to the 12 sequences of ITS and in several cases the availability of two or more strains for the same species.

CHAPTER FOUR

4.0 RESULTS

4.1 AFLP polymorphism

A total of 643 AFLP loci were generated from 84 samples of *Pleurotus* species using five primer combinations (Table 4). The primer combinations used produced 330 polymorphic loci across all the species accounting for 51% of the total scorable loci (Table 4). The number of scorable loci generated by each AFLP primer pair varied from 20 to 228. The number of polymorphic loci for each primer pair varied from 16 to 116. The loci ranged in size from 51 to 497 bp as generated by GeneMapper version 4.1. The number of loci varied for different primer combinations. The primer combination of 5'*Eco* +AGG- *Mse* +CTC 3' gave the smallest number of both scorable (20) and polymorphic (16) loci, respectively while 5'*Eco* +AAC- *Mse* +CTG 3' gave the highest number of both scorable (228) and polymorphic (116) loci. Five primer pairs used generated an average of 7 scorable and 4 polymorphic loci across all the 84 species studied.

Table 4. AFLP primers and polymorphism

Primer pair	Total number of loci	Polymorphic loci	Polymorphism (%)
E-AAC/M-CTC	228	116	52
E-ACA/M-CAT	115	102	89
E-AT/M-CTG	160	40	25
E-AGG/M-CTG	20	16	80
E-AGG/CAT	120	56	47
Total	643	330	

4.2 Genetic diversity of Kenyan *Pleurotus* species

The genetic diversity among the studied *Pleurotus* species was very small as revealed by the estimates of Nei's unbiased genetic diversity in Table 5. The genetic diversity values ranged from 0.27 to 0.24 between species obtained from Arabuko Sokoke and Mt. Kenya. Cultivated species had similar levels of genetic diversity with the wild species from Mt. Kenya. The same order of gene diversity was revealed by Shannon's information index (I) and heterozygosity values in which wild species from Arabuko Sokoke was the most heterozygous (H=0.26) while both the JKUAT and Mt. Kenya population had the least heterozygosity values (H=0.23) each. The percentage polymorphic loci were also in close agreement with the diversity estimates. Wild species from Arabuko sokoke had high diversity estimates and percentage polymorphic loci. The observed number of alleles (na) and the effective number of alleles (ne) were also high in populations with high diversity (Arabuko Sokoke) and low in those with low diversity (JKUAT and Mt. Kenya). Nei's unbiased diversity values among the studied species were very small. This was the case with Shannon Information Index, percentage polymorphic loci and heterozygosity values.

Table 5. Genetic diversity estimates among 4 populations of *Pleurotus* species

Population ID	Sample size	na	ne	h	I	% loci	H
AS	34	1.98	1.42	0.27	0.41	99.1	0.26
KK	25	1.88	1.39	0.25	0.39	92.1	0.25
MK	11	1.73	1.37	0.24	0.37	83.1	0.23
Mean	23	1.86	1.39	0.25	0.39	91.43	0.25
JKUAT	13	1.72	1.37	0.24	0.35	82.1	0.23

na= Observed number of alleles; ne= Effective number of alleles; h = Nei's unbiased measure of genetic diversity; I = Shannon's Information index; % loci = Percentage polymorphic loci; H-mean heterozygosity. JKUAT represents population of cultivated species. Populations of wild species are represented by AS-Arabuko Sokoke, KK-Kakamega, MK-Mt. Kenya

4.3 Analysis of Molecular Variance

Summaries of analyses of molecular variance (AMOVA) are represented in Table 6. Of the total observed allele frequency variations, the majority was found from within populations (89 %). The remaining 11% could be attributed to frequency variations among populations. The contributions from each of these sources were significantly greater than 0, indicating statistically significant ($P<0.001$) genetic differentiations within rather than among populations. The degree of gene differentiation among populations in terms of allele frequency (F_{ST}) was also moderately low (0.125).

Table 6. Summary results of AMOVA

Source of variation	Variance components	% variation	P-values	F_{st}
Among population	6.611	11%	<0.001	
Within Population	52.848	89%	<0.001	0.125
Total	59.459	100%		

4.4 Cluster analysis

A dendrogram based on Nei's genetic distance (D) is illustrated in Figure 4. The dendrogram clustered 71 wild and 13 cultivated *Pleurotus* species into 3 major clades Clade I and II consisted mainly of wild species (KK, MK, AS) with bootstrap values of 38% and 67% respectively. The cultivated species (JK) formed a distinct cluster with a bootstrap support of 66%. Distribution of the wild species within each cluster did not correspond to their geographical origin.

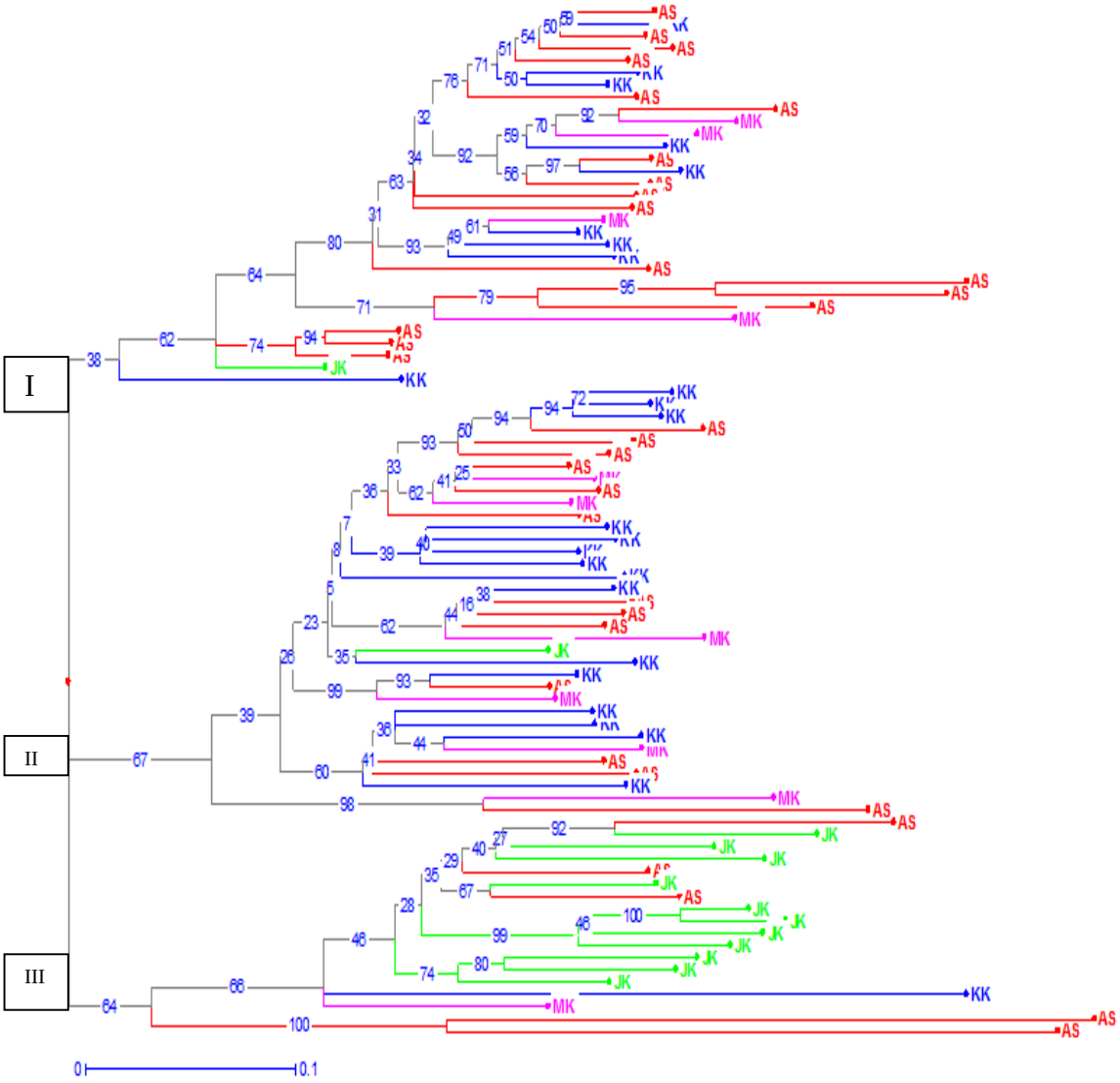

Figure 3. Dendogram of clustering analysis of 84 *Pleurotus* species.
KK(blue) AS(red) MK(pink) and JK(green) represents species from Kakamega, Arabuko Sokoke, Mt. Kenya and cultivated species from JKUAT respectively

4.5 Internal transcribed spacer sequence data

The phylogenetic relationships based on the ITS sequences was obtained by the neighbor joining (NJ) tree (Figure 4).

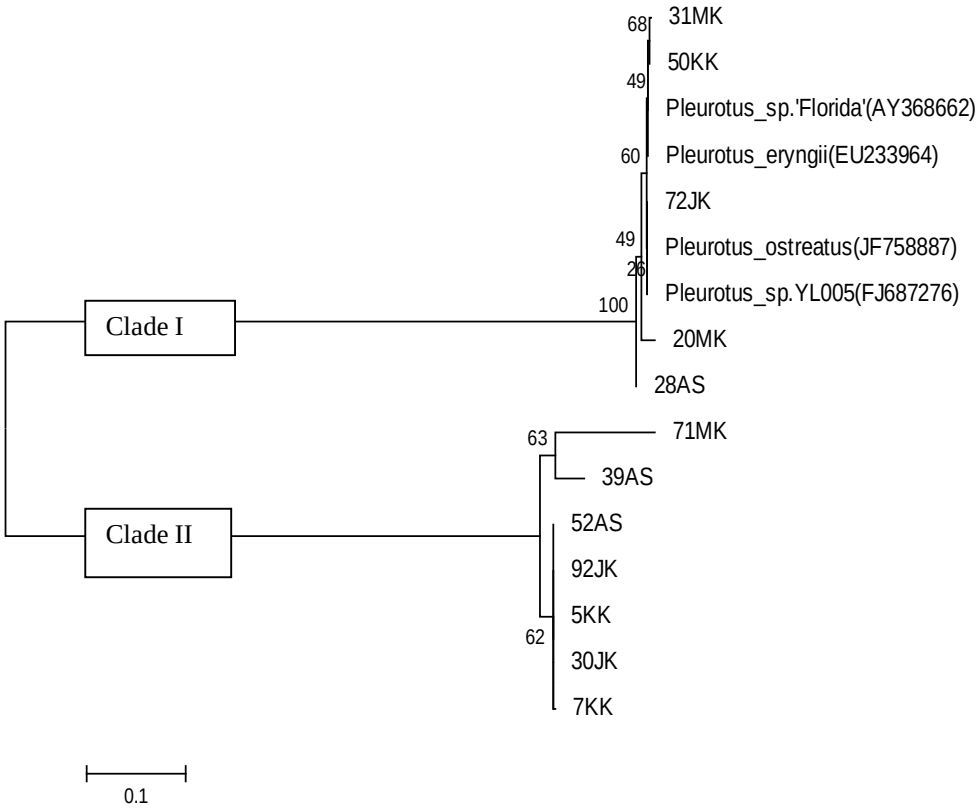

Figure 4. Neighbor-joining tree of 12 *Pleurotus* species based on ITS sequences AS, KK and MK represent species collected from Arabuko Sokoke, Kakamega and Mt. Kenya respectively. JK are cultivated species obtained from JKUAT.

Phylogenetic tree revealed little genetic differences within the species studied; indicating that these species were very similar. Two clades were identified within 12 *Pleurotus* species with clade I consisting of 50KK, 28AS, 20MK, 31MK, 72JK whereas clade II consisted of 5KK, 7KK, 39AS, 52AS, 71MK, 31MK and 92JK, 30JK. Species

in clade I grouped with the reference strains with well supported bootstrap value (100%).

Sequences (Appendix 1) for a possible match to the rDNA ITS sequence of 12 strains of *Pleurotus* species yielded 1098 hits on the query sequence in the nucleotide databases at the NCBI. The highest match was *Pleurotus* sp. 'Florida' (AY368662), ITS-1, 5.8 S, and ITS-2 nuclear rDNA sequence. The score for this match is 1110 bits with an E value of zero. The alignment (Appendix 2) of 604 total nucleotides showed 88% minimum and 100% maximum identities. The identified sequences had associated species identification in the GenBank database, belonging to *Pleurotus ostreatus* (JF75887), *Pleurotus eryngii* (EU233964), and *Pleurotus* sp. YL005 (FJ687276).

CHAPTER FIVE

5.0 DISCUSSIONS, CONCLUSIONS AND RECOMMENDATIONS

5.1 General discussions

The five AFLP primer pairs (E + ACA/M + CTC, E + AT/M + CAT, E + ACA/M + CTA, E + ACC/M + CTC, and E + AT/M + CTC) used revealed 330 polymorphic loci. This confirmed the suitability of AFLP markers to evaluate genetic variability in mushroom lineages at species level (Terefework *et al.*, 2001). The use of AFLP markers in assaying genetic variations among Basidiomycetes fungi has been widely reported in many mushroom lineages (Mueller and Wolfenbarger, 1999). The used primer pairs can be good candidate primer combinations to verify genetic diversity of other *Pleurotus* species.

Closely related or similar *Pleurotus* species were distributed across Kakamega (KK), Mt. Kenya (MK) and Arabuko Sokoke (AS) Forests (Figure 3). Close similarity within and among the populations (Table 5) suggested the likelihood of a small degree of variability in terms of growth characteristics, colour, size, Biological efficiency (BE) and susceptibility to both pests and diseases. Minimal gene flow events could be responsible for the observed low genetic variations within the wild species. Similar studies reported low levels of variations in species with a restricted distribution, or those that have long been cultivated for commercial purposes, or in populations with just a

few individuals, or in species that reproduce exclusively asexually (Old *et al.*, 1984; Burdon and Roelfs, 1985).

The cultivated species (JK) formed a distinct cluster with a few species distributed within the wild populations. Production of many cultivars from limited number of elite lines could be responsible for low diversity observed within the cultivated species. Distribution of a few cultivated species within the populations of wild species could be due to human-mediated spore dispersal following the increased setting up of several mushroom units in many parts of the country.

The identification of *Pleurotus ostreatus, Pleurotus eryngii*, *Pleurotus* sp. 'Florida' and *Pleurotus* sp. YL005 as part of the genetic diversity of *Pleurotus* species in Kenya suggests that efforts to domesticate wild genotypes could save local farmers from the burden of importing similar species. Close similarity between the wild and cultivated species also indicated close phylogenetic relationships between the wild and cultivated species. It therefore implies that the wild species have great potential for commercial cultivation.

5.2 Conclusions

Wild *Pleurotus* species from different parts of Kenya are similar to the cultivated species. However, diversity within species is high. The high genetic diversity within populations can be used for selection of more commercially suitable *Pleurotus* species. AFLP markers revealed polymorphism across the 84 samples used. AFLP

markers and rDNA ITS sequence analysis showed that the wild and cultivated *Pleurotus* species were closely related.

5.3 Recommendations

More analysis of multiple additional genes from larger collections of wild and cultivated *Pleurotus* species from different locations in Kenya is needed to fully understand their diversity and molecular phylogeny. Conservation of wild *Pleurotus* species is also necessary to maintain the genetic diversity of this species in nature. Selection of commercial mushrooms from the wild population is also possible.

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APPENDICES

Appendix 1: Sequence alignment of ITS region from 12 strains of *Pleurotus* species

Sequence alignment from genotypes of *Pleurotus* species Sequence alignments were performed using the Clustal_W algorithm (Higgins *et al.*, 1991) available at the European Molecular Biology Laboratory (EMBL-EBI). Identical regions that were aligned across all genotypes are indicated with asterisk.

```
Pleurotus_ostreatus_JF758887_       TGAATTCACTATGGAGTTGTTGCTGGCCTCTAGGGGCATGTGCACGCTTC 50
Pleurotus_sp.YL005_FJ687276_        TGAATTCACTATGGAGTTGTTGCTGGCCTCTAGGGGCATGTGCACGCTTC 50
72JK                                TGAATTCACTATGGAGTTGTTGCTGGCCTCTAGGGGCATGTGCACGCTTC 50
Pleurotus_eryngii_EU233964_         TGAATTCACTATGGAGTTGTTGCTGGCCTCTAGGGGCATGTGCACGCTTC 50
Pleurotus_sp.'Florida'_AY36866      TGAATTCACTATGGAGTTGTTGCTGGCCTCTAGGGGCATGTGCACGCTTC 50
28AS                                TTAATTC-CTATGGAGTTGTTGCTGGCCTCTAGGGGCATGTGCACGCTTC 49
31MK                                TGAATTCACTATGGAGTTGTTGCTGGCCTCTAGGGGCATGTGCACGCTTC 50
50KK                                TGAATTCACTATGGAGTTGTTGCTGGCCTCTAGGGGCATGTGCACGCTTC 50
20MK                                TGGAT--ACTATGGAGTTGTTGCTGGCCTCTAGGGGCATGTGCACGCTTC 48
52AS                                TCAAATTGTCAAATTGTCCTTGCGGACGATTAGAGAGCTGGACTCTATTC 50
92JK                                TCAAATTGTCAAATTGTCCTTGCGGACGATTAGAGAGCTGGACTCTATTC 50
5KK                                 TCAAATTGTCAAATTGTCCTTGCGGACGATTAGAGAGCTGGACTCTATTC 50
30JK                                TCAAATTGTCAAATTGTCCTTGCGGACGATTAGAGAGCTGGACTCTATTC 50
7KK                                 TCAAATTGTCAAATTGTCCTTGCGGACGATTAGAGAGCTGGACTCTATTC 50
71MK                                TCAA-TTGTCAAATTGTCCTTGCGGACGATTAGAGAGCTGGACTCTATTC 49
39AS                                TCAAATTGTCAAATTGTCCTTGCGGACGATTAGAGAGCTGGACTCTATTC 50
                                    *  *      *    **  **** * *   *** *   **  * *  ***

Pleurotus_ostreatus_JF758887_       ACTAGT-CTTTCAACCACCTGTGA-ACTTTTGATAG-ATCTGTGAAGTCG 97
Pleurotus_sp.YL005_FJ687276_        ACTAGT-CTTTCAACCACCTGTGA-ACTTTTGATAG-ATCTGTGAAGTCG 97
72JK                                ACTAGT-CTTTCAACCACCTGTGA-ACTTTTGATAG-ATCTGTGAAGTCG 97
Pleurotus_eryngii_EU233964_         ACTAGT-CTTTCAACCACCTGTGA-ACTTTTGATAG-ATCTGTGAAGTCG 97
Pleurotus_sp.'Florida'_AY36866      ACTAGT-CTTTCAACCACCTGTGA-ACTTTTGATAG-ATCTGTGAAGTCG 97
28AS                                ACTAGT-CTTTCAACCACCTGTGA-ACTTTTGATAG-ATCTGTGAAGTCG 96
31MK                                ACTAGT-CTTTCAACCACCTGTGA-ACTTTTGATAG-ATCTGTGAAGTCG 97
50KK                                ACTAGT-CTTTCAACCACCTGTGA-ACTTTTGATAG-ATCTGTGAAGTCG 97
20MK                                ACTAGT-CTTTCAACCACCTGTGA-ACTTTTGATAG-ATCTGTGAAGTCG 95
52AS                                ATGCGTGCTATTGATGA---GTGATAATTATCACATCATGCGCAGAGGCA 97
92JK                                ATGCGTGCTATTGATGA---GTGATAATTATCACATCATGCGCAGAGGCA 97
5KK                                 ATGCGTGCTATTGATGA---GTGATAATTATCACATCATGCGCAGAGGCA 97
30JK                                ATGCGTGCTATTGATGA---GTGATAATTATCACATCATGCGCAGAGGCA 97
7KK                                 ATGCGTGCTATTGATGA---GTGATAATTATCACATCATGCGCAGAGGCA 97
71MK                                ATGCGTGCTATTGATGA---GTGATAATTATCACATCATGCGCAGAGGCA 96
39AS                                ATGCGTGCTATTGATGA---GTGATAATTAACACATCATGCGCAAAGGCA 97
                                    *   ** ** *  *  *   **** * **   * *  **  *   ** *
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Pleurotus_ostreatus_JF758887_       TCTCTCAAGTCGT-CAGACTTGGTTGCTGGGATTTAAACGTCTCGGTGTG 146
Pleurotus_sp.YL005_FJ687276_        TCTCTCAAGTCGT-CAGACTTGGTTGCTGGGATTTAAACGTCTCGGTGTG 146
72JK                                TCTCTCAAGTCGT-CAGACTTGGTTGCTGGGATTTAAACGTCTCGGTGTG 146
Pleurotus_eryngii_EU233964_         TCTCTCAAGTCGT-CAGACTTGGTTGCTGGGATTTAAACGTCTCGGTGTG 146
Pleurotus_sp.'Florida'_AY36866      TCTCTCAAGTCGT-CAGACTTGGTTGCTGGGATTTAAACGTCTCGGTGTG 146
28AS                                TCTCTCAAGTCGT-CAGACTTGGTTGCTGGGATTTAAACGTCTCGGTGTG 145
31MK                                TCTCTCAAGTCGT-CAGACTTGGTTGCTGGGATATAAACGTCTCGGTGTG 146
50KK                                TCTCTCAAGTCGT-CAGACTTGGTTGCTGGGATATAAACGTCTCGGTGTG 146
20MK                                TCTCTCAAGTCGT-CAGACTTGGTTGCTGGGATTTAAACGTCTCGGTGTG 144
52AS                                A-TGAGAAGTCCTGCTAATGCATTTAAGAGGA----GCCGACCTGTCAAG 142
92JK                                A-TGAGAAGTCCTGCTAATGCATTTAAGAGGA----GCCGACCTGTCAAG 142
5KK                                 A-TGAGAAGTCCTGCTAATGCATTTAAGAGGA----GCCGACCTGTCAAG 142
30JK                                A-TGAGAAGTCCTGCTAATGCATTTAAGAGGA----GCCGACCTGTCAAG 142
7KK                                 A-TGAGAAGTCCTGCTAATGCATTTAAGAGGA----GCCGACCTGTCAAG 142
71MK                                A-TGAGAAGTCCTGCTAATGCATTTAAGAGGA----GCCGACCTGTCAAG 141
39AS                                A-TGAGAAGTCCTGCTAATGCATTTAAGAGGA----GCCGACCTGTCAAG 142
                                      *   ***** * *  *     **    ***      ** *  *    *

Pleurotus_ostreatus_JF758887_       ACTACGCAGTCT---ATTTACTTACACACCCCAAATGTATGTCTACGAAT 193
Pleurotus_sp.YL005_FJ687276_        ACTACGCAGTCT---ATTTACTTACACACCCCAAATGTATGTCTACGAAT 193
72JK                                ACTACGCAGTCT---ATTTACTTACACACCCCAAATGTATGTCTACGAAT 193
Pleurotus_eryngii_EU233964_         ACTACGCAGTCT---ATTTACTTACACACCCCAAATGTATGTCTACGAAT 193
Pleurotus_sp.'Florida'_AY36866      ACTACGCAGTCT---ATTTACTTACACACCCCAAATGTATGTCTACGAAT 193
28AS                                ACTACGCAGTCT---ATTTACTTACACACCCCAAATGTATGTCTACGAAT 192
31MK                                ACTACGCAGTCT---ATTTACTTACACACCCCAAATGTATGTCTACGAAT 193
50KK                                ACTACGCAGTCT---ATTTACTTACACACCCCAAATGTATGTCTACGAAT 193
20MK                                ACTACCCAGTCT---ATTTACTTACACACCCCAAATGTATGTCTACAAAT 191
52AS                                GCC-AGCAGCCCCCAAC-AATCCAAACATCACAATTGGAAAGAAACCAAA 190
92JK                                GCC-AGCAGCCCCCAAC-AATCCAAACATCACAATTGGAAAGAAACCAAA 190
5KK                                 GCC-AGCAGCCCCCAAC-AATCCAAACATCACAATTGGAAAGAAACCAAA 190
30JK                                GCC-AGCAGCCCCCAAC-AATCCAAACATCACAATTGGAAAGAAACCAAA 190
7KK                                 GCC-AGCAGCCCCCAAC-AATCCAAACATCACAATTGGAAAGAAACCAAA 190
71MK                                GCC-AGCAGCCCCCAAC-AATCCAAACATCACAATTGGAAAAAAACCAAA 189
39AS                                GCCCAGCAGCCCCCAACCAATCCAAACATCAAAATTGGAAAAAAACCAAA 192
                                     *    *** *    *   *   * *** *  ** ** *     ** **

Pleurotus_ostreatus_JF758887_       GTCATTTA--ATGGGCCTTGTG--CCTTTAAACCATAATACAACTTTCAA 239
Pleurotus_sp.YL005_FJ687276_        GTCATTTA--ATGGGCCTTGTG--CCTTTAAACCATAATACAACTTTCAA 239
72JK                                GTCATTTA--ATGGGCCTTGTG--CCTTTAAACCATAATACAACTTTCAA 239
Pleurotus_eryngii_EU233964_         GTCATTTA--ATGGGCCTTGTG--CCTATAAACCATAATACAACTTTCAA 239
Pleurotus_sp.'Florida'_AY36866      GTCATTTA--ATGGGCCTTGTG--CCTATAAACCATAATACAACTTTCAA 239
28AS                                GTCATTTA--ATGGGCCTTGTG--CCTATAAACCATAATACAACTTTCAA 238
31MK                                GTCATTTA--ATGGGCCTTGTG--CCTATAAACCATAATACAACTTTCAA 239
50KK                                GTCATTTA--ATGGGCCTTGTG--CCTATAAACCATAATACAACTTTCAA 239
20MK                                GTCATTTA--ATGGGCCTTGTG--CCTATAAACCATAATACAACTTTCAA 237
52AS                                GTGAGTTT--GAGAATTTAATG-ACACTCAAACAGGCATGCCCCTCGGAA 237
92JK                                GTGAGTTT--GAGAATTTAATG-ACACTCAAACAGGCATGCCCCTCGGAA 237
5KK                                 GTGAGTTT--GAGAATTTAATG-ACACTCAAACAGGCATGCCCCTCGGAA 237
30JK                                GTGAGTTT--GAGAATTTAATG-ACACTCAAACAGGCATGCCCCTCGGAA 237
7KK                                 GTGAGTTT--GAGAATTTAATG-ACACTCAAACAGGCATGCCCCTCGGAA 237
71MK                                -TGAATTT--GAAAAATTAATGGACCTCCAACCAGGATGGCCCTCCGAAC 236
39AS                                GGGAGTGTTAGAAAATTTAA-GCACCCTCAAACAGGCATGCCCCCCGAGA 241
                                      * *          *   *  *    ** *      *
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Pleurotus_ostreatus_JF758887_       CAACGGATCTCTTG-GCTCTCGCATCGATGAAGAACGCAGCGAAATGCGA 288
Pleurotus_sp.YL005_FJ687276_        CAACGGATCTCTTG-GCTCTCGCATCGATGAAGAACGCAGCGAAATGCGA 288
72JK                                CAACGGATCTCTTG-GCTCTCGCATCGATGAAGAACGCAGCGAAATGCGA 288
Pleurotus_eryngii_EU233964_         CAACGGATCTCTTG-GCTCTCGCATCGATGAAGAACGCAGCGAAATGCGA 288
Pleurotus_sp.'Florida'_AY36866      CAACGGATCTCTTG-GCTCTCGCATCGATGAAGAACGCAGCGAAATGCGA 288
28AS                                CAACGGATCTCTTG-GCTCTCGCATCGATGAAGAACGCAGCGAAATGCGA 287
31MK                                CAACGGATCTCTTG-GCTCTCGCATCGATGAAGAACGCAGCGAAATGCGA 288
50KK                                CAACGGATCTCTTG-GCTCTCGCATCGATGAAGAACGCAGCGAAATGCGA 288
20MK                                CAACGGATCTCTTG-GCTCTCGCATCGATGAAAAACGCAGCGAAATGCGA 286
52AS                                CAAGGGGCGCAAGGTGCGTTCAAA--GATTCGAGATTCACTGAATT-CTG 284
92JK                                CAAGGGGCGCAAGGTGCGTTCAAA--GATTCGAGATTCACTGAATT-CTG 284
5KK                                 CAAGGGGCGCAAGGTGCGTTCAAA--GATTCGAGATTCACTGAATT-CTG 284
30JK                                CAAGGGGCGCAAGGTGCGTTCAAA--GATTCGAGATTCACTGAATT-CTG 284
7KK                                 CAAGGGGCGCAAGGTGCGTTCAAA--GATTCGAGATTCACTGAATT-CTG 284
71MK                                CAAGGGG-GCAAGGGGGGTTCCAAA-AATCCAAAAT-CAATTAATT-CTG 282
39AS                                CAAGGGGCGCAAGGGGGGTTCAAAA-GATTCAAAATTCAGAGATTTGCAG 290
                                    *** **       * *   **  *   **     *  **   *  * *
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Pleurotus_ostreatus_JF758887_       TAAGTAATGTGA----ATTGCAGAATTCAGTGAAT-CATCGAATCTTTGA 333
Pleurotus_sp.YL005_FJ687276_        TAAGTAATGTGA----ATTGCAGAATTCAGTGAAT-CATCGAATCTTTGA 333
72JK                                TAAGTAATGTGA----ATTGCAGAATTCAGTGAAT-CATCGAATCTTTGA 333
Pleurotus_eryngii_EU233964_         TAAGTAATGTGA----ATTGCAGAATTCAGTGAAT-CATCGAATCTTTGA 333
Pleurotus_sp.'Florida'_AY36866      TAAGTAATGTGA----ATTGCAGAATTCAGTGAAT-CATCGAATCTTTGA 333
28AS                                TAAGTAATGTGA----ATTGCAGAATTCAGTGAAT-CATCGAATCTTTGA 332
31MK                                TAAGTAATGTGA----ATCGCAGAATTCAGTGAAT-CATCGAATCTTTGA 333
50KK                                TAAGTAATGTGA----ATTGCAGAATTCAGTGAAT-CATCGAATCTTTGA 333
20MK                                TAAGTAATGTGA----ATTGCAGAATTCAGTGAAT-CATCGAATCTTTGA 331
52AS                                CAATTCACATTA-CTTATCGC--ATTTCGCTGCGTTCTTC--ATCGATGC 329
92JK                                CAATTCACATTA-CTTATCGC--ATTTCGCTGCGTTCTTC--ATCGATGC 329
5KK                                 CAATTCACATTA-CTTATCGC--ATTTCGCTGCGTTCTTC--ATCGATGC 329
30JK                                CAATTCACATTA-CTTATCGC--ATTTCGCTGCGTTCTTC--ATCGATGC 329
7KK                                 CAATTCACATTA-CTTATCGC--ATTTCGCTGCGTTCTTC--ATCGATGC 329
71MK                                CCATTTACCTTAACTAATC-C--AATTCCCTTCCTTCCTC--CTCCATGC 327
39AS                                CAATTCACATTAACTAATCGC--ATTCCCCTCCTTTCTTC--ATCAATGC 336
                                      * * *  * *    **  *  * * * *  *   * * **   **  **
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```
Pleurotus_ostreatus_JF758887_       ACGCACCTT---GCGCCCCTTGGTATTCCGAGGGGCATGCCTGTTTGAGT 380
Pleurotus_sp.YL005_FJ687276_        ACGCACCTT---GCGCCCCTTGGTATTCCGAGGGGCATGCCTGTTTGAGT 380
72JK                                ACGCACCTT---GCGCCCCTTGGTATTCCGAGGGGCATGCCTGTTTGAGT 380
Pleurotus_eryngii_EU233964_         ACGCACCTT---GCGCCCCTTGGTATTCCGAGGGGCATGCCTGTTTGAGT 380
Pleurotus_sp.'Florida'_AY36866      ACGCACCTT---GCGCCCCTTGGTATTCCGAGGGGCATGCCTGTTTGAGT 380
28AS                                ACGCACCTT---GCGCCCCTTGGTATTCCGAGGGGCATGCCTGTTTGAGT 379
31MK                                ACGCACCTT---GCGCCCCTTGGTATTCCGAGGGGCATGCCTGTTTGAGT 380
50KK                                ACGCACCTT---GCGCCCCTTGGTATTCCGAGGGGCATGCCTGTTTGAGT 380
20MK                                ACGCACCTT---GCGCCCCTTGGTATTCCGAGGGGCATGCCTGTTTGAGT 378
52AS                                GAGAGCCA--AGAGATCCGTTGTTG---AAAGTTGTA-TTATGGTTTATA 373
92JK                                GAGAGCCA--AGAGATCCGTTGTTG---AAAGTTGTA-TTATGGTTTATA 373
5KK                                 GAGAGCCA--AGAGATCCGTTGTTG---AAAGTTGTA-TTATGGTTTATA 373
30JK                                GAGAGCCA--AGAGATCCGTTGTTG---AAAGTTGTA-TTATGGTTTATA 373
7KK                                 GAGAGCCA--AGAGATCCGTTGTTG---AAAGTTGTA-TTATGGTTTAAA 373
71MK                                CAAAACCC--AAAAATCCCTTGGTT---AAAGTTGGAATAATGTTTAATA 372
39AS                                AAGAGACACAAGATATCCGTTGGAG---AAAGTGGTAATTATGGTTAATA 383
                                          *        ** ***       **  * *    ** ** *
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Pleurotus_ostreatus_JF758887_       GTCATTAAATTC-TCAAATCACTTTGGTTTCTTTCCAATTGTGATGTTTG 429
Pleurotus_sp.YL005_FJ687276_        GTCATTAAATTC-TCAAATCACTTTGGTTTCTTTCCAATTGTGATGTTTG 429
72JK                                GTCATTAAATTC-TCAAATCACTTTGGTTTCTTTCCAATTGTGATGTTTG 429
Pleurotus_eryngii_EU233964_         GTCATTAAATTC-TCAAATCACTTTGGTTTCTTTCCAATTGTGATGTTTG 429
Pleurotus_sp.'Florida'_AY36866      GTCATTAAATTC-TCAAATCACTTTGGTTTCTTTCCAATTGTGATGTTTG 429
28AS                                GTCATTAAATTC-TCAAATCACTTTGGTTTCTTTCCAATTGTGATGTTTG 428
31MK                                GTCATTAAATTC-TCAAATCACTTTGGTTTCTTTCCAATTGTGATGTTTG 429
50KK                                GTCATTAAATTC-TCAAATCACTTTGGTTTCTTTCCAATTGTGATGTTTG 429
20MK                                GTCATTAAATTC-TCAAATCACTTTGGTTTCTTTCCAATTGTGATGTTTG 427
52AS                                GGCACAAGGCCCATTAAATGACATTCGTAGACATACATTTGGGGTGTGTA 423
92JK                                GGCACAAGGCCCATTAAATGACATTCGTAGACATACATTTGGGGTGTGTA 423
5KK                                 GGCACAAGGCCCATTAAATGACATTCGTAGACATACATTTGGGGTGTGTA 423
30JK                                GGCACAAGGCCCATTAAATGACATTCGTAGACATACATTTGGGGTGTGTA 423
7KK                                 GGCACAAGGCCCATTAAATGACATTCGTAGACATACATTTGGGGTGTGTA 423
71MK                                GGCCCAAGGCC-ATTAAATGACCTTTCTAAAACTTCCTTTGGGGGGGGTA 421
39AS                                GGCACAAGGCCCATTAAATCACTTTCGTAAACATACATTTGGGGTGTGTA 433
                                    * *   *      * **** ** **  *     * *  *** *  *  *
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Pleurotus_ostreatus_JF758887_       GATTGTTGGGGGCTGCTGGCCTTGACAGGTCGGCTCCTCTTAAATGCATT 479
Pleurotus_sp.YL005_FJ687276_        GATTGTTGGGGGCTGCTGGCCTTGACAGGTCGGCTCCTCTTAAATGCATT 479
72JK                                GATTGTTGGGGGCTGCTGGCCTTGACAGGTCGGCTCCTCTTAAATGCATT 479
Pleurotus_eryngii_EU233964_         GATTGTTGGGGGCTGCTGGCCTTGACAGGTCGGCTCCTCTTAAATGCATT 479
Pleurotus_sp.'Florida'_AY36866      GATTGTTGGGGGCTGCTGGCCTTGACAGGTCGGCTCCTCTTAAATGCATT 479
28AS                                GATTGTTGGGGGCTGCTGGCCTTGACAGGTCGGCTCCTCTTAAATGCATT 478
31MK                                GATTGTTGGGGGCTGCTGGCCTTGACAGGTCGGCTCCTCTTAAATGCATT 479
50KK                                GATTGTTGGGGGCTGCTGGCCTTGACAGGTCGGCTCCTCTTAAATGCATT 479
20MK                                GATTGTTGGGGGCTGCTGGCCTTGACAGGTCGGCTCCTCTTAAATGCATT 477
52AS                                AGTAAATAGA--CTGCG----TAGTCACACCGAGACGTTTAAATCCCAGC 467
92JK                                AGTAAATAGA--CTGCG----TAGTCACACCGAGACGTTTAAATCCCAGC 467
5KK                                 AGTAAATAGA--CTGCG----TAGTCACACCGAGACGTTTAAATCCCAGC 467
30JK                                AGTAAATAGA--CTGCG----TAGTCACACCGAGACGTTTAAATCCCAGC 467
7KK                                 AGTAAATAGA--CTGCG----TAGTCACACCGAGACGTTTAAATCCCAGC 467
71MK                                AGTAAATAGA--CTGCG----TAGTCACACCGAGACGTTTAA--TCCAGC 463
39AS                                AGTAAATAGA--CTGCGG---TAGTCACACCGAGACGTTTAATTCCCACC 478
                                      *   * *   ****     * * **   **   * * * *    **
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Pleurotus_ostreatus_JF758887_       AGC--AG---GACTTCTCATTGCCTCTGCGCATGATGTGATAATTATCAC 524
Pleurotus_sp.YL005_FJ687276_        AGC--AG---GACTTCTCATTGCCTCTGCGCATGATGTGATAATTATCAC 524
72JK                                AGC--AG---GACTTCTCATTGCCTCTGCGCATGATGTGATAATTATCAC 524
Pleurotus_eryngii_EU233964_         AGC--AG---GACTTCTCATTGCCTCTGCGCATGATGTGATAATTATCAC 524
Pleurotus_sp.'Florida'_AY36866      AGC--AG---GACTTCTCATTGCCTCTGCGCATGATGTGATAATTATCAC 524
28AS                                AGC--AG---GACTTCTCATTGCCTCTGCGCATGATGTGATAATTATCAC 523
31MK                                AGC--AG---GACTTCTCATTGCCTCTGCGCATGATGTGATAATTATCAC 524
50KK                                AGC--AG---GACTTCTCATTGCCTCTGCGCATGATGTGATAATTATCAC 524
20MK                                AAC--AG---GACTTCTCATTGCCTCTGCGCATGATGTGATAATTATCAC 522
52AS                                AACCAAGTCTGACGACTTGACGACTTCACAGATC-TATCAAAAGT-TCAC 515
92JK                                AACCAAGTCTGACGACTTGACGACTTCACAGATC-TATCAAAAGT-TCAC 515
5KK                                 AACCAAGTCTGACGACTTGACGACTTCACAGATC-TATCAAAAGT-TCAC 515
30JK                                AACCAAGTCTGACGACTTGACGACTTCACAGATC-TATCAAAAGT-TCAC 515
7KK                                 AACCAAGTCTGACGACTTGACGACTTCACAGATC-TATCAAAAGT-TCAC 515
71MK                                AAC-AAATCTGAACACTTGA-GACTTCACAGATC-TATCAAAAGT-TCAC 509
39AS                                AACCAAGTCTGACCACTTGACGACTTCACAGATC-TATCAAAAGT-TCAC 526
                                    * *  *    **   **    * **   *  **  * * * ** * ****
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Pleurotus_ostreatus_JF758887_       ---TCATCAATAGCACGCATGAATAGAGTCCAGCTCTCTAATCGTCCGCA 571
Pleurotus_sp.YL005_FJ687276_        ---TCATCAATAGCACGCATGAATAGAGTCCAGCTCTCTAATCGTCCGCA 571
72JK                                ---TCATCAATAGCACGCATGAATAGAGTCCAGCTCTCTAATCGTCCGCA 571
Pleurotus_eryngii_EU233964_         ---TCATCAATAGCACGCATGAATAGAGTCCAGCTCTCTAATCGTCCGCA 571
Pleurotus_sp.'Florida'_AY36866      ---TCATCAATAGCACGCATGAATAGAGTCCAGCTCTCTAATCGTCCGCA 571
28AS                                ---TCATCAATAGCACGCATGAATAGAGTCCAGCTCTCTAATCGTCCGCA 570
31MK                                ---TCATCAATAGCACGCATGAATAGAGTCCAGCTCTCTAATCGTCCGCA 571
50KK                                ---TCATCAATAGCACGCATGAATAGAGTCCAGCTCTCTAATCGTCCGCA 571
20MK                                ---TCATCAATAGCACGCATGAATAGAGTCCAGCTCTCTAATCGTCCGCA 569
52AS                                AGGTGGTTGAAAG-ACTAGTGAAGCGTGCACATGCCCCTAGAGGCCAGCA 564
92JK                                AGGTGGTTGAAAG-ACTAGTGAAGCGTGCACATGCCCCTAGAGGCCAGCA 564
5KK                                 AGGTGGTTGAAAG-ACTAGTGAAGCGTGCACATGCCCCTAGAGGCCAGCA 564
30JK                                AGGTGGTTGAAAG-ACTAGTGAAGCGTGCACATGCCCCTAGAGGCCAGCA 564
7KK                                 AGGTGGTTGAAAG-ACTAGTGAAGCGTGCACATGCCCCTAGAGGCCAGCA 564
71MK                                AGGTGGTTGAAAG-ACTAGTGAAGCGTGCACATGCCCCTAGAGGCCAGCA 558
39AS                                AGGTGGTTGAAAG-ACTAGTGAAGCGTGCACATGCCCCTAGAGGCCAGCA 575
                                       *  *  * ** **   ****  * *  **   * ***   * * ***
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Pleurotus_ostreatus_JF758887_       AGGACAATTTGACAATTTGACCTCA-----AATC 600
Pleurotus_sp.YL005_FJ687276_        AGGACAATTTGACAATT-GACCTCA-----AATC 599
72JK                                AGGACAATTTGACAATTTGACCTCA-----AATC 600
Pleurotus_eryngii_EU233964_         AGGACAATTTGACAATTTGACCTCA-----AATC 600
Pleurotus_sp.'Florida'_AY36866      AGGACAATTTGACAATTTGACCTCA-----AATC 600
28AS                                AGGACAATTTGACAATTTGACCTCA-----AATC 599
31MK                                AGGACAATTTGACAATTTGACCTCA-----AATC 600
50KK                                AGGACAATTTGACAATTTGACCTCA-----AATC 600
20MK                                AGGACAATTTGACAATTTGACCTCA-----AATC 598
52AS                                A---CAACTCCATAGT--GAATTCATTAATGATC 593
92JK                                A---CAACTCCATAGT--GAATTCATTAATGATC 593
5KK                                 A---CAACTCCATAGT--GAATTCATTAATGATC 593
30JK                                A---CAACTCCATAGT--GAATTCATTAATGATC 593
7KK                                 A---CAACTCCATAGT--GAATTCATTAATGATC 593
71MK                                A---CA-CTCCATAGT--GAATTCATTAATGATC 586
39AS                                A---CAACTCCATAGT--GAATTCATTAATGATC 604
                                    *   **  *  * * *  **  ***      ***
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Appendix 2: Blast search results for 12 *Pleurotus* species

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72JK>  gb|JF758887.1|  Pleurotus ostreatus strain pl.n0037 18S ribosomal
RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal
RNA gene, and internal transcribed spacer 2, complete sequence;
and 28S ribosomal RNA gene, partial sequence Length=659

 Score = 1110 bits (601),  Expect = 0.0  Identities = 601/601 (100%), Gaps =
0/601 (0%)  Strand=Plus/Plus

Query  1    TGAATTCACTATGGAGTTGTTGCTGGCCTCTAGGGGCATGTGCACGCTTCACTAGTCTTT  60
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  34   TGAATTCACTATGGAGTTGTTGCTGGCCTCTAGGGGCATGTGCACGCTTCACTAGTCTTT  93

Query  61   CAACCACCTGTGAACTTTTGATAGATCTGTGAAGTCGTCTCTCAAGTCGTCAGACTTGGT  120
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  94   CAACCACCTGTGAACTTTTGATAGATCTGTGAAGTCGTCTCTCAAGTCGTCAGACTTGGT  153

Query  121  TGCTGGGATTTAAACGTCTCGGTGTGACTACGCAGTCTATTTACTTACACACCCCAAATG  180
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  154  TGCTGGGATTTAAACGTCTCGGTGTGACTACGCAGTCTATTTACTTACACACCCCAAATG  213

Query  181  TATGTCTACGAATGTCATTTAATGGGCCTTGTGCCTTTAAACCATAATACAACTTTCAAC  240
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  214  TATGTCTACGAATGTCATTTAATGGGCCTTGTGCCTTTAAACCATAATACAACTTTCAAC  273

Query  241  AACGGATCTCTTGGCTCTCGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGA  300
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  274  AACGGATCTCTTGGCTCTCGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGA  333

Query  301  ATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACCTTGCGCCCCTTGGTATTCCG  360
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  334  ATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACCTTGCGCCCCTTGGTATTCCG  393

Query  361  AGGGGCATGCCTGTTTGAGTGTCATTAAATTCTCAAACTCACTTTGGTTTCTTTCCAATT  420
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  394  AGGGGCATGCCTGTTTGAGTGTCATTAAATTCTCAAACTCACTTTGGTTTCTTTCCAATT  453

Query  421  GTGATGTTTGGATTGTTGGGGGCTGCTGGCCTTGACAGGTCGGCTCCTCTTAAATGCATT  480
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  454  GTGATGTTTGGATTGTTGGGGGCTGCTGGCCTTGACAGGTCGGCTCCTCTTAAATGCATT  513

Query  481  AGCAGGACTTCTCATTGCCTCTGCGCATGATGTGATAATTATCACTCATCAATAGCACGC  540
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  514  AGCAGGACTTCTCATTGCCTCTGCGCATGATGTGATAATTATCACTCATCAATAGCACGC  573

Query  541  ATGAATAGAGTCCAGCTCTCTAATCGTCCGCAAGGACAATTTGACAATTTGACCTCAAAT  600
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  574  ATGAATAGAGTCCAGCTCTCTAATCGTCCGCAAGGACAATTTGACAATTTGACCTCAAAT  633

Query  601  C  601
            |
Sbjct  634  C  634
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52AS>  gb|AY368662.1|  Pleurotus sp. 'Florida' strain ASI 2181 18S ribosomal
RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal
RNA gene and internal transcribed spacer 2, complete
sequence; and large subunit ribosomal RNA gene, partial sequence
Length=638

 Score = 1110 bits (601),  Expect = 0.0  Identities = 601/601 (100%), Gaps =
0/601 (0%) Strand=Plus/Minus

Query  1    TCAAATTGTCAAATTGTCCTTGCGGACGATTAGAGAGCTGGACTCTATTCATGCGTGCTA  60
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  604  TCAAATTGTCAAATTGTCCTTGCGGACGATTAGAGAGCTGGACTCTATTCATGCGTGCTA  545

Query  61   TTGATGAGTGATAATTATCACATCATGCGCAGAGGCAATGAGAAGTCCTGCTAATGCATT  120
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  544  TTGATGAGTGATAATTATCACATCATGCGCAGAGGCAATGAGAAGTCCTGCTAATGCATT  485

Query  121  TAAGAGGAGCCGACCTGTCAAGGCCAGCAGCCCCCAACAATCCAAACATCACAATTGGAA  180
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  484  TAAGAGGAGCCGACCTGTCAAGGCCAGCAGCCCCCAACAATCCAAACATCACAATTGGAA  425

Query  181  AGAAACCAAAGTGAGTTTGAGAATTTAATGACACTCAAACAGGCATGCCCCTCGGAATAC  240
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  424  AGAAACCAAAGTGAGTTTGAGAATTTAATGACACTCAAACAGGCATGCCCCTCGGAATAC  365

Query  241  CAAGGGGCGCAAGGTGCGTTCAAAGATTCGATGATTCACTGAATTCTGCAATTCACATTA  300
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  364  CAAGGGGCGCAAGGTGCGTTCAAAGATTCGATGATTCACTGAATTCTGCAATTCACATTA  305

Query  301  CTTATCGCATTTCGCTGCGTTCTTCATCGATGCGAGAGCCAAGAGATCCGTTGTTGAAAG  360
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  304  CTTATCGCATTTCGCTGCGTTCTTCATCGATGCGAGAGCCAAGAGATCCGTTGTTGAAAG  245

Query  361  TTGTATTATGGTTTATAGGCACAAGGCCCATTAAATGACATTCGTAGACATACATTTGGG  420
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  244  TTGTATTATGGTTTATAGGCACAAGGCCCATTAAATGACATTCGTAGACATACATTTGGG  185

Query  421  GTGTGTAAGTAAATAGACTGCGTAGTCACACCGAGACGTTTAAATCCCAGCAACCAAGTC  480
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  184  GTGTGTAAGTAAATAGACTGCGTAGTCACACCGAGACGTTTAAATCCCAGCAACCAAGTC  125

Query  481  TGACGACTTGAGAGACGACTTCACAGATCTATCAAAAGTTCACAGGTGGTTGAAAGACTA  540
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  124  TGACGACTTGAGAGACGACTTCACAGATCTATCAAAAGTTCACAGGTGGTTGAAAGACTA  65

Query  541  GTGAAGCGTGCACATGCCCCTAGAGGCCAGCAACAACTCCATAGTGAATTCATTAATGAT  600
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  64   GTGAAGCGTGCACATGCCCCTAGAGGCCAGCAACAACTCCATAGTGAATTCATTAATGAT  5

Query  601  C  601
            |
Sbjct  4    C  4
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31MK>  gb|EU233964.1|  Pleurotus eryngii isolate D2308.1 18S ribosomal
RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal
RNA gene, and internal transcribed spacer 2, complete sequence;
and large subunit ribosomal RNA gene, partial sequence Length=607

 Score = 1099 bits (595),  Expect = 0.0  Identities = 599/601 (99%),
Gaps = 0/601 (0%)  Strand=Plus/Plus

Query  1    TGAATTCACTATGGAGTTGTTGCTGGCCTCTAGGGGCATGTGCACGCTTCACTAGTCTTT  60
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  7    TGAATTCACTATGGAGTTGTTGCTGGCCTCTAGGGGCATGTGCACGCTTCACTAGTCTTT  66

Query  61   CAACCACCTGTGAACTTTTGATAGATCTGTGAAGTCGTCTCTCAAGTCGTCAGACTTGGT  120
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  67   CAACCACCTGTGAACTTTTGATAGATCTGTGAAGTCGTCTCTCAAGTCGTCAGACTTGGT  126

Query  121  TGCTGGGATATAAACGTCTCGGTGTGACTACGCAGTCTATTTACTTACACACCCCAAATG  180
            ||||||||| ||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  127  TGCTGGGATTTAAACGTCTCGGTGTGACTACGCAGTCTATTTACTTACACACCCCAAATG  186

Query  181  TATGTCTACGAATGTCATTTAATGGGCCTTGTGCCTATAAACCATAATACAACTTTCAAC  240
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  187  TATGTCTACGAATGTCATTTAATGGGCCTTGTGCCTATAAACCATAATACAACTTTCAAC  246

Query  241  AACGGATCTCTTGGCTCTCGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGA  300
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  247  AACGGATCTCTTGGCTCTCGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGA  306

Query  301  ATCGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACCTTGCGCCCCTTGGTATTCCG  360
            || ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  307  ATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACCTTGCGCCCCTTGGTATTCCG  366

Query  361  AGGGGCATGCCTGTTTGAGTGTCATTAAATTCTCAAACTCACTTTGGTTTCTTTCCAATT  420
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  367  AGGGGCATGCCTGTTTGAGTGTCATTAAATTCTCAAACTCACTTTGGTTTCTTTCCAATT  426

Query  421  GTGATGTTTGGATTGTTGGGGGCTGCTGGCCTTGACAGGTCGGCTCCTCTTAAATGCATT  480
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  427  GTGATGTTTGGATTGTTGGGGGCTGCTGGCCTTGACAGGTCGGCTCCTCTTAAATGCATT  486

Query  481  AGCAGGACTTCTCATTGCCTCTGCGCATGATGTGATAATTATCACTCATCAATAGCACGC  540
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  487  AGCAGGACTTCTCATTGCCTCTGCGCATGATGTGATAATTATCACTCATCAATAGCACGC  546

Query  541  ATGAATAGAGTCCAGCTCTCTAATCGTCCGCAAGGACAATTTGACAATTTGACCTCAAAT  600
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  547  ATGAATAGAGTCCAGCTCTCTAATCGTCCGCAAGGACAATTTGACAATTTGACCTCAAAT  606

Query  601  C  601
            |
Sbjct  607  C  607
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28AS>  gb|EU233964.1|  Pleurotus eryngii isolate D2308.1 18S ribosomal RNA
gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal
RNA gene, and internal transcribed spacer 2, complete sequence;
and large subunit ribosomal RNA gene, partial sequence Length=607

 Score = 1099 bits (595),  Expect = 0.0  Identities = 598/599 (99%), Gaps =
1/599 (0%)  Strand=Plus/Plus

Query  3    AATTC-CTATGGAGTTGTTGCTGGCCTCTAGGGGCATGTGCACGCTTCACTAGTCTTTCA  61
            ||||| ||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  9    AATTCACTATGGAGTTGTTGCTGGCCTCTAGGGGCATGTGCACGCTTCACTAGTCTTTCA  68

Query  62   ACCACCTGTGAACTTTTGATAGATCTGTGAAGTCGTCTCTCAAGTCGTCAGACTTGGTTG  121
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  69   ACCACCTGTGAACTTTTGATAGATCTGTGAAGTCGTCTCTCAAGTCGTCAGACTTGGTTG  128

Query  122  CTGGGATTTAAACGTCTCGGTGTGACTACGCAGTCTATTTACTTACACACCCCAAATGTA  181
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  129  CTGGGATTTAAACGTCTCGGTGTGACTACGCAGTCTATTTACTTACACACCCCAAATGTA  188

Query  182  TGTCTACGAATGTCATTTAATGGGCCTTGTGCCTATAAACCATAATACAACTTTCAACAA  241
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  189  TGTCTACGAATGTCATTTAATGGGCCTTGTGCCTATAAACCATAATACAACTTTCAACAA  248

Query  242  CGGATCTCTTGGCTCTCGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAAT  301
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  249  CGGATCTCTTGGCTCTCGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAAT  308

Query  302  TGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACCTTGCGCCCCTTGGTATTCCGAG  361
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  309  TGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACCTTGCGCCCCTTGGTATTCCGAG  368

Query  362  GGGCATGCCTGTTTGAGTGTCATTAAATTCTCAAACTCACTTTGGTTTCTTTCCAATTGT  421
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  369  GGGCATGCCTGTTTGAGTGTCATTAAATTCTCAAACTCACTTTGGTTTCTTTCCAATTGT  428

Query  422  GATGTTTGGATTGTTGGGGGCTGCTGGCCTTGACAGGTCGGCTCCTCTTAAATGCATTAG  481
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  429  GATGTTTGGATTGTTGGGGGCTGCTGGCCTTGACAGGTCGGCTCCTCTTAAATGCATTAG  488

Query  482  CAGGACTTCTCATTGCCTCTGCGCATGATGTGATAATTATCACTCATCAATAGCACGCAT  541
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  489  CAGGACTTCTCATTGCCTCTGCGCATGATGTGATAATTATCACTCATCAATAGCACGCAT  548

Query  542  GAATAGAGTCCAGCTCTCTAATCGTCCGCAAGGACAATTTGACAATTTGACCTCAAATC  600
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  549  GAATAGAGTCCAGCTCTCTAATCGTCCGCAAGGACAATTTGACAATTTGACCTCAAATC  607
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71MK>  gb|FJ687276.1|  Pleurotus sp. YL005 18S ribosomal RNA gene, partial
sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and
internal transcribed spacer 2, complete sequence; and 28S ribosomal
RNA gene, partial sequence Length=647

 Score =  704 bits (381),  Expect = 0.0  Identities = 541/613 (88%), Gaps =
32/613 (5%) Strand=Plus/Minus

Query  1    TCAATTGTCAAATTGTCCTTGCGGACGATTAGAGAGCTGGACTCTATTCATGCGTGCTAT  60
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  635  TCAATTGTCAAATTGTCCTTGCGGACGATTAGAGAGCTGGACTCTATTCATGCGTGCTAT  576

Query  61   TGATGAGTGATAATTATCACATCATGCGCAGAGGCAATGAGAAGTCCTGCTAATGCATTT  120
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  575  TGATGAGTGATAATTATCACATCATGCGCAGAGGCAATGAGAAGTCCTGCTAATGCATTT  516

Query  121  AAGAGGAGCCGACCTGTCAAGGCCAGCAGCCCCCAACAATCCAAACATCACAATTGGaaa  180
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  515  AAGAGGAGCCGACCTGTCAAGGCCAGCAGCCCCCAACAATCCAAACATCACAATTGGAAA  456

Query  181  aaaaCCAAA-TGAATTTGAAAAATTAATGGAC-CTCCAACCAGG-ATGGCCCTCCGAATA  237
             |||||||| ||| ||||| || |||||| || ||| ||| ||| ||| ||||| |||||
Sbjct  455  GAAACCAAAGTGAGTTTGAGAATTTAATG-ACACTCAAAC-AGGCATGCCCCTCGGAATA  398

Query  238  ACCAAGGGG-GCAAGGGGGGTTCCAAAAATCCAATAAT-CAATTAATTCTGCCATTTACC  295
             |||||||| |||||| | |||| ||| || | || || || | |||||||| ||| ||
Sbjct  397  -CCAAGGGGCGCAAGGTGCGTTC-AAAGATTCGATGATTCACTGAATTCTGCAATTCACA  340

Query  296  TTAACTAATC-CAATTCCCTTCCTTCCTCCTCCATGCCAAAACCCAAAA-ATCCCTTGGT  353
            ||| || ||| || ||| || | ||| || || |||| | | || || | |||| ||| |
Sbjct  339  TTA-CTTATCGCATTTCGCTGCGTTCTTCATCGATGCGAGAGCC-AAGAGATCCGTTG-T  283

Query  354  T-AAAGTTGGAATAATG-TTTAATAGGCCCAAGGCC-ATTAAATGACCTTTC-TAAAACT  409
            | ||||||| | || || ||||| |||| ||||||| |||||||||| || | || | |
Sbjct  282  TGAAAGTTGTATTA-TGGTTTAA-AGGCACAAGGCCCATTAAATGACATT-CGTAGA-CA  227

Query  410  TCC-TTTGGGGTGGGGTAAGTAAATAGACTGCGTAGTCACACCGAGACGTTTAA-TCC-A  466
            | | |||||||||| | ||||||||||||||||||||||||||||||||||| ||| |
Sbjct  226  TACATTTGGGGTGTG-TAAGTAAATAGACTGCGTAGTCACACCGAGACGTTTAAATCCCA  168

Query  467  GCAACAAA-TCTGAAC-ACTTG-GAGA-GACTTCACAGATCTATCAAAAGTTCACAGGTG  522
            ||||| || ||||| | ||||| |||| ||||||||||||||||||||||||||||||||
Sbjct  167  GCAACCAAGTCTGA-CGACTTGAGAGACGACTTCACAGATCTATCAAAAGTTCACAGGTG  109

Query  523  GTTGAAAGACTAGTGAAGCGTGCACATGCCCCTAGAGGCCAGCAACA-CTCCATAGTGAA  581
            |||||||||||||||||||||||||||||||||||||||||||||||| ||||||||||||
Sbjct  108  GTTGAAAGACTAGTGAAGCGTGCACATGCCCCTAGAGGCCAGCAACAACTCCATAGTGAA  49

Query  582  TTCATTAATGATC  594
            |||||||||||||
Sbjct  48   TTCATTAATGATC  36
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20MK>  gb|EU233964.1|  Pleurotus eryngii isolate D2308.1 18S ribosomal RNA
gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal
RNA gene, and internal transcribed spacer 2, complete sequence;
and large subunit ribosomal RNA gene, partial sequence Length=607

 Score = 1075 bits (582),  Expect = 0.0  Identities = 590/594 (99%), Gaps =
0/594 (0%) Strand=Plus/Plus

Query  6    ACTATGGAGTTGTTGCTGGCCTCTAGGGGCATGTGCACGCTTCACTAGTCTTTCAACCAC  65
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  14   ACTATGGAGTTGTTGCTGGCCTCTAGGGGCATGTGCACGCTTCACTAGTCTTTCAACCAC  73

Query  66   CTGTGAACTTTTGATAGATCTGTGAAGTCGTCTCTCAAGTCGTCAGACTTGGTTGCTGGG  125
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  74   CTGTGAACTTTTGATAGATCTGTGAAGTCGTCTCTCAAGTCGTCAGACTTGGTTGCTGGG  133

Query  126  ATTTAAACGTCTCGGTGTGACTACCCAGTCTATTTACTTACACACCCCAAATGTATGTCT  185
            |||||||||||||||||||||||| ||||||||||||||||||||||||||||||||||||
Sbjct  134  ATTTAAACGTCTCGGTGTGACTACGCAGTCTATTTACTTACACACCCCAAATGTATGTCT  193

Query  186  ACAAATGTCATTTAATGGGCCTTGTGCCTATAAACCATAATACAACTTTCAACAACGGAT  245
            || ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  194  ACGAATGTCATTTAATGGGCCTTGTGCCTATAAACCATAATACAACTTTCAACAACGGAT  253

Query  246  CTCTTGGCTCTCGCATCGATGAAAAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAG  305
            ||||||||||||||||||||||| ||||||||||||||||||||||||||||||||||||
Sbjct  254  CTCTTGGCTCTCGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAG  313

Query  306  AATTCAGTGAATCATCGAATCTTTGAACGCACCTTGCGCCCCTTGGTATTCCGAGGGGCA  365
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  314  AATTCAGTGAATCATCGAATCTTTGAACGCACCTTGCGCCCCTTGGTATTCCGAGGGGCA  373

Query  366  TGCCTGTTTGAGTGTCATTAAATTCTCAAACTCACTTTGGTTTCTTTCCAATTGTGATGT  425
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  374  TGCCTGTTTGAGTGTCATTAAATTCTCAAACTCACTTTGGTTTCTTTCCAATTGTGATGT  433

Query  426  TTGGATTGTTGGGGGCTGCTGGCCTTGACAGGTCGGCTCCTCTTAAATGCATTAACAGGA  485
            |||||||||||||||||||||||||||||||||||||||||||||||||||| |||||
Sbjct  434  TTGGATTGTTGGGGGCTGCTGGCCTTGACAGGTCGGCTCCTCTTAAATGCATTAGCAGGA  493

Query  486  CTTCTCATTGCCTCTGCGCATGATGTGATAATTATCACTCATCAATAGCACGCATGAATA  545
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  494  CTTCTCATTGCCTCTGCGCATGATGTGATAATTATCACTCATCAATAGCACGCATGAATA  553

Query  546  GAGTCCAGCTCTCTAATCGTCCGCAAGGACAATTTGACAATTTGACCTCAAATC  599
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  554  GAGTCCAGCTCTCTAATCGTCCGCAAGGACAATTTGACAATTTGACCTCAAATC  607
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50KK>  gb|EU233964.1|  Pleurotus eryngii isolate D2308.1 18S ribosomal RNA
gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal
RNA gene, and internal transcribed spacer 2, complete sequence;
and large subunit ribosomal RNA gene, partial sequence Length=607

 Score = 1105 bits (598),  Expect = 0.0  Identities = 600/601 (99%), Gaps =
0/601 (0%)  Strand=Plus/Plus

Query  1    TGAATTCACTATGGAGTTGTTGCTGGCCTCTAGGGGCATGTGCACGCTTCACTAGTCTTT  60
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  7    TGAATTCACTATGGAGTTGTTGCTGGCCTCTAGGGGCATGTGCACGCTTCACTAGTCTTT  66

Query  61   CAACCACCTGTGAACTTTTGATAGATCTGTGAAGTCGTCTCTCAAGTCGTCAGACTTGGT  120
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  67   CAACCACCTGTGAACTTTTGATAGATCTGTGAAGTCGTCTCTCAAGTCGTCAGACTTGGT  126

Query  121  TGCTGGGATATAAACGTCTCGGTGTGACTACGCAGTCTATTTACTTACACACCCCAAATG  180
            ||||||||| ||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  127  TGCTGGGATTTAAACGTCTCGGTGTGACTACGCAGTCTATTTACTTACACACCCCAAATG  186

Query  181  TATGTCTACGAATGTCATTTAATGGGCCTTGTGCCTATAAACCATAATACAACTTTCAAC  240
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  187  TATGTCTACGAATGTCATTTAATGGGCCTTGTGCCTATAAACCATAATACAACTTTCAAC  246

Query  241  AACGGATCTCTTGGCTCTCGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGA  300
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  247  AACGGATCTCTTGGCTCTCGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGA  306

Query  301  ATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACCTTGCGCCCCTTGGTATTCCG  360
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  307  ATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACCTTGCGCCCCTTGGTATTCCG  366

Query  361  AGGGGCATGCCTGTTTGAGTGTCATTAAATTCTCAAACTCACTTTGGTTTCTTTCCAATT  420
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  367  AGGGGCATGCCTGTTTGAGTGTCATTAAATTCTCAAACTCACTTTGGTTTCTTTCCAATT  426

Query  421  GTGATGTTTGGATTGTTGGGGGCTGCTGGCCTTGACAGGTCGGCTCCTCTTAAATGCATT  480
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  427  GTGATGTTTGGATTGTTGGGGGCTGCTGGCCTTGACAGGTCGGCTCCTCTTAAATGCATT  486

Query  481  AGCAGGACTTCTCATTGCCTCTGCGCATGATGTGATAATTATCACTCATCAATAGCACGC  540
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  487  AGCAGGACTTCTCATTGCCTCTGCGCATGATGTGATAATTATCACTCATCAATAGCACGC  546

Query  541  ATGAATAGAGTCCAGCTCTCTAATCGTCCGCAAGGACAATTTGACAATTTGACCTCAAAT  600
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  547  ATGAATAGAGTCCAGCTCTCTAATCGTCCGCAAGGACAATTTGACAATTTGACCTCAAAT  606

Query  601  C  601
            |
Sbjct  607  C  607
```

92JK> gb|AY368662.1| Pleurotus sp. 'Florida' strain ASI 2181 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and large subunit ribosomal RNA gene, partial sequence Length=638

Score = 1110 bits (601), Expect = 0.0 Identities = 601/601 (100%), Gaps = 0/601 (0%) Strand=Plus/Minus

```
Query  1    TCAAATTGTCAAATTGTCCTTGCGGACGATTAGAGAGCTGGACTCTATTCATGCGTGCTA  60
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  604  TCAAATTGTCAAATTGTCCTTGCGGACGATTAGAGAGCTGGACTCTATTCATGCGTGCTA  545

Query  61   TTGATGAGTGATAATTATCACATCATGCGCAGAGGCAATGAGAAGTCCTGCTAATGCATT  120
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  544  TTGATGAGTGATAATTATCACATCATGCGCAGAGGCAATGAGAAGTCCTGCTAATGCATT  485

Query  121  TAAGAGGAGCCGACCTGTCAAGGCCAGCAGCCCCCAACAATCCAAACATCACAATTGGAA  180
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  484  TAAGAGGAGCCGACCTGTCAAGGCCAGCAGCCCCCAACAATCCAAACATCACAATTGGAA  425

Query  181  AGAAACCAAAGTGAGTTTGAGAATTTAATGACACTCAAACAGGCATGCCCCTCGGAATAC  240
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  424  AGAAACCAAAGTGAGTTTGAGAATTTAATGACACTCAAACAGGCATGCCCCTCGGAATAC  365

Query  241  CAAGGGGCGCAAGGTGCGTTCAAAGATTCGATGATTCACTGAATTCTGCAATTCACATTA  300
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  364  CAAGGGGCGCAAGGTGCGTTCAAAGATTCGATGATTCACTGAATTCTGCAATTCACATTA  305

Query  301  CTTATCGCATTTCGCTGCGTTCTTCATCGATGCGAGAGCCAAGAGATCCGTTGTTGAAAG  360
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  304  CTTATCGCATTTCGCTGCGTTCTTCATCGATGCGAGAGCCAAGAGATCCGTTGTTGAAAG  245

Query  361  TTGTATTATGGTTTATAGGCACAAGGCCCATTAAATGACATTCGTAGACATACATTTGGG  420
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  244  TTGTATTATGGTTTATAGGCACAAGGCCCATTAAATGACATTCGTAGACATACATTTGGG  185

Query  421  GTGTGTAAGTAAATAGACTGCGTAGTCACACCGAGACGTTTAAATCCCAGCAACCAAGTC  480
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  184  GTGTGTAAGTAAATAGACTGCGTAGTCACACCGAGACGTTTAAATCCCAGCAACCAAGTC  125

Query  481  TGACGACTTGAGAGACGACTTCACAGATCTATCAAAAGTTCACAGGTGGTTGAAAGACTA  540
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  124  TGACGACTTGAGAGACGACTTCACAGATCTATCAAAAGTTCACAGGTGGTTGAAAGACTA  65

Query  541  GTGAAGCGTGCACATGCCCCTAGAGGCCAGCAACAACTCCATAGTGAATTCATTAATGAT  600
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  64   GTGAAGCGTGCACATGCCCCTAGAGGCCAGCAACAACTCCATAGTGAATTCATTAATGAT  5

Query  601  C  601
            |
Sbjct  4    C  4
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39AS>  gb|AY368662.1|  Pleurotus sp. 'Florida' strain ASI 2181 18S ribosomal
RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal
RNA gene and internal transcribed spacer 2, complete sequence; and large
subunit ribosomal RNA gene, partial sequence Length=638

 Score =  813 bits (440),  Expect = 0.0  Identities = 566/623 (91%), Gaps =
23/623 (4%) Strand=Plus/Minus

Query  1    TCAAATTGTCAAATTGTCCTTGCGGACGATTAGAGAGCTGGACTCTATTCATGCGTGCTA  60
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  604  TCAAATTGTCAAATTGTCCTTGCGGACGATTAGAGAGCTGGACTCTATTCATGCGTGCTA  545

Query  61   TTGATGAGTGATAATTAACACATCATGCGCAAAGGCAATGAGAAGTCCTGCTAATGCATT  120
            |||||||||||||||| |||||||||||| ||||||||||||||||||||||||||||||
Sbjct  544  TTGATGAGTGATAATTATCACATCATGCGCAGAGGCAATGAGAAGTCCTGCTAATGCATT  485

Query  121  TAAGAGGAGCCGACCTGTCAAGGCCCAGCAGCCCCCAACCAATCCAAACATCAAAATTGG  180
            |||||||||||||||||||||| ||||||||||||| ||||||||||||| ||||||
Sbjct  484  TAAGAGGAGCCGACCTGTCAAGG-CCAGCAGCCCCCAA-CAATCCAAACATCACAATTGG  427

Query  181  aaaaaaaCCAAAGGGAGTGTTAGAAAATTTAA-GCACCCTCAAACAGGCATGCCCCCCGA  239
            ||| ||||||||| |||| || || ||||||| | || ||||||||||||||||||| ||
Sbjct  426  AAAGAAACCAAAGTGAGT-TT-GAGAATTTAATG-ACACTCAAACAGGCATGCCCCTCG-  371

Query  240  GAATCACCAAGGGGCGCAAGGGGGGTTCAAAAGATTCAAGTAATTCAGAGATTTGCAGCA  299
            |||| |||||||||||||||| | |||| |||||||| | | |||||  || || | |||
Sbjct  370  GAAT-ACCAAGGGGCGCAAGGTGCGTTC-AAAGATTCGA-TGATTCACTGAATT-CTGCA  315

Query  300  ATTCACATTAACTAATCGCATTCCCCTCCTTTCTTCATCAATGCAAGAGACACAAGATAT  359
            ||||||||| ||| |||||||| | || | ||||||||| |||| |||| | ||||| ||
Sbjct  314  ATTCACATT-ACTTATCGCATTTCGCTGCGTTCTTCATCGATGCGAGAG-C-CAAGAGAT  258

Query  360  CCGTTGGAAGAAAGTGGTAATTATGGTTAATAGGCACAAGGCCCATTAAAGATCACTTTC  419
            ||||| |  |||||| || ||||||||| |||||||||||||||||| || || || |||
Sbjct  257  CCGTT-GTTGAAAGTTGT-ATTATGGTTTATAGGCACAAGGCCCATT-AA-ATGACATTC  202

Query  420  GTAAACACTTACATTTGGGGGGTGTGTAAGTAAATAGACTGCGGTAGTCACACCGAGACG  479
            ||| |||  |||||||  |||||||||||||||||||||| ||||||||||||||||||
Sbjct  201  GTAGACA--TACATTT--GGGGTGTGTAAGTAAATAGACTGC-GTAGTCACACCGAGACG  147

Query  480  TTTAATTCCCACCAACCAAGTCTGACCACTTGAGAGACGACTTCACAGATCTATCAAAAG  539
            ||||| ||||| ||||||||||||||| |||||||||||||||||||||||||||||||
Sbjct  146  TTTAAATCCCAGCAACCAAGTCTGACGACTTGAGAGACGACTTCACAGATCTATCAAAAG  87

Query  540  TTCACAGGTGGTTGAAAGACTAGTGAAGCGTGCACATGCCCCTAGAGGCCAGCAACAACT  599
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  86   TTCACAGGTGGTTGAAAGACTAGTGAAGCGTGCACATGCCCCTAGAGGCCAGCAACAACT  27

Query  600  CCATAGTGAATTCATTAATGATC  622
            |||||||||||||||||||||||
Sbjct  26   CCATAGTGAATTCATTAATGATC  4
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5KK>  gb|AY368662.1|  Pleurotus sp. 'Florida' strain ASI 2181 18S ribosomal
RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal
RNA gene and internal transcribed spacer 2, complete
sequence; and large subunit ribosomal RNA gene, partial sequence
Length=638

 Score = 1110 bits (601),  Expect = 0.0  Identities = 601/601 (100%), Gaps =
0/601 (0%)  Strand=Plus/Minus

Query  1    TCAAATTGTCAAATTGTCCTTGCGGACGATTAGAGAGCTGGACTCTATTCATGCGTGCTA  60
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  604  TCAAATTGTCAAATTGTCCTTGCGGACGATTAGAGAGCTGGACTCTATTCATGCGTGCTA  545

Query  61   TTGATGAGTGATAATTATCACATCATGCGCAGAGGCAATGAGAAGTCCTGCTAATGCATT  120
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  544  TTGATGAGTGATAATTATCACATCATGCGCAGAGGCAATGAGAAGTCCTGCTAATGCATT  485

Query  121  TAAGAGGAGCCGACCTGTCAAGGCCAGCAGCCCCCAACAATCCAAACATCACAATTGGAA  180
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  484  TAAGAGGAGCCGACCTGTCAAGGCCAGCAGCCCCCAACAATCCAAACATCACAATTGGAA  425

Query  181  AGAAACCAAAGTGAGTTTGAGAATTTAATGACACTCAAACAGGCATGCCCCTCGGAATAC  240
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  424  AGAAACCAAAGTGAGTTTGAGAATTTAATGACACTCAAACAGGCATGCCCCTCGGAATAC  365

Query  241  CAAGGGGCGCAAGGTGCGTTCAAAGATTCGATGATTCACTGAATTCTGCAATTCACATTA  300
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  364  CAAGGGGCGCAAGGTGCGTTCAAAGATTCGATGATTCACTGAATTCTGCAATTCACATTA  305

Query  301  CTTATCGCATTTCGCTGCGTTCTTCATCGATGCGAGAGCCAAGAGATCCGTTGTTGAAAG  360
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  304  CTTATCGCATTTCGCTGCGTTCTTCATCGATGCGAGAGCCAAGAGATCCGTTGTTGAAAG  245

Query  361  TTGTATTATGGTTTATAGGCACAAGGCCCATTAAATGACATTCGTAGACATACATTTGGG  420
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  244  TTGTATTATGGTTTATAGGCACAAGGCCCATTAAATGACATTCGTAGACATACATTTGGG  185

Query  421  GTGTGTAAGTAAATAGACTGCGTAGTCACACCGAGACGTTTAAATCCCAGCAACCAAGTC  480
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  184  GTGTGTAAGTAAATAGACTGCGTAGTCACACCGAGACGTTTAAATCCCAGCAACCAAGTC  125

Query  481  TGACGACTTGAGAGACGACTTCACAGATCTATCAAAAGTTCACAGGTGGTTGAAAGACTA  540
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  124  TGACGACTTGAGAGACGACTTCACAGATCTATCAAAAGTTCACAGGTGGTTGAAAGACTA  65

Query  541  GTGAAGCGTGCACATGCCCCTAGAGGCCAGCAACAACTCCATAGTGAATTCATTAATGAT  600
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  64   GTGAAGCGTGCACATGCCCCTAGAGGCCAGCAACAACTCCATAGTGAATTCATTAATGAT  5

Query  601  C  601
            |
Sbjct  4    C  4
```

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30JK>  gb|AY368662.1|  Pleurotus sp. 'Florida' strain ASI 2181 18S ribosomal
RNA gene,partial sequence; internal transcribed spacer 1, 5.8S ribosomal
RNA gene and internal transcribed spacer 2, complete
sequence; and large subunit ribosomal RNA gene, partial sequence
Length=638

 Score = 1110 bits (601),  Expect = 0.0  Identities = 601/601 (100%), Gaps =
0/601 (0%) Strand=Plus/Minus

Query  1    TCAAATTGTCAAATTGTCCTTGCGGACGATTAGAGAGCTGGACTCTATTCATGCGTGCTA  60
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  604  TCAAATTGTCAAATTGTCCTTGCGGACGATTAGAGAGCTGGACTCTATTCATGCGTGCTA  545

Query  61   TTGATGAGTGATAATTATCACATCATGCGCAGAGGCAATGAGAAGTCCTGCTAATGCATT  120
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  544  TTGATGAGTGATAATTATCACATCATGCGCAGAGGCAATGAGAAGTCCTGCTAATGCATT  485

Query  121  TAAGAGGAGCCGACCTGTCAAGGCCAGCAGCCCCCAACAATCCAAACATCACAATTGGAA  180
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  484  TAAGAGGAGCCGACCTGTCAAGGCCAGCAGCCCCCAACAATCCAAACATCACAATTGGAA  425

Query  181  AGAAACCAAAGTGAGTTTGAGAATTTAATGACACTCAAACAGGCATGCCCCTCGGAATAC  240
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  424  AGAAACCAAAGTGAGTTTGAGAATTTAATGACACTCAAACAGGCATGCCCCTCGGAATAC  365

Query  241  CAAGGGGCGCAAGGTGCGTTCAAAGATTCGATGATTCACTGAATTCTGCAATTCACATTA  300
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  364  CAAGGGGCGCAAGGTGCGTTCAAAGATTCGATGATTCACTGAATTCTGCAATTCACATTA  305

Query  301  CTTATCGCATTTCGCTGCGTTCTTCATCGATGCGAGAGCCAAGAGATCCGTTGTTGAAAG  360
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  304  CTTATCGCATTTCGCTGCGTTCTTCATCGATGCGAGAGCCAAGAGATCCGTTGTTGAAAG  245

Query  361  TTGTATTATGGTTTATAGGCACAAGGCCCATTAAATGACATTCGTAGACATACATTTGGG  420
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  244  TTGTATTATGGTTTATAGGCACAAGGCCCATTAAATGACATTCGTAGACATACATTTGGG  185

Query  421  GTGTGTAAGTAAATAGACTGCGTAGTCACACCGAGACGTTTAAATCCCAGCAACCAAGTC  480
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  184  GTGTGTAAGTAAATAGACTGCGTAGTCACACCGAGACGTTTAAATCCCAGCAACCAAGTC  125

Query  481  TGACGACTTGAGAGACGACTTCACAGATCTATCAAAAGTTCACAGGTGGTTGAAAGACTA  540
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  124  TGACGACTTGAGAGACGACTTCACAGATCTATCAAAAGTTCACAGGTGGTTGAAAGACTA  65

Query  541  GTGAAGCGTGCACATGCCCCTAGAGGCCAGCAACAACTCCATAGTGAATTCATTAATGAT  600
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  64   GTGAAGCGTGCACATGCCCCTAGAGGCCAGCAACAACTCCATAGTGAATTCATTAATGAT  5

Query  601  C  601
            |
Sbjct  4    C  4
```

7KK> gb|JF758887.1| Pleurotus ostreatus strain pl.n0037 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence Length=659

Score = 1110 bits (601), Expect = 0.0 Identities = 601/601 (100%), Gaps = 0/601 (0%) Strand=Plus/Minus

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Query  1    TCAAATTGTCAAATTGTCCTTGCGGACGATTAGAGAGCTGGACTCTATTCATGCGTGCTA  60
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  625  TCAAATTGTCAAATTGTCCTTGCGGACGATTAGAGAGCTGGACTCTATTCATGCGTGCTA  566

Query  61   TTGATGAGTGATAATTATCACATCATGCGCAGAGGCAATGAGAAGTCCTGCTAATGCATT  120
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  565  TTGATGAGTGATAATTATCACATCATGCGCAGAGGCAATGAGAAGTCCTGCTAATGCATT  506

Query  121  TAAGAGGAGCCGACCTGTCAAGGCCAGCAGCCCCCAACAATCCAAACATCACAATTGGAA  180
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  505  TAAGAGGAGCCGACCTGTCAAGGCCAGCAGCCCCCAACAATCCAAACATCACAATTGGAA  446

Query  181  AGAAACCAAAGTGAGTTTGAGAATTTAATGACACTCAAACAGGCATGCCCCTCGGAATAC  240
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  445  AGAAACCAAAGTGAGTTTGAGAATTTAATGACACTCAAACAGGCATGCCCCTCGGAATAC  386

Query  241  CAAGGGGCGCAAGGTGCGTTCAAAGATTCGATGATTCACTGAATTCTGCAATTCACATTA  300
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  385  CAAGGGGCGCAAGGTGCGTTCAAAGATTCGATGATTCACTGAATTCTGCAATTCACATTA  326

Query  301  CTTATCGCATTTCGCTGCGTTCTTCATCGATGCGAGAGCCAAGAGATCCGTTGTTGAAAG  360
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  325  CTTATCGCATTTCGCTGCGTTCTTCATCGATGCGAGAGCCAAGAGATCCGTTGTTGAAAG  266

Query  361  TTGTATTATGGTTTAAAGGCACAAGGCCCATTAAATGACATTCGTAGACATACATTTGGG  420
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  265  TTGTATTATGGTTTAAAGGCACAAGGCCCATTAAATGACATTCGTAGACATACATTTGGG  206

Query  421  GTGTGTAAGTAAATAGACTGCGTAGTCACACCGAGACGTTTAAATCCCAGCAACCAAGTC  480
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  205  GTGTGTAAGTAAATAGACTGCGTAGTCACACCGAGACGTTTAAATCCCAGCAACCAAGTC  146

Query  481  TGACGACTTGAGAGACGACTTCACAGATCTATCAAAAGTTCACAGGTGGTTGAAAGACTA  540
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  145  TGACGACTTGAGAGACGACTTCACAGATCTATCAAAAGTTCACAGGTGGTTGAAAGACTA  86

Query  541  GTGAAGCGTGCACATGCCCCTAGAGGCCAGCAACAACTCCATAGTGAATTCATTAATGAT  600
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  85   GTGAAGCGTGCACATGCCCCTAGAGGCCAGCAACAACTCCATAGTGAATTCATTAATGAT  26

Query  601  C  601
            |
Sbjct  25   C  25
```