The Pennsylvania State University

The Graduate School

MOLECULAR PHYLOGENETIC ANALYSIS OF *GRIFOLA FRONDOSA* (MAITAKE) AND RELATED SPECIES AND THE INFLUENCE OF SELECTED NUTRIENT SUPPLEMENTS ON MUSHROOM YIELD

A Thesis in

Plant Pathology

by

Qing Shen

Submitted in Partial Fulfillment
of the Requirements
for the Degree of

Doctor of Philosophy

May 2001

We approve the thesis of Qing Shen.

Date of Signature

_____ _____
Daniel J. Royse
Professor of Plant Pathology
Thesis Advisor
Chair of Committee

_____ _____
John E. Ayers
Professor of Plant Pathology

_____ _____
C. Peter Romaine
Professor of Plant Pathology

_____ _____
David M. Geiser
Assistant Professor of Plant Pathology

_____ _____
David R. Huff
Associate Professor of Turfgrass Breeding

_____ _____
Elwin L. Stewart
Professor of Plant Pathology
Head of the Department of Plant Pathology

Abstract

Grifola frondosa (Dickson: Fr.) S.F.Gray (maitake) is a choice edible mushroom and has been marketed in Asia in recent years for medicinal use. Production and consumption of this mushroom is increasing rapidly in the world. There was a 41-fold increase from 1988-97 worldwide and a 38% increase in the United States (1999-2000).

While this mushroom has enjoyed increased popularity among consumers, scientists know little about the genetics and life history of this species. I, therefore, sought to quantify genetic diversity among available isolates and to establish a taxonomic position for this mushroom and some of its allies. To accomplish these goals, the internal transcribed spacers 1 and 2 (ITS-1 and 2) and 5.8S regions of the nuclear ribosomal DNA (rDNA) transcriptional unit and a portion of the β-tubulin gene were PCR-amplified. Then, rDNA and β-tubulin amplicons from 52 isolates of *Grifola frondosa*, one isolate of *Grifola sordulenta*, *Polyporus umbellatus* and *Meripilus giganteus* were sequenced. In both rDNA and β-tubulin sequences, nucleotide variation within isolates of *G. frondosa* (5.4% in rDNA and 12.2% in β-tubulin) was relatively small compared to that observed between isolates of *G. frondosa* and *G. sordulenta* (14.3% in rDNA and 30.2% in β-tubulin). Molecular phylogenetic analysis of rDNA, β-tubulin and

combined sequences revealed two major clades within *G. frondosa*. Clade I (U.S. clade) included all the U.S. isolates, while Clade II (Asian clade) consisted only of Asian isolates. The major commercial isolate (unknown origin) used on most U.S. mushroom farms grouped within the Asian clade and is apparently of Asian decent. Based on partial β-tubulin sequence, phylogenetic analyses indicated that *G. frondosa* and its allies (*G. sordulenta* and *P. umbellatus*) share a common ancestor.

To assist with the demand of commercial growers for isolates with improved yield and quality characteristics, I evaluated 23 genotypes of *Grifola frondosa* for crop cycle time, biological efficiency (BE; ratio of fresh weight of mushroom harvested to dry weight of substrate x 100) and quality. Significant differences among lines were found for these parameters when mushrooms were produced on nutrient supplemented sawdust substrates. Four isolates (WC828, M036, M037 and M040) were found to have the most consistent and highest BEs (38.5%, 39.5%, 35.8% and 38.9%, respectively) and quality (1.2, 1.3, 1.4 and 1.2, respectively).

In order to determine the effects of nutrient supplements on mushroom crop cycle time, BE and quality, different combinations of wheat bran, rye, millet and corn meal were evaluated. Combinations of two or three nutrients selected from wheat bran, millet and rye were found to be the most desirable formulations with short crop cycle, high quality and high BE.

Significant differences for crop cycle times, BEs and quality also were found when different combinations of wheat bran, rye and millet at different levels (10%, 20% and 30% of total dry substrate wt) were used. The combination of 10% wheat bran, 10% millet and 10% rye (BE 47.1%, quality 1.8 and crop cycle 12 weeks) and the combination of 10% wheat bran plus 20% rye (BE 44%, quality 1.7 and crop cycle 10 weeks) gave the most consistent yields and basidiome quality over time.

Table of Contents

List of Figures

List of Tables

Acknowledgements

I am grateful to many people who have assisted me the completion of this project. Foremost amongst those are my advisor, Dr. Daniel J. Royse, and my family. Dr. Royse provided more than just technical assistance and funding support. His invaluable support, direction, encouragement, teaching and constructive criticisms have been critical to the completion of this thesis. I would like to thank Wenfeng Sun for her patience and love that made this work possible. I wish to express my great appreciation to my committee members, Dr. John E. Ayers, Dr. David Geiser, Dr. David Huff and Dr. C. Peter Romaine for their thoughtful review of this work and numerous suggestions regarding molecular phylogenetic analyses, PCR, sequencing and statistical analyses. My sincerest acknowledgement also goes to Patrick D. Collopy, Dr. Xi Chen, Dr. Manuel Ospina-Giraldo and Dr. Zhaowei Lu, my colleagues here in Buckhout Laboratory, for their scientific advice and friendship.

Chapter 1: Introduction

1.1 Cultivation of *Grifola frondosa*

Grifola frondosa (Dickson: Fr.) S.F.Gray, also known as hen-of-the-woods or maitake, is a choice edible mushroom (Lincoff 1981a,b). It produces large basidiomes (it can weigh up to 45kg) and is one of the most popular edible mushrooms collected in the fall in United States. It somewhat resembles a small hen, is often found covered with leaves and grows at the base of hardwoods (especially oaks) and some conifers.

1.1.1 World production

Until about 20 years ago, maitake was only available from the wild. The first cultivation techniques were developed for maitake in 1979 (Hobbs 1996). Commercial production of maitake began in 1981 in Japan (Takama *et al.* 1981). Since then, Japan has become the major producer of maitake accounting for 98% of worldwide production (Chang 1999). Only 325 tons were produced in 1981. However, by 1986, production reached 2,203 tons, a 6.8-fold increase in five years. By 1991, annual production reached 7,950 tons, another 3.6-fold increase over the 1986-91 period. This increase is much higher than the increase (96.4%) for all cultivated edible mushrooms from 1986 (2176 tons) to 1991 (4273 tons) (Chang 1999). During the 1990s, commercial production of maitake increased rapidly not only in Japan, but also in China and the U.S. By 1997, world production of maitake reached 331,000 tons (an increase of 40.6 fold compared with 1991) (Figure 1.1).

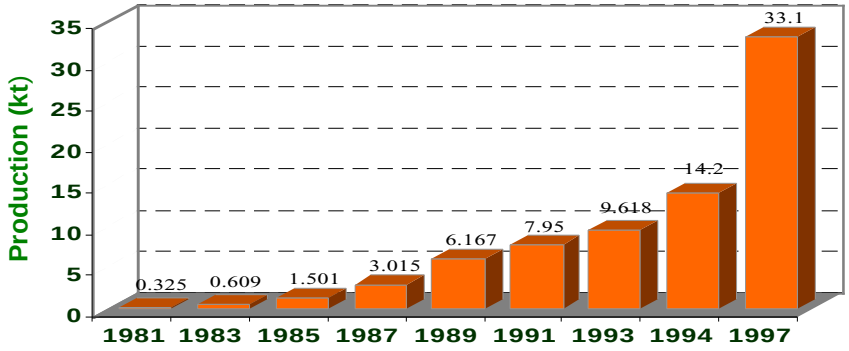


Figure 1.1. World production of *Grifola frondosa* (maitake) from 1981 through 1997. (Source: Chang 1999, Ohmasa 1994, Yamanaka 1997).

1.1.2 Nutrition and physiology

Matsumoto and Ohira (1982) found that glucose, soluble starch, maltose, mannose and fructose, respectively, were the most effective carbon sources utilized for mycelial growth by maitake. Peptone was the "best" nitrogen source among those tested by Matsumoto and Ohira (1982). These researchers also found that the absence of Mn^{2+}, Fe^{2+} and Cu^{2+} in the basal medium resulted in an apparently decreased growth rate of the mycelium.

1.1.3 Methods of commercial production

Relatively little literature is available for growers contemplating maitake cultivation when compared with information available for other edible mushrooms. A common substrate used for commercial production of maitake is supplemented sawdust. Hardwood sawdust (oak, beech, larch, poplar, cottonwood, elm, willow and alder) is generally used by commercial growers. Oak (Lee 1994, D.J. Royse, unpublished data) is the most popular choice in the

U.S. and Japan, while beech and larch are also used in Japan (Kirchhoff 1996, Yoshizawa *et al*. 1997, Stamets 2000). In China, cottonseed hulls were used as a substitute for sawdust and provided an acceptable yield (Zhao *et al*. 1983). Bran derived from cereal grains, such as rice bran (Takama *et al*. 1981), wheat bran (Mayuzumi and Mizuno 1997), oat bran and corn bran, are widely used as nutrient supplements. Other nutrient supplements used for maitake production include millet (D.J. Royse, unpublished data), corn meal (Kirchhoff 1996), and soybean cake (Mizuno and Zhuang 1995).

To date, three basic methods of cultivation have been established. These include bag culture, bottle culture and outdoor bed culture (Mayuzumi and Mizuno 1997).

Bag culture is used most frequently by commercial producers. For bag culture, the moistened substrate (2.5 kg) is filled into polypropylene or high density polyethylene bags. After sterilization and cooling, the substrate is inoculated with maitake spawn that is through-mixed by shaking. A spawn run lasts about 30 to 45 days depending on isolate and substrate formulation (Royse 1997). Expected yields are in the range of 0.35 to 0.68kg per 2.5kg bag of moist (58%) substrate (Royse 1997).

For bottle production, the containers are filled with moistened (55 to 60%) substrate and sterilized or pasteurized prior to inoculation (Royse 1997). The size of the harvested mushroom is smaller than those from bags because there is less substrate in bottles. However, bottle culture is suitable for year-round production and is more suitable for mechanization with minimum labor requirements. In Japan, it is anticipated that automatic mechanized bottle culture systems in large-scale facilities soon will become the dominant method for maitake production (Yamanaka 1997).

Outdoor bed culture on colonized substrate in moist soil was first attempted in Japan under natural climatic conditions (Mayuzumi and Mizuno 1997). This method requires about 6 months from inoculation to basidiome formation, and yields are much lower than those of bag or bottle methods. Therefore, this method only is used on a very limited scale today in Japan.

1.2 Geographic distribution and host range of *Grifola frondosa*

Grifola frondosa grows on dead or dying trees in the temperate regions of many countries. It was first discovered and reported from Europe (England, Norway, Denmark and Finland). It is commonly found in Eastern Canada and the eastern, midwestern, and southeastern United States, but rarely in the Pacific Northwest. *Grifola frondosa* also is indigenous to the temperate hardwood regions of China, and has been reported from the northeastern regions of Japan, Australia and other countries (Gilbertson and Ryvarden 1986, Hobbs 1996, Stamets 2000, Zhao and Zhang 1992).

Maitake is found on stumps or at the base of dead or dying deciduous hardwoods (Stamets 2000). It also may grow on roots and in the heartwood of living hardwoods causing butt rot disease (Farr *et al.* 1987, Webster 1980). *Grifola frondosa* is considered a white rot fungus decomposing both lignin and cellulose (Mizuno and Zhuang 1995).

The host range of *Grifola frondosa* includes oak (*Quercus*), elm (*Ulmus*), maple (*Acer*), blackgum (*Nyssa*), larch (*Larix*) and beech (*Fagus*). It is also found occasionally on species of *Castanea, Pinus, Prunus,* and *Pseudotsuga* (Farr *et al.* 1987).

1.3 Nutritional components

As shown in Table 1.1, fresh basidiomes of maitake at harvest contain approximately 91% moisture. Protein, carbohydrate, lipid, and fiber are the major components. Maitake also contains other nutritive components including minerals, free amino acids, organic acids, nucleotides, vitamins B_1, B_2 and ergosterol (provitamin D). Nucleotides, free carbohydrates, organic acids and amino acids are the main flavor components.

Table 1.1. Nutritional components of basidiomes of *Grifola frondosa* (Imazeki and Hong 1989).

Component	Fresh (%)	Dry wt. basis (%)
Moisture	91	-
Protein	3.7	40.7
Lipid	0.7	7.8
Ash	0.8	8.9
Carbohydrate	2.4	26.6
Fiber	1.4	15.5

Basidiomes of maitake are rich in potassium (K) and phosphorous (P), and contain lesser amounts of Mg, Ca, Na and Zn (Tmazeki 1989). The types and amounts of amino acids present in the basidiomes are an important contributor to flavor. Glutamic acid, which contributes substantially to culinary desirability, is present in the greatest amount in maitake basidiomes, followed by alanine, threonine, aspartic acid, valine, lysine, and arginine (Mizuno and Zhuang 1995). The free carbohydrates in maitake include trehalose, glucose, and mannitol (Mizuno and Zhuang 1995). Many free acids have been detected in maitake basidiomes. These include pyroglutamic acid, lactic acid, acetic acid, formic acid, malic acid, citric acid, succinic acid, oxalic acid, and fumaric acid (Kagawa 1989, Mizuno and Kim 1996). Lipids found in maitake include neutral lipids (triglycerides, TG), sterol lipids (acylsterol) and sphingolipids (ceramide and cerebroside) (Mizuno and Kim 1996).

1.4 Medicinal value

Maitake has gained in popularity among consumers, not only because of its excellent favor, but also because of its reported medicinal value. In the last 25 years, over 70% of scientific articles on maitake have dealt with some aspect of its medicinal properties.

Approximately 2000 years ago, maitake was used as a Chinese medicine called "Keisho". *Shen Nong's Herbal* (Wu 1955) describes the use of Keisho for improving the health of the spleen and stomach, calming nerves and treating hemorrhoids. In recent animal experiments and human clinical trials, maitake was shown to have both anti-tumor and anti-viral properties (Hobbs 1996, Jong *et al.* 1991, Mizuno and Zhuang 1995). Other medicinal uses of this mushroom include blood pressure regulation, control of diabetes, reduction of cholesterol, treatment of chronic fatigue syndrome (CFS), and anti-HIV activity (Adachi *et al.* 1988, Arakawa *et al.* 1977, Jong and Birmingham 1990, Kabir *et al.* 1987, Kubo and Nanba 1997, Mizuno and Zhuang 1995, Nanba 1993, Yagishita *et al.* 1977, 1978).

The majority of the references in recent years concern the anti-tumor activities of maitake. The major anti-tumor substances, which have been obtained from extracts of the basidiome and liquid-cultured mycelium, include polysaccharides and lectins (for example, anti-tumor polysaccharides - FI_0-α-β_1, FIII-1a, LMCA and *Grifola frondosa* lectin - GFL) (Mizuno and Kim 1996).

1.4.1 Anti-tumor polysaccharides

Both the basidiome and liquid-cultured mycelium of *G. frondosa* contain polysaccharides that inhibit growth of tumors (Ying *et al.* 1987). More than 20 anti-tumor polysaccharides have been isolated and purified from *G. frondosa*. Each active polysaccharide has a basic structure of a (1-6)-β-branched (1-3)-β-

D-glucan and heteroglycan or heteroglycan-protein complex as the major component (Mizuno *et al.* 1995).

Glucans are some of the major constituents in fungal cell walls. Miyazaki *et al.* (1978) proposed that the anti-tumor activity of glucans was influenced by the type of carbohydrate linkage, branch length and frequency, and molecular size and configuration.

Several glucans isolated from *G*. *frondosa* have been patented in Japan as potential anti-tumor, anti-cancer and immunodulating agents. These include GF-1, Grifolan-N and Grifolan NMF-5N (anonymous 1983, 1985, Oikawa *et al.* 1987, Takeyama *et al.* 1987). Scientists have concentrated on how these polysaccharides stimulate the mammalian immune system. They appear to enhance the cytolytic and interleukin-1 productivity of macrophages or T cells and to potentiate the delayed-type hypersensitivity response associated with tumor growth suppression (Jong and Birmingham 1990). However, the relationship between these polysaccharides and the medical effects is not fully elucidated.

1.4.2 Lectins

Lectins, carbohydrate-proteins of nonimmune origin, which agglutinate cells or precipitate polysaccharides or glycoconjugates, are widely distributed in living organisms including animals, plants, fungi, bacteria and viruses (Wang 1998). *Ischnoderma resinosum* agglutinin (IRA), a β-galactosyl-specific lectin, was the first lectin isolated from fungi (Kawagishi 1995). *Grifola frondosa* lectin (GFL) was isolated from basidiomes by affinity chromatography on acid-treated sepharose CL-4B and subsequently on GalNAc-toyopearl (Kawagishi 1990). It is N-acetylgalactosamine-specific with more than three subunits per molecule and over 100 kDa in size. GFL was cytotoxic against HeLa cells (cervical cancer cells). The minimum concentration of lectin leading to death of all cells was 25

µg/ml (Kawagishi 1995). However, lectins were only isolated from basidiomes; neither maitake mycelium nor its culture filtrate showed any lectin activity (Mizuno and Zhuang 1995).

1.5 Morphology and classical taxonomy

The genus *Grifola* S.F.Gray is characterized by its large compound basidiomes emerging from soil at the base of trees or stumps (Figure 1.2). In 1821, *G. frondosa* was described as "having stem lateral and cap semicircular" and was placed in the family *Hymenotheceae* by Gray (1821). Recently, the genus *Grifola* S.F.Gray was placed in the phylum *Basidiomycota*, order *Aphyllophorales*, and family *Polyporaceae* (Alexopoulos 1996).

Figure 1.2. Morphology of *Grifola frondosa* (maitake)

Among the monographs about polypores, Gilbertson and Ryvarden's *North American Polypores* (1986) may be the most widely accepted with regard to taxonomy of *Grifola*. Another monograph about polypores is the work of Zhao and Zhang (1992) "*The Polypores of China*". Zhao and Zhang (1992) accepted most of the taxonomic work of Gilbertson and Ryvarden (1986) when they described Chinese polypores. Gilbertson and Ryvarden (1986) described the basidiomes of *Grifola* as annual, stipitate, stipe simple or branched to give rise to large numbers of petaloid pilei, upper surface gray to brownish, finely tomentose to glabrous, pore surface white to cream colored, the pores angular, 2-4 per mm, context white to pale buff, tubes decurrent on stipe. The hyphal system is dimitic containing generative hyphae with clamp connections. Basidiospores are ovoid to ellipsoid. Zhao and Zhang (1992) accepted these morphological descriptions and taxonomy concerning *Grifola*.

However, they also added some additional characters: thick-walled, yellowish skeletal hyphae and hyaline, smooth basidiospores.

Gilbertson and Ryvarden (1986) and Zhao and Zhang (1992) included only one species - *Grifola frondosa* (Dicks: Fr.) S.F. Gray in this genus. The history of *G. frondosa* dates to 1785; Dickson (1785) named this species *Boletus frondosus* Dicks. Fries (1821) changed it to *Polyporus frondosus* Dicks.: Fr. and Gray (1821) changed it to *Grifola frondosa* (Dicks.: Fr.) S.F. Gray. Gray (1821) described *Grifola frondosa* as "thallus much branched, caps numerous, halved, smoke grey, at the foot of oak-trees in autumn, about a foot wide and edible."

Gilbertson and Ryvarden (1986) examined morphological details of specimens collected in the United States. Basidiomes were described as annual, stipitate, and reaching to 40 cm wide. Stipe much branched from a thick base, cream colored, up to 10 cm or more in diameter at the base, giving rise to large numbers of imbricate, petaloid or flabelliform and often confluent pilei up to 8 cm wide and 8 mm thick. Upper surface pale lavender-gray at first, becoming darker and finally a dull dark brown on older specimens. Margin is concolorous, thin, often undulate or curled. Pore surface is ivory white. Pores are angular, 2-4 per mm, with thin, lacerate dissepiments. The context is ivory white, up to 2 mm thick in individual pilei, up to several centimetre thick at base and in main branches of the stipe. The tube layer is decurrent on the stipe, often to the ground line, distinct from context, pale tan on older dried specimens, brittle and shattering easily when dried, up to 5 mm thick. Odor is pleasant and nutlike. The contextual generative hyphae of *G. frondosa* are hyaline, thin-walled, with clamp connections, rarely branched, 2.5-5 nm in diameter. Contextual skeletal hyphae are moderately thick-walled, nonseptate, with infrequent branching, 2.5-6 nm in diameter, but generative hyphae are more frequently branched. Basidia are clavate, 4-sterigmate, 22-26 x 7-8 µm, with a basal clamp connection. Basidiospores (6-7 x 4-4.5 µm) are negative in Meizer's reagent.

Zhao and Zhang (1992) based their descriptions on Chinese specimens. The morphological characters are very similar to those described by Gilbertson and Ryvarden (1986) except that some differences were noted regarding color and size. Basidiomes are 2-7 x 3-7 cm, 2-7 mm thick with a white to gray-white upper surface. The context is white, 1-3 mm thick. Tubes are 1-4 mm long, whitish or milky yellow. Pore surfaces are white to yellowish with 1-3 pores per mm. The generative hyphae are 2.3-5.2µm in diameter, less branched, with clamp connections. The skeletal hyphae are colorless, or faintly yellowish, 3.5-5 µm in diameter. Basidiospore size is 5-7.5 x 3.5-4.5 µm.

1.6 Taxonomic discrepancies

1.6.1 Family level

Grifola frondosa is widely accepted in the family *Polyporaceae.* However, there are discrepancies regarding family placement. Hawksworth *et al.* (1995) placed this species in the family *Coriolaceae* in his *Dictionary of the Fungi*. Jülich (1981) proposed a new family *Grifolaceae*. In the National Center for Biotechnology Information (NCBI) taxonomy database (1999), *Grifola* was placed in the family *Schizophyllaceae*.

1.6.2 Genus level

Fries (1821) described the macroscopic characters of *Polyporus frondosus* in his *Systema Mycologicum*. Saccardo and Traverso's (1882) *Sylloge Fungorum Omnium Hucusque Cognitorum* did not include the genus *Grifola*. Hobles (1965) published a multiple-choice key for the identification of cultures of 149 species of wood-inhabiting Hymenomycetes based on comprehensive characters including microscopic characters, hyphal systems, extracellular oxidase and cultural characters. He also did not use the genus *Grifola* for *Polyporus frondosus* Dicks. ex Fr.

The genus *Grifola* was listed in the *Check List of European Polypores* (Donk 1974). Corner (1989) identified several South Asian *Grifola* species in his *Ad Polyporaceae*. The use of *G. frondosa* seems not to have been fully understood (Corner 1989) and, even now, *Polyporus frondosus*, the synonym of *Grifola frondosa*, still is widely used. The genus *Grifola* has not been accepted by all mycologists, so, the taxonomy of *Grifola* remains problematical. Corner (1989) found *Grifola* varied from multipileate to unipileate when he described Malesian species, so his description of *Grifola* is "fruit-bodies multipileate to unipileate". He also used a different description of the hyphal system. In his illustration of a Bornean (Malaysia) collection, hyphae of *G. frondosa* were described as monomitic, clamped, inflating in the stem and pileus and becoming copiously secondarily septate without clamps. And, these microscopic details agree with collections that he had made in England. Corner wrote: "The secondary septation of the hyphae of the stem and pileus of *G. frondosa*, which is so prominent that the species has been described as without clamps, might be considered a good generic distinction. It is described, in error it seems, by Gilbertson and Ryvarden as dimitic with uninflating hyphae" (Corner 1989). Ying *et al.* (1987) also observed "thin-walled, branched, septate hyphae without clamp connections" when he described Chinese specimens.

1.6.3 Species level

Gilbertson and Ryvarden (1987), Farr *et al.* (1989) and Zhao and Zhang (1992) accepted *Grifola frondosa* as the only species in this genus. But several other species of *Grifola* have been identified and/or used as tradition by many authors.

Gray (1821) identified five species of *Grifola* other than *frondosa.* They are *Grifola platypora* (wide-pored grifola), *Grifola cristata* (crested grifola), *Grifola lucida* (shining grifola), *Grifola badia* (bay grifola), and *Grifola varia* (changing

grifola). Singer (1969) identified an Argentine specimen as *Grifola sordulenta* (Montagne) Singer and a Chilean specimen as *Grifola gargal* Sing. Spec. nov. Donk (1974) listed one more species: *Grifola umbellata* (Pers. per Fr.) Pilát. Corner (1989) identified three new Singapore and Malaysian species: *Grifola armeniaca* sp. nov., *Grifola eos* sp. nov. and *Grifola* sp.A. Mizuno and Zhuang (1995) recognized three species of *Grifola* other than *G. frondosa* (maitake). They are *G. albicans* (shiromaitake), *G. gigantea* (choreimaitake), and *G. umbellata* (tonbimaitake).

Among all of these species mentioned by different authors, at least three species are thought to be very similar to *G. frondosa* based on morphological characters. They are *G. sordulenta* (Mont.) Singer, *G. gigantea* (Pers.) Pilét and *G. umbellata* (Pers. ex Fr.) Pilát.

***Grifola sordulenta* (Montagne) Singer**

Singer (1969) described *Grifola sordulenta* as multipileate polypore. Its stipes rise from a large common trunk-like base where secondary stipes branch off. Its spores vary from subglobose to short ellipsoid or ellipsoid. And more recently, Rajchenberg and Greslebin (1995) identified *Grifola sordulenta*, which decays standing and/or fallen trees of several *Nothofagus* species (the main native forest resource in Patagonia) in southern Argentina. It is similar to *G. frondosa* (Dicks.: Fr.) S.F. Gray. However, it was found that *G. frondosa* differs with *G. sordulenta* in culture by a faster growth rate, by the formation of chlamydospores, and by readily sporulating in culture according to the description of Nobles (1965).

***Grifola gigantea* (Pers.) Pilét**

Grifola gigantea is a synonym of *Meripilus giganteus* (Fr.) Karst and *Polyporus giganteus* Fr. Gilbertson and Ryvarden (1986) and Zhao and Zhang

(1992) both accepted *Meripilus giganteus* (Fr.) Karst. as a legatee species. They suggested that the genus *Grifola* was closely related to *M*. *gigantea*. For both genera, the large compound basidiomes, the ability to cause white rot, and the production of very similar spores are three characteristics that are nearly identical for the two genera. In contrast to *Grifola*, however, *Meripilus* has generative hyphae with simple septa, and has thin to thick walls without clamp connections (Gilbertson and Ryvarden 1986, Zhao and Zhang 1992).

Grifola gigantea is called choreimaitake in Japan, is edible when young but has an offensive odor (Mizuno and Zhuang 1995). It is found on the ground near broadleaf trees (Ying *et al.* 1987, Jong and Birmingham 1990). A water extract of the basidiomes of *G*. *gigantea* was shown to inhibit Ehrlich cancer development in white mice while similar extracts have shown inhibition rates as high as 90% against sarcoma 180 (Ying *et al.* 1987).

***Grifola umbellata* (Pers. ex Fr.) Pilét**

Grifola umbellata is a synonym of *Polyporus umbellatus* (Pers.) Fr. (Arora 1986, Donk 1974, Farr *et al.* 1989) and *Dendropolyporus umbellatus* (Pers.: Fr.) Jül. (Breitenbach and Kränzlin 1991). It has a profusely branched basidiome with many petaloid pilei and differs from *G. frondosa* in basidiome form, colors and spores (Gilbertson and Ryvarden 1986, Zhao and Zhang 1992). The primordia of *G. frondosa* are rich, dark gray brown to gray black in color whereas the basidiome initials of *G. umbellata* are light gray. Macroscopically, these two mushrooms are easily distinguished by their form. Specimens of *G. umbellata* have central branched stipes. Microscopically, the spores of *G. umbellata* are substantially larger and more cylindrically shaped than the spores of *G. frondosa* (Stamets 2000).

Grifola umbellata is referred to as tonbimaitake in Japan (Mizuno and Zhuang 1995). In nature, this mushroom is found on the ground around tree

roots, at the base of stumps or from underground wood in broadleaf and coniferous forests. It is distributed primarily in the Northern Hemisphere (Farr *et al.* 1989). The portion found above ground is edible, while the portion under ground has purported medicinal value (Jong and Birmingham 1990, Ying *et al.* 1987). It has desirable culinary characteristics (Arora 1986, Bessette *et al.* 1997) and its medicinal value has been utilized in Chinese medicine from ancient times. A β-(l-3)-D-glucan showing antitumor activity against cancer has been isolated from its basidiomes (Mizuno and Zhuang 1995).

The taxonomic position of *Grifola* and some of its close allies is not clear. In the last century, Gray (1821) recognized six species of *Grifola* using only simple macroscopic characters. However, most other authors regard Gray's species designations species of *Polyporus.* For example, *G*. *badia* Pers.: S.F. Gray is *P*. *badius* (Pers.:S.F.Gray) Schweinitz (Bessette *et al.* 1997, Farr *et al.* 1989, Gilbertson and Ryvarden 1987). While *G*. *varia* is accepted as *P*. *varius* Fr.: Fr. (Farr *et al.* 1989, Gilbertson and Ryvarden 1987). Gray's shining grifola – *G*. *lucida* is thought to be *P*. *lucidium* (the synonym of *Ganoderma lucidium*). Recently, although more details on morphological characters have been used to describe this group of fungi, there remain close similarities among the genera *Grifola*, *Meripilus* and *Polyporus*.

1.7 Molecular systematics

1.7.1 Ribosomal RNA genes

Recent advances in molecular genetics have provided techniques that allow researchers to study relationships among organisms at the molecular level. DNA sequence analysis has been exploited extensively in recent years by mycologists for systematic and phylogenetic studies on the various groups of fungi. Molecular data were useful in cases where morphological characters

alone are insufficient for the delineation of clear taxonomic groups. Most of these studies have focused on the analysis of ribosomal RNA genes (rDNA).

Ribosomal RNA genes exist in most genomes as multiple copies arranged in tandem repeats along one or more chromosomes. In eukaryotes, each repeat is composed of a transcription unit that codes for three RNAs: a small subunit RNA (SSU), a large subunit RNA (LSU) and a 5.8S RNA. The three genes are separated by two transcribed spacers, the ITS 1 and ITS 2. Each repeat is separated by a non-transcribed spacer, also called an intergenic spacer (IGS) (Figure 1.3). The gene coding for a 5S RNA may be found within the non-transcribed spacer or comprising its own tandem repeat unit elsewhere in the genome.

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). For example, ITS regions are very suitable for phylogenetic analysis on both the species and genus levels.

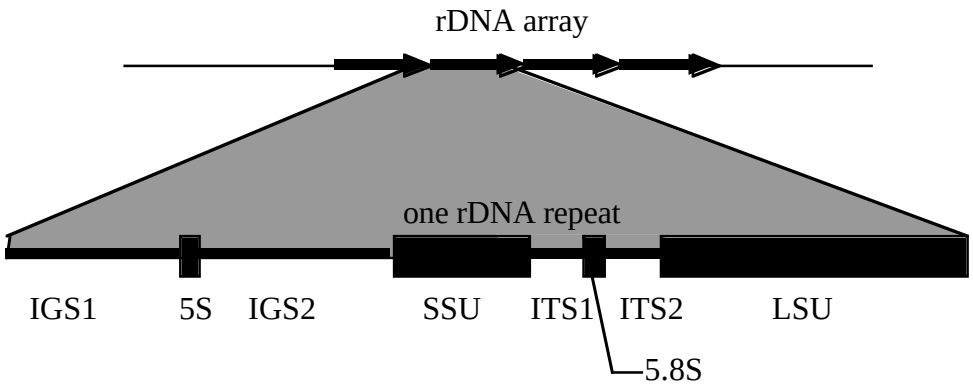


Figure 1.3. Common gene arrangement within a eukaryotic rDNA unit. IGS= Intergenic spacer; ITS= internal transcribed spacer; 5S, 5.8S, SSU and LSU= coding regions, 'S' refers to the sedimentation coefficient in Swedberg unit.

1.7.2 Molecular phylogenetic studies with multiple genes

Although rDNA has been used widely in phylogenetic studies, the evolution of one gene may not represent the evolution of an entire genome. Therefore, gene trees reconstructed from sequences of a single gene may not infer authentic phylogenetic relationships among taxa (Li and Graur 1991). One way to increase the confidence in the phylogenetic assumption that a given gene tree reflects the underlying organism tree is to sample additional, independent genes. Each gene can be analyzed separately and the resulting phylogenies can be compared to see if they support or conflict with each other. Phylogenies can be inferred from the combined datasets if two different gene trees are not in conflict (Geiser *et al.* 2000).

Genes encoding for metabolic and structural proteins are conserved and have advantages over rDNA in that the alignment of the sequence is less ambiguous (Bruns *et al.* 1991). Moreover, degrees of variation in intron, exon, and protein sequences may provide valuable information for fungal systematic and evolutionary studies at different taxonomic levels.

1.7.3 β-tubulin genes

β-tubulin genes encode components of microtubules, which are major components of the cytoskeleton, mitotic spindles, and flagella of eukaryotic cells (Sullivan 1988). In fungi, mutations within the β-tubulin gene may confer resistance to the fungicide benomyl (Goldman *et al.* 1993, Koenraadt *et al.* 1992, Yarden and Katan 1993). This has led to an interest in studying this gene for the characterization of benomyl resistance in plant pathogens as well as for the development of selectable markers for transformation systems (May *et al.* 1987, Panaccione and Hanau 1990).

β-tubulin genes are useful for phylogenetic analysis at a variety of taxonomic levels. It has conserved exons and many introns. For example, the benA β-tubulin gene of *Aspergillus* spp. and *Schizophyllum commune* (Figure 1.4) usually have eight introns and the β-tubulin gene of *Neurospora crassa* has six introns (Figure 1.5). Several primers are available for use in amplifying specific regions of the gene (Thon and Royse 1999a).

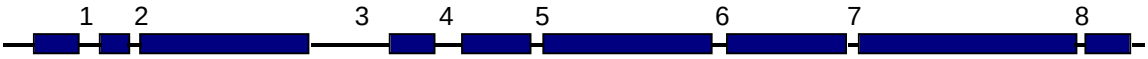


Figure 1.4. β-tubulin gene structure of *Schizophyllum commune* (Russo *et al.* 1992). Regions marked with numbers are introns.

Figure 1.5. Schematic structure of *Neurospora crassa* β-tubulin gene (Orbach *et al.* 1986). Regions marked with numbers are introns.

Fungi such as *Saccharomyces cerevisiae* Hansen (Neff *et al.* 1983), *Septoria nodorum* Berk. (Cooley *et al.* 1991), and *Neurospora crassa* Shear et Dodge (Orbach *et al.* 1986) have one copy of the β-tubulin gene. Fungi, like

Aspergillus nidulans (Eid.) Wint. (May *et al.* 1987), *Colletotrichum graminicola* (Ces.) Wils. (Panaccione and Hanau 1990), and probably *Schizophyllum commune* Fr. (Russo *et al.* 1992) have two β-tubulin genes. But these two divergent genes have distinct functions. Thus, they are easily distinguished at the sequence level.

In recent years, β-tubulin gene sequences have been used in phylogenetic studies of slime molds (Baldauf and Doolittle 1997), protozoa (Edlind *et al.* 1996), Ascomycetes, such as *Fusarium* (Donaldson *et al.* 1995, O'Donnell *et al.* 1998) and *Aspergillus* (Geiser *et al.* 1998b) and Basidiomycetes, such as *Lentinula* and *Pleurotus* (Thon and Royse 1999a).

1.8 Grifola genome

Heim (1954), using microscopic observation, reported that *Grifola frondosa* has eight chromosomes. However, very little is known about the genome of *G*. *frondosa*, including the structure, distribution, and organization of ribosomal and β-tubulin genes in this genus. In GenBank, the only reported sequences of *G. frondosa* are partial ribosomal RNA 25S large subunit sequences (Accession numbers: AF287863, M98641, M98611, M98580) (Hibbett and Vilgalys 1993, Hibbett *et al.* 2000), trehalose phosphorylase encoding cDNA sequences (Accession numbers:E17395, E17394) (Horinouchi *et al.* 1999), and trehalose synthase mRNA sequences (Accession numbers: AB010105, AB010104) (Ohnishi *et al.* 1998).

Chapter 2: Genetic variation present in ITS-1, ITS-2 and 5.8S of the ribosomal DNA repeat and in selected regions of the β-tubulin gene for *Grifola frondosa* and related species

2.1 Introduction

Grifola frondosa (Dickson: Fr.) S.F.Gray is a white rot fungus widely distributed in Asia, North America and Europe. Commonly called maitake or hen-of-the-woods, it is considered a choice edible mushroom with exotic taste and medicinal qualities. Maitake is marketed throughout Asia and, because of increased consumer demand, its commercial production has grown dramatically in Asia and the United States.

Grifola frondosa was first described by S. F. Gray based on the production of unique, large compound basidiomes (Gray 1821). Recently, Gilbertson and Ryvarden (1986) identified *G. frondosa* found in North America, while Zhao and Zhang (1992) identified this species from China. Gilbertson and Ryvarden (1986) and Zhao and Zhang (1992) only accepted one species (*G. frondosa*) in the genus *Grifola*. However, Singer (1969) identified another *Grifola* species *G. sordulenta*. *Polyporus umbellata* (Pers.) Fr. and *Meripilus gigantea* (Fr.) Karst are also considered as close allies of *G. frondosa* based on morphological characters (Gilbertson and Ryvarden 1986, Zhao and Zhang 1992). These two species have synonyms of *G. umbellata* (Pers. ex Fr.) Pilét and *G. gigantea* (Pers.) Pilét, respectively.

No previous work concerning the genetic variability within *G. frondosa* and related species has been conducted. A clearer understanding of genetic variability is needed for classification and delineation of phylogenetic relationships of these fungi. Genetic selection and improvement of cultivated

isolates in commercial mushroom production also may be facilitated by such a study. Because of the morphological similarities of *G. frondosa* and its allies, molecular data would provide more information on genetic variability. In recent years, molecular techniques have been exploited extensively by mycologists for systematic studies on the various groups of fungi. Many of these studies have focused on DNA sequence analysis of ribosomal RNA genes (rDNA) and β-tubulin genes.

Ribosomal RNA genes are conserved and well characterized with many primers available to amplify and sequence different regions of the rDNA repeat (White *et al.* 1990). In addition, Thon and Royse (1999a) have provided primer sequences that can be used for amplification of partial β-tubulin sequences in basidiomycetes.

In this study, partial regions of rDNA and β-tubulin genes were analyzed to examine the amount of genetic variability within isolates of *G. frondosa* and its allies. Specific primers were developed for amplification of regions of the β-tubulin gene of *G. frondosa*. Sequences of a portion of the β-tubulin gene including three introns and exons at the 3' half were analyzed. Regions sequenced for analyzing genetic variability in rDNA included internal transcribed spacers-1 and -2 (ITS-1 & -2) and the 5.8 S rRNA gene.

2.2 Materials and Methods

2.2.1 Cultures

A list of mushroom isolates used in this study are shown in Table 2.1 along with their geographic origin, locations and hosts. Fifty-two isolates of *G. frondosa*, including all isolates available from The American Type Culture Collection (ATCC) and The Pennsylvania State University Mushroom Culture

Collection (PSUMCC), were examined for genetic variability. All cultures were maintained by subculturing on potato dextrose agar supplemented with 1.5g/L of yeast extract (PDYA).

Table 2.1. List of species, isolate code, source, geographic origin, substrate and locality of *Grifola frondosa* and related species used for this study.

Species	Isolate code	Source [a]	Geographic origin	Host/Substrate	Locality
G. frondosa	WC248	L.C. Schisler	PSU, PA	N/A [b]	N/A
G. frondosa	WC364	L.C. Schisler	PSU, PA	N/A	N/A
G. frondosa	WC367	Jodon	PSU, PA	N/A	Hort. Woods
G. frondosa	WC483	ATCC 11936	Maryland	Oak stump	N/A
G. frondosa [c]	WC484	ATCC 48688	N/A	*Acer saccharum*	N/A
G. frondosa	WC493	ATCC 48141	Norway	*Quercus robur*	N/A
G. frondosa	WC555	Y.H.Park	Korea	N/A	N/A
G. frondosa	WC556	Y.H.Park	Korea	N/A	N/A
G. frondosa	WC557	Y.H.Park	Korea	N/A	N/A
G. frondosa	WC581	Y.H.Park	Korea	N/A	N/A
G. frondosa	WC582	Y.H.Park	Korea	N/A	N/A
G. frondosa	WC583	Y.H.Park	Korea	N/A	N/A
G. frondosa	WC659	Y.H.Park	Korea	N/A	N/A
G. frondosa	WC685	B.W.Yoo	N/A	N/A	N/A
G. frondosa	WC808	Bill Shanley	Tidioute, PA	White Oak	N/A
G. frondosa	WC828	D.J.Royse	N/A	Commercial isolate	N/A
G. frondosa	WC834	NGF 001	Nara Prefecture, Japan	*Castanopsis* spp.	Lowlands
G. frondosa	WC835	Hokken M-1	Japan	Commercial isolate on oak	Highlands
G. frondosa	WC836	Mori 51	Japan	Commercial isolate on oak	Highlands
G. frondosa	M001	USDA FP-101988-T	Cooksville (Rock), WI	Soil near downed hardwood log (*Quercus*?)	Edge of Old Mill Pond at Hwy 59, Fire #638
G. frondosa	M002	USDA FP-102464-Sp	Madison, WI	*Quercus*, soil at base of dead stump.	N/A
G. frondosa	M003	USDA FP-102464-T	Madison(Dane), WI	*Quercus*, soil at base of dead stump.	Picnic Point, UW campus
G. frondosa	M004	USDA FP-103424-T	Athens(Georgia), GA	*Quercus nigra*, at base	N/A
G. frondosa	M005	USDA FP-105867-Sp	Beltsville (Prince George), MD	*Quercus coccinea* (scarlet oak) living, at base	Forest Disease Lab Station, Agr. Res. Center
G. frondosa	M006	USDA FP-134675-Sp	Madison (Dane), WI	*Quercus*, underneath	UW Arboretum
G. frondosa	M007	USDA FP-47462	WV	*Quercus alba* (white oak)	Plat #55, at 1500 feet elevation, Devil's Hole
G. frondosa	M008	USDA RLG-6889-Sp	Syracuse, NY	*Quercus alba*	N/A
G. frondosa	M009	USDA LOO-14980-T	LA	*Quercus* snag, inside of hollow (oak)	N/A

Species	Isolate code	Source [a]	Geographic origin	Host/Substrate	Locality
G. frondosa	M010	USDA OKM-4954-T	Beltsville (Prince George), MD	N/A	Ground, Beltsville Expt Forest
G. frondosa	M011	USDA OKM-6133-Sp	Washington(District of Columbia), DC	N/A	Rock Creek Park
G. frondosa	M012	USDA RLG-14995-T	Baton Rouge, LA	*Quercus virginiana*	Memorial Grove, LA State U campus
G. frondosa	M013	USDA L-15552-Sp	Syracuse, NY	N/A	N/A
G. frondosa	M014	USDA RLG-6889-T	Syracuse, NY	*Quercus alba*	Oakwood Cemetery
G. frondosa	M015	USDA TJV-93-130-T	Madison(Dane), WI	*Quercus macrocarpa*, base of live	Turville Pt. Woods
G. frondosa	M016	FIRDI 36283	Taiwan	N/A	N/A
G. frondosa	M017	FIRDI 36286	Taiwan	N/A	N/A
G. frondosa	M018	FIRDI 36355	Taiwan	N/A	N/A
G. frondosa	M019	FIRDI 36356	Taiwan	N/A	N/A
G. frondosa	M020	FIRDI 36357	Taiwan	N/A	N/A
G. frondosa	M021	FIRDI 36434	Taiwan	N/A	N/A
G. frondosa	M029	PSUMCC 600	Taiwan	Commercial strain	N/A
G. frondosa	M030	PSUMCC 601	Taiwan	Commercial strain	N/A
G. frondosa	M031	PSUMCC 602	Taiwan	Commercial strain	N/A
G. frondosa	M032	PSUMCC 604	Taiwan	Commercial strain	N/A
G. frondosa	M033	PSUMCC 630	Taiwan	Commercial strain	N/A
G. frondosa	M034	PSUMCC 644	Taiwan	Commercial strain	N/A
G. frondosa	M035	USDA RLG-6889-Sp	Syracuse, NY	*Quercus alba*	Oakwood Cemetery
G. frondosa	M036	X.W.Chen	China	N/A	N/A
G. frondosa	M037	X.W.Chen	China	N/A	N/A
G. frondosa	M038	ATCC 60891	China	N/A	N/A
G. frondosa	M039	Tan 0206	He Bei, China	N/A	N/A
G. frondosa	M40	M. Chen	China	Commercial strain	N/A
G. sordulenta	G01	ATCC 200416	Argentina	*Nothofagus dombeyi* trunk	N/A
P. umbellatus [d]	G02	ATCC 60546	N/A	N/A	N/A
M. giganteus [e]	G06	USDA FP-135344-Sp	England	Carpinus spp.	Virginia Waters

[a] ATCC = American Type Culture Collection; USDA = The Unitied States Department of Agriculture; FIRDI = Food Industry Research and Development Institute, Taiwan; PSUMCC = Pennsylvania State University Mushroom Culture Collection.

[b] N/A = not available

[c] Culture misidentified. Found 98% match as *Spongipellis delectans* by blast search of ITS rDNA in this study.

[d] *Polyporus umbellatus*, synonym of *Grifola umbellata*.

[e] *Meripilus giganteus*, synonym of *Grifola giganteus* and *Polyporus giganteus*.

2.2.2 DNA extraction

Cultures were grown in 50 ml of potato dextrose yeast broth (PDYB) for 20 to 30 days at room temperature. Mycelium was harvested by vacuum filtration on Whatman qualitative filter paper, and washed once with distilled water. Fresh mycelium (100mg) was used to isolate DNA following the LETS extraction procedure (Chen *et al*. 1999). DNA preparations were diluted with sterile water and used as template for PCR amplification.

2.2.3 Primer design

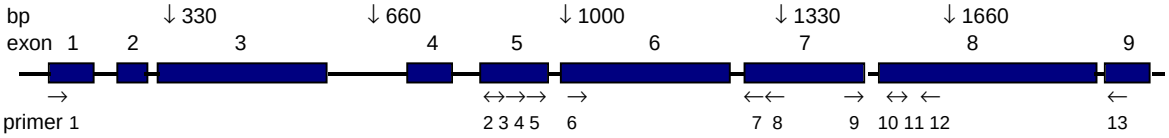
Nine primers, targeting exons 5 to 9 of the β-tubulin genes of *Schizophyllum commune*, were prepared as outlined by Thon and Royse (1999a). I designed another four primers based on β-tubulin exon alignments of *S. commune* (Russo *et al.* 1992), *Pleurotus pulmonarius* (Kim and Magae 1999) and *Coprinus cinereus* (Matsuo *et al.* 1999). Sequences were targeted at exon 1, 5 and 7 of the β-tubulin gene, respectively. A total of 13 specific primers (Figure 2.1) were screened in 15 different combinations (Table 2.2) using 7 isolates of *Grifola frondosa* (WC556, WC828, WC835, M009, M036, WC484, WC493), *G. sordulenta* G01, and *Polyporus umbellatus* G02. The selected isolates were assumed genetically diverse because of their taxonomy and isolation from substrates found in different geographic sites.

None of the primer pairs provided a single band of predicted size for all isolates tested. However, strong bands of around 680bp and 500bp were consistently found with primer pairs BT1.1/B34R and B43F/B41R, respectively, from all isolates (Figure 2.2). PCR products were subjected to electrophoresis at 70 V for 3 hours in 2% low melting point agarose prepared in TAE buffer (40 mM

Tris-acetate, 1 mM EDTA, pH 8.4). Amplicons (~680bp and ~500bp bands) were excised from the agarose gels and purified using the Wizard PCR Preps system according to instructions provided by the manufacturer (Promega Corp., Madison, WI).

Purified PCR products were sequenced using primers BT1.1, B34R, B43F and B41R. Sequences including the 3' end of exon 5 to exon 9 of the β-tubulin gene were aligned, and the specific primers BTG5F (5'-CGTTGTGCCCAGTCCTAAGGTG-3') and BTG8R (5'-GTTCTTGCTCTGCACGTTCTG-3') were designed to target the exon 5 to 8 region of the β-tubulin gene. These specific primers amplified a single strong band for all isolates of *Grifola frondosa*, except isolate WC484. All oligonucleotides were synthesized in an Oligo 1000M DNA synthesizer (Beckman Instruments, Inc; PSU Nucleic Acid Facility).

a.



b.

Primer Number	Name	Sequence 5' to 3'	Location in *S. Commune* sequence
1	BT1F	ATGCGTGAAATCGTCCACCT	1
2	BT5F	ATTCGTGAGGAATACCCCGAC	727
3	BT5R	CGGTCGGGGTATTCCTCACGA	730
4	B36F*	CACCCACTCCCTCGGTGGTG	833
5	BT1.1*	CTGGTATGGGTACTCTCCTGATCT	853
6	S11F*	CTTTCTGCATTGATAACGAG	1076
7	BT7R	CCATCATGTTCTTGGCGTCGAAC	1247
8	B12R*	CATGAAGAAGTGAAGACGCGGGAA	1309
9	B43F*	CCAAGAACATGATGGCTGC	1418
10	B42F*	CTTGGTCTCCATGAAGGAGG	1536
11	B34R*	CCTTCATGGAGACCTTGCCAC	1536
12	S12R*	CACTCGACGAAGTAGGCAGAGT	1591
13	B41R*	CTGGTACTGCTGGTACTCG	1890

* Thon and Royse 1999a

Figure 2.1. Locations (a) and sequences (b) of 13 primers according to the *Schizophyllum commune* β-tubulin gene sequence reported by Russo *et al.* (1992).

Table 2.2. Combinations (15) of primer (13) pairs and their PCR amplification sizes in the *Schizophyllum commune* β-tubulin sequence tested for amplification in eight isolates of *Grifola frondosa, G. sordulenta* and *Polyporus umbellatus*.

Primer pair	Size (bp) in *S. commune* sequence*
BT1F/BT5R	748
BT5F/BT7R	542
BT5F/B12R	606
B36F/ B12R	500
B36F/ B34R	724
B36F/ S12R	780
BT1.1/ B12R	480
BT1.1/ B34R	704
BT1.1/ S12R	760
S11F/ B12R	257
S11F/ B34R	481
S11F/ S12R	537
S11F/ B41R	833
B43F/ B41R	490
B42F/ B41R	373

*. Russo *et al.* 1992.

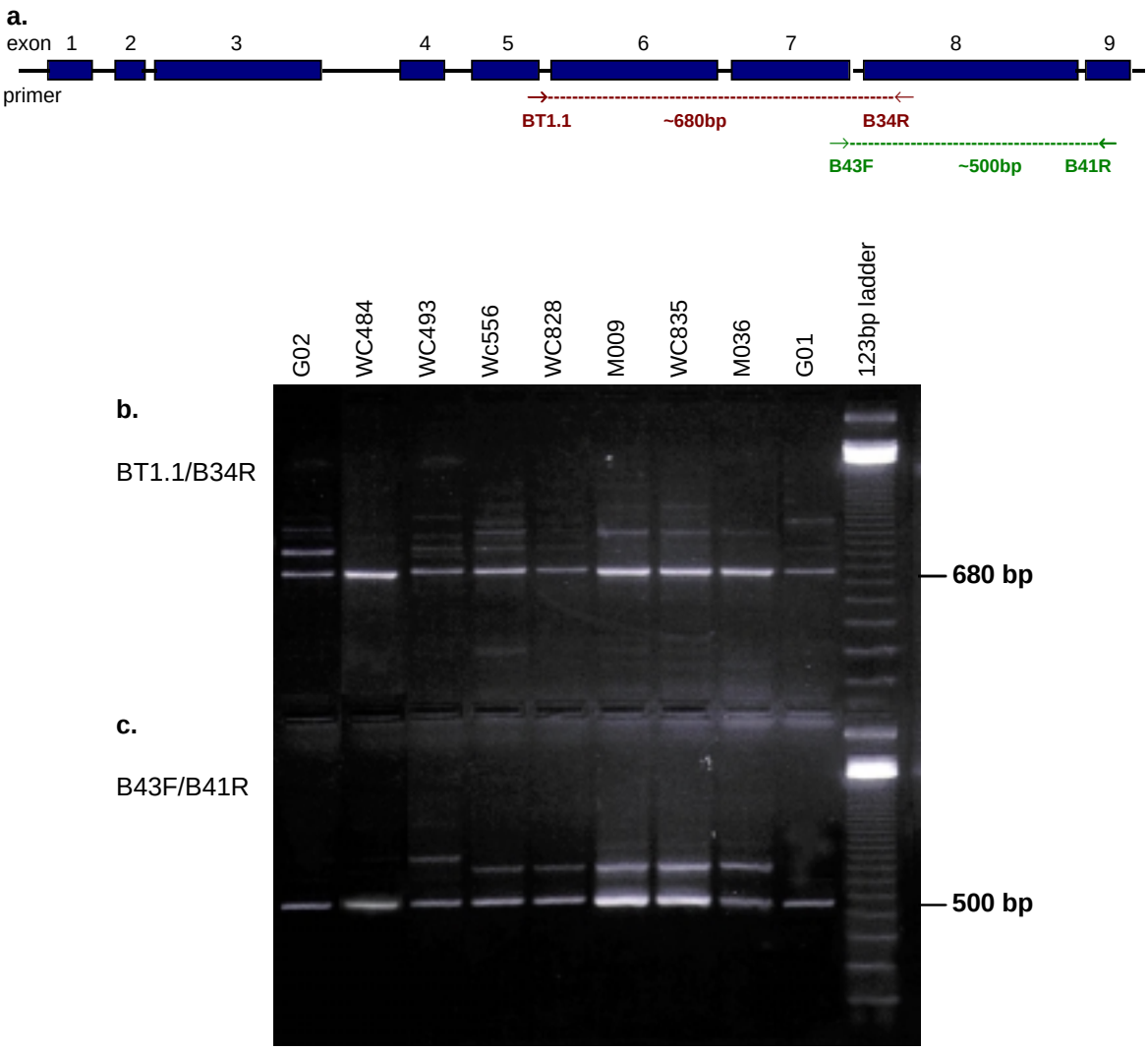


Figure 2.2. PCR amplification products by primer pairs BT1.1/B34R (b) and B43F/B41R (c) corresponding to β-tubulin gene sequence (a) reported by Russo *et al.* (1992) from seven isolates of *Grifola frondosa*, one *G. sordulenta* G01 and *Polyporus umbellatus* G02. Isolate numbers are listed above each lane.

2.2.4 PCR amplification and sequencing of rDNA and β-tubulin

PCR was performed in 25 µl reactions on a 96-well PCR cycler (PTC-100 Programmable Thermal Controller, MJ Research, Inc.), using 10mg DNA template, 1 Unit of *Taq* DNA polymerase (Promega, Madison, WI), 0.2 mM of each dNTP, 2 mM $MgCl_{2,}$ 0.1% Triton, as well as 0.5 µM of each primer.

2.2.4.1 rDNA

Amplification of ITS-1, ITS-2, and 5.8 S rDNA was performed for all isolates utilizing primers ITS1AF (5'-TCCGTAGGTGAACCTGCGG-3') (White *et al.* 1990) and ALR0 (5'-CATATGCTTAAGTTCAGCGGG-3') (Figure 2.3). PCR reactions for ITS regions were performed with the following parameters: 94°C/1 min; 35 cycles of 94°C/15 s, 60°C/30 s, 72°C/1 min; and 72°C/5 min.

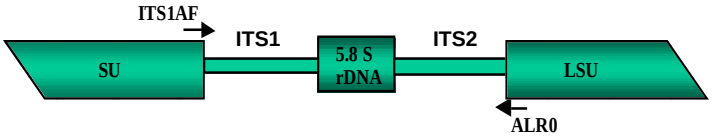


Figure 2.3. Locations of the primers for amplification of ITS-1, ITS-2 and 5.8s rDNA gene regions of the rDNA repeat.

2.2.4.2 β-tubulin

PCR reactions for β-tubulin regions were performed using two primer pairs BTG5F/BTG8R and BT1.1/B34R (Figure 2.4) with the following parameters: 94°C/2 min; 35 cycles of 94°C/15 s, 57°C/30 s, 72°C/1 min; and 72°C/7 min. Primers BTG5F (5'-CGTTGTGCCCAGTCCTAAGGTG-3') and BTG8R (5'-GTTCTTGCTCTGCACGTTCTG-3') were used to amplify 51 isolates of *G. frondosa,* and one isolate of *G. sordulenta* G01 and *Polyporus umbellatus* G02. Isolate WC484 was amplified using primers BT1.1 and B34R. Reactions were optimized by adjusting concentrations of template DNA and other reagents and

primer annealing temperatures. Amplification products were electrophoresed on a 1.0% agarose gel and checked to ensure that a single DNA band was produced of the expected size (~600bp for ITS PCR products and ~680bp for β-tubulin PCR products).

For sequencing, PCR products were purified directly from reactions using the wizard PCR Preps System (Promega Corp., Madison, WI) and adjusted to a concentration of 20ng/μl. Sequencing reactions were performed using the PCR primers and an ABI dye-terminator kit (ABI/Perkin-Elmer). Sequences were resolved using an ABI Prism® Model 377 automated sequencing system (Applied Biosystems, Foster City, CA) located in the Department of Plant Pathology (Buckhout Lab.).

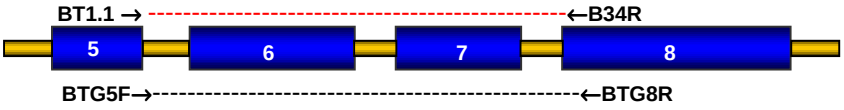


Figure 2.4. Locations of primer pairs (BTG5F/BTG8R and BT1.1/B34R) and the size (~680bp) of amplicons for portions of the β-tubulin gene. Numbers indicate exons.

2.2.5 Sequence data analysis

Sequences were edited and initially aligned using the clustal W algorithm (Higgins *et al.* 1991) in the Lasergene package (DNAStar, Inc. Madison, WI) and then optimized visually. Nucleotide variations occurred in rDNA and β-tubulin genes were calculated by kimura 2-parameter model using PAUP, version 4.0b4a (Swofford 2000).

For rDNA, variable nucleotide sites within ITS-1, ITS-2 and the 5.8 S regions were calculated respectively among 51 isolates of *Grifola frondosa*, 52 isolates of *Grifola* spp. (*G. sordulenta* G01and 51 isolates of *G. frondosa*), and 12

isolates of *G. frondosa* and its allies (eight isolates of *G. frondosa,* WC484, *G. sordulenta* G01, *Polyporus umbellatus* G02, and *Meripilus giganteus* G06). For the β-tubulin gene, variable nucleotide sites within each exon and intron from exon 5 to exon 8 were calculated, respectively, among 51 isolates of *Grifola frondosa*, 52 isolates of *Grifola* spp. (*G. sordulenta* G01 and 51 isolates of *G. frondosa*), and 11 isolates of *G. frondosa* and its allies (eight isolates of *G. frondosa,* WC484, *G. sordulenta* G01, and *Polyporus umbellatus* G02). Nucleotide variations within these regions were compared and the highly variable and highly conserved domains within the regions were determined.

2.2.6 Database search

The database search of sequences for a possible match to the rDNA sequence of isolate WC484 was conducted using the Blastn algorithm (Altschul *et al.* 1997) available at the National Center for Biotechnology Information (NCBI, Bethesda, MD).

2.3 Results

2.3.1 Analysis of ITS sequences

Amplification of the ITS-1, ITS-2 and 5.8S ribosomal DNA repeat yielded fragments of approximately 600 bp as estimated by agarose gel electrophoresis. Characteristics of nucleotide variation present in these regions of *Grifola frondosa* and its allies are summarized in Table 2.3.

An alignment (Appendix A) with total sites of 574bp was used to compare nucleotide variation within *Grifola frondosa* and *G. sordulenta*. The alignment included the sequences of all isolates of *Grifola frondosa* (except WC484) and the only isolate of *G. sordulenta*. Isolate WC484 was excluded because of extreme variation compared with other *G. frondosa* isolates. Nucleotide variation

within isolates of *Grifola frondosa* was 5.4%. Thus, variation was relatively small compared to that observed between isolates of *G. frondosa* and *G. sordulenta* (14.3%).

Table 2.3. Site variation within the ITS-1, 5.8 S, and ITS-2 gene region of *Grifola frondosa* and its allies.

		ITS-1			5.8S			ITS-2			Total		
Isolates	Number [a]	Sites [b]	Total [c]	V(%)[d]	Sites	Total	V(%)	Sites	Total	V(%)	Sites	Total	V(%)
G. frondosa [e]	51	7	199	3.5	0	158	0	24	217	11.1	31	574	5.4
Grifola spp. [f]	52	30	199	15.1	0	158	0	52	217	24.0	82	574	14.3
Grifola frondosa and *allies* [g]	11	116	220	52.7	0	158	0	119	222	53.6	235	600	39.2

[a] Number of isolates included.

[b] Number of variable sites.

[c] Total number of sites compared.

[d] Percentage of sequence variation.

[e] All sequences of *G. frondosa* (excluding WC484).

[f] Isolates included *G. frondosa* (excluding WC484) and *G. sordulenta.*

[g] Isolates included eight *G. frondosa*, *G. sordulenta, Polyporus umbellatus* G01, and *Meripilus giganteus* G06.

The same pattern of variation occurred within the ITS-1, 5.8S, and ITS-2 gene regions. Isolates of *G. frondosa* showed the lowest nucleotide variation in ITS-1 (3.5%), 5.8S (0%), and ITS-2 (11.1%) gene regions. Nucleotide variation between isolates of *G. frondosa* and *G. sordulenta* in ITS-1, 5.8S, and ITS-2 gene regions were 15.1%, 0%, and 24.0% respectively.

Sequences of eight isolates of *G. frondosa* (M009, M004, WC493, WC659, M030, WC835, WC828 and M037), which have different geographic origins, and isolates of *G. sordulenta*, *Polyporus umbellatus* and *Meripilus giganteus* were aligned to compare and calculate nucleotide variation. Total number of sites of the alignment (Appendix B) were 600bp. Among isolates of *Grifola frondosa* and its allies, the nucleotide variation (39.2%) was much higher.

In the 5.8S gene, no nucleotide differences were found among isolates of *G. frondosa* and it allies. Thus, most of the variation observed was attributed to nucleotide differences within the ITS-1 and ITS-2 regions. Because of the high nucleotide variation in the ITS-1 (52.7 %) and ITS-2 (53.6%) gene regions, the alignment (Appendix B) is ambiguous.

The blast search of sequences for a possible match to the rDNA sequence of isolate WC484 yielded 998 hits on the query sequence in the nucleotide databases at the NCBI. The highest match was *Spongipellis delectans* ITS-1, 5.8 S, and ITS-2 nuclear rDNA sequence (Yao *et al.* 1998). The score for this match is 1088 bits with an E value of zero. The alignment (Appendix C) of 573 total nucleotides showed 98% identities. The scores for the remainder of the matches were lower than 335 bits with E values higher than 1.0E-89. This indicated that *Grifola frondosa* isolate WC484 from the ATCC (American Type Culture Collection) is probably a misidentified isolate of *Spongipellis delectans*.

2.3.2 Analysis of partial β-tubulin sequences

Amplified fragments of the partial β-tubulin sequences were approximately 680bp and contained 40bp of the 3'-end of exon 5, exon 6, 7, 72bp of the 5'-end of exon 8 and intron 5, 6, 7, which were identified based on their reported locations in the *Schizophyllum commune* β-tubulin gene sequence (Russo *et al.* 1992) and the identity of GT- -AG splice junctions for introns. Isolate of *Meripilus giganteus* G06 was not included in the analysis because several attempts to sequence PCR products were unsuccessful.

The characteristics of nucleotide variation present in partial β-tubulin sequence regions of *Grifola frondosa* and its allies are summarized in Table 2.4. The nucleotide variations within *Grifola frondosa* and *G. sordulenta* were analyzed by aligning 51 sequences of G. *frondosa* and one isolate of *G.*

sordulenta (Appendix D). The total number of sites was 587bp and included 4bp of 3'-end of exon 5, 58bp of intron 5, 229bp of exon 6, 62bp of intron 6, 164bp of exon 7, 62bp of intron 7 and 8bp of the 5'-end of exon 8 of the β-tubulin gene. An alignment (Appendix E) with total sites of 401bp was used to compare nucleotide variation within *Grifola frondosa* (M009, M004, WC493, WC659, M030, WC835, WC828 and M037) and related species (*G. sordulenta* and *Polyporus umbellatus*). This alignment included end base pairs (AG) of intron 5 and 6, 229bp of exon 6, 164bp of exon 7, and start base pairs (GT) of intron 6 and 7 of the β-tubulin gene sequence. Intron 5, 6 and 7 were excluded from the analysis because of an ambiguous alignment.

Within isolates of *Grifola frondosa*, nucleotide variation was 12.2% for total sites. Most of the variation occurred in introns with 14.8% for intron 5, 23.7% for intron 6, and 32.3% for intron 7. Lesser variation was observed in exons with 8.3% for exon 6, and 5.5% for exon 7. The sequences of *G. frondosa* and *G. sordulenta* showed much higher variation for the total alignment (30.2%) and across the introns and exons. The most variable region was intron 5 (56.9%) and exon 7 was the most conserved region (18.3%) within the β-tubulin gene sequence regions analyzed.

Among isolates of *Grifola frondosa* and its allies, sequence variances within introns 5 and 7 were too high to allow an unambiguous alignment. The nucleotide variation of exon 6 (23.6%) and exon 7 (21.3%) were much higher than that within *G. frondosa*. No insertions and deletions were observed within exons.

Table 2.4. Site variation within the partial β-tubulin gene region of *Grifola* and its allies.

		Exon 5			Intron 5			Exon 6			Intron 6		
Isolates	number [a]	Sites [b]	Total [c]	V(%) [d]	Sites	Total	V(%)	Sites	Total	V(%)	Sites	Total	V(%)
G. frondosa [e]	51	0	4	0	8	54	14.8	19	229	8.3	14	59	23.7
Grifola spp. [f]	52	0	4	0	33	58	56.9	45	229	19.7	35	62	56.5
Grifola frondosa and *allies* [g]	10	-	-	-	0	2	0	54	229	23.6	0	4	0

		Exon 7			Intron 7			Exon 8			Total		
Isolates	number	Sites	Total	V(%)	Sites	Total	V(%)	Sites	Total	V(%)	Sites	Total	V(%)
G. frondosa [e]	51	9	164	5.5	20	62	32.3	1	8	12.5	71	580	12.2
Grifola spp. [f]	52	30	164	18.3	33	62	53.2	1	8	12.5	177	587	30.2
Grifola frondisa and *allies* [g]	10	35	164	21.3	0	2	0	-	-	-	89	401	22.2

[a] Number of isolates included.

[b] Number of variable sites.

[c] Total number of sites compared.

[d] Percentage of sequence variation

[e] All sequences of *G. frondosa* (excluding WC484)

[f] Isolates included *G. frondosa* (excluding WC484) and *G. sordulenta*

[g] Isolates included eight *G. frondosa*, *G. sordulenta* G01, *Polyporus umbellatus* G02.

2.4 Discussion

Nearly 30 years ago, researchers first observed that rRNA genes from closely related species are highly evolutionarily conserved, while ITS and intergenic regions are much more variable (Brown *et al.* 1972). The findings regarding the genetic variability in ITS and 5.8S rDNA regions of the *G. frondosa* and its related species are similar. Most of the nucleotide variation was found in the ITS regions, while 5.8S shared significant sequence similarity (Table 2.3).

Similar to other protein encoding genes, nucleotide variation of exons in β-tubulin genes was much less than that of introns. Thus, β-tubulin gene

sequences may be used successfully for phylogenetic studies because alignments in exons are less ambiguous, and sufficient phylogenetic information is available from introns. The protein encoding regions (exons) of β-tubulin genes are conserved because they encode components of microtubules that are major structural components of eukaryotic cells. No amino acid changes were observed within isolates of *G. frondosa*. Two amino acid changes were observed between isolates of *G. frondosa* and *G. sordulenta*. Most nucleotide substitutions were observed in the third codon of amino acids.

Comparison of sequence divergence of ITS and β-tubulin revealed a higher level of nucleotide variation in the regions of β-tubulin than in ITS regions. Within G. frondosa species, nucleotide variation in exons of β-tubulin was even higher than in ITS-1 and ITS-2. Thus, the β-tubulin gene provided more phylogenetic information at the species level.

DNA sequence data indicated that *Grifola frondosa* isolate WC484 is probably misidentified. Morphological differences in culture and spawn between WC484 and *G. frondosa* were observed. It was also found that WC484 formed a unique red color primordium on supplemented sawdust substrates (Figure 2.5). This is different than the dark gray color primordium formed by *G. frondosa* (Chapter 4, this thesis) and can be considered a significant morphological difference. Unfortunately, the production of fruitbodies from WC484 was not successful.

Figure 2.5. Primordium of WC484 grown on sawdust substrate.

Chapter 3: Phylogenetic analysis of *Grifola frondosa* and its related species based on ITS-1, ITS-2 and 5.8S rDNA and partial β-tubulin gene sequences

3.1 Introduction

Grifola frondosa is a white rot fungus usually found on stumps or at the bases of dead or dying deciduous hardwoods (Farr *et al.* 1987, Stamets 2000, Webster 1980). It is considered a choice edible mushroom and has proven effective as an antiviral and antitumor agent (Hobbs 1996, Jong *et al.* 1991). Production and consumption of this mushroom is increasing rapidly in the world and the United States because of its excellent taste and its nutritional and medicinal value.

Traditional classification of *G. frondosa* was based solely on morphological characters. The genus *Grifola* S.F.Gray was first applied by Gray (1821) and described as a polypore with large compound basidiomes. Later, microscopic characters, such as the hyphal system and spore morphology, were used for classification. However, even with these simple descriptions many taxonomic discrepancies remain for *G. frondosa* at the species, genus and family levels.

Wild populations of *G. frondosa* are widely distributed in Asia, North America and Europe. Previous taxonomic investigations (Gilbertson and Ryvarden 1986, Zhao and Zhang 1992) have revealed similar morphological characters between North American and Asian isolates. They recognized *G. frondosa* (Dicks.:Fr.) S.F.Gray as the only species in the genus *Grifola*. However, Singer (1969) identified another *Grifola* species - *G. sordulenta*. Based on morphological characters, *Polyporus umbellatus* (Pers.) Fr. and *Meripilus*

gigantea (Fr.) Karst are close allies of *G. frondosa* (Gilbertson and Ryvarden 1987). They also are considered species of *Grifola* by some researchers (Mizuno and Zhuang 1995). Alexopoulos *et al.* (1996) accepted *G. frondosa* as a member of the *Polyporaceae*; however, it also was placed in the family *Grifolaceae* (Jülich 1981) and *Coriolaceae* (Hawksworth *et al.* 1995) by other researchers. Thus, the taxonomic position of *Grifola* and some of its allies is not clear.

Few studies have been undertaken to investigate genetic diversity, evolutionary relationships, and systematics in the genus *Grifola*. A thorough understanding of these relationships are necessary to effectively carry out efforts aimed at developing improved commercial lines of *G. frondosa* and understanding the taxonomic and phylogenetic relationships among *Grifola* and its allies.

Molecular approaches have been used extensively for examining phylogenetic relationships among other edible fungi (for example see Hibbett *et al.* 1995, Thon and Royse 1999b and Vilgalys and Sun 1994). However, no such work has been reported on *Grifola* until the recent studies by Hibbett *et al.* (1997, 2000) based on mitochondrial and nuclear small and large subunit ribosomal RNA sequences. They presented a phylogenetic analysis of a board sampling of basidiomycetes, which included one isolate of *G. frondosa* and one isolate of a related species (*Meripilus giganteus*). Their result showed that *G. frondosa* was far related to *M. giganteus*.

In this study, a phylogenetic analysis using DNA sequences from two gene regions was undertaken to clarify the relationships of *G. frondosa* and its allies. I used the internal transcribed spacers 1 and 2 (ITS-1 and 2) and 5.8S gene of the nuclear ribosomal DNA transcriptional unit and a portion of the β-tubulin gene encoding for 3 introns and 3 exons.

The rDNA regions were chosen because the small and large subunit regions are conserved and well characterized and many primers are available for PCR. In addition, the variability observed in the ITS regions supplied sufficient sequence data for analyzing taxa at both the species and genus levels. The β-tubulin gene is a structural protein gene with many introns. The conserved protein encoding regions provided less ambiguous alignment than the ITS regions of rDNA.

3.2 Materials and Methods

3.2.1 Cultures

A total of 51 isolates of *G. frondosa*, one isolate of *G. sordulenta*, one isolate of *Polyporus umbellatus*, one isolate of *Meripilus giganteus*, and one isolate of *Ganoderma lucidium* were used in this study (Table 3.1). The isolates of *G. frondosa* included all available isolates from the American Type Culture Collection (ATCC) and the Pennsylvania State University Mushroom Culture Collection (PSUMCC). Isolates represented various geographic origins including Asia (27), United States (21), Europe (1), and unknown origin (3). All cultures are maintained on potato dextrose agar supplemented with 1.5g/L of yeast extract (PDYA).

Table 3.1. List of species, isolate code, source, geographic origin, substrate and locality of *Grifola frondosa* and related species used for this study.

Species	Isolate code	Source [a]	Geographic origin	Host/Substrate	Locality
G. frondosa	WC248	L.C. Schisler	PSU, PA	N/A [b]	N/A
G. frondosa	WC364	L.C. Schisler	PSU, PA	N/A	N/A
G. frondosa	WC367	Jodon	PSU, PA	N/A	Hort. Woods
G. frondosa	WC483	ATCC 11936	Maryland	Oak stump	N/A
G. frondosa	WC493	ATCC 48141	Norway	*Quercus robur*	N/A
G. frondosa	WC555	Y.H.Park	Korea	N/A	N/A
G. frondosa	WC556	Y.H.Park	Korea	N/A	N/A
G. frondosa	WC557	Y.H.Park	Korea	N/A	N/A
G. frondosa	WC581	Y.H.Park	Korea	N/A	N/A
G. frondosa	WC582	Y.H.Park	Korea	N/A	N/A
G. frondosa	WC583	Y.H.Park	Korea	N/A	N/A

Species	Isolate code	Source [a]	Geographic origin	Host/Substrate	Locality
G. frondosa	WC659	Y.H.Park	Korea	N/A	N/A
G. frondosa	WC685	B.W.Yoo	N/A	N/A	N/A
G. frondosa	WC808	Bill Shanley	Tidioute, PA	White Oak	N/A
G. frondosa	WC828	D.J.Royse	N/A	Commercial isolate	N/A
G. frondosa	WC834	NGF 001	Nara Prefecture, Japan	*Castanopsis* spp.	Lowlands
G. frondosa	WC835	Hokken M-1	Japan	Commercial isolate on oak	Highlands
G. frondosa	WC836	Mori 51	Japan	Commercial isolate on oak	Highlands
G. frondosa	M001	USDA FP-101988-T	Cooksville (Rock), WI	Soil near downed hardwood log (*Quercus*?)	Edge of Old Mill Pond at Hwy 59, Fire #638
G. frondosa	M002	USDA FP-102464-Sp	Madison, WI	*Quercus*, soil at base of dead stump.	N/A
G. frondosa	M003	USDA FP-102464-T	Madison(Dane), WI	*Quercus*, soil at base of dead stump.	Picnic Point, UW campus
G. frondosa	M004	USDA FP-103424-T	Athens(Georgia), GA	*Quercus nigra*, at base	N/A
G. frondosa	M005	USDA FP-105867-Sp	Beltsville (Prince George), MD	*Quercus coccinea* (scarlet oak) living, at base	Forest Disease Lab Station, Agr. Res. Center
G. frondosa	M006	USDA FP-134675-Sp	Madison (Dane), WI	*Quercus*, underneath	UW Arboretum
G. frondosa	M007	USDA FP-47462	WV	*Quercus alba* (white oak)	Plat #55, at 1500 feet elevation, Devil's Hole
G. frondosa	M008	USDA RLG-6889-Sp	Syracuse, NY	*Quercus alba*	N/A
G. frondosa	M009	USDA LOO-14980-T	LA	*Quercus* snag, inside of hollow (oak)	N/A
G. frondosa	M010	USDA OKM-4954-T	Beltsville (Prince George), MD	N/A	Ground, Beltsville Expt Forest
G. frondosa	M011	USDA OKM-6133-Sp	Washington(District of Columbia), DC	N/A	Rock Creek Park
G. frondosa	M012	USDA RLG-14995-T	Baton Rouge, LA	*Quercus virginiana*	Memorial Grove, LA State U campus
G. frondosa	M013	USDA L-15552-Sp	Syracuse, NY	N/A	N/A
G. frondosa	M014	USDA RLG-6889-T	Syracuse, NY	*Quercus alba*	Oakwood Cemetery
G. frondosa	M015	USDA TJV-93-130-T	Madison(Dane), WI	*Quercus macrocarpa*, base of live	Turville Pt. Woods
G. frondosa	M016	FIRDI 36283	Taiwan	N/A	N/A
G. frondosa	M017	FIRDI 36286	Taiwan	N/A	N/A
G. frondosa	M018	FIRDI 36355	Taiwan	N/A	N/A
G. frondosa	M019	FIRDI 36356	Taiwan	N/A	N/A
G. frondosa	M020	FIRDI 36357	Taiwan	N/A	N/A
G. frondosa	M021	FIRDI 36434	Taiwan	N/A	N/A
G. frondosa	M029	PSUMCC 600	Taiwan	Commercial strain	N/A
G. frondosa	M030	PSUMCC 601	Taiwan	Commercial strain	N/A
G. frondosa	M031	PSUMCC 602	Taiwan	Commercial strain	N/A
G. frondosa	M032	PSUMCC 604	Taiwan	Commercial strain	N/A
G. frondosa	M033	PSUMCC 630	Taiwan	Commercial strain	N/A
G. frondosa	M034	PSUMCC 644	Taiwan	Commercial strain	N/A

Species	Isolate code	Source [a]	Geographic origin	Host/Substrate	Locality
G. frondosa	M035	USDA RLG-6889-Sp	Syracuse, NY	*Quercus alba*	Oakwood Cemetery
G. frondosa	M036	X.W.Chen	China	N/A	N/A
G. frondosa	M037	X. W.Chen	China	N/A	N/A
G. frondosa	M038	ATCC 60891	China	N/A	N/A
G. frondosa	M039	Tan 0206	He Bei, China	N/A	N/A
G. frondosa	M40	M. Chen	China	Commercial strain	N/A
G. sordulenta	G01	ATCC 200416	Argentina	*Nothofagus dombeyi* trunk	N/A
P. umbellatus [c]	G02	ATCC 60546	N/A	N/A	N/A
M. giganteus [d]	G06	USDA FP-135344-Sp	England	Carpinus spp.	Virginia Waters
Ganoderma lucidium	WC724	T. Mitchel	PSU, PA		Lawn PSU Forest

[a] ATCC = American Type Culture Collection; USDA = The Unitied States Department of Agriculture; FIRDI = Food Industry Research and Development Institute, Taiwan; PSUMCC = Pennsylvania State University Mushroom Culture Collection.
[b] N/A = not available.
[c] *Polyporus umbellatus*, synonym of *Grifola umbellata*.
[d] *Meripilus giganteus*, synonym of *Grifola giganteus* and *Polyporus giganteus*.

3.2.2 DNA extraction

Cultures were grown in 50 ml of potato dextrose yeast broth (PDYB) for 20 to 30 days at room temperature. Mycelium was harvested by vacuum filtration on Whatman (grade #1) filter paper, and washed once with distilled water. Fresh mycelium (100 mg) was used to isolate DNA following the LETS extraction procedure (Chen *et al*. 1999). DNA preparations were diluted with sterile water and used as template for PCR amplification.

3.2.3 PCR amplification and sequencing

PCR was performed in 25 µl reactions with a 96-well PCR cycler (PTC-100 Programmable Thermal Controller, MJ Research, Inc.), using 10mg DNA template, one Unit of *Taq* DNA polymerase (Promega, Madison, WI), 0.2 mM of each dNTP, 2 mM $MgCl_{2,}$ 0.1% Triton, as well as 0.5 µM of each primer. Amplification of ITS-1, ITS-2, and 5.8 S rDNA was performed for all isolates utilizing primers ITS1AF (5'-TCCGTAGGTGAACCTGCGG-3') (White *et al.* 1990)

and ALR0 (5'-CATATGCTTAAGTTCAGCGGG-3') (Figure 3.1). PCR reactions for ITS regions were performed with the following parameters: 94°C/1 min; 35 cycles of 94°C/15 s, 60°C/30 s, 72°C/1 min; and 72°C/5 min. PCR Reactions for β-tubulin regions were performed with two primer pairs BTG5F/BTG8R and BT1.1/B34R (Figure 3.2) with the following parameters: 94°C/2 min; 35 cycles of 94°C/15 s, 57°C/30 s, 72°C/1 min; and 72°C/7 min. Primers BTG5F (5'-CGTTGTGCCCAGTCCTAAGGTG-3') and BTG8R (5'-GTTCTTGCTCTGCACGTTCTG-3') were used to amplify 51 isolates of *G. frondosa*, one isolate of *G. sordulenta* (G01), and one isolate of *Polyporus umbellatus* (G02). Isolate of *Ganoderma lucidium* were amplified using primers BT1.1 and B34R. Reactions were optimized by adjusting the concentration of template DNA and other reagents, and primer annealing temperature. Amplification products were electrophoresed on a 1.0% agarose gel and checked to ensure that a single DNA band was produced of the expected size (~600bp for ITS PCR products and ~680bp for β-tubulin PCR products). For sequencing, the PCR products were purified directly from reactions using the wizard PCR Preps System (Promega Corp., Madison, WI) with the concentration adjusted to 20ng/μl. Sequencing reactions were performed using the PCR primers and an ABI dye-terminator kit (ABI/Perkin-Elmer) and resolved using an ABI Prism® Model 377 automated sequencing system (Applied Biosystems, Foster City, CA).

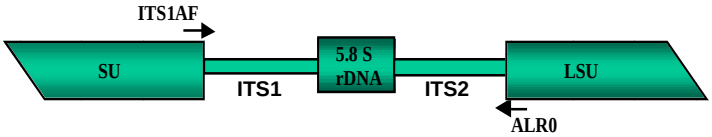


Figure 3.1. Locations of the primers for amplification of ITS-1, ITS-2 and 5.8s rDNA gene regions of the rDNA repeat.

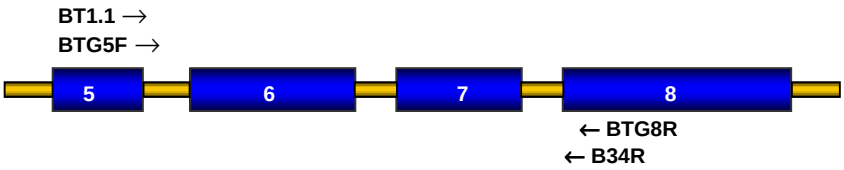


Figure 3.2. Locations of primers used for PCR-amplification of the β-tubulin gene in *Grifola frondosa, G. sordulenta* (G01), *Polyporus umbellatus* (G02) and *Ganoderma lucidium*. Numbers indicate exons.

3.2.4 Sequence data analysis

Sequence ends were trimmed using the SeqMan II module in the Lasergene package (DNAStar, Inc. Madison, WI) and adjusted manually. All sequences then were edited and initially aligned using the clustal W algorithm (Higgins *et al.* 1991) in the Lasergene package (DNAStar, Inc. Madison, WI). Multiple alignment parameters used were gap penalty = 10 and gap length penalty = 10. Final alignments then were optimized visually.

Phylogenetic analyses were completed using PAUP Version 4.0b4a (Swofford 2000). A neighbor-joining (NJ) tree was constructed using the Kimura 2-parameter model. The stability of clades was evaluated by bootstrap tests with 1000 replications (Felsenstein 1985, Hills and Bull 1993). A maximum parsimony (MP) analysis was performed using heuristic searches with 1000 random addition searches. Other indices for the generated topology, including tree length, a consistency index (CI), as well as retention index (RI) were calculated. A strict consensus of the minimum length MP trees was calculated.

The partition homogeneity test (PHT) option in PAUP was used to determine whether the ITS and β-tubulin datasets are in conflict, using only

phylogenetically informative characters, with 1000 replicates. Gaps were considered missing data for all analyses. The combined data were used to find a consensus phylogeny. *Ganoderma lucidium* was chosen as an outgroup because the available partial *Grifola* ribosomal RNA large subunit sequence (Hibbett and Vilgalys 1992) indicated that *Grifola* is a member of the polyporoid clade, and that it is particularly closely related to *Ganoderma/Lentinus* clade (D. Geiser, personal communication, Hibbett *et al.* 1997).

3.3 Results

3.3.1 Phylogenetic relationship within *Grifola frondosa*

3.3.1.1 Analysis of ITS sequence data

Amplification of the ITS-1, ITS-2 and 5.8S ribosomal DNA repeat yielded fragments of approximately 600 bp as estimated by agarose gel electrophoresis. An alignments (Appendix A) including sequences of the isolate of *G. sordulenta* and all isolates of *Grifola frondosa* was generated. *Grifola sordulenta* was used as an outgroup in the phylogenetic analysis.

A Neighbor-Joining tree (Figure 3.3) was generated based on the alignment. Two major clades were identified within *G. frondosa*. Clade I included all of the U.S. isolates, while Clade II consisted of Asian isolates. The European isolate (WC493) was grouped with the U.S. isolates. Within the Asian clade, most of the isolates from China (M037, M038 and M039) and Japan (WC834, WC835 and WC835) tended to group together, while isolates from Taiwan and Korea were dispersed throughout this clade. Two Taiwan isolates (M019, M021) were distinct from all other Asian isolates and formed a subgroup. Two isolates of unknown origin (WC828 and WC685) were located in the Asian clade. This indicated that the major commercial isolate WC828 used on most

mushroom farms in the United States is probably of Asian decent. Within the U.S. clade, isolates did not show any distribution pattern related to their geographic state of origin.

The maximum parsimony (MP) analysis produced 424 equally parsimonious trees (length = 113 steps, consistency index=0.796, retention index=0.911). The MP tree (Figure 3.4) retained a similar topology for most of the NJ trees.

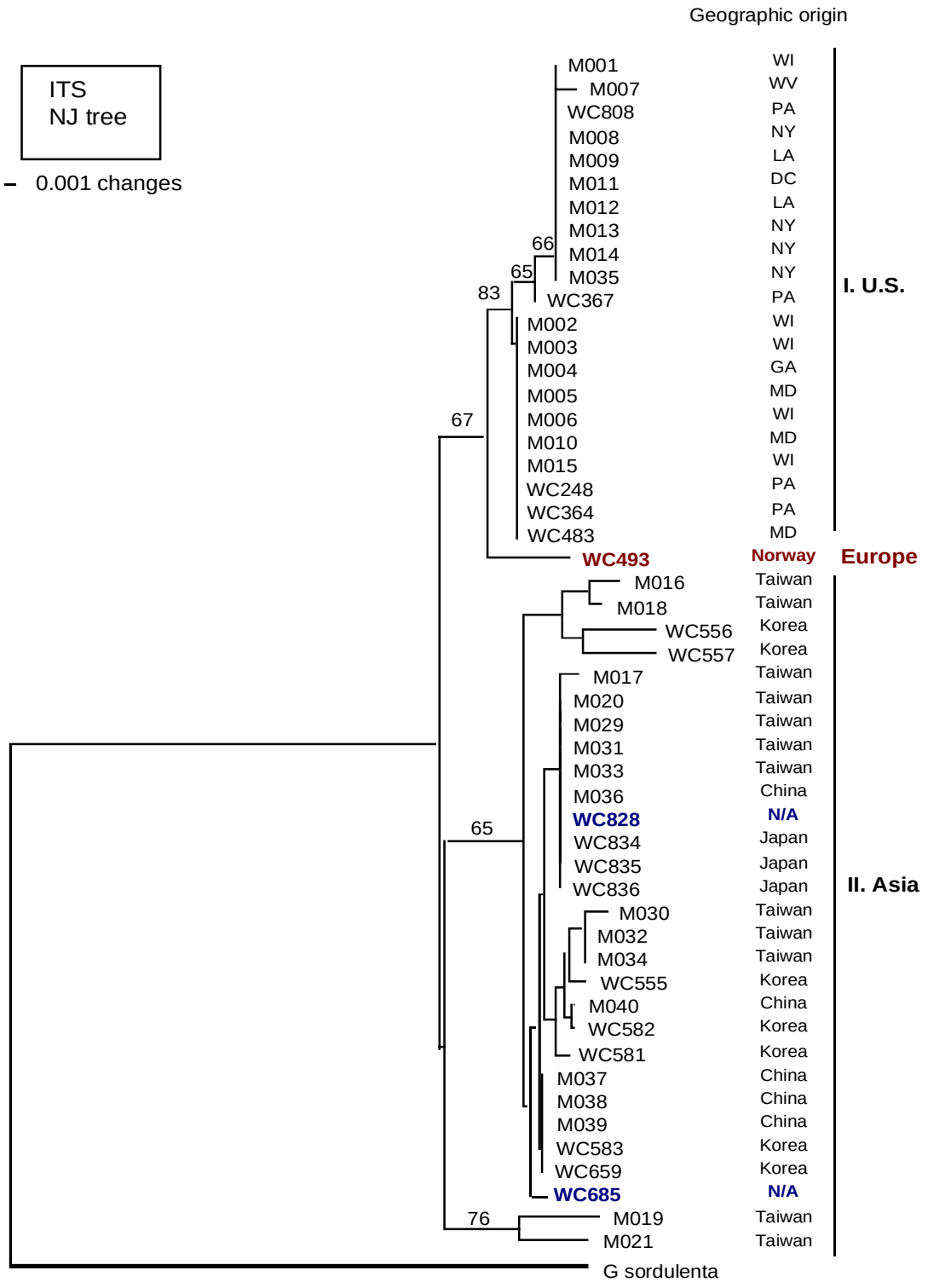


Figure 3.3. Phylogenetic analysis of 51 *Grifola frondosa* isolates based on rDNA ITS sequences using the neighbor-joining method with distance analysis calculated by the Kimura 2-parameter model. Geographic origin is listed beside isolate codes. Numbers on branches represent bootstrap values obtained from 1,000 replications (values greater than 60% were shown). Sidebars represent inferred clades based on geographic origin.

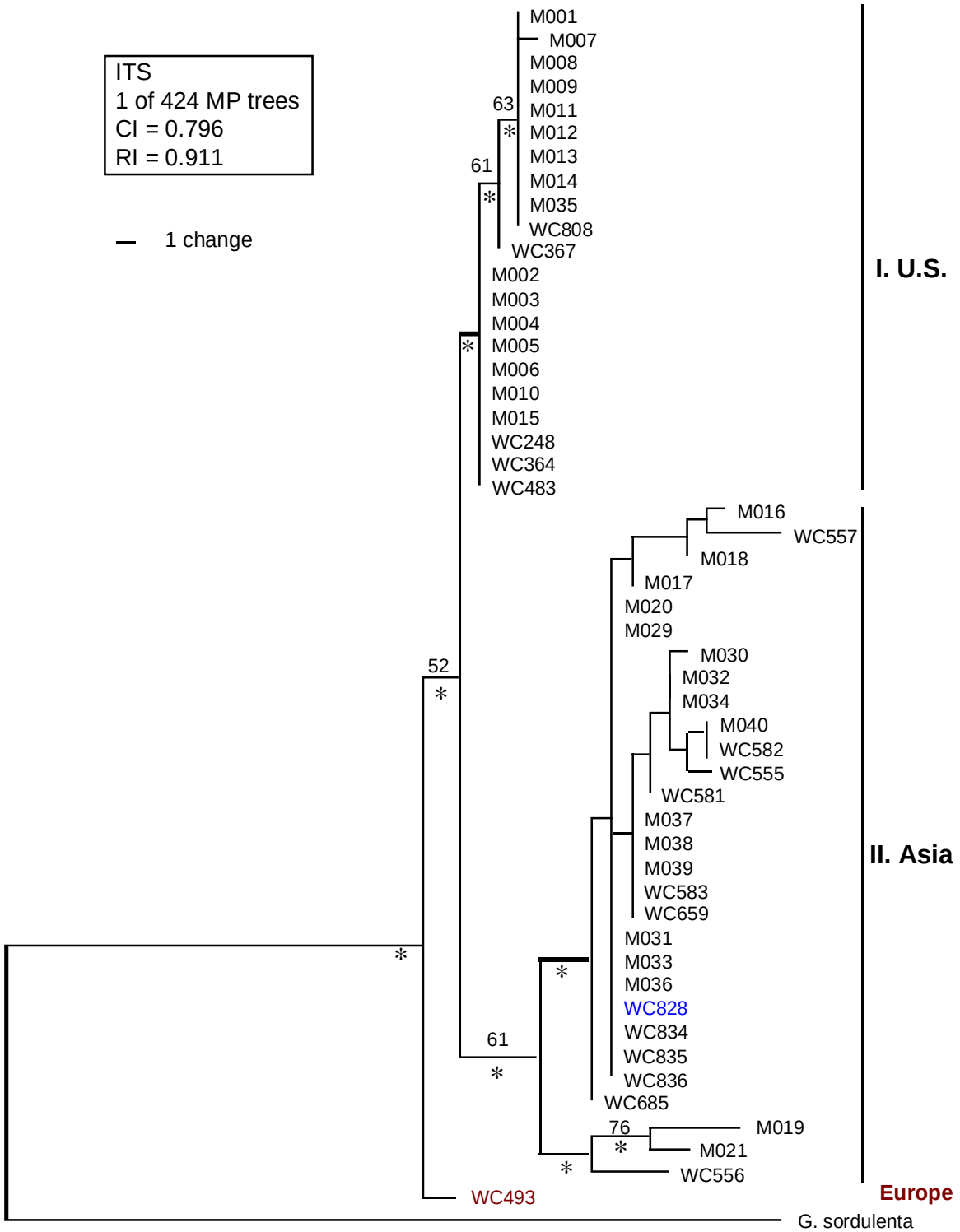


Figure 3.4. Maximum parsimony (MP) analysis of rDNA ITS sequences of *Grifola frondosa* isolates. One of 424 MP trees is shown. Braches with asterisks are maintained in the strict consensus tree. Numbers on the branches represent bootstrap values obtained from 1,000 replications (only values greater than 50% are shown). Sidebars represent inferred clades based on geographic origin.

3.3.1.2 Analysis of partial β-tubulin sequence data

Amplicons (680bp) of the partial β-tubulin sequences contained 40bp of the 3'-end of exons 5, 6 and 7, and 72bp of the 5'-end of exon 8 and introns 5, 6, and 7. These positions were identified based on their reported locations in *Schizophyllum commune* β-tubulin gene sequence and the identity of GT- -AG splice junctions for introns. Isolate of *Meripilus giganteus* were not included in the analysis because of unsuccessful sequences.

The phylogenetic relationships of *Grifola frondosa* isolates were examined by aligning a sequence of *G. sordulenta* and sequences from 51 isolates of *G. frondosa*. The alignment (Appendix D) started 4bp of the 3'-end of exon 5 to 8bp of the 5'-end to exon 8 of the partial β-tubulin gene sequence. *Grifola sordulenta* was used as an outgroup in the analysis.

A Neighbor-Joining tree (Figure 3.5) was generated based on partial β-tubulin gene sequences. Two distinct clades, with strong bootstrap support (99% and 74%), were identified within *G. frondosa.* Clade I included all of the U.S. isolates, while clade II consisted of Asian isolates. The European isolate (WC493) was grouped within the Asian lineage with 95% bootstrap support. Two isolates of unknown origin (WC828 and WC685) were grouped with the Asian isolates. In the Asian clade, isolate WC828, used for commercial cultivation, was closely related to Japanese isolate WC835 with 78% bootstrap support. Other strong bootstrap-supported clusters included 3 Korean isolates (WC556, WC557 and WC582) and 3 Taiwan isolates (M019, M020 and M021). A Korean isolate, WC555 constituted a unique branch in this clade. In the U.S. clade, 3 isolates (M007, M101 and WC248) formed a sister group with other U.S. isolates (94% bootstrap support).

The maximum parsimony (MP) analysis based on partial β-tubulin gene sequence alignment (Appendix D) produced 48,100 trees until PAUP was aborted because of lack of memory. One MP tree (length=251 steps, consistency index=0.793, retention index=0.932) from 5,000 equally parsimonious trees is shown in Figure 3.6. Despite the large number of equally parsimonious trees, the strict consensus tree was highly resolved.

Similar to the NJ tree, two clades (U.S. and Asian clades) were observed within isolates of *G. frondosa* with strong bootstrap support (97% and 91%, respectively). The European isolate (WC493) formed a collapsed branch within the Asian clade and the commercially-used isolate (WC828) of unknown origin grouped with the Asian isolates. The MP tree also showed the same subgroups in the U.S. and Asian clades as did the NJ tree.

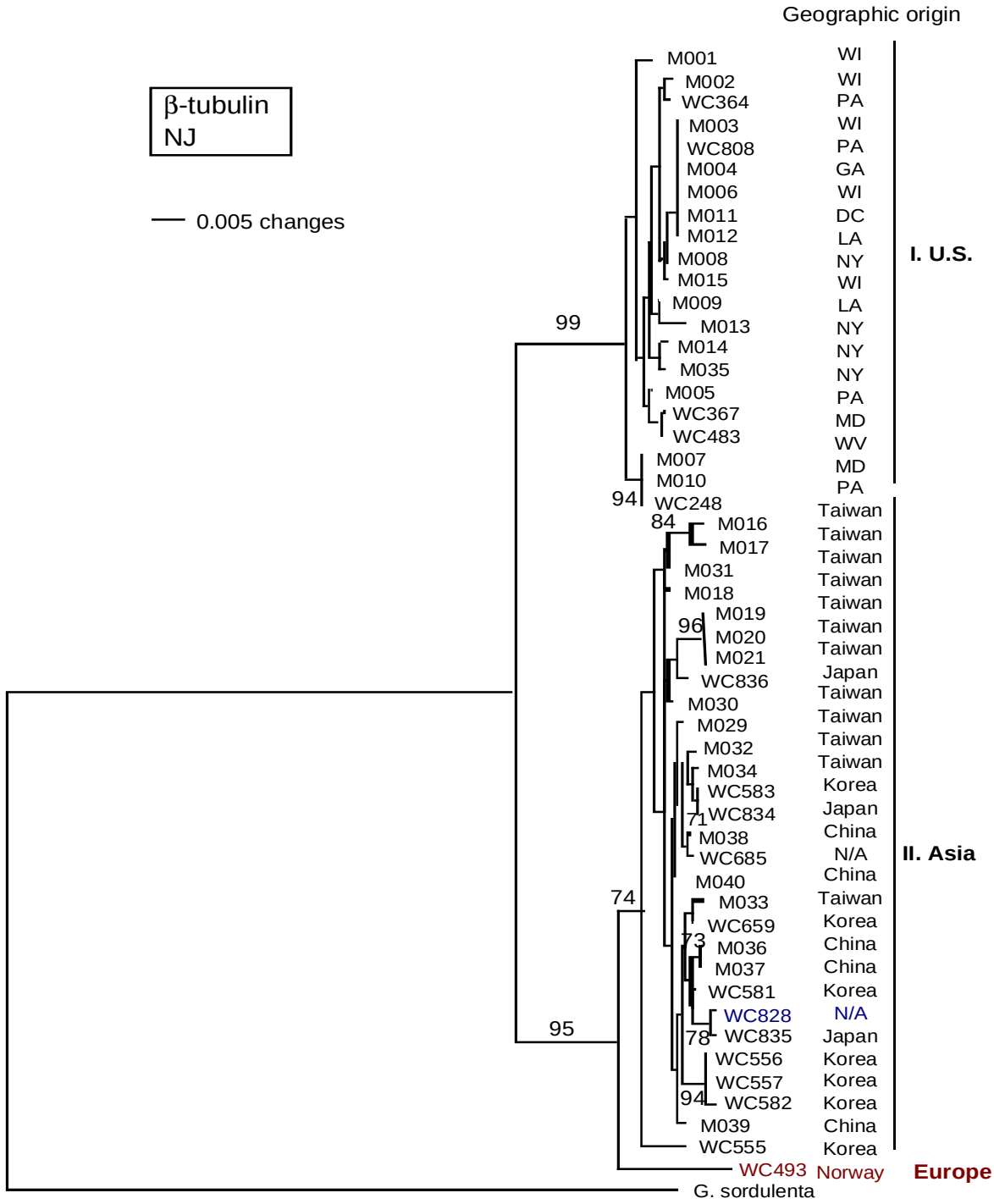


Figure 3.5. Phylogenetic analysis of 51 *Grifola frondosa* isolates based on partial β-tubulin gene sequences using the neighbor-joining method with distance analysis calculated by the Kimura 2-parameter model. Geographic origin is shown beside isolate codes. Numbers on branches represent bootstrap values obtained from 1,000 replications (values greater than 70% were shown). Sidebars represent inferred clades based on geographic origin.

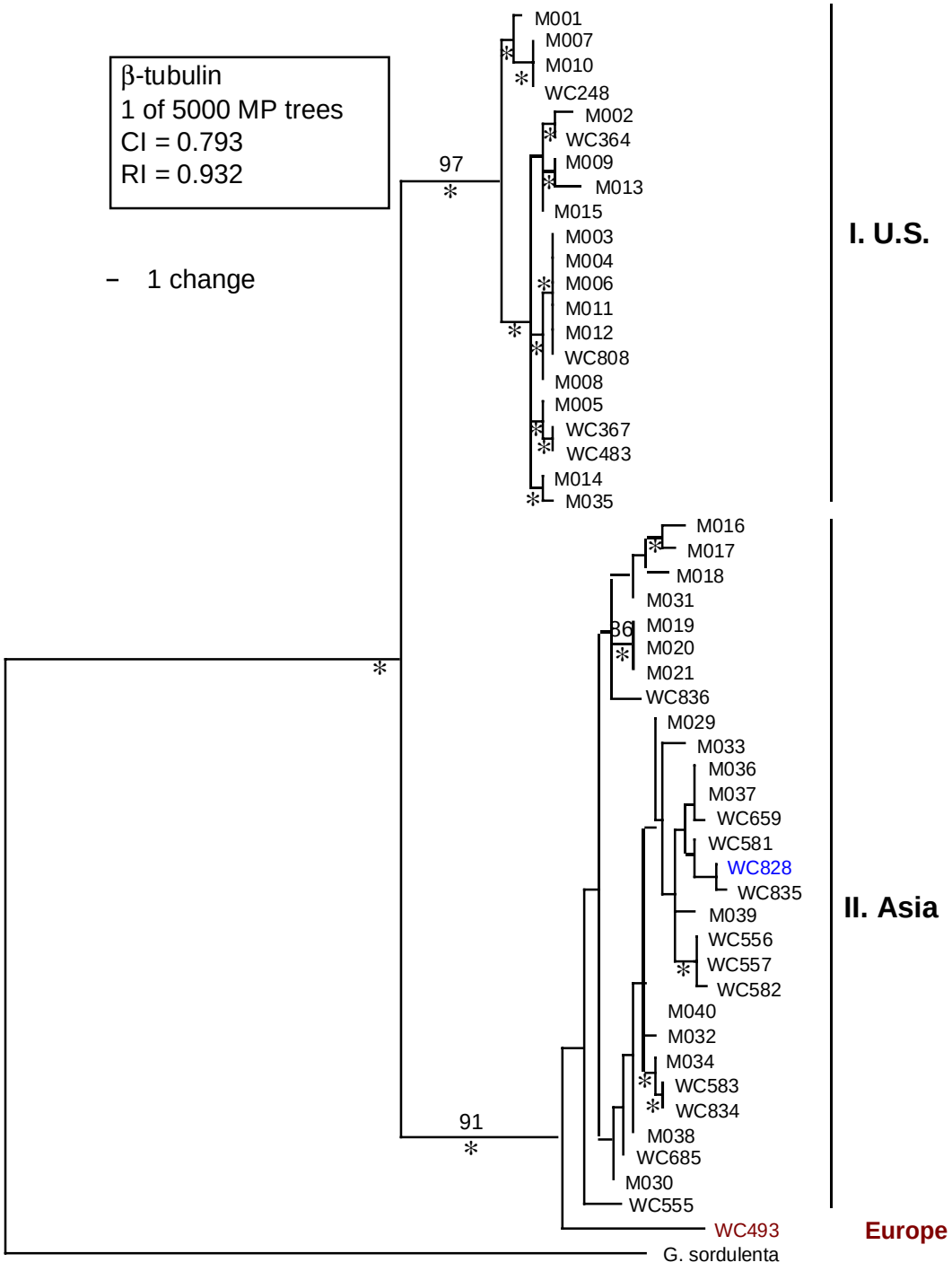


Figure 3.6. Maximum parsimony (MP) analysis of partial β-tubulin gene sequences of 51 *Grifola frondosa* isolates. One of 5,000 equally parsimonious trees is shown. Braches with asterisks maintain in the strict consensus tree. Numbers on the branches represent bootstrap values obtained from 1,000 replications (only values greater than 70% are shown). Sidebars represent inferred clades based on geographic origin.

3.3.1.3 Analysis of combined rDNA and partial β-tubulin gene sequence data

A DNA sequence alignment was generated by combining both datasets of rDNA and partial β-tubulin gene sequences. A Neighbor-Joining tree (Figure 3.7) was generated based on the combined data. It supported most of the results produced by rDNA and β-tubulin separately. Two independent clades - the U.S. and Asian clades were strongly supported by high bootstrap values (100% and 98%, respectively). The European isolate (WC493) was grouped in the Asian clade with 89% bootstrap support, and agrees with the results from the β-tubulin data alone. Two of the unknown origin isolates (WC828 and WC685) were grouped in the Asian clade. Isolate WC828 was observed closely related to Japanese isolate WC835 with 82% bootstrap support. Two Taiwanese isolates (M019, M021) grouped strongly and distinct from other Asian isolates. In the U.S. clade, 3 isolates (M007, M010 and WC248) clustered together.

The maximum parsimony (MP) analysis, based on combined dataset produced 405 equally parsimonious trees. One MP tree (length=382 steps, consistency index=0.741, retention index=0.901) is shown in Figure 3.8. It retained a similar topology, for the most part, to the NJ trees. Two clades, that separated U.S. and Asian isolates, were observed with strong bootstrap support.

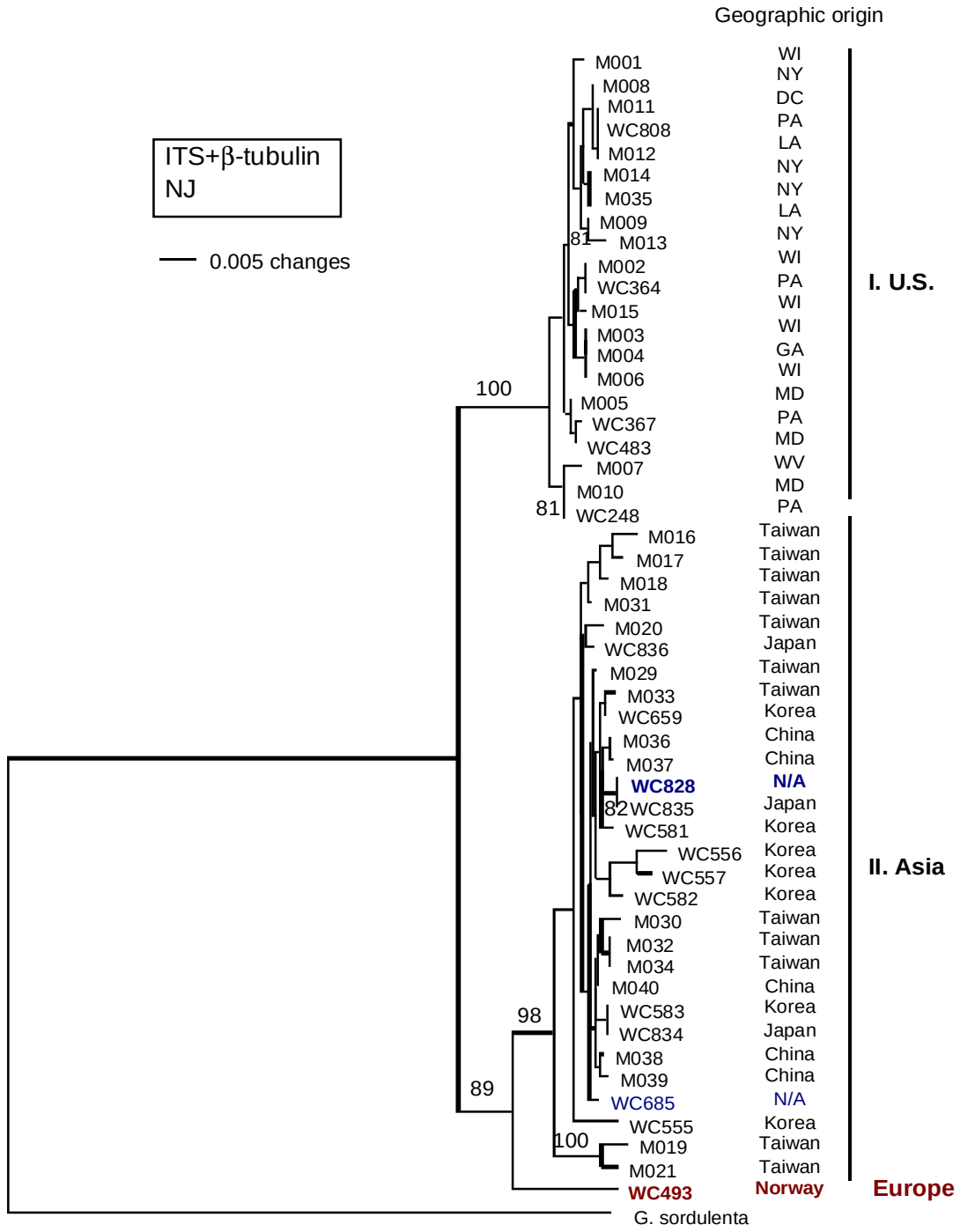


Figure 3.7. Phylogenetic analysis of 51 *Grifola frondosa* isolates based on a combination of rDNA and partial β-tubulin gene sequences using the neighbor-joining method with distance analysis calculated by the Kimura 2-parameter model. Geographic origin is shown beside isolate codes. Numbers on branches represent bootstrap values obtained from 1,000 replications (values greater than 80% were shown). Sidebars represent inferred clades based on geographic origin.

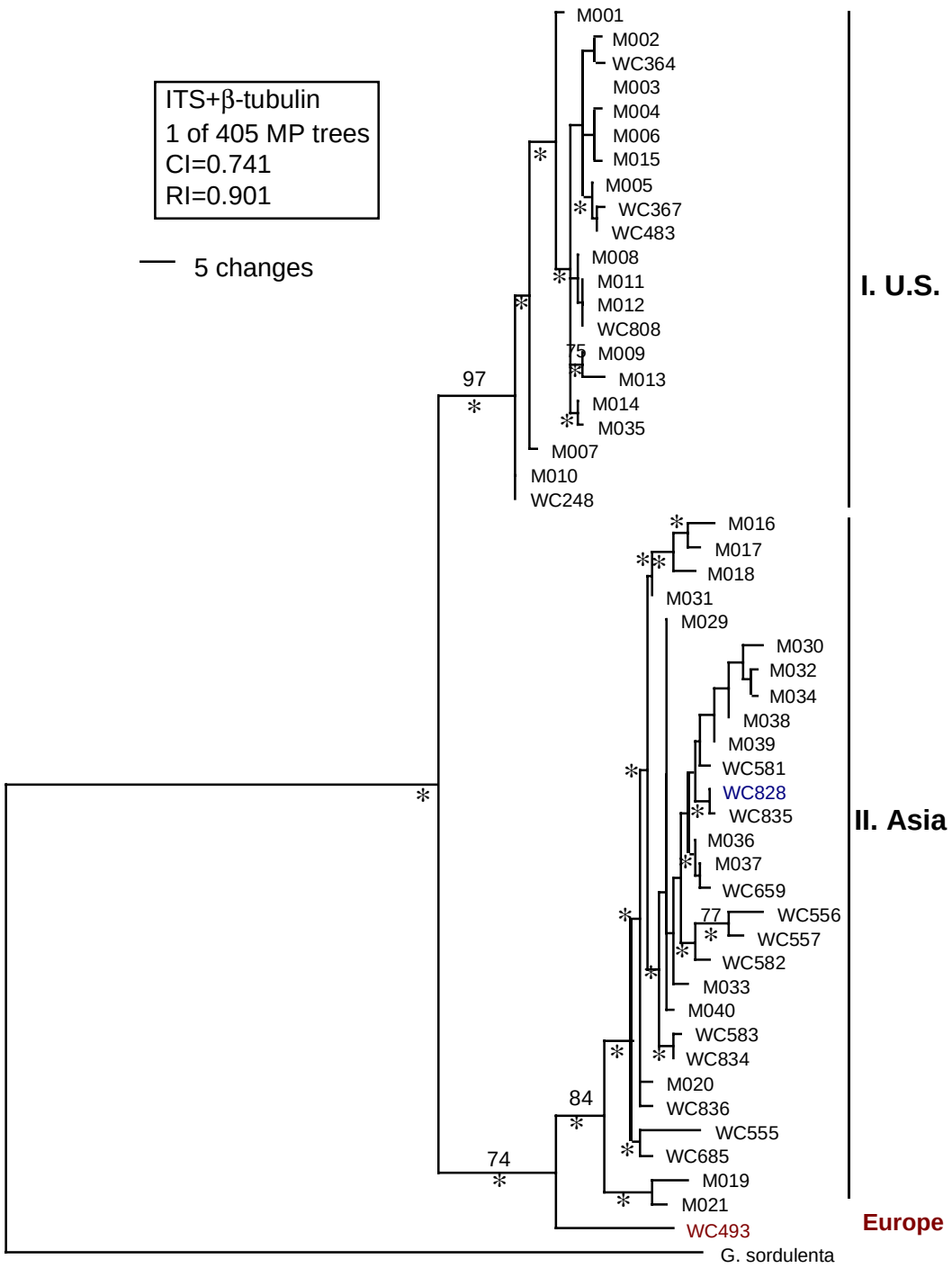


Figure 3.8. Maximum parsimony (MP) analysis of combined rDNA and partial β-tubulin gene sequences of 51 *Grifola frondosa* isolates. One of 405 equally parsimonious trees is shown. Braches with asterisks are maintained in the strict consensus tree. Numbers on the branches represent bootstrap values obtained from 1,000 replications (only values greater than 70% are shown). Sidebars represent inferred clades based on geographic origin.

3.3.2 Phylogenetic relationship among *Grifola frondosa* and allies

The phylogenetic relationship among *G. frondosa* and its allies was analyzed by aligning partial β-tubulin gene sequences alone. An unambiguous alignment was not achieved because of the high nucleotide variation in ITS regions between *G. frondosa* and its allies (Chapter 2, this thesis). The alignment (Appendix E) included exons 6 and 7 of β-tubulin gene sequences of *Ganoderma lucidium*, eight isolates of *G. frondosa* (M009, M004, WC493, WC659, M030, WC835, WC828 and M037; all with different geographic origins) and one isolate each of *G. sordulenta* (G01) and *Polyporus umbellatus* (G02). *Ganoderma lucidium* was included for rooting purposes. Introns were excluded from the analysis because of an ambiguous alignment.

Both neighbor joining (NJ) and maximum parsimony (MP) analyses revealed a distinct *G. frondosa* clade with strong bootstrap support (100%). A NJ tree is shown in Figure 3.9. MP analysis produced 4 trees. One MP tree (length=155 steps, consistency index=0.845, retention index=0.704) is shown in Figure 3.10. Isolate *P. umbellatus* G02 showed a closer relationship with *G. frondosa* than with isolate *G. sordulenta* (G01). Based on these data, it appears that *P. umbellatus* and *G. sordulenta* share a common ancestor with *G. frondosa* and should be grouped into *Grifola*.

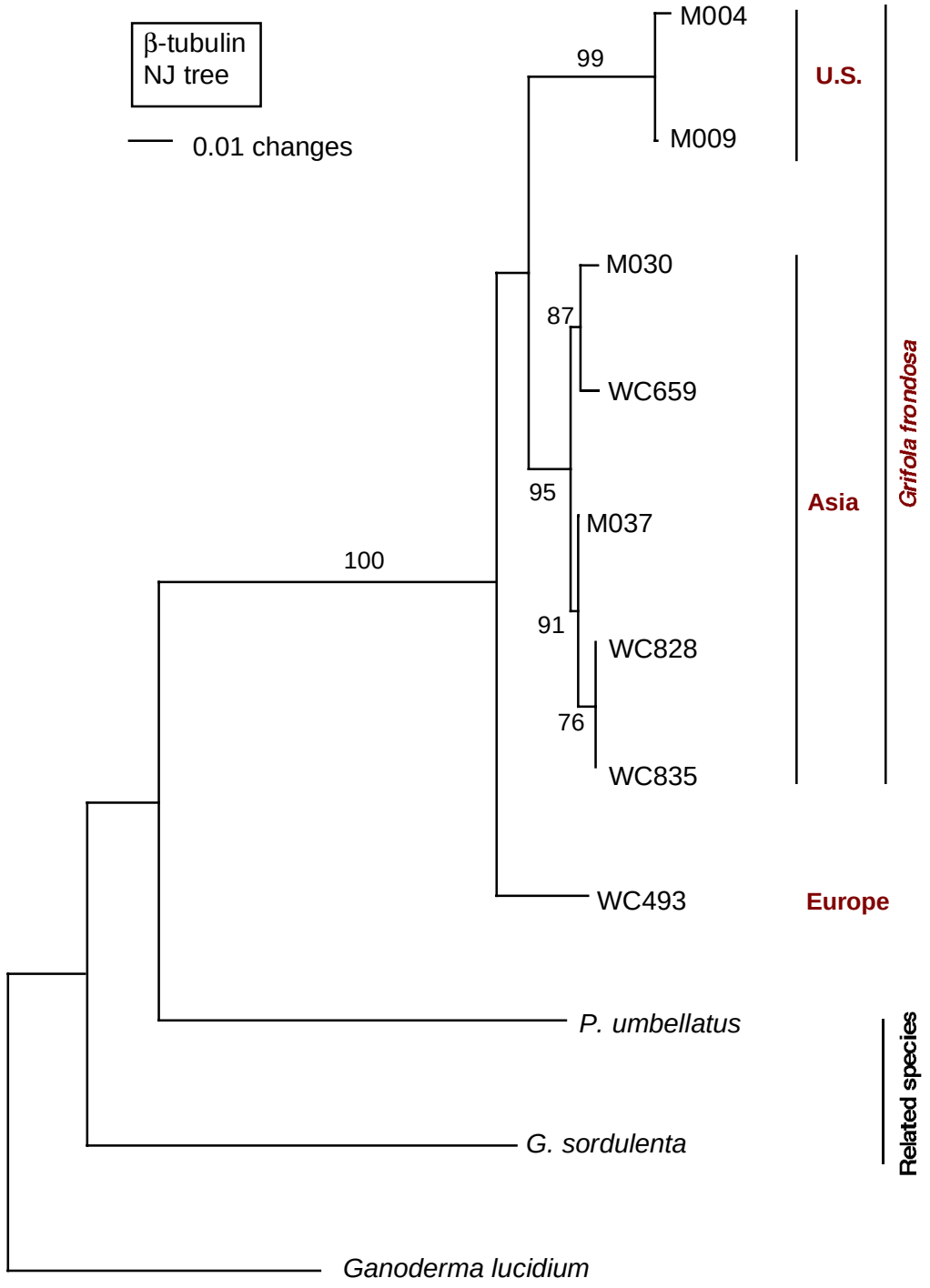


Figure 3.9. Phylogenetic analysis of *Grifola frondosa* and its allies based on partial β-tubulin gene sequences using the neighbor-joining method with distance analysis calculated by the Kimura 2-parameter model. Numbers on branches represent bootstrap values obtained from 1,000 replications (values greater than 70% were shown). Sidebars represent inferred clades based on geographic origin.

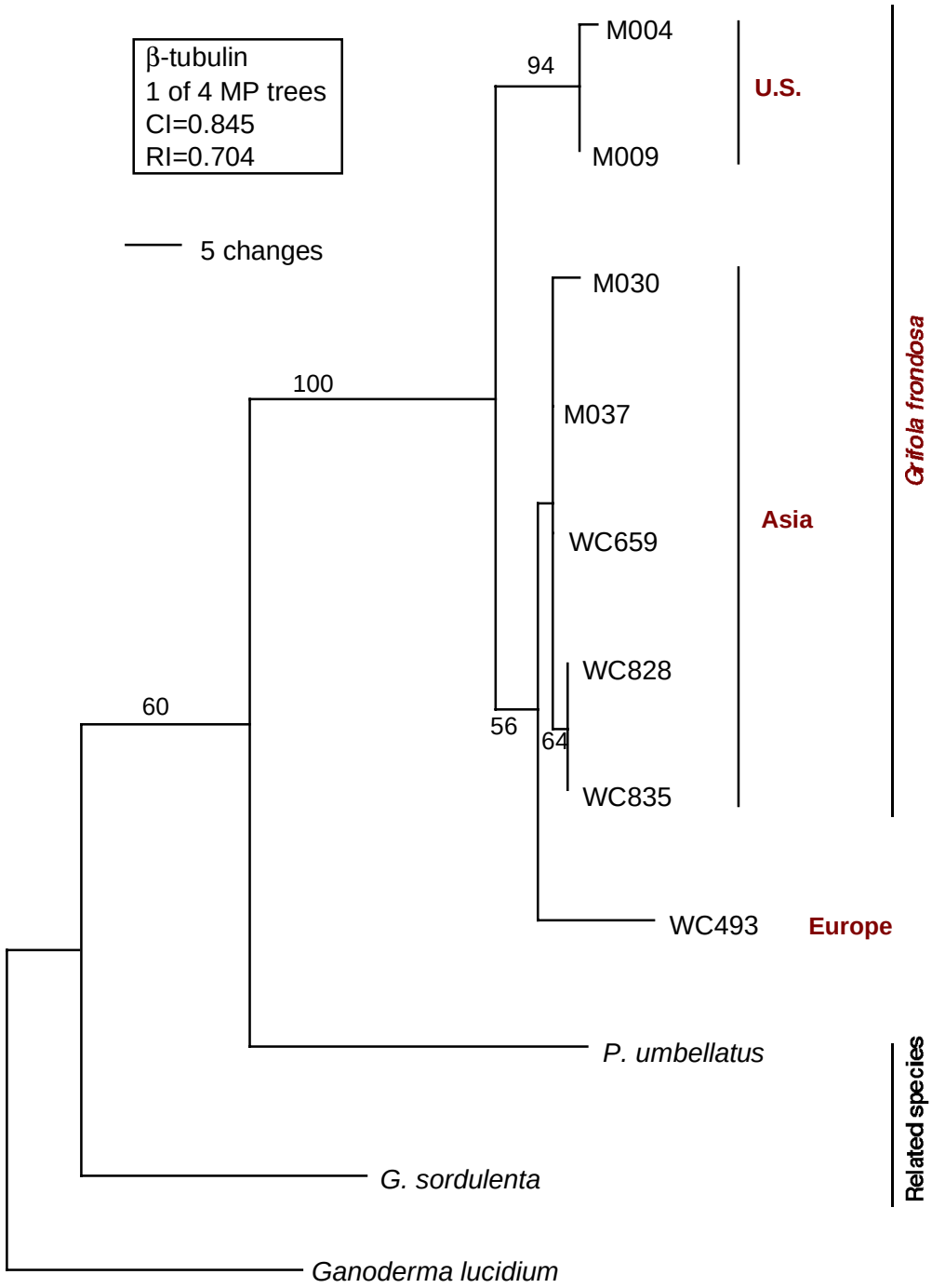


Figure 3.10. Maximum parsimony (MP) analysis of partial β-tubulin gene sequences of eight *Grifola frondosa* isolates and its allies. One of four equally parsimonious trees is shown. Numbers on the branches represent bootstrap values obtained from 1,000 replications (only values greater than 50% are shown). Sidebars represent inferred clades based on geographic origin.

3.4 Discussion

3.4.1 Relationships within *Grifola frondosa*

Isolates of *G. frondosa* (51) used in this study clearly clustered into two clades (U.S. and Asian clade) in both NJ and MP analysis based on either rDNA, β-tubulin, or combined sequence analyses. Two clades were well supported by the bootstrap test in both β-tubulin and combined datasets. Based on combined data, the NJ analysis showed 100% bootstrap support for the U.S. clade and 89% for the Asian clade (Figure 3.7). The MP analysis supported the U.S. clade with 97% and the Asian clade with 84% bootstrap values (Figure 3.8). A partition homogeneity test (PHT) (Farris *et al*. 1995, Huelsenbeck *et al*. 1996), which was used to determine whether the rDNA and partial β-tubulin gene sequence data were in significant conflict, was not successful because PAUP ran out of memory. Although the PHT test was not performed, the strong bootstrap results suggest that U.S. and Asian clades are distinct groups and may be evolving independently.

Previous taxonomic studies on *G. frondosa* were mostly based on isolates collected by authors from certain geographic areas. For example, Zhao and Zhang (1992) examined only Chinese isolates, while Gilbertson and Ryvarden (1986) examined North American isolates. No morphological differences were found according to the character descriptions in their monographs, which include basidiome, basidiospores, habitat and context hyphal system. No mating tests between the U.S. and Asian isolates have been conducted to determine if they are different biological species. Using a molecular phylogenetic species concept potentially would allow recognition of changes in gene sequences long before changes in mating behavior or morphology became evident (Taylor *et al.* 2000).

Previously, *Grifola frondosa* was primarily found in Europe and was named *Boletus frondosus* Dicks. (Dickson 1785). Later, *Polyporus frondosus* (Dicks.) Fr. (Fries 1821) and *G. frondosa* (Dicks.: Fr.) S.F. Gray (Gray 1821) were identified (both based on European isolates). However, the phylogenetic relationship of the single European isolate (WC493) to other isolates is not clearly resolved. Based on combined ITS and β-tubulin and β-tubulin sequence data, both NJ and MP analysis showed WC493 shared a common ancestor with Asian isolates with high bootstrap support. However, rDNA sequence data did not support this result and NJ analysis indicated that WC493 has a closer relationship with the U.S. isolates (Figure 3.3). This grouping was not strongly supported by bootstrap analysis (67%), however.

3.4.2 Relationships between *Grifola frondosa* and its allies

Traditional taxonomic studies (Gilbertson and Ryvarden 1986, Zhao and Zhang 1992) show that *Grifola sordulenta, P. umbellatus* and *M. giganteus* share many common characters and suggest that they are very closely related species (Table 3.2). However, the influence of environmental conditions on morphological characteristics of mushroom-forming fungi is usually significant and often makes identification difficult. Figure 3.11 shows the basidiome formed by *M. giganteus* under our cultivation conditions. Apparently, our environmental conditions were different than the conditions found in the wild and produced a totally different mushroom. Unfortunately, *P. umbellatus* G02 and *G. sordulenta* did not fruit in our cultivation rooms.

Figure 3.11. Basidiomes of *M. giganteus* formed on sawdust substrate.

Table 3.2. Morphological characters or traits of *Grifola frondosa*, *Meripilus giganteus*, *Polyporus umbellatus* and *Grifola sordulenta*.

Character or Trait	*G. frondosa*	*M. giganteus*	*P. umbellatus*	*G. sordulenta*
Stipe	lateral branched	lateral branched	central branched	Lateral or central branched
Spore	smooth	smooth	smooth, larger and more cylindrically shaped	smooth, subglobose to short ellipsoid or ellipsoid
Generative hyphae	clamp connections	simple septate	clamp connections	clamp connections
Hyphal system	dimitic with skeletal hypha	monomitic	dimitic with binding hypha	not available
Rot Type	white			
Basidiome	stipitate, profusely branched with many petaloid pilei			
Color of context or pore surface	white-pale brown			
Hymenophore	poroid			

Source: Gilbertson and Ryvarden (1987), Singer (1969).

Recent molecular evidence based on nuclear small subunit (nuc-ssu) rDNA, nuclear large subunit (nuc-lsu) rDNA and mitochondrial small subunit (mt-ssu) rDNA (Hibbett 2000) showed that *G. frondosa* has a closer phylogenetic relationship with other members of the *Polyporaceae*, such as *Laetiporus porteutosus* than with *M. giganteus*. No previous reports have centered on the evolutionary history of *P. umbellatus* and *G. sordulenta.* My results suggest that this relationship still is unresolved. Based only on β-tubulin gene dataset, *P. umbellatus* was grouped within the *Grifola* clade. However, no strong bootstrap values supported this relationship. *Meripilus giganteus* was not included in the phylogenetic analysis bacause of the high nucleotide variation in ITS and β-tubulin gene between it and *G. frondosa.* More sampling of isolates and the use of more conservative genes may help to better understand the phylogenetic relationships between *G. frondosa* and its allies.

In view of medicinal qualities of these mushrooms, there are many similarities between *G. frondosa*, *P. umbellatus* and *M. giganteus. Polyporus umbellatus* and *M . giganteus* are called tonbimaitake and choreimaitake,

respectively, in Japan (Mizuno and Zhuang 1995). They are edible (Arora 1986, Bessette *et al.* 1997) and have been documented for use as Chinese medicine 2000 years ago (Ying *et al.* 1987). Similar to *G. frondosa,* both are known to produce antitumor activity against cancer when ingested orally. Their medicinal qualities have been utilized in Chinese medicine from ancient times (Jong and Birmingham 1990, Ying *et al.* 1987). A clearer understanding of the evolutionary relationships of these fungi may be useful in broadening the search for additional anti-cancer substances.

3.4.3 Molecular phylogenetic analysis based on combined gene sequences

Use of a single gene to construct phylogenetic trees may not result in true representation of the phylogeny of the taxa under study (Nei 1987). To help alleviate this problem, more than one gene may be used to reconstruct a phylogeny (Li 1997). In this study, rDNA and β-tubulin were used. The characteristics of the rDNA, β-tubulin and combined regions summarized in Table 3.3. When the relationship within isolates of *G. frondosa* was analyzed, 87 phylogenetically informative nucleotide sites in a combined sequence (25 in rDNA and 62 in β-tubulin) were used (Table 3.3). The total number of sites of rDNA (574) and β-tubulin (587) were similar. However, β-tubulin has more variable sites (177) than rDNA (82). β-tubulin also has more phylogenetically informative sites (62) than rDNA (25).

Table 3.3. Summary of sequence alignments[a] of rDNA, β-tubulin and combined datasets for *Grifola frondosa*.

	rDNA	β-tubulin	Combined
Total nucleotide (nt) sites	574	587	1161
nt sites in variable gene regions (introns for β-tubulin and ITS1&2 for rDNA)	416	182	598
nt sites in conserved gene regions (exons for β-tubulin and 5.8S for rDNA)	158	405	563
Variable nt sites	82	177	259
Phylogenetically informative nt sites	25	62	87

[a] Alignments including 51 sequences of *G. frondosa* and *G. sordulenta*.

3.4.4 Phylogeny of commercial mushroom cultivars

Both neighbor joining (NJ) and most parsimonious (MP) trees derived from all DNA datasets revealed a consistent grouping of U.S. commercial cultivar WC828 in the Asian clade. This suggested that WC828 has an Asian origin and is closely related to Asian commercial cultivars. It is known that molecular data can be effectively used to select and improve commercial lines of edible mushrooms (Thon and Royse 1999b). So, a better understanding of the phylogenetic relationships of *Grifola frondosa* may help the selection and breeding of commercial lines and help to improve commercial cultivation of these mushrooms.

Chapter 4: Effects of germplasm and selected nutrient supplements on mushroom yield

4.1 Introduction

Grifola frondosa (Dicks.:Fr.) S.F. Gray, commonly known as hen-of-the-woods or maitake, is considered one of the most popular choice edible mushrooms (Lincoff 1981a,b). It is a white rot, wood decay fungus that naturally inhabits many hardwood species in Asia, North America, and Europe. The annual commercial production (Figure 1.1) of maitake has continued to increase dramatically because of its excellent taste, and nutritional and medicinal values. Presently, most maitake is marketed as food. Powdered fruitbodies are also used in the production of many health foods such as maitake tea, whole powder, granules, drinks, and tablets (Royse 1997).

Maitake is well known for centuries in China and Japan both as a health food and as a medicine (Mizuno and Zhuang 1995). It has received increasing attention in recent years because of documented anti-tumor and anti-viral properties (Hobbs 1996, Jong *et al.* 1991). Strong consumer demand has stimulated increased production world wide. Maitake production and consumption also is increasing rapidly in the United States (up 38% 1999-2000). Figure 4.1 shows world annual production increases of maitake in the last 20 years. Commercial production of maitake began in 1981 in Japan (Takama *et al.* 1981). It increased 577.8% from 1981 to 1986. In the next 5 years (1986-1990), production increased 250.1%, and then 84.1% from 1990-1994. The most recent published production data is 33,100 tons in 1997, which is a 133.1% increase compared with 1994. Production increases for maitake are much higher than that of total world mushroom production and especially the most common cultivated edible mushroom (*Agaricus bisporus*). The production increases of

maitake are also higher than the most popular cultivated specialty mushroom - shiitake (*Lentinula edodes*), except in the 1990 to 1994 period.

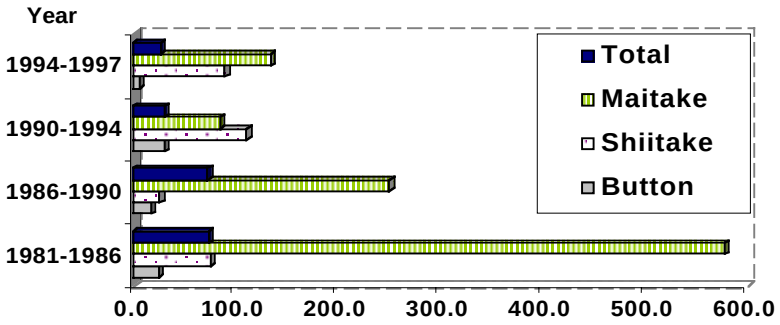


Figure 4.1. Percentage increases of world production of maitake (*Grifola frondosa*), shiitake (*Lentinula edodes*), button (*Agaricus bisporus*) and all mushrooms during various periods from 1981 to 1997. (Source: Chang 1999)

Bag, bottle and outdoor bed cultivation are the three basic methods of commercial production of maitake (Mayuzumi and Mizuno 1997). Commercial production of most maitake is on synthetic substrate contained in polypropylene bags (Figure 4.2). After sterilization and cooling, the moistened substrate is inoculated with maitake spawn. Spawn run lasts about 30 to 60 days depending on isolate and substrate formulation. Temperatures then are lowered from about 22°C to 14°C to induce fruiting and fruitbody maturation (Royse 1997). For bottle production, the containers are filled with moistened substrate and sterilized or pasteurized prior to inoculation (Royse 1997). Most growers use automated inoculation equipment thereby saving on labor costs (Yamanaka

Figure 4.2. Cultivated *Grifola frondosa* (maitake) emerging from nutrient supplemented sawdust contained in polypropylene bags.

1997). However, the size of the harvested mushroom is smaller than those harvested from bags because there is less substrate in bottles. Different from bag and bottle cultivation, outdoor bed cultivation is on colonized substrate buried in moist soil (Mayuzumi and Mizuno 1997). It requires a long crop cycle (about six months), and yields are much lower than those of bag or bottle methods.

There still are limited reference texts available for producing maitake. Presently, the techniques used to grow maitake are mostly adopted from other specialty mushroom cultivation, such as shiitake. Fortunately, extensive research has been done on efficient methods, genotypes and nutritional formulation of specialty mushrooms other than maitake (Douglas and Royse 1986, Royse and Bahler 1988, Royse *et al.* 1990). A common substrate used for commercial production of maitake is supplemented sawdust. Oak (Lee 1994, D.J. Royse, unpublished data) is the most popular choice in the United States and Japan, while beech (Kirchhoff 1996, Yoshizawa *et al*. 1997) and larch (Stamets 2000) are also preferred to a lesser extent in Japan. In China, cottonseed hulls were used as a substitute for sawdust with acceptable yields (Zhao *et al*. 1983). Bran derived from cereal grains, such as rice bran (Takama *et al*. 1981), wheat bran (Mayuzumi and Mizuno 1997), oat bran and corn bran, are widely used as nutrient supplements. Other nutrient supplements used for maitake cultivation include millet (D.J. Royse, unpublished data), corn meal (Kirchhoff 1996), and soybean cake (Mizuno and Zhuang 1995).

No published research on genotypes of maitake used for commercial production was found, partially because of the short cultivation history of this mushroom. It was found that the presently used commercial lines in Japan, China, Taiwan and the U.S. have different geographic origins, with different levels of genetic variation (Chapter 3, this thesis).

The rapid growth of maitake production has focused the need for additional research in two areas. First, a search is needed for isolates with improved yield and quality characteristics. To assist with this search, I evaluated 23 genotypes of *Grifola frondosa* for crop cycle time, biological efficiency (BE), yield and quality. Significant differences among lines were found for these parameters when mushrooms were produced on a nutrient supplemented sawdust substrate. Second, there is a desire to develop more efficient substrate formulas to improve yield and quality and to shorten the crop cycle. In this study, nine experiments were conducted to determine the effects of selected nutrient supplements and their levels on maitake crop cycle time, BE, yield and quality. Significant differences among different formulations were found and the best combinations of nutrient supplements among those tested were identified. For continued growth of the commercial industry, efforts directed toward improving biological efficiency, yield, quality, and reduced time to primordium formation and harvest are desirable.

4.2 Materials and Methods

4.2.1 Substrates and preparation

The major substrate ingredient - mixed oak sawdust (mostly *Quercus rubra* L.) was obtained from a local sawmill in Centre Country, Pennsylvania with approximately 30% moisture. The general substrate formulation (dry weight basis) consisted of 74.8% mixed oak sawdust, 15% white millet (*Panicum miliaceum* L.), 10% wheat bran (*Triticum aestivum* L.) and 0.2% gypsum ($CaSO_4$). Moisture content of the substrate was adjusted to 55%-58% of the fresh weight. This formula was initially chosen to determine the effects of genotypes on mushroom yield because previous experiments indicated reasonable growth (D.J. Royse, unpublished data). All ingredients were combined, mixed, pasteurized, cooled, inoculated, and bagged with an

autoclaving paddle mixer described previously by Royse (1985). Dry matter contents of the processed substrates were determined by drying 100g of the processed substrates in an oven for 24 hours at 105°C.

4.2.2 Genotypes

Twenty three isolates (Table 4.1) of maitake (*G. frondosa*) from our collection (Table 2.1) were evaluated for the effects of genotypes on crop cycle time, yield and quality. Four isolates were selected because they are commercially used cultivars in the U.S. (WC828), China (M040) and Japan (WC835 and WC836). The isolates M019, M036, M037, M038, M039, WC582, WC659 and WC834 were selected from the same clade as the commercial isolates as determined by phylogenetic analysis (Figure 4.3). The U.S. isolates M002, M004, M007, M009, M011, M013, M014, M015, WC248 and W483 were selected because they are most genetically distinct from commercial isolates (Figure 4.3) and each represented the geographic origin from a state of the U.S. Isolate WC493 is a European isolate without a cultivation history. The isolates were maintained on potato-dextrose-yeast extract agar (PDYA) as outlined by Jodon and Royse (1979).

Table 4.1. Isolates and geographic origin of *Grifola frondosa* (maitake) used for genotype selection experiments.

Isolates	Geographic origin
M002	U.S.-WI
M004	U.S.-GA
M007	U.S.-WV
M009	U.S.-LA
M011	U.S.-DC
M013	U.S.-NY
M014	U.S.-NY
M015	U.S.-WI
M019	Taiwan
M036	China
M037	China
M038	China
M039	China
M040	China/commercial
W483	U.S.-MD
WC248	U.S.-PA
WC493	Norway
WC582	Korea
WC659	Korea
WC828	unknown/commercial in U.S.
WC834	Japan
WC835	Japan/commercial
WC836	Japan/commercial

The isolates were screened on a general formula (dwt basis) containing 74.8% mixed oak sawdust, 15% millet, 10% wheat bran, and 0.2% $CaSO_4$. Isolate WC828 was selected for the nutrient experiments based on the evaluation of crop cycle time, quality, yield and biological efficiency (Results, chapter 4, this thesis).

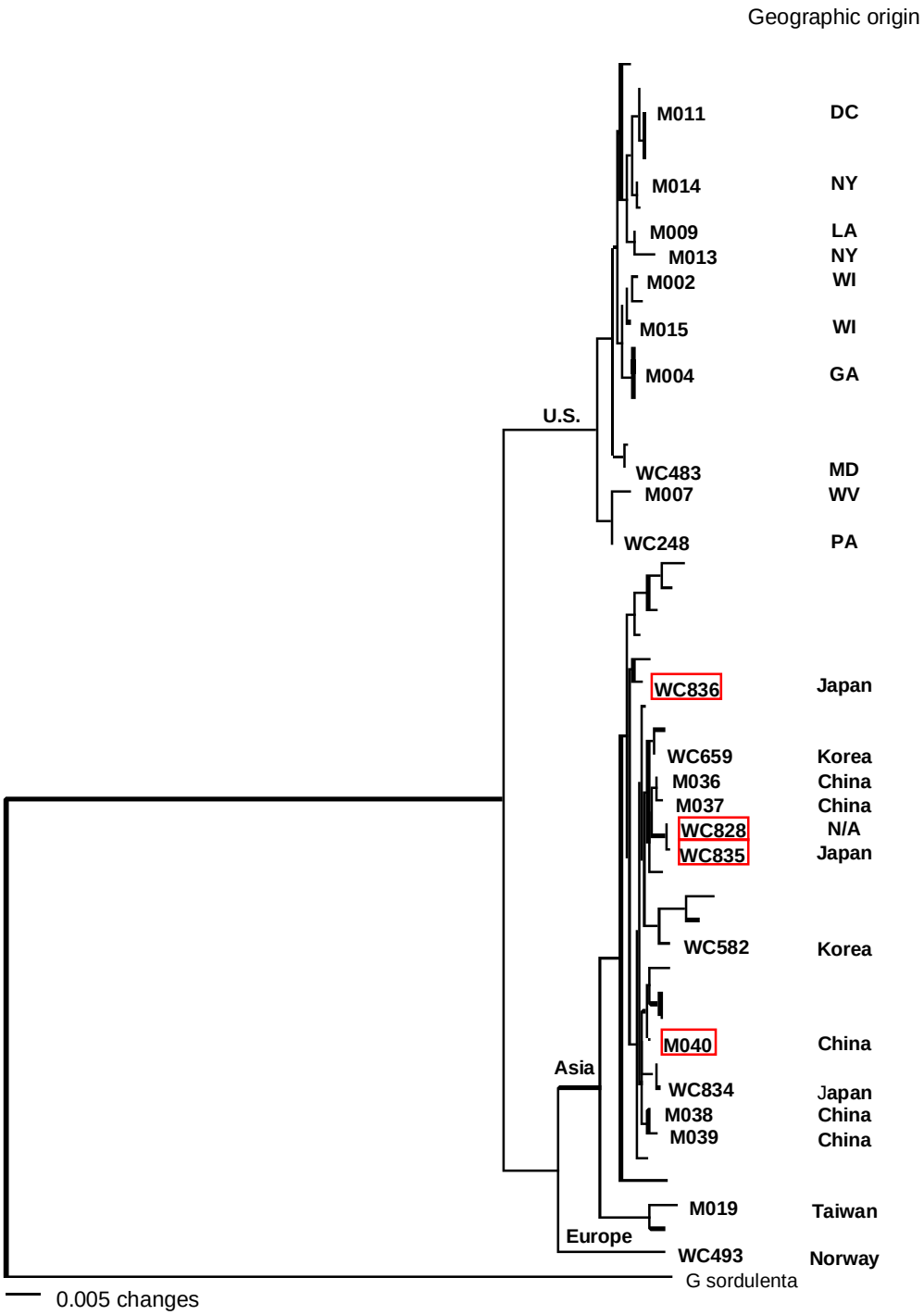


Figure 4.3. The neighbor-joining (NJ) tree containing 23 *Grifola frondosa* isolates selected for genotype selection experiments based on combined rDNA and partial β-tubulin gene sequences. Geographic origin is presented beside isolate codes. Isolate numbers highlighted in boxes are of commercial origin.

4.2.3 Spawn, spawn run, primordial development and fruitbody development

Spawn was prepared in 500ml flasks following a spawn formula (100 ml beaker level full of Stanford mushroom rye grain, 50 ml beaker of hardwood sawdust, one-half teaspoon $CaSO_4$, and 120 ml of warm tap water, D.J. Royse, unpublished) known to support good growth of maitake mycelium. After inoculation with spawn, polyethylene bags were used to contain moist (55-58%) substrates (2650 grams per bag) for incubation. Spawn run temperature was maintained at 20±1°C. The bags were sealed with a twist tie and, after the spawned substrate was incubated for one week, 20 slits (5mm long) were made at the top of each bag with a sharp scalpel to provide for gas exchange. Spawn run is the period from the beginning of inoculation to primordia formation. After primordia formation, two holes were cut in the polyethylene bags exposing the developing primordia. The top of the bag was folded over, exposing only the developing primordia to the fruiting environment. Taped bags (Figure 4.4) then were moved to a production room for fruiting. The period of fruitbody development was initiated when the primordia began to grow and differentiate to form small pilei and stipes. A crop cycle of 12 weeks or less was considered short based on our experience and compared to the 15-week crop cycle reported by Stamets (2000).

Figure 4.4. Taped polypropylene bags with basidiomes developing from substrate directly under holes cut in bags. Mushrooms maturing approximately 70 days after inoculation.

4.2.4 Experimental design

All experiments (Table 4.2) were conducted as completely randomized designs and carried out at the Mushroom Research Center of the Pennsylvania State University under the same conditions. Environmental conditions were as described by Royse (1985). Relative humidity (90 to 95%) was maintained by water atomizers placed in air handling ducts, 4 hours of light were provided daily by cool-white fluorescent bulbs, and temperature was maintained at 17±2°C. Sufficient air changes were maintained to hold CO_2 concentrations below 700 ppm (µl/l).

SAS program JMP (SAS Institute 1997) was used to analyze data. The general linear models procedure was used to perform an analysis of variance. Treatments with zero value were excluded from the data analysis. Tukey-Kramer Honestly Significant Difference (HSD) was used to separate treatment means (SAS Institute 1997). When two crops were performed for one experiment, the significant difference between two crops was determined by a two-way analysis of variance. Both crops were analyzed separately and the combined data were analyzed if there were no significant differences between two crops for BE and quality.

Table 4.2. Description of 13 experiments performed to determine effects of genotypes and nutrient supplements on crop cycle time and mushroom yield and quality.

Experiment number	Number of crops	Number of replicates	Supplementation level (%)	Description/Purpose
1	2	15	25	Genotypes
2	1	15	25	
3	1	15	25	
4	2	15	25	
5	2	10	20	Selected nutrient supplements
6	1	10	10	Different levels of wheat bran and millet
7	1	10	20	
8	1	10	30	
9	1	10	10	Different levels of wheat bran and rye
10	1	10	20	
11	1	10	30	
12	2	10	20	Different levels of wheat bran, millet and rye
13	2	10	30	

Four genotype experiments (Experiment #1 - #4) were conducted to determine the effects of genotypes on crop cycle time, yield and quality with 15 replicates for each treatment while Experiment #1 evaluated 5 isolates (WC828, WC835, WC836 WC659 and WC834). Experiment #2 evaluated seven isolates (M040, M007, M014, WC493, WC248 and WC582). Seven isolates (M036, M002, M009, M011, M013, M019, WC483) were evaluated in Experiment #3. Experiment #4 tested seven isolates (WC828, M036, M37, M038, M039, M040 and M015). Two crops were performed for Experiments #1 and #4 with the same experimental design and similar environmental conditions.

One nutrient experiment (Experiment #5) was designed to determine the influence of selected nutrient supplements on mushroom yield with 10 replicates for each treatment. Two crops were performed and 20% total selected nutrient supplements were used. Selected nutrient supplements included wheat bran and different types of cereal grain, such as millet, rye, and corn meal. Different combinations of these supplements were tested by adding them to a basal substrate of sawdust which included 79.8% mixed red and white oak plus 0.2% $CaSO_4$. Table 4.3 shows the treatments with proportions of selected nutrient supplements in a basal substrate according to a simplex centroid mixture design (SAS Institute, 1997).

Table 4.3. Simplex centroid mixture design listing treatment numbers and supplements added to 79.8% oak sawdust plus 0.2% gypsum ($CaSO_4$) used for production performance of isolate WC828 of *Grifola frondosa* (Experiment #5).

	Supplement (%)			
Treatment	Wheat Bran	Millet	Rye	Corn meal
1	20	0	0	0
2	0	20	0	0
3	0	0	20	0
4	0	0	0	20
5	10	10	0	0
6	10	0	10	0
7	10	0	0	10
8	0	10	10	0
9	0	10	0	10
10	0	0	10	10
11	6.7	6.7	6.7	0
12	6.7	6.7	0	6.7
13	6.7	0	6.7	6.7
14	0	6.7	6.7	6.7
15	5	5	5	5

In order to evaluate the effects of different levels of selected nutrient supplements on mushroom crop cycle, yield and quality, eight nutrient level experiments (#6 - #13) were performed with 10 replicates for each treatment. The combinations of selected nutrient supplements of wheat bran, millet and rye were used in these experiments based on the results of nutrient Experiment #5 (Result, chapter 4, this thesis). The selected nutrient supplements used in experiments #6, #7 and #8 were wheat bran plus millet. In experiments #9, #10 and #11, wheat bran plus rye were used as the selected nutrient supplements. Three levels of total nutrient supplements (10%, 20%, and 30%) were tested for each combination. The combinations of wheat bran, millet plus rye were used as nutrient supplements in experiments #12, #13. Two levels of total nutrient supplements (20% and 30%) were tested for each experiment. Table 4.4 to Table 4.11 list the treatments in each of the 9 experiments. Only one crop was conducted for Experiments #6, #7, #8, #9, #10 and #11, while two crops were conducted for Experiments #12 and #13.

Table 4.4. Treatment numbers and nutrient mixtures for production of *Grifola frondosa* (WC828) on oak sawdust (89.8%) and gypsum (0.2%) at the Mushroom Research Center (Experiment #6).

	Supplement (%)	
Treatment	Wheat bran	Millet
1	0	10
2	2.5	7.5
3	5	5
4	7.5	2.5
5	10	0

Table 4.5. Treatment numbers and nutrient mixtures for production of *Grifola frondosa* (WC828) on oak sawdust (79.8%) and gypsum (0.2%) at the Mushroom Research Center (Experiment #7).

	Supplement (%)	
Treatment	Wheat bran	Millet
1	0	20
2	5	15
3	10	10
4	15	5
5	20	0

Table 4.6. Treatment numbers and nutrient mixtures for production of *Grifola frondosa* (WC828) on oak sawdust (69.8%) and gypsum (0.2%) at the Mushroom Research Center (Experiment #8).

	Supplement (%)	
Treatment	Wheat bran	Millet
1	0	30
2	7.5	22.5
3	15	15
4	22.5	7.5
5	30	0

Table 4.7. Treatment numbers and nutrient mixtures for production of *Grifola frondosa* (WC828) on oak sawdust (89.8%) and gypsum (0.2%) at the Mushroom Research Center (Experiment #9).

	Supplement (%)	
Treatment	Wheat bran	Rye
1	0	10
2	2.5	7.5
3	5	5
4	7.5	2.5
5	10	0

Table 4.8. Treatment numbers and nutrient mixtures for production of *Grifola frondosa* (WC828) on oak sawdust (79.8%) and gypsum (0.2%) at the Mushroom Research Center (Experiment #10).

	Supplement (%)	
Treatment	Wheat bran	Rye
1	0	20
2	5	15
3	10	10
4	15	5
5	20	0

Table 4.9. Treatment numbers and nutrient mixtures for production of *Grifola frondosa* (WC828) on oak sawdust (69.8%) and gypsum (0.2%) at the Mushroom Research Center (Experiment #11).

	Supplement (%)	
Treatment	Wheat bran	Rye
1	0	30
2	7.5	22.5
3	15	15
4	22.5	7.5
5	30	0

Table 4.10. Treatment numbers and nutrient mixtures for production of *Grifola frondosa* (WC828) on oak sawdust (79.8%) and gypsum (0.2%) at the Mushroom Research Center (Experiment #12).

	Supplement (%)		
Treatment	Wheat bran	Millet	Rye
1	0	0	20
2	0	6.7	13.3
3	0	13.3	6.7
4	0	20	0
5	6.7	0	13.3
6	6.7	6.7	6.7
7	6.7	13.3	0
8	13.3	0	6.7
9	13.3	6.7	0
10	20	0	0

Table 4.11. Treatment numbers and nutrient mixtures for production of *Grifola frondosa* (WC828) on oak sawdust (69.8%) and gypsum (0.2%) at the Mushroom Research Center (Experiment #13).

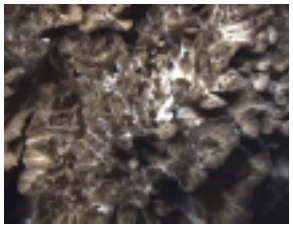
	Supplement (%)		
Treatment	Wheat bran	Millet	Rye
1	0	0	30
2	0	10	20
3	0	20	10
4	0	30	0
5	10	0	20
6	10	10	10
7	10	20	0
8	20	0	10
9	20	10	0
10	30	0	0

4.2.5 Harvesting and determination of BE and quality.

Mushrooms were harvested from the substrate when the caps were fully mature. The substrate clinging to the main stipe was removed and the clusters of mushrooms were weighed. The biological efficiency (BE) was determined as the ratio of kg fresh mushrooms harvested per kg dry substrate and expressed as a percentage (Royse 1992). The quality of maitake was evaluated by the

shape and color of the basidiome and rated as 1 to 4 (Table 4.12) (Kunitomo 1992).

Table 4.12. The quality scale (1-4) for maitake.

Rating	Description	Photograph
1	The best quality mushrooms with black to dark gray color, uniform and no misshapen pilei.	
2	Mushrooms with gray to light gray color and mostly uniform shape.	
3	Mushrooms with more than half of thepilei misshapen.	
4	Mushrooms with misshapen, immature and undeveloped pilei.	

4.3 Results

4.3.1 Effects of germplasm on crop cycle time

A total of 23 isolates were evaluated in four experiments. The results are shown in Table 4.13 (Appendix F). Five isolates - two Korean isolates (WC582, WC659) and four U.S. isolates (M014, M011 and M007) colonized the substrate,

but the mycelium grew poorly and did not form primordia. Two U.S. isolates (M002 and WC248), one Taiwan isolate (M019) and one Japanese isolate (WC834) formed primordia, but did not produce mushrooms. Four U.S. isolates (M004, M009, M013 and WC483) and the European isolate (WC493) produced mushrooms, but consistent fruiting was not achieved. Some replicates in each treatment did not produce mushrooms while some replicates completed the whole crop cycle. In most cases, more than half of the replicates did not produce mature mushrooms. Mushrooms were consistently produced by five Chinese isolates (M036, M037, M038, M039 and M040), two Japanese isolates (WC835 and WC836), one U.S. isolate (M015) and an U.S. commercial isolate with unknown origin (WC828). For a better understanding of the genetic variation among these isolates, a phylogenetic tree is presented in Figure 4.5.

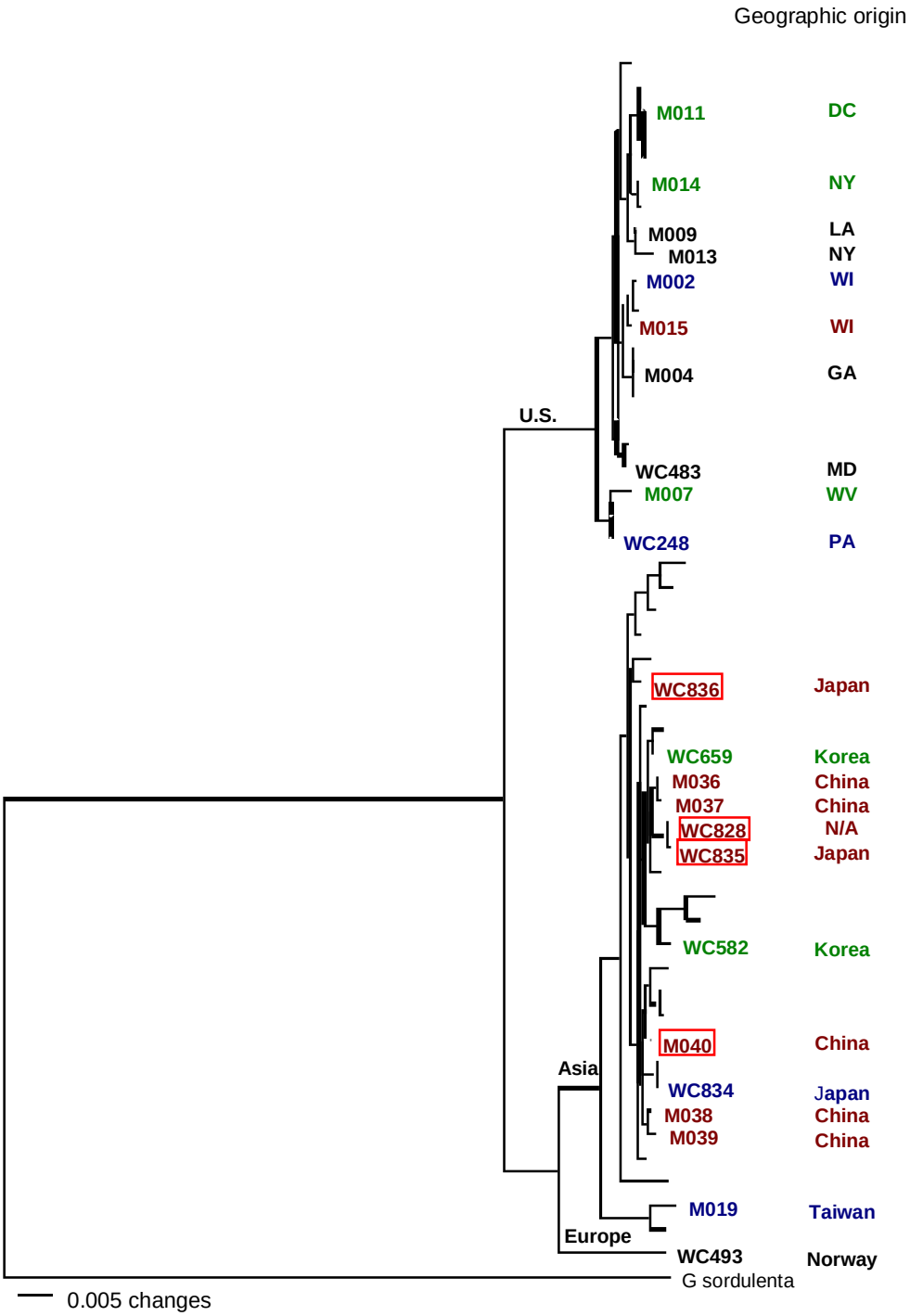


Figure 4.5. Phylogenetic relationships of 23 *Grifola frondosa* isolates selected for fruiting trials. Phylogenies are based on combined rDNA and partial β-tubulin gene sequences. Geographic origin is shown beside the isolate codes. Cultures highlighted in box are of commercial origin. Colors indicate: a) red - isolates which completed whole crop cycle and consistently fruited, b) black - isolates which did not consistently fruit, but some completed the crop cycle, c) Blue - isolates that only formed primordia, but did not fruit, d) Green - isolates that did not form primordia.

In Experiment #1, WC828 showed the shortest time to primordium formation (5 weeks) and fruiting (8 weeks). In Experiment #4, M039 showed the shortest crop cycle of 8 weeks, with 4 weeks to form primordia and 6 weeks to produce fruitbodies. Results of experiments #1 and #4 are summarized in Figure 4.6.

Experiments #2 and #3 were discontinued and a second crop was not conducted because most of the isolates did not complete the crop cycle and had no yield data. Further evaluation of 14 isolates (WC493, WC483, M004, M009, M013, WC834, WC248, M019, M002, WC582, WC659, M014, M011 and M007) in Experiments #2 and #3 was terminated, except for isolates M036 and M040, which were also included in Experiment #4. Thus, yield performance and quality assessment of these two isolates were evaluated in Experiment #4.

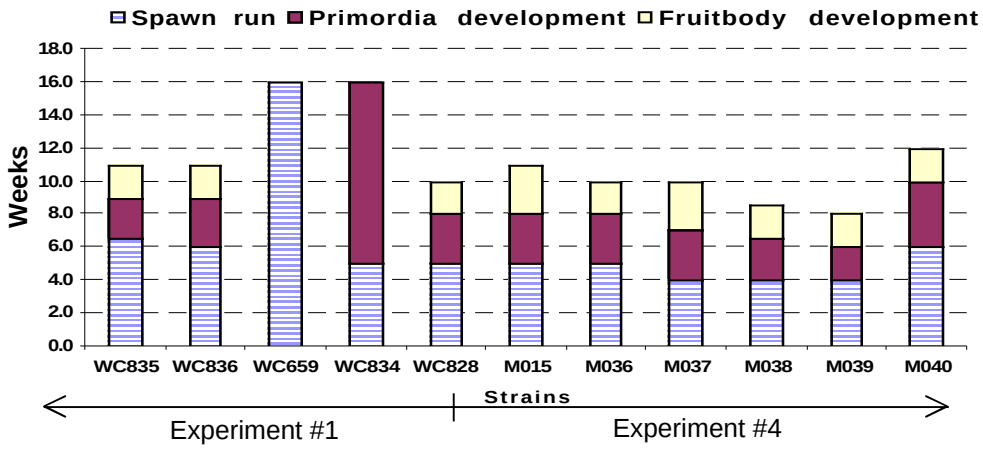


Figure 4.6. Summary of crop cycle time (wk) showing spawn run time and primordia and fruitbody development of 11 isolates of *Grifola frondosa* grown on sawdust substrate supplemented with 25% nutrient at The Mushroom Research Center (Experiment #1 and #4). Treatment descriptions are given in Table 4.13 (Appendix F).

4.3.2 Effects of germplasm on quality and yield

Statistical analysis of BE and quality data for two crops of Experiment #1 and #4 were shown in Table 4.14 (Appendix F). Isolates WC828, WC835, WC836, M015, M036, M037, M038, M039 and M040 were included in this analysis.

Significant differences in BEs and quality were found among the three isolates evaluated in Experiment #1 (Table 4.14A) (Appendix F). In crop I, BEs ranged from 35.4% (WC828) to 21.4% (WC836). Quality ranged from 1.2 (WC836) to 2.9 (WC835). In crop II, BEs ranged from 33.5% (WC828) to 21.2% (WC835). Quality ranged from 1.1 (WC836) to 3.0 (WC835). Although the actual BEs and quality were different in two crops, isolate WC828 had the highest BEs and significantly higher than WC835 and WC836 in crop I and only significantly higher than WC835 in crop II. The quality of WC835 was significantly lower than other two isolates. There was no significant difference of quality between WC828 and WC836 in two crops. Combined data of crop I and II were analyzed because no significant differences for BE and quality between two crops were found. The results are shown in Figure 4.7. Isolate WC828 had the highest BE (34.5%). Isolates WC836 and WC828 had the highest qualities (1.2 and 1.4, respectively).

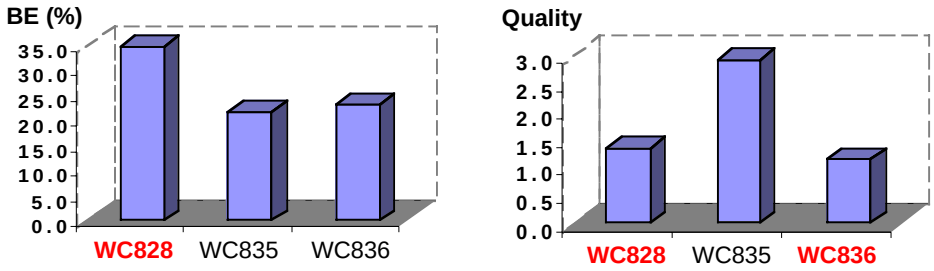


Figure 4.7. Graphic summary of means of BEs (left) and quality (right) of combined data of two crops of *Grifola frondosa* to determine the effect of three genotypes (Experiment #1). Treatments highlighted in bold font showed significantly higher BEs or quality in the experiment. Quality was rated 1-4 with 1 being the highest quality while 4 was the lowest quality rating. Treatment descriptions are shown in Table 4.14 (Appendix F).

In Experiment #4, significant differences in BEs and quality were found among seven isolates evaluated (Table 4.14B) (Appendix F). In crop I, BEs ranged from 37.6% (M040) to 31.3% (M038). Quality ranged from 1.2 (M038 and M040) to 2.0 (M039). Isolates WC828, M036, M037, M038 and M040 had the best quality. Isolate M038 produced the lowest BE of all isolates tested. Isolate M015 produced the lowest quality mushroom. In crop II, BEs ranged from 42.6% (M036) to 30.1% (M015). Quality ranged from 1.1 (WC828, M038 and M040) to 3.0 (M015). Significant differences for BE and quality were found between crop I and II. However, the results from two crops are not in conflict. It was found that BEs and qualities of crop II are overall higher than those of crop I. Total means of BE for crop I and II are 34.6% and 37.7%, respectively. Total means of quality for crop I and II are 1.7 and 1.5, respectively. WC828, M036, M037 and M040 had the significantly higher BEs and quality than the other lines tested in both crops (Figure 4.8).

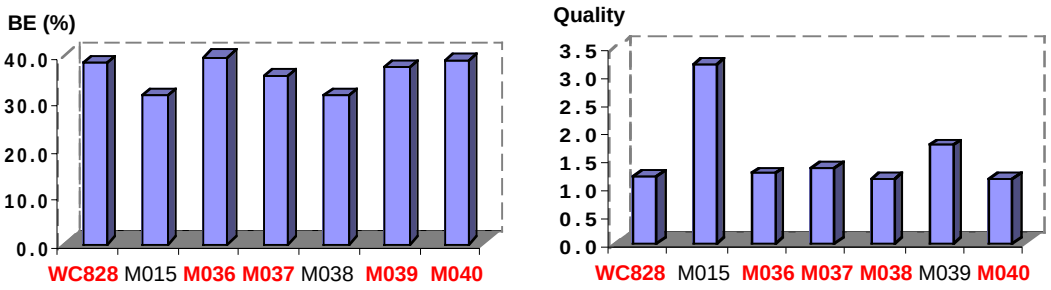


Figure 4.8. Graphic summary of means of BEs (left) and quality (right) of two crops of *Grifola frondosa* (WC828) to determine the effect of seven genotypes (Experiment #4). Treatments highlighted in red bold font showed significantly higher BEs or quality in both crops in the experiment. Quality was rated 1-4 with 1 being the highest quality while 4 was the lowest quality rating. Treatment descriptions are shown in Table 4.14 (Appendix F).

These was a 7% difference in BE for WC828 between Experiment #1 and #4 for Crop II. When crop means for BEs were compared for WC828 between experiments, however, there was a difference of only 4%. Thus, overall yields

were similar from experiment to experiment. In summary, isolates WC828, M036, M037 and M040 had both consistently high BEs and quality in both crops, with no significant difference between isolates. Since WC828 is the commercial isolate used in the U.S., it was chosen to perform the nutrient experiments.

4.3.3 Effects of nutrient supplements on crop cycle time

Isolate WC828 performed differently on sawdust supplemented with different combinations of nutrients. The results are shown in Table 4.15 (Appendix F) and Figure 4.9. The crop cycle was the shortest (10 weeks) when combinations of 10% wheat bran plus 10% rye (treatment #6) were used. This treatment also had the shortest spawn run (4 weeks). Combinations of wheat bran and rye appeared to allow shortest spawn run time and shortest crop cycle time. Formulations with only 20% wheat bran (treatment #1) and only 20% rye (treatment #3) had relatively short spawn run times (5.5 and 6 weeks, respectively). When only millet (Experiment #2), corn meal (Experiment #4) or the combination of millet and corn meal (Experiment #9) were used, the crop cycle could not be completed due to arrested development and maturation of fruitbodies. However, the combinations of millet and corn meal with either wheat bran or rye resulted in a completed, but delayed crop cycle.

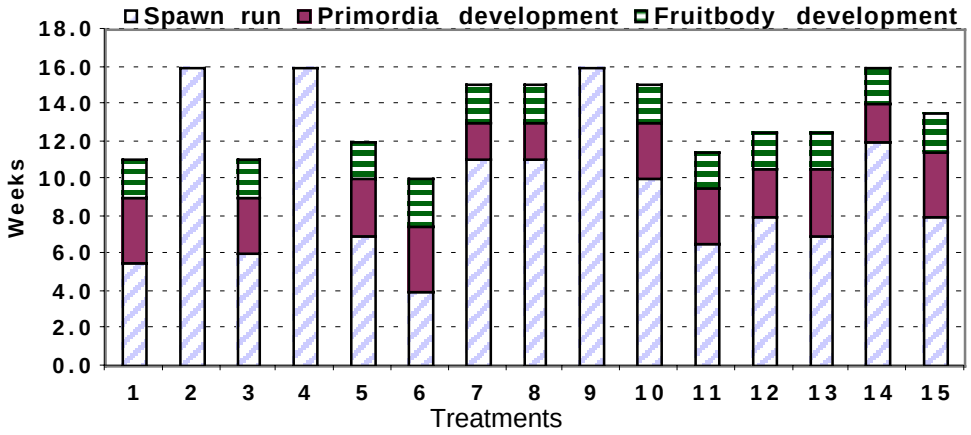


Figure 4.9. Graphic summary (15 treatments) of crop cycle time of *Grifola frondosa* (WC828) as influenced by selected nutrient supplements (total 20% of substrate) used alone or in various combinations (Experiment #5). Treatment descriptions are shown in Table 4.15 (Appendix F).

4.3.4 Effects of nutrient supplements on quality and yield

Significant differences in BEs and quality were found among 15 treatments in crop I and crop II (Table 4.16) (Appendix F). In crop I, BEs ranged from 40.5% (treatment #6) to zero (treatments #2, 4 and 9). Quality ranged from 1.2 (treatment #6) to 3.0 (treatment #8). A combination of 10% wheat bran plus 10% rye (treatment #6) added to substrate produced the highest BE and best quality. However, there was no significant difference in BE between treatments #5, #6 and #11. Treatments with combinations of 10% millet plus 10% rye (treatment #8), 6.7% wheat bran, 6.7% millet plus 6.7% corn meal (treatment #12), and 6.7% millet, 6.7% rye plus 6.7% corn meal (treatment #14), respectively, were significantly lower in BEs than all other nutrient combinations tested. In Crop II, BEs ranged from 40.1% (treatment #11) to zero (treatments #2, 4 and 9). Quality ranged from 1.1 (treatment #6) to 3.1 (treatment #12). The combination of 6.7% wheat bran, 6.7% millet plus 6.7% rye (treatment #11),

along with treatment #3 #5, #6, and #10 had the highest BEs with no significant difference. The highest quality mushrooms were produced by substrates from treatments #3 #5, #6, #11, and #13.

No significant differences for BE and quality were found between crop I and II. The results based on combined data are summarized in Figure 4.10. The combination of 10% wheat bran plus 10% rye (treatment #6), 10% wheat bran plus 10% millet (treatment #5), and 6.7% wheat bran, 6.7% millet plus 6.7% rye (treatment #11) had both the highest BEs and quality.

Comparison of all substrate formulations evaluated in this experiment revealed that for isolate WC828, wheat bran, rye and millet produced better fruiting and yields than corn meal. In general, combinations of more than one type of nutrient were better than only one nutrient. Combinations of two or three nutrients selected from wheat bran, rye or millet was the most desirable formulation with shortest crop cycle, best quality and highest BE.

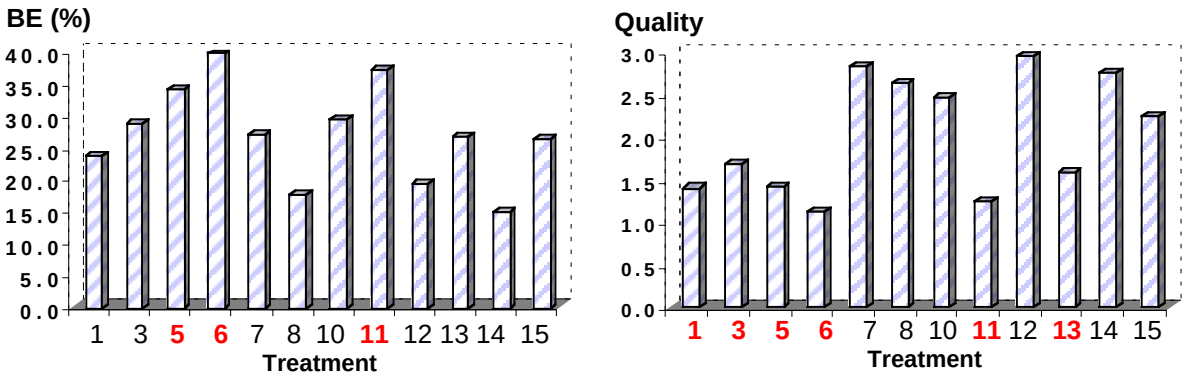


Figure 4.10. Graphic summary (12 out of 15 total treatments) of means of BEs (left) and quality (right) of combined data of two crops of *Grifola frondosa* (WC828) to determine the effect of selected nutrient supplements (total 20% of substrate) used alone or in various combinations (Experiment #5). Treatments highlighted in red bold font showed significantly higher BEs or quality in both crops in the experiment. Quality was rated 1-4 with 1 being the highest quality while 4 was the lowest quality rating. Treatment descriptions are shown in Table 4.16 (Appendix F).

4.3.5 Effects of different levels of selected nutrient supplements on crop cycle time

Different levels of wheat bran and millet significantly influenced mushroom crop cycle time. The results are shown in Table 4.17 (Appendix F) and summarized in Figure 4.11. There was no primordium formation for any levels of millet used alone. When the 10% nutrient supplement level was used, mycelial growth was weak and none of the treatments completed the crop cycle. Three treatments, that contained 10% wheat bran plus 10% millet (treatment #3), 15% wheat bran plus 5% millet (treatment #4) and 20% wheat bran (treatment #5), completed the whole crop cycle. The shortest crop cycle (11 weeks) was observed when 20% wheat bran was used as supplement. At the 30% total supplement level, crop cycles for each treatment were shorter compared to the 20% level. The shortest crop cycle was 9 weeks observed for 30% wheat bran.

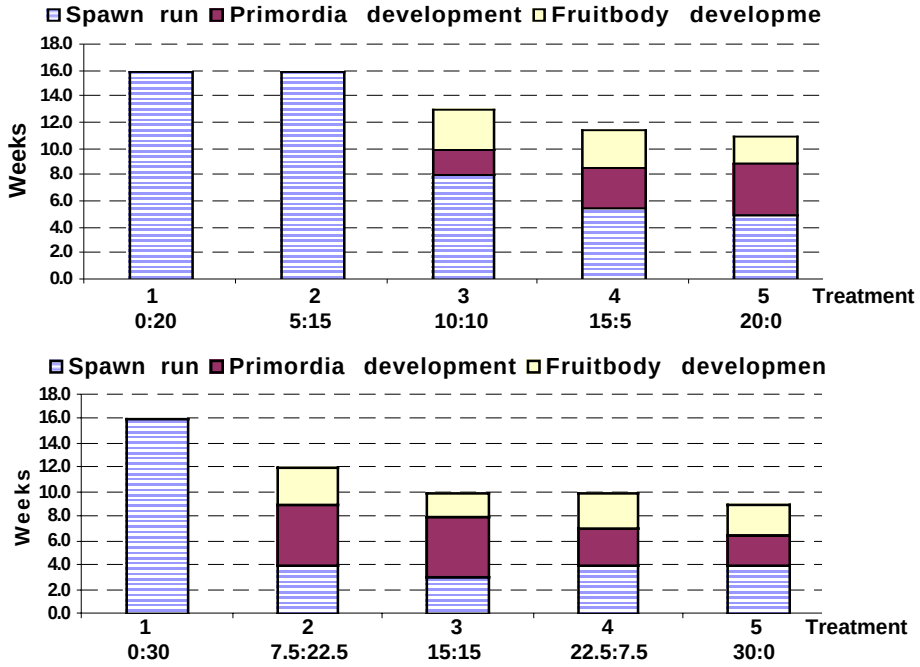


Figure 4.11. Graphic summary (5 treatments) of crop cycle time of *Grifola frondosa* (WC828) as influenced by 20% (Experiment #7) (top) and 30% (Experiment #8) (below) levels of wheat bran and millet used alone or in various combinations. Ratios shown below each treatment number indicate percentages of wheat bran : millet.

The results for different levels of wheat bran and rye are shown in Table 4.18 (Appendix F) and summarized in Figure 4.12. All treatments produced mushrooms, except those at the 10% total supplement level. At the 20% level, there was not much difference in crop cycle time for each treatment (Table 4.18A). Crop cycle time at the 30% level ranged from 8 weeks to 14 weeks, with the shortest crop cycle (8 weeks) comprising a combination of 22.5% wheat bran plus 7.5% rye (Table 4.18B). Primordia developed sooner at the 30% level than those at the 20% level. This indicated that higher levels of wheat bran and rye stimulated primordia formation and development. However, spawn run for treatment #1 (rye only) was much longer at the 30% level (14 weeks) than at the 20% level (11 weeks).

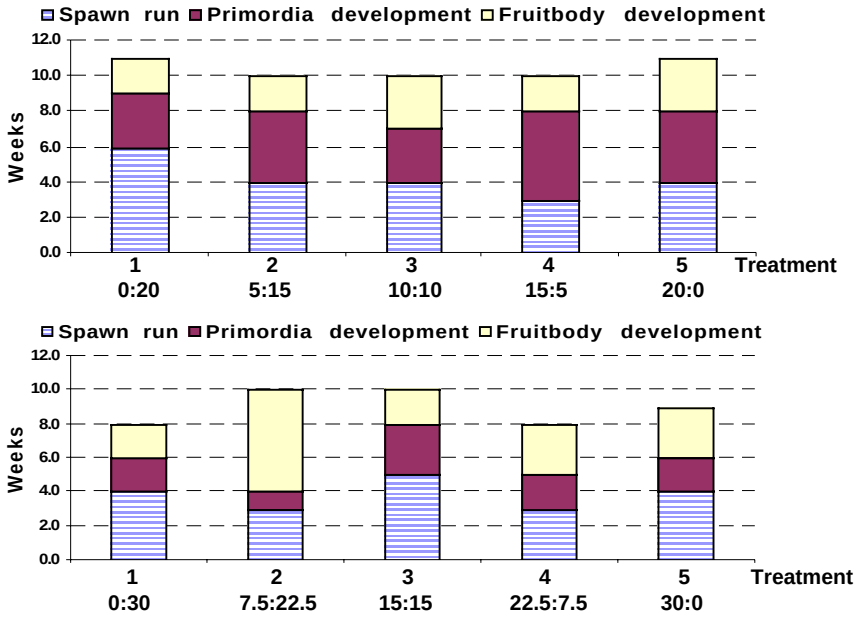


Figure 4.12. Graphic summary (5 treatments) of crop cycle time of *Grifola frondosa* (WC828) as influenced by 20% (Experiment #10) (top) and 30% (Experiment #11) (below) levels of wheat bran and rye used alone or in various combinations. Ratios shown below each treatment number indicate percentages of wheat bran : rye.

Only the 20% and 30% levels of wheat bran, millet and rye and their mixtures were evaluated because the 10% level of nutrient was not sufficient for optimum mushroom growth. The results are shown in Table 4.19 (Appendix F) and summarized in Figure 4.13. All treatments resulted in completion of the crop cycle, except treatment #4 (millet only) at both levels, treatment #2, #3 and #7 at the 20% level. At 20% level, crop cycle time for treatment #8 (wheat bran : millet : rye = 13.3% : 0% : 6.7%) and treatment #5 (wheat bran : millet : rye = 6.7% : 0 : 13.3%) was the shortest (10 weeks). At the 30% level, 9 weeks was the shortest crop cycle achieved (treatment #8 and #10).

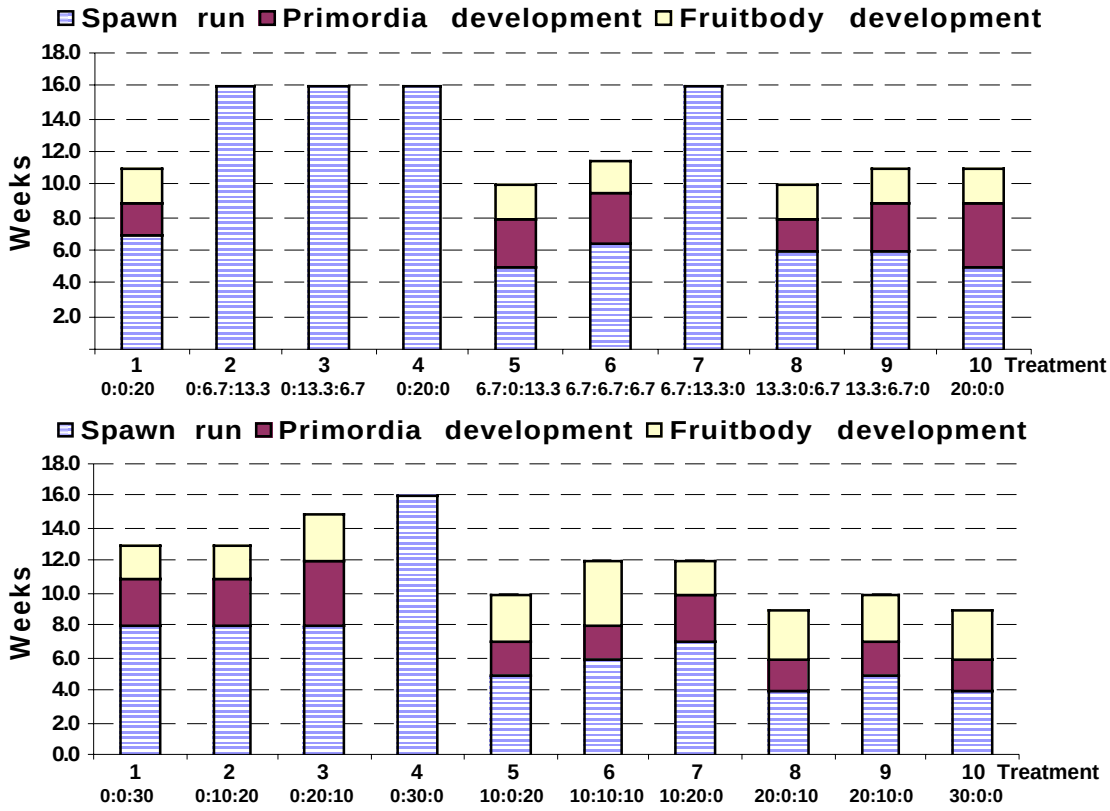


Figure 4.13. Graphic summary (10 treatments) of crop cycle time of *Grifola frondosa* (WC828) as influenced by 20% (Experiment #12) (top) and 30% (Experiment #13) (below) levels of wheat bran, millet and rye used alone or in various combinations. Ratios shown below each treatment number indicate percentages of wheat bran : millet : rye.

4.3.6 Effects of different levels of selected nutrient supplements on quality and yield

4.3.6.1 Wheat bran and millet

The BEs and quality data for the combinations of wheat bran and millet are shown in Table 4.20 (Appendix F) and summarized in Figure 4.14. At the 20% level, BEs ranged from 30.3% (treatment #3) to zero (treatments #1 and 2), and quality ranged from 1.6 (treatment #3) to 1.9 (treatment #4). No significant differences were found between treatments for BE and quality. At the 30% level, BEs ranged from 40.2% (treatment #3) to zero (treatment #1), and quality ranged from 1.8 (treatment #3) to 2.4 (treatment #5). Treatment #3 (15% wheat bran and 15% millet) had the highest BE and quality. There were no significant differences in BE between treatments #2 (7.5% wheat bran and 22.5% millet) and #4 (22.5% wheat bran and 7.5% millet).

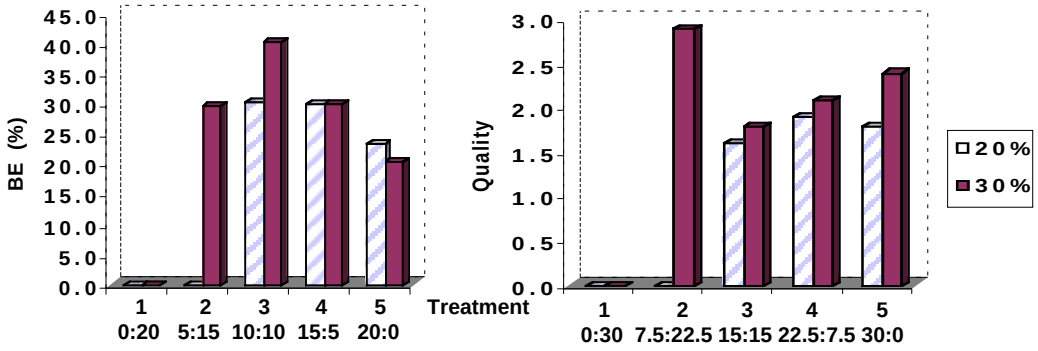


Figure 4.14. Graphic summary (5 treatments) of means of BEs (left) and quality (right) of *Grifola frondosa* (WC828) to determine 20% (Experiment #7) and 30% (Experiment #8) levels of wheat bran and millet used alone or in various combinations. Quality was rated 1-4 with 1 being the highest quality while 4 was the lowest quality rating. Ratios shown below each treatment number indicate percentages of wheat bran : millet.

4.3.6.2 Wheat bran and rye

When wheat bran and rye were used, no significant differences in quality were found at both the 20% and 30% total supplement level, while there were significant differences in BEs between treatments. The results are shown in Table 4.21 (Appendix F) and summarized in Figure 4.15. The BEs ranged from 41.5% (treatment #3) to 24.1% (treatment #5) at the 20% level. Treatment #2 (5% wheat bran and 15% rye), #3 (10% wheat bran and 10% rye) and #4 (15% wheat bran and 5% rye) had higher BEs and were significantly different (P = 0.05) than the other two treatments (#1 and 5). Combinations of wheat bran plus rye at a 10%:10% rate (treatment #3) had the highest BE (41.5%) and the best quality (1.3). At the 30% level, BEs ranged from 45.6% (treatment #3) to 22.3% (treatment #5). Treatment 5 (30% wheat bran) had a significantly lower BE (22.3%) and all the others were not significantly different. The highest BE was 45.6% achieved from substrate of treatment #3 (15% wheat bran and 15% rye); this result is the same as that found for the 20% level. Comparison of the two levels indicated that, in general, higher nutrient levels increased yield, but lowered mushroom quality.

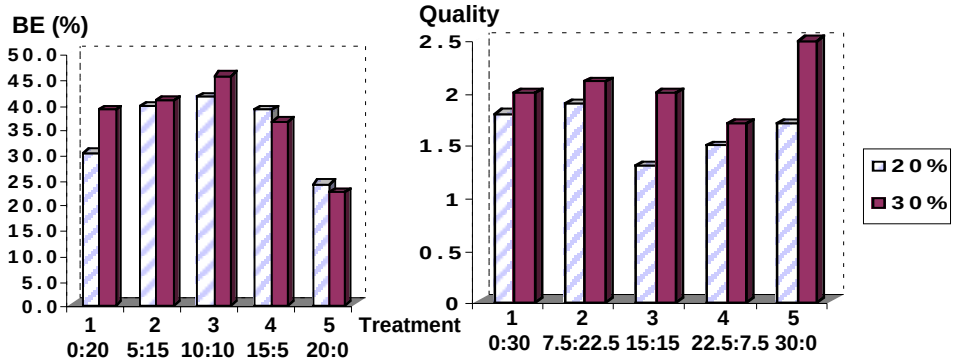


Figure 4.15. Graphic summary (5 treatments) of means of BEs (left) and quality (right) of *Grifola frondosa* (WC828) to determine 20% (Experiment #10) and 30% (Experiment #11) levels of wheat bran and rye used alone or in various combinations. Quality was rated 1-4 with 1 being the highest quality while 4 was the lowest quality rating. Ratios shown below each treatment number indicate percentages of wheat bran : rye.

4.3.6.3 Wheat bran, millet and rye at 20% level

The BEs and quality of two crops for the 20% level of combined wheat bran, millet and rye are shown in Table 4.22 (Appendix F) and summarized in Figure 4.16. In crop I, no significant differences in quality were found among the treatments. BEs ranged from 42% (treatment #8) to zero (treatments #2, 3, 4 and 7). Treatment #8 (13.3% wheat bran and 6.7% rye), treatment #6 (6.7% each of wheat bran, millet and rye), treatment #5 (6.7%wheat bran and 13.3% rye) and treatment #9 (13.3% wheat bran and 6.7% millet) had the highest BEs (42%, 38.5%, 36.1% and 32.9%, respectively).

A significant difference for BE was found between crops I and II. However, the results from two crops are not in conflict. Results for crop II were similar to crop I although the actual values of BE and quality were lower. Total means for BE for crop I and II were 33.9% and 31.2%, respectively. There was no significant difference in mushroom quality for any of the treatments in crop II. When wheat bran and rye were used, both combinations of 6.7%:13.3% (treatment #5) and 13.3%:6.7% (treatment #8) produced high BEs. When wheat bran and millet were used, only the combination of 13.3%:6.7% (treatment #9) produced high BEs. This suggests that a higher level of wheat bran may provide additional yield increases. The combination of wheat bran, millet and rye also was effective in stimulating mushroom yield.

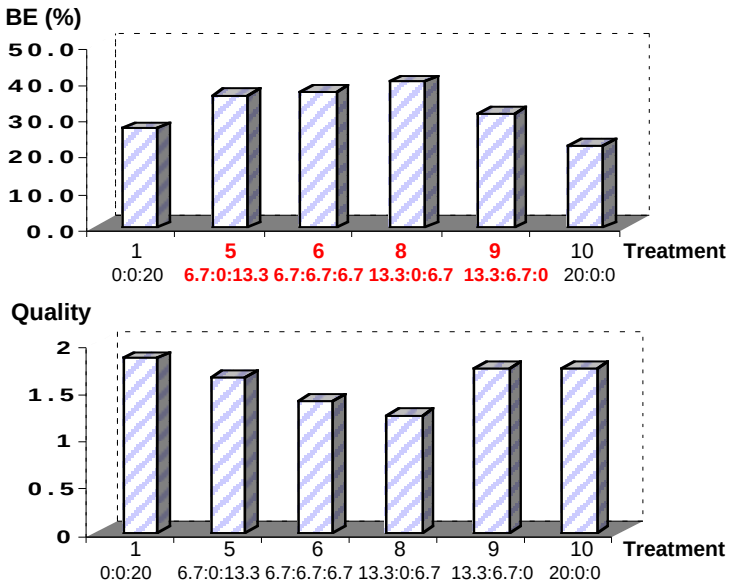


Figure 4.16. Graphic summary (6 out of 10 total treatments) of means of BEs (top) and quality (below) of two crops of *Grifola frondosa* (WC828) to determine the effect of 20% wheat bran, millet and rye used alone or in various combinations (Experiment #12). Treatments highlighted in red bold font showed significantly higher BEs or quality in both crops in the experiment. Quality was rated 1-4 with 1 being the highest quality while 4 was the lowest quality rating. Ratios shown below each treatment number indicate percentages of wheat bran : millet : rye.

4.3.6.4 Wheat bran, millet, and rye at the 30% level

The BEs and quality for two crops for the 30% level of wheat bran, millet and rye are shown in Table 4.23 (Appendix F) and summarized in Figure 4.17. Significant differences in BEs and quality were found in crops I and II. In crop I, BEs ranged from 48.9% (treatment #6) to zero (treatment #4), and quality ranged from 1.6 (treatment #3) to 2.5 (treatment #10). The BEs for treatment #6 (10% each of wheat bran, millet and rye) (48.9%) and treatment #5 (10% wheat bran and 20% rye) (44.1%) were significantly higher than the other treatments. Treatment #10 (30% wheat bran only) resulted in lower mushroom quality. There was no significant difference in quality for the other treatments.

In crop II, BEs ranged from 45.2% (treatment #6) to zero (treatment #4), and quality ranged from 1.4 (treatment #3) to 2.3 (treatment #10). Similar to crop I, a combination of 10% each of wheat bran, millet and rye (treatment #6) and 10% wheat bran plus 20% rye (treatment #5) had the highest BEs. In this crop, combinations of 20% wheat bran plus 10% rye (treatment #8) and 20% wheat bran plus 10% millet (treatment #9) also were significantly higher. Quality results for crop II were the same as crop I with treatment #10 (30% wheat bran only) significantly lower than the others. Significant differences for BE were found between crops I and II. However, the results from the two crops were not in conflict. The overall BEs for crop I were higher than those for crop II. Total means of BEs for crop I and II were 36.6% and 33.4%, respectively. There was no significant difference for quality between the two crops. Overall results for combined data were the same as those for each individual crop.

Comparison of the 20% and 30% levels of added nutrient showed that, in general, increasing the nutrient level increased yield although this was not always the case. For example, BE for treatments 7, 8 and 10 were higher for the 20% level than for the 30% level. In addition, some treatments did not produce mushrooms at the 20% level of supplementation, while at the 30% level, relatively high yields were obtained (treatment #2, 3 and 7). The BEs of treatment 6 (wheat bran : millet : rye = 10%:10%:10%) and treatment #5 (wheat bran : rye = 10%:20%) were significantly higher than the other treatments at the 30% and 20% levels, and thus, could be considered one of the best substrate formulas for WC828.

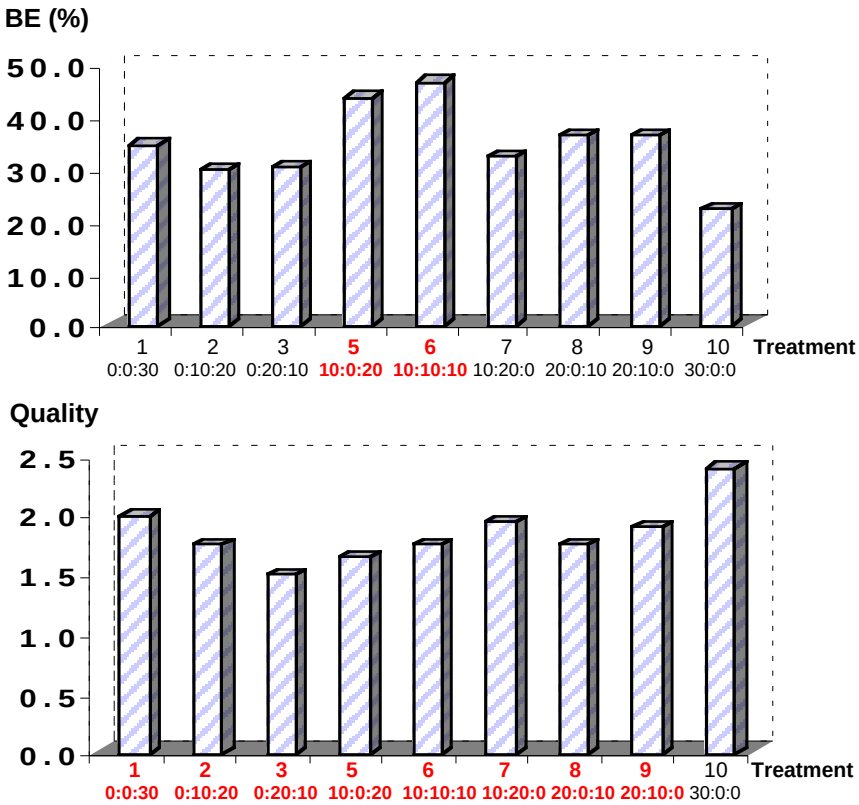


Figure 4.17. Graphic summary (9 out of 10 total treatments) of means of BEs (top) and quality (below) of two crops of *Grifola frondosa* (WC828) to determine the effect of 30% of wheat bran, millet and rye used alone or in various combinations (Experiment #13). Treatments highlighted in red bold font showed significantly higher BEs or quality in both crops in the experiment. Quality was rated 1-4 with 1 being the highest quality while 4 was the lowest quality rating. Ratios shown below each treatment number indicate percentage of wheat bran : millet : rye.

4.4 Discussion

Grifola frondosa (maitake) is a relatively new cultivated mushroom when compared with the 1400-year cultivation history for *Auricularia auricula*, 1000-year history for *Lentinula edodes*, and 400-year history for *Agaricus bisporus* (Chang 1999). It is anticipated that consumer demand for maitake will increase substantially in the next few years (D.J. Royse, personal communication). However, knowledge currently is very limited as to how different genotypes perform under various conditions and how different nutrient types and levels influence the crop cycle, and mushroom yield and quality. Therefore, I sought to examine the effects of genotype and selected nutrient supplements on the

mushroom crop cycle, yield and quality were evaluated to provide additional information to growers on cultivation of this mushroom.

Significant differences in crop cycle performance, and mushroom yield and quality among lines was observed. I also found substantial genetic variability among the isolates examined. Among 23 isolates from the wild I tested, nine (39%) did not fruit under our conditions.

I evaluated 10 isolates of U.S origin for fruiting capacity and only one (10%) isolate (M015) produced mushrooms consistently. Of the 12 isolates of Asian origin, eight (67%) isolates (WC836, WC828, WC835, M036, M037, M038, M039, and M040) produced mushroom consistently, while only four did not. The only European isolate available (WC493) did not fruit consistently in my tests and mushroom quality was good to excellent. While it may seem that Asian isolates have a higher proportion of strains that fruit relative to U.S. isolates, it should be pointed out that only one of the eight Asian isolates tested was a known wild isolate. At least four of the 12 isolates of Asian origin were/are used commercially. Therefore, fruiting capacities of wild isolates may be similar from continent to continent, but it is not possible to determine this with the available data.

Among the nine isolates of *G. frondosa* that produced mushrooms consistently, significant differences in BE and quality were observed. Isolates WC828, M036, M037 and M040 would appear to have the greatest potential for commercial production since they consistently produced the highest BEs and quality. Other isolates may be useful to breeders, however, because some of them had high BE (i.e. M039 and M015), with reasonable quality (i.e. WC836 and M038) and a short crop cycle (i.e. M039). Thus, genetic variability found in these isolates may be utilizable by breeders wishing to combine several desirable traits in a single line. Relatively little effort has been directed to breeding and selection

of lines for commercial cultivation in this species due to the short history of cultivation. My work should assist these efforts by providing initial data on fruiting capacity on various nutrient supplemented substrates and initial selection of strains for use in the breeding program. In addition, I have assembled an extensive collection of lines that is available to commercial companies that may want to pursue genetic improvement of lines. I should point out that isolates from Europe are under-represented in this collection and additional efforts should be directed toward increasing availability of lines from this area of the world. It might also be worthwhile to increase efforts to obtain representatives from South American and perhaps, New Zealand to determine if additional genetic variability would be available from these areas.

The results of my experiments clearly indicate that type and quality of nutrient supplements influence crop cycle and mushroom yield and quality. Combinations of two or three nutrients selected from wheat bran, rye or millet are the most desirable formulations found to date.

Nutrient levels of 10% proved too low for optimum growth and development of basidiomes. In fact, no mushrooms were produced when total nutrient levels were this low regardless of nutrient combination. Wheat bran is one of the most important factors for reducing crop cycle time. Formulations with only rye produced mushrooms, but were significantly lower in BEs than formulations with combinations of wheat bran and rye. Comparison of the supplement levels of 20% and 30% showed that as the nutrient levels increased, BEs in most cases, increased as well. The combination of 10% wheat bran, 10% millet and 10% rye and the combination of 10% wheat bran plus 20% rye were the best overall formulations for isolate WC828.

I found that better quality mushrooms and more consistent yields were produced from a more nutritionally balanced substrate. For example, higher

levels of wheat bran significantly shortened the crop cycle, but produced poorer quality mushrooms and lowered BEs. On the other hand, increasing wheat bran levels in sawdust substrates containing millet and rye or both, increased productivity and, often times, improved mushroom quality. The use of millet in substrate is another good example of poor yield and quality when millet is used alone. However, when it was used together with wheat bran and rye, significantly higher BEs and quality were achieved. Additional work evaluating the effects of other types and quantities of nutrients on mushroom BE and quality may reveal more productive combinations than I found in this study. I also would suggest that additional nutritional investigations be completed with two or more strains of diverse genetic origin. This would help minimize the potential independent effect of germplasm on crop cycle time and mushroom yield and quality.

Chapter 5: General Conclusions

DNA sequences of internal transcribed spacers 1 and 2 (ITS-1 and 2) and 5.8S regions of the nuclear ribosomal DNA transcriptional unit and the partial β-tubulin gene were obtained to compare nucleotide variation within 51 isolates of *Grifola frondosa* and its allies (*G. sordulenta*, *Polyporus umbellatus* and *Meripilus giganteus*). For rDNA and partial β-tubulin gene sequences, interspecies nucleotide variation for *Grifola frondosa* (5.4% for rDNA and 12.2% for β-tubulin) was relatively small compared to intraspecies variation (*G. frondosa* and *G. sordulenta*; 14.3% for rDNA and 30.2% for β-tubulin). Among isolates of *Grifola frondosa* and its allies, the nucleotide variation was much higher and unambiguous sequence alignments were not achievable. Most of the variation observed was attributable to nucleotide differences within ITS-1 and ITS-2 regions of rDNA and introns of β-tubulin gene sequences. I found that isolate WC484 from ATCC (American Type Culture Collection) is a misidentified isolate with a 98% match of rDNA (ITS-1, ITS-2 and 5.8s) sequences to *Spongipellis detectans*).

Molecular phylogenetic analyses of rDNA, β-tubulin and combined analyses revealed two major clades within *Grifola frondosa* by both neighbor joining (NJ) and maximum parsimony (MP) analyses. Clade I (U.S. clade) included all the U.S. isolates, while Clade II (Asian clade) consisted only of Asian isolates. The strong bootstrap support in β-tubulin and combined datasets suggested that the U.S. clade and the Asian clade are distinct groups that are evolving independently.

The single European isolate (WC493) shared a common ancestor with Asian isolates (with significant bootstrap support) based on combined and partial

β-tubulin gene sequence data in both NJ and MP analyses. However, ITS rDNA sequence data indicated that WC493 has a closer relationship with U.S. isolates (NJ analysis) or is the most recent ancestor of U.S. and Asian isolates in MP analysis. These groupings were not strongly supported by bootstrap analysis, however. Based on partial β-tubulin gene sequence and morphological data, *G. frondosa* and its allies (*G. sordulenta* and *P. umbellatus*) are independent lineages and also share a common ancestor. However, this was not fully resolved by ITS and β-tubulin sequence data.

In this study, genetic variation and phylogenetic information inferred from molecular data were of benefit in the attempts to select improved commercial lines of maitake. The phylogenetic analyses revealed that the major line WC828 (unknown origin) used commercially on most mushroom farms is of Asian decent. Furthermore, 23 isolates were selected based on their genetic diversity and then evaluated for the effects of genotypes on crop cycle time and biological efficiency (BE) and mushroom quality. Significant differences among lines were found for these parameters when mushrooms were produced on a nutrient supplemented sawdust substrate. Among 23 isolates tested, nine did not fruit. Among nine isolates that produced mushrooms consistently, significant differences in BEs and quality were found. Isolates WC828, M036, M037 and M040 were the most consistent with highest BEs (38.5%, 39.5%, 35.8% and 38.9%, respectively) and quality (1.2, 1.3, 1.4 and 1.2, respectively) than the other isolates with a shorter crop cycle (10, 9, 10 and 12 weeks, respectively).

In order to determine the effects of nutrient supplements on crop cycle time, BE and mushroom quality, experiments were conducted using various combinations of wheat bran, rye, millet and corn meal at 20% total supplement level. Statistical analyses indicated that different types of nutrient supplements have significant effects on crop cycle time and mushroom yield and quality. Combinations of two or three nutrients selected from wheat bran, millet and rye

were the most desirable formulation with short crop cycle, high quality and high BE.

Different crop cycle times and significant differences for BEs and quality were found for various combinations of wheat bran, rye and millet at 10%, 20% and 30% total supplement levels. A total nutrient supplement level of 10% was insufficient for mushroom production. Wheat bran was necessary for a consistent, relatively short crop cycle. Increased nutrient levels were positively correlated with increased BE. The combinations of 10% wheat bran, 10% millet and 10% rye (BE 47.1%, quality 1.8 and crop cycle 12 weeks) and 10% wheat bran plus 20% rye (BE 44%, quality 1.7 and crop cycle 10 weeks) were rated as the best formulations for isolate WC828 under our environmental conditions.

In conclusion, I believe this work has led to improved commercial production potential for maitake. Some of my best lines and formulas are already being used by commercial maitake growers in the United States. I expect my work will be adopted by other commercial growers worldwide.

Appendix A: Sequence alignment of ITS-1, 5.8 S, and ITS-2 rDNA sequences from isolates of *Grifola frondosa* and *G. sordulenta*

Sequence alignments were performed using the Clustal W algorithm (Higgins *et al.* 1991) in the MegAlign application of the Wisconsin Package (Genetics Computer Group, Madison, WI). Numbers at the top of each sequence block designate site location within the sequence alignment. The 5.8S region is labeled and underlined for the first isolate (M001).

```
[                1                                                          60]
M001            AGTTCAGAAAAGGGTTGTAGCTGGCCTCAAA-TCCGGGGCATGTGCACACCCTGCTCATC
M002            AGTTCAGAAAAGGGTTGTAGCTGGCCTCAAA-TCCGGGGCATGTGCACACCCTGCTCATC
M003            AGTTCAGAAAAGGGTTGTAGCTGGCCTCAAA-TCCGGGGCATGTGCACACCCTGCTCATC
M004            AGTTCAGAAAAGGGTTGTAGCTGGCCTCAAA-TCCGGGGCATGTGCACACCCTGCTCATC
M005            AGTTCAGAAAAGGGTTGTAGCTGGCCTCAAA-TCCGGGGCATGTGCACACCCTGCTCATC
M006            AGTTCAGAAAAGGGTTGTAGCTGGCCTCAAA-TCCGGGGCATGTGCACACCCTGCTCATC
M007            AGTACAGAAAAGGGTTGTAGCTGGCCTCAAA-TCCGGGGCATGTGCACACCCTGCTCATC
M008            AGTTCAGAAAAGGGTTGTAGCTGGCCTCAAA-TCCGGGGCATGTGCACACCCTGCTCATC
M009            AGTTCAGAAAAGGGTTGTAGCTGGCCTCAAA-TCCGGGGCATGTGCACACCCTGCTCATC
M010            AGTTCAGAAAAGGGTTGTAGCTGGCCTCAAA-TCCGGGGCATGTGCACACCCTGCTCATC
M011            AGTTCAGAAAAGGGTTGTAGCTGGCCTCAAA-TCCGGGGCATGTGCACACCCTGCTCATC
M012            AGTTCAGAAAAGGGTTGTAGCTGGCCTCAAA-TCCGGGGCATGTGCACACCCTGCTCATC
M013            AGTTCAGAAAAGGGTTGTAGCTGGCCTCAAA-TCCGGGGCATGTGCACACCCTGCTCATC
M014            AGTTCAGAAAAGGGTTGTAGCTGGCCTCAAA-TCCGGGGCATGTGCACACCCTGCTCATC
M015            AGTTCAGAAAAGGGTTGTAGCTGGCCTCAAA-TCCGGGGCATGTGCACACCCTGCTCATC
M016            AGTTCAGAAAAGGGTTGTCGCTGGCCTCAAAATTCGGGGCATGTGCACACCCTGCTCATC
M017            AGTTCAGAAAAGGGTTGTCGCTGGCCTCAAAATCCGGGGCATGTGCACACCCTGCTCATC
M018            AGTTCAGAAAAGGGTTGTCGCTGGCCTCAAAATCCGGGGCATGTGCACACCCTGCTCATC
M019            AGTTCAGAAAAGGGTTGTCGCTGGCCTCAAAATTCGGGGCATGTGCACACCCTGCTCATC
M020            AGTTCAGAAAAGGGTTGTCGCTGGCCTCAAAATCCGGGGCATGTGCACACCCTGCTCATC
M021            AGTTCAGAAAAGGGTTGTCGCTGGCCTCAAAATTCGGGGCATGTGCACACCCTGCTCATC
M029            AGTTCAGAAAAGGGTTGTCGCTGGCCTCAAAATCCGGGGCATGTGCACACCCTGCTCATC
M030            AGTACAGAAATGGGTTGTCGCTGGCCTCAAAATCCGGGGCATGTGCACACCCTGCTCATC
M031            AGTTCAGAAAAGGGTTGTCGCTGGCCTCAAAATCCGGGGCATGTGCACACCCTGCTCATC
M032            AGTTCAGAAATGGGTTGTCGCTGGCCTCAAAATCCGGGGCATGTGCACACCCTGCTCATC
M033            AGTTCAGAAAAGGGTTGTCGCTGGCCTCAAAATCCGGGGCATGTGCACACCCTGCTCATC
M034            AGTTCAGAAATGGGTTGTCGCTGGCCTCAAAATCCGGGGCATGTGCACACCCTGCTCATC
M035            AGTTCAGAAAAGGGTTGTAGCTGGCCTCAAA-TCCGGGGCATGTGCACACCCTGCTCATC
M036            AGTTCAGAAAAGGGTTGTCGCTGGCCTCAAAATCCGGGGCATGTGCACACCCTGCTCATC
M037            AGTTCAGAAAAGGGTTGTCGCTGGCCTCAAAATCCGGGGCATGTGCACACCCTGCTCATC
M038            AGTTCAGAAAAGGGTTGTCGCTGGCCTCAAAATCCGGGGCATGTGCACACCCTGCTCATC
M039            AGTTCAGAAAAGGGTTGTCGCTGGCCTCAAAATCCGGGGCATGTGCACACCCTGCTCATC
M040            AGTTCAGAAATGGGTTGTCGCTGGCCTCAAAATCCGGGGCATGTGCACACCCTGCTCATC
WC248           AGTTCAGAAAAGGGTTGTAGCTGGCCTCAAA-TCCGGGGCATGTGCACACCCTGCTCATC
WC364           AGTTCAGAAAAGGGTTGTAGCTGGCCTCAAA-TCCGGGGCATGTGCACACCCTGCTCATC
WC367           AGTTCAGAAAAGGGTTGTAGCTGGCCTCAAA-TCCGGGGCATGTGCACACCCTGCTCATC
WC483           AGTTCAGAAAAGGGTTGTAGCTGGCCTCAAA-TCCGGGGCATGTGCACACCCTGCTCATC
WC493           AGTTCAGAAAAGGGTTGTAGCTGGCCTCAAA-TCCGGGGCATGTGCACACCCTGCTCATC
WC555           AGTTCAGAAATGGGTTGTCGCTGGCCTCAAAATCCGGGGCATGTGCACACCCTGCTCATC
WC556           AGTTCAGANAAGGGTTGTCGCTGGCCTCAAAATTCGGGGCATGTGCACACCCTGCTCATC
WC557           AGTACAGAAAAGGGTTGTCGCTGGCCTCAAAATTCGGGGCATGTGCACACCCTGCTCATC
WC581           AGTTCAGAAATGGGTTGTCGCTGGCCTCAAAATCCGGGGCATGTGCACACCCTGCTCATC
WC582           AGTTCAGAAATGGGTTGTCGCTGGCCTCAAAATCCGGGGCATGTGCACACCCTGCTCATC
WC583           AGTTCAGAAAAGGGTTGTCGCTGGCCTCAAAATCCGGGGCATGTGCACACCCTGCTCATC
```

```
WC659             AGTTCAGAAAAGGGTTGTCGCTGGCCTCAAAATCCGGGGCATGTGCACACCCTGCTCATC
WC685             AGTTCAGAAAAGGGTTGTCGCTGGCCTCAAAATCCGGGGCATGTGCACACCCTGCTCATC
WC808             AGTTCAGAAAAGGGTTGTAGCTGGCCTCAAA-TCCGGGGCATGTGCACACCCTGCTCATC
WC828             AGTTCAGAAAAGGGTTGTCGCTGGCCTCAAAATCCGGGGCATGTGCACACCCTGCTCATC
WC834             AGTTCAGAAAAGGGTTGTCGCTGGCCTCAAAATCCGGGGCATGTGCACACCCTGCTCATC
WC835             AGTTCAGAAAAGGGTTGTCGCTGGCCTCAAAATCCGGGGCATGTGCACACCCTGCTCATC
WC836             AGTTCAGAAAAGGGTTGTCGCTGGCCTCAAAATCCGGGGCATGTGCACACCCTGCTCATC
G_sordulenta_G01  AGTTCTGAAACGGGTTGTAGCTGGCCTTA-----CGAGGCATGTGCACGCCCTGCTCATC

[                  61                                                    120]
M001              CACTCTCACACCTGTGCACTTTCTGTAGGTCGGTTCGGGATCTGGTCCCTCGCGGGGTCG
M002              CACTCTCACACCTGTGCACTTTCTGTAGGTCGGTTCGGGATCTGGTCCCTCGCGGGGTCG
M003              CACTCTCACACCTGTGCACTTTCTGTAGGTCGGTTCGGGATCTGGTCCCTCGCGGGGTCG
M004              CACTCTCACACCTGTGCACTTTCTGTAGGTCGGTTCGGGATCTGGTCCCTCGCGGGGTCG
M005              CACTCTCACACCTGTGCACTTTCTGTAGGTCGGTTCGGGATCTGGTCCCTCGCGGGGTCG
M006              CACTCTCACACCTGTGCACTTTCTGTAGGTCGGTTCGGGATCTGGTCCCTCGCGGGGTCG
M007              CACTCTCACACCTGTGCACTTTCTGTAGGTCGGTTCGGGATCTGGTCCCTCGCGGGGTCG
M008              CACTCTCACACCTGTGCACTTTCTGTAGGTCGGTTCGGGATCTGGTCCCTCGCGGGGTCG
M009              CACTCTCACACCTGTGCACTTTCTGTAGGTCGGTTCGGGATCTGGTCCCTCGCGGGGTCG
M010              CACTCTCACACCTGTGCACTTTCTGTAGGTCGGTTCGGGATCTGGTCCCTCGCGGGGTCG
M011              CACTCTCACACCTGTGCACTTTCTGTAGGTCGGTTCGGGATCTGGTCCCTCGCGGGGTCG
M012              CACTCTCACACCTGTGCACTTTCTGTAGGTCGGTTCGGGATCTGGTCCCTCGCGGGGTCG
M013              CACTCTCACACCTGTGCACTTTCTGTAGGTCGGTTCGGGATCTGGTCCCTCGCGGGGTCG
M014              CACTCTCACACCTGTGCACTTTCTGTAGGTCGGTTCGGGATCTGGTCCCTCGCGGGGTCG
M015              CACTCTCACACCTGTGCACTTTCTGTAGGTCGGTTCGGGATCTGGTCCCTCGCGGGGTCG
M016              CACTCTCACACCTGTGCACTTTCTGTAGGTCGGTTCGGGATCGGGTCCCTCGCGGGGTCG
M017              CACTCTCACACCTGTGCACTTTCTGTAGGTCGGTTCGGGATCTGGTCCCTCGCGGGGTCG
M018              CACTCTCACACCTGTGCACTTTCTGTAGGTCGGTTCGGGATCNGGTCCCTCGCGGGGTCG
M019              CACTCTCACACCTGTGCACTTTCTGTAGGTCGGTTCGGGATCGGGTCCCTCGCGGGGTCG
M020              CACTCTCACACCTGTGCACTTTCTGTAGGTCGGTTCGGGATCTGGTCCCTCGCGGGGTCG
M021              CACTCTCACACCTGTGCACTTTCTGTAGGTCGGTTCGGGATCTGGTCCCTCGCGGGGTCG
M029              CACTCTCACACCTGTGCACTTTCTGTAGGTCGGTTCGGGATCTGGTCCCTCGCGGGGTCG
M030              CACTCTCACACCTGTGCACTTTCTGTAGGTCGGTTCGGGATCGGGTCCCTCGCGGGGTCG
M031              CACTCTCACACCTGTGCACTTTCTGTAGGTCGGTTCGGGATCTGGTCCCTCGCGGGGTCG
M032              CACTCTCACACCTGTGCACTTTCTGTAGGTCGGTTCGGGATCGGGTCCCTCGCGGGGTCG
M033              CACTCTCACACCTGTGCACTTTCTGTAGGTCGGTTCGGGATCTGGTCCCTCGCGGGGTCG
M034              CACTCTCACACCTGTGCACTTTCTGTAGGTCGGTTCGGGATCGGGTCCCTCGCGGGGTCG
M035              CACTCTCACACCTGTGCACTTTCTGTAGGTCGGTTCGGGATCTGGTCCCTCGCGGGGTCG
M036              CACTCTCACACCTGTGCACTTTCTGTAGGTCGGTTCGGGATCTGGTCCCTCGCGGGGTCG
M037              CACTCTCACACCTGTGCACTTTCTGTAGGTCGGTTCGGGATCTGGTCCCTCGCGGGGTCG
M038              CACTCTCACACCTGTGCACTTTCTGTAGGTCGGTTCGGGATCTGGTCCCTCGCGGGGTCG
M039              CACTCTCACACCTGTGCACTTTCTGTAGGTCGGTTCGGGATCTGGTCCCTCGCGGGGTCG
M040              CACTCTCACACCTGTGCACTTTCTGTAGGTCGGTTCGGGATCNGGTCCCTCGCGGGGTCG
WC248             CACTCTCACACCTGTGCACTTTCTGTAGGTCGGTTCGGGATCTGGTCCCTCGCGGGGTCG
WC364             CACTCTCACACCTGTGCACTTTCTGTAGGTCGGTTCGGGATCTGGTCCCTCGCGGGGTCG
WC367             CACTCTCACACCTGTGCACTTTCTGTAGGTCGGTTCGGGATCTGGTCCCTCGCGGGGTCG
WC483             CACTCTCACACCTGTGCACTTTCTGTAGGTCGGTTCGGGATCTGGTCCCTCGCGGGGTCG
WC493             CACTCTCACACCTGTGCACTTTCTGTAGGTCGGTTCGGGATCTGGTCCCTCGCGGGGTCG
WC555             CACTCTCACACCTGTGCACTTTCTGTAGGTCGGTTCGGGATCGGGTCCCTCGCGGGGTCG
WC556             CACTCTCACACCTGTGCACTTTCTGTAGGTCGGTTCGGGATCTGGTCCCTCGCGGGGTCG
WC557             CACTCTCACACCTGTGCACTTTCTGTAGGTCGGTTCGGGATCGGGTCCCTCGCGGGGTCG
WC581             CACTCTCACACCTGTGCACTTTCTGTAGGTCGGTTCGGGATCTGGTCCCTCGCGGGGTCG
WC582             CACTCTCACACCTGTGCACTTTCTGTAGGTCGGTTCGGGATCTGGTCCCTCGCGGGGTCG
WC583             CACTCTCACACCTGTGCACTTTCTGTAGGTCGGTTCGGGATCTGGTCCCTCGCGGGGTCG
WC659             CACTCTCACACCTGTGCACTTTCTGTAGGTCGGTTCGGGATCTGGTCCCTCGCGGGGTCG
WC685             CACTCTCACACCTGTGCACTTTCTGTAGGTCGGTTCGGGATCTGGTCCCTCGCGGGGTCG
WC808             CACTCTCACACCTGTGCACTTTCTGTAGGTCGGTTCGGGATCTGGTCCCTCGCGGGGTCG
WC828             CACTCTCACACCTGTGCACTTTCTGTAGGTCGGTTCGGGATCTGGTCCCTCGCGGGGTCG
WC834             CACTCTCACACCTGTGCACTTTCTGTAGGTCGGTTCGGGATCTGGTCCCTCGCGGGGTCG
WC835             CACTCTCACACCTGTGCACTTTCTGTAGGTCGGTTCGGGATCTGGTCCCTCGCGGGGTCG
WC836             CACTCTCACACCTGTGCACTTTCTGTAGGTCGGTTCGGGATCTGGTCCCTCGCGGGGTCG
G_sordulenta_G01  CACTCT-ACACCTGTGCACCATCTGTAGGTCGGTTTGGGTTCGGATGCTTCGCGGCGTTC

[                  121                                                   180]
M001              GGTTCTGTGCCTTCCTATGTACAATCACAAACGCTTCAGTATTCAGAATGTCATTGCGAT
M002              GGTTCTGTGCCTTCCTATGTACAATCACAAACGCTTCAGTATTCAGAATGTCATTGCGAT
M003              GGTTCTGTGCCTTCCTATGTACAATCACAAACGCTTCAGTATTCAGAATGTCATTGCGAT
M004              GGTTCTGTGCCTTCCTATGTACAATCACAAACGCTTCAGTATTCAGAATGTCATTGCGAT
```

```
M005              GGTTCTGTGCCTTCCTATGTACAATCACAAACGCTTCAGTATTCAGAATGTCATTGCGAT
M006              GGTTCTGTGCCTTCCTATGTACAATCACAAACGCTTCAGTATTCAGAATGTCATTGCGAT
M007              GGTTCTGTGCCTTCCTATGTACAATCACAAACGCTTCAGTATTCAGAATGTCATTGCGAT
M008              GGTTCTGTGCCTTCCTATGTACAATCACAAACGCTTCAGTATTCAGAATGTCATTGCGAT
M009              GGTTCTGTGCCTTCCTATGTACAATCACAAACGCTTCAGTATTCAGAATGTCATTGCGAT
M010              GGTTCTGTGCCTTCCTATGTACAATCACAAACGCTTCAGTATTCAGAATGTCATTGCGAT
M011              GGTTCTGTGCCTTCCTATGTACAATCACAAACGCTTCAGTATTCAGAATGTCATTGCGAT
M012              GGTTCTGTGCCTTCCTATGTACAATCACAAACGCTTCAGTATTCAGAATGTCATTGCGAT
M013              GGTTCTGTGCCTTCCTATGTACAATCACAAACGCTTCAGTATTCAGAATGTCATTGCGAT
M014              GGTTCTGTGCCTTCCTATGTACAATCACAAACGCTTCAGTATTCAGAATGTCATTGCGAT
M015              GGTTCTGTGCCTTCCTATGTACAATCACAAACGCTTCAGTATTCAGAATGTCATTGCGAT
M016              GGTTCTGCGCCTTCCTATGTACAATCACAAACGCTTCAGTATTCAGAATGTCATTGCGAT
M017              GGTTCTGCGCCTTCCTATGTACAATCACAAACGCTTCAGTATTCAGAATGTCATTGCGAT
M018              GGTTCTGCGCCTTCCTATGTACAATCACAAACGCTTCAGTATTCAGAATGTCATTGCGAT
M019              GGTTCTGCGCCTTCCTATGTACAATCACAAACGCTTCAGTATTCAGAATGTCATCGCGAT
M020              GGTTCTGCGCCTTCCTATGTACAATCACAAACGCTTCAGTATTCAGAATGTCATTGCGAT
M021              GGTTCTGCGCCTTCCTATGTACAATCACAAACGCTTCAGTATTCAGAATGTCATTGCGAT
M029              GGTTCTGCGCCTTCCTATGTACAATCACAAACGCTTCAGTATTCAGAATGTCATTGCGAT
M030              GGTTCTGCGCCTTCCTATGTACAATCACAAACGCTTCAGTATTCAGAATGTCATTGCGAT
M031              GGTTCTGCGCCTTCCTATGTACAATCACAAACGCTTCAGTATTCAGAATGTCATTGCGAT
M032              GGTTCTGCGCCTTCCTATGTACAATCACAAACGCTTCAGTATTCAGAATGTCATTGCGAT
M033              GGTTCTGCGCCTTCCTATGTACAATCACAAACGCTTCAGTATTCAGAATGTCATTGCGAT
M034              GGTTCTGCGCCTTCCTATGTACAATCACAAACGCTTCAGTATTCAGAATGTCATTGCGAT
M035              GGTTCTGTGCCTTCCTATGTACAATCACAAACGCTTCAGTATTCAGAATGTCATTGCGAT
M036              GGTTCTGCGCCTTCCTATGTACAATCACAAACGCTTCAGTATTCAGAATGTCATTGCGAT
M037              GGTTCTGCGCCTTCCTATGTACAATCACAAACGCTTCAGTATTCAGAATGTCATTGCGAT
M038              GGTTCTGCGCCTTCCTATGTACAATCACAAACGCTTCAGTATTCAGAATGTCATTGCGAT
M039              GGTTCTGCGCCTTCCTATGTACAATCACAAACGCTTCAGTATTCAGAATGTCATTGCGAT
M040              GGTTCTGCGCCTTCCTATGTACAATCACAAACGCTTCAGTATTCAGAATGTCATTGCGAT
WC248             GGTTCTGTGCCTTCCTATGTACAATCACAAACGCTTCAGTATTCAGAATGTCATTGCGAT
WC364             GGTTCTGTGCCTTCCTATGTACAATCACAAACGCTTCAGTATTCAGAATGTCATTGCGAT
WC367             GGTTCTGTGCCTTCCTATGTACAATCACAAACGCTTCAGTATTCAGAATGTCATTGCGAT
WC483             GGTTCTGTGCCTTCCTATGTACAATCACAAACGCTTCAGTATTCAGAATGTCATTGCGAT
WC493             GGTTCTGTGCCTTCCTATGTACAATCACAAACGCTTCAGTATTCAGAATGTCATTGCGAT
WC555             GGTTCTGCGCCTTCCTATGTACAATCACAAACGCTTCAGTATTCAGAATGTCATTGCGAT
WC556             GGTTCTGCGCCTTCCTATGTACAATCACAAACGCTTCAGTATTCAGAATGTCATTGCGAT
WC557             GGTTCTGCGCCTTCCTATGTACAATCACAAACGCTTCAGTATTCAGAATGTCATTGCGAT
WC581             GGTTCTGCGCCTTCCTATGTACAATCACAAACGCTTCAGTATTCAGAATGTCATTGCGAT
WC582             GGTTCTGCGCCTTCCTATGTACAATCACAAACGCTTCAGTATTCAGAATGTCATTGCGAT
WC583             GGTTCTGCGCCTTCCTATGTACAATCACAAACGCTTCAGTATTCAGAATGTCATTGCGAT
WC659             GGTTCTGCGCCTTCCTATGTACAATCACAAACGCTTCAGTATTCAGAATGTCATTGCGAT
WC685             GGTTCTGCGCCTTCCTATGTACAATCACAAACGCTTCAGTATTCAGAATGTCATTGCGAT
WC808             GGTTCTGTGCCTTCCTATGTACAATCACAAACGCTTCAGTATTCAGAATGTCATTGCGAT
WC828             GGTTCTGCGCCTTCCTATGTACAATCACAAACGCTTCAGTATTCAGAATGTCATTGCGAT
WC834             GGTTCTGCGCCTTCCTATGTACAATCACAAACGCTTCAGTATTCAGAATGTCATTGCGAT
WC835             GGTTCTGCGCCTTCCTATGTACAATCACAAACGCTTCAGTATTCAGAATGTCATTGCGAT
WC836             GGTTCTGCGCCTTCCTATGTACAATCACAAACGCTTCAGTATTCAGAATGTCATTGCGAT
G_sordulenta_G01  GGGCTCGGGCCTTCCTATGTACT-TCACACACGCTTTAGTAT-CAGAATGTAATTGCGA-

[                  181              5.8S→                                240]
M001              AATTAAAACGCATCTTATACAACTTTCAGCAACGGATCTCTTGGCTCTCGCATCGATGAA
M002              AATTAAAACGCATCTTATACAACTTTCAGCAACGGATCTCTTGGCTCTCGCATCGATGAA
M003              AATTAAAACGCATCTTATACAACTTTCAGCAACGGATCTCTTGGCTCTCGCATCGATGAA
M004              AATTAAAACGCATCTTATACAACTTTCAGCAACGGATCTCTTGGCTCTCGCATCGATGAA
M005              AATTAAAACGCATCTTATACAACTTTCAGCAACGGATCTCTTGGCTCTCGCATCGATGAA
M006              AATTAAAACGCATCTTATACAACTTTCAGCAACGGATCTCTTGGCTCTCGCATCGATGAA
M007              AATTAAAACGCATCTTATACAACTTTCAGCAACGGATCTCTTGGCTCTCGCATCGATGAA
M008              AATTAAAACGCATCTTATACAACTTTCAGCAACGGATCTCTTGGCTCTCGCATCGATGAA
M009              AATTAAAACGCATCTTATACAACTTTCAGCAACGGATCTCTTGGCTCTCGCATCGATGAA
M010              AATTAAAACGCATCTTATACAACTTTCAGCAACGGATCTCTTGGCTCTCGCATCGATGAA
M011              AATTAAAACGCATCTTATACAACTTTCAGCAACGGATCTCTTGGCTCTCGCATCGATGAA
M012              AATTAAAACGCATCTTATACAACTTTCAGCAACGGATCTCTTGGCTCTCGCATCGATGAA
M013              AATTAAAACGCATCTTATACAACTTTCAGCAACGGATCTCTTGGCTCTCGCATCGATGAA
M014              AATTAAAACGCATCTTATACAACTTTCAGCAACGGATCTCTTGGCTCTCGCATCGATGAA
M015              AATTAAAACGCATCTTATACAACTTTCAGCAACGGATCTCTTGGCTCTCGCATCGATGAA
M016              AATTAAAACGCATCTTATACAACTTTCAGCAACGGATCTCTTGGCTCTCGCATCGATGAA
M017              AATTAAAACGCATCTTATACAACTTTCAGCAACGGATCTCTTGGCTCTCGCATCGATGAA
M018              AATTAAAACGCATCTTATACAACTTTCAGCAACGGATCTCTTGGCTCTCGCATCGATGAA
```

```
M019              AATTAAAACGCATCTTATACAACTTTCAGCAACGGATCTCTTGGCTCTCGCATCGATGAA
M020              AATTAAAACGCATCTTATACAACTTTCAGCAACGGATCTCTTGGCTCTCGCATCGATGAA
M021              AATTAAAACGCATCTTATACAACTTTCAGCAACGGATCTCTTGGCTCTCGCATCGATGAA
M029              AATTAAAACGCATCTTATACAACTTTCAGCAACGGATCTCTTGGCTCTCGCATCGATGAA
M030              AATTAAAACGCATCTTATACAACTTTCAGCAACGGATCTCTTGGCTCTCGCATCGATGAA
M031              AATTAAAACGCATCTTATACAACTTTCAGCAACGGATCTCTTGGCTCTCGCATCGATGAA
M032              AATTAAAACGCATCTTATACAACTTTCAGCAACGGATCTCTTGGCTCTCGCATCGATGAA
M033              AATTAAAACGCATCTTATACAACTTTCAGCAACGGATCTCTTGGCTCTCGCATCGATGAA
M034              AATTAAAACGCATCTTATACAACTTTCAGCAACGGATCTCTTGGCTCTCGCATCGATGAA
M035              AATTAAAACGCATCTTATACAACTTTCAGCAACGGATCTCTTGGCTCTCGCATCGATGAA
M036              AATTAAAACGCATCTTATACAACTTTCAGCAACGGATCTCTTGGCTCTCGCATCGATGAA
M037              AATTAAAACGCATCTTATACAACTTTCAGCAACGGATCTCTTGGCTCTCGCATCGATGAA
M038              AATTAAAACGCATCTTATACAACTTTCAGCAACGGATCTCTTGGCTCTCGCATCGATGAA
M039              AATTAAAACGCATCTTATACAACTTTCAGCAACGGATCTCTTGGCTCTCGCATCGATGAA
M040              AATTAAAACGCATCTTATACAACTTTCAGCAACGGATCTCTTGGCTCTCGCATCGATGAA
WC248             AATTAAAACGCATCTTATACAACTTTCAGCAACGGATCTCTTGGCTCTCGCATCGATGAA
WC364             AATTAAAACGCATCTTATACAACTTTCAGCAACGGATCTCTTGGCTCTCGCATCGATGAA
WC367             AATTAAAACGCATCTTATACAACTTTCAGCAACGGATCTCTTGGCTCTCGCATCGATGAA
WC483             AATTAAAACGCATCTTATACAACTTTCAGCAACGGATCTCTTGGCTCTCGCATCGATGAA
WC493             AATTAAAACGCATCTTATACAACTTTCAGCAACGGATCTCTTGGCTCTCGCATCGATGAA
WC555             AATTAAAACGCATCTTATACAACTTTCAGCAACGGATCTCTTGGCTCTCGCATCGATGAA
WC556             AATTAAAACGCATCTTATACAACTTTCAGCAACGGATCTCTTGGCTCTCGCATCGATGAA
WC557             AATTAAAACGCATCTTATACAACTTTCAGCAACGGATCTCTTGGCTCTCGCATCGATGAA
WC581             AATTAAAACGCATCTTATACAACTTTCAGCAACGGATCTCTTGGCTCTCGCATCGATGAA
WC582             AATTAAAACGCATCTTATACAACTTTCAGCAACGGATCTCTTGGCTCTCGCATCGATGAA
WC583             AATTAAAACGCATCTTATACAACTTTCAGCAACGGATCTCTTGGCTCTCGCATCGATGAA
WC659             AATTAAAACGCATCTTATACAACTTTCAGCAACGGATCTCTTGGCTCTCGCATCGATGAA
WC685             AATTAAAACGCATCTTATACAACTTTCAGCAACGGATCTCTTGGCTCTCGCATCGATGAA
WC808             AATTAAAACGCATCTTATACAACTTTCAGCAACGGATCTCTTGGCTCTCGCATCGATGAA
WC828             AATTAAAACGCATCTTATACAACTTTCAGCAACGGATCTCTTGGCTCTCGCATCGATGAA
WC834             AATTAAAACGCATCTTATACAACTTTCAGCAACGGATCTCTTGGCTCTCGCATCGATGAA
WC835             AATTAAAACGCATCTTATACAACTTTCAGCAACGGATCTCTTGGCTCTCGCATCGATGAA
WC836             AATTAAAACGCATCTTATACAACTTTCAGCAACGGATCTCTTGGCTCTCGCATCGATGAA
G_sordulenta_G01  ---TAAAACGCACCTTATACAACTTTCAGCAACGGATCTCTTGGCTCTCGCATCGATGAA

[                  241                                                    300]
M001              GAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTT
M002              GAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTT
M003              GAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTT
M004              GAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTT
M005              GAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTT
M006              GAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTT
M007              GAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTT
M008              GAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTT
M009              GAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTT
M010              GAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTT
M011              GAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTT
M012              GAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTT
M013              GAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTT
M014              GAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTT
M015              GAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTT
M016              GAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTT
M017              GAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTT
M018              GAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTT
M019              GAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTT
M020              GAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTT
M021              GAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTT
M029              GAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTT
M030              GAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTT
M031              GAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTT
M032              GAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTT
M033              GAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTT
M034              GAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTT
M035              GAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTT
M036              GAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTT
M037              GAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTT
M038              GAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTT
M039              GAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTT
```

```
M040               GAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTT
WC248              GAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTT
WC364              GAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTT
WC367              GAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTT
WC483              GAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTT
WC493              GAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTT
WC555              GAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTT
WC556              GAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTT
WC557              GAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTT
WC581              GAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTT
WC582              GAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTT
WC583              GAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTT
WC659              GAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTT
WC685              GAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTT
WC808              GAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTT
WC828              GAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTT
WC834              GAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTT
WC835              GAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTT
WC836              GAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTT
G_sordulenta_G01   GAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTT

[                   301                                                ←5.8S 360]
M001               TGAACGCACCTTGCGCTCCTTGGTATTCCGAGGAGCATGCCTGTTTGAGTGTCATGGAAT
M002               TGAACGCACCTTGCGCTCCTTGGTATTCCGAGGAGCATGCCTGTTTGAGTGTCATGGAAT
M003               TGAACGCACCTTGCGCTCCTTGGTATTCCGAGGAGCATGCCTGTTTGAGTGTCATGGAAT
M004               TGAACGCACCTTGCGCTCCTTGGTATTCCGAGGAGCATGCCTGTTTGAGTGTCATGGAAT
M005               TGAACGCACCTTGCGCTCCTTGGTATTCCGAGGAGCATGCCTGTTTGAGTGTCATGGAAT
M006               TGAACGCACCTTGCGCTCCTTGGTATTCCGAGGAGCATGCCTGTTTGAGTGTCATGGAAT
M007               TGAACGCACCTTGCGCTCCTTGGTATTCCGAGGAGCATGCCTGTTTGAGTGTCATGGAAT
M008               TGAACGCACCTTGCGCTCCTTGGTATTCCGAGGAGCATGCCTGTTTGAGTGTCATGGAAT
M009               TGAACGCACCTTGCGCTCCTTGGTATTCCGAGGAGCATGCCTGTTTGAGTGTCATGGAAT
M010               TGAACGCACCTTGCGCTCCTTGGTATTCCGAGGAGCATGCCTGTTTGAGTGTCATGGAAT
M011               TGAACGCACCTTGCGCTCCTTGGTATTCCGAGGAGCATGCCTGTTTGAGTGTCATGGAAT
M012               TGAACGCACCTTGCGCTCCTTGGTATTCCGAGGAGCATGCCTGTTTGAGTGTCATGGAAT
M013               TGAACGCACCTTGCGCTCCTTGGTATTCCGAGGAGCATGCCTGTTTGAGTGTCATGGAAT
M014               TGAACGCACCTTGCGCTCCTTGGTATTCCGAGGAGCATGCCTGTTTGAGTGTCATGGAAT
M015               TGAACGCACCTTGCGCTCCTTGGTATTCCGAGGAGCATGCCTGTTTGAGTGTCATGGAAT
M016               TGAACGCACCTTGCGCTCCTTGGTATTCCGAGGAGCATGCCTGTTTGAGTGTCATGGAAT
M017               TGAACGCACCTTGCGCTCCTTGGTATTCCGAGGAGCATGCCTGTTTGAGTGTCATGGAAT
M018               TGAACGCACCTTGCGCTCCTTGGTATTCCGAGGAGCATGCCTGTTTGAGTGTCATGGAAT
M019               TGAACGCACCTTGCGCTCCTTGGTATTCCGAGGAGCATGCCTGTTTGAGTGTCATGGAAT
M020               TGAACGCACCTTGCGCTCCTTGGTATTCCGAGGAGCATGCCTGTTTGAGTGTCATGGAAT
M021               TGAACGCACCTTGCGCTCCTTGGTATTCCGAGGAGCATGCCTGTTTGAGTGTCATGGAAT
M029               TGAACGCACCTTGCGCTCCTTGGTATTCCGAGGAGCATGCCTGTTTGAGTGTCATGGAAT
M030               TGAACGCACCTTGCGCTCCTTGGTATTCCGAGGAGCATGCCTGTTTGAGTGTCATGGAAT
M031               TGAACGCACCTTGCGCTCCTTGGTATTCCGAGGAGCATGCCTGTTTGAGTGTCATGGAAT
M032               TGAACGCACCTTGCGCTCCTTGGTATTCCGAGGAGCATGCCTGTTTGAGTGTCATGGAAT
M033               TGAACGCACCTTGCGCTCCTTGGTATTCCGAGGAGCATGCCTGTTTGAGTGTCATGGAAT
M034               TGAACGCACCTTGCGCTCCTTGGTATTCCGAGGAGCATGCCTGTTTGAGTGTCATGGAAT
M035               TGAACGCACCTTGCGCTCCTTGGTATTCCGAGGAGCATGCCTGTTTGAGTGTCATGGAAT
M036               TGAACGCACCTTGCGCTCCTTGGTATTCCGAGGAGCATGCCTGTTTGAGTGTCATGGAAT
M037               TGAACGCACCTTGCGCTCCTTGGTATTCCGAGGAGCATGCCTGTTTGAGTGTCATGGAAT
M038               TGAACGCACCTTGCGCTCCTTGGTATTCCGAGGAGCATGCCTGTTTGAGTGTCATGGAAT
M039               TGAACGCACCTTGCGCTCCTTGGTATTCCGAGGAGCATGCCTGTTTGAGTGTCATGGAAT
M040               TGAACGCACCTTGCGCTCCTTGGTATTCCGAGGAGCATGCCTGTTTGAGTGTCATGGAAT
WC248              TGAACGCACCTTGCGCTCCTTGGTATTCCGAGGAGCATGCCTGTTTGAGTGTCATGGAAT
WC364              TGAACGCACCTTGCGCTCCTTGGTATTCCGAGGAGCATGCCTGTTTGAGTGTCATGGAAT
WC367              TGAACGCACCTTGCGCTCCTTGGTATTCCGAGGAGCATGCCTGTTTGAGTGTCATGGAAT
WC483              TGAACGCACCTTGCGCTCCTTGGTATTCCGAGGAGCATGCCTGTTTGAGTGTCATGGAAT
WC493              TGAACGCACCTTGCGCTCCTTGGTATTCCGAGGAGCATGCCTGTTTGAGTGTCATGGAAT
WC555              TGAACGCACCTTGCGCTCCTTGGTATTCCGAGGAGCATGCCTGTTTGAGTGTCATGGAAT
WC556              TGAACGCACCTTGCGCTCCTTGGTATTCCGAGGAGCATGCCTGTTTGAGTGTCATGGAAT
WC557              TGAACGCACCTTGCGCTCCTTGGTATTCCGAGGAGCATGCCTGTTTGAGTGTCATGGAAT
WC581              TGAACGCACCTTGCGCTCCTTGGTATTCCGAGGAGCATGCCTGTTTGAGTGTCATGGAAT
WC582              TGAACGCACCTTGCGCTCCTTGGTATTCCGAGGAGCATGCCTGTTTGAGTGTCATGGAAT
WC583              TGAACGCACCTTGCGCTCCTTGGTATTCCGAGGAGCATGCCTGTTTGAGTGTCATGGAAT
WC659              TGAACGCACCTTGCGCTCCTTGGTATTCCGAGGAGCATGCCTGTTTGAGTGTCATGGAAT
WC685              TGAACGCACCTTGCGCTCCTTGGTATTCCGAGGAGCATGCCTGTTTGAGTGTCATGGAAT
```

```
WC808             TGAACGCACCTTGCGCTCCTTGGTATTCCGAGGAGCATGCCTGTTTGAGTGTCATGGAAT
WC828             TGAACGCACCTTGCGCTCCTTGGTATTCCGAGGAGCATGCCTGTTTGAGTGTCATGGAAT
WC834             TGAACGCACCTTGCGCTCCTTGGTATTCCGAGGAGCATGCCTGTTTGAGTGTCATGGAAT
WC835             TGAACGCACCTTGCGCTCCTTGGTATTCCGAGGAGCATGCCTGTTTGAGTGTCATGGAAT
WC836             TGAACGCACCTTGCGCTCCTTGGTATTCCGAGGAGCATGCCTGTTTGAGTGTCATGGAAT
G_sordulenta_G01  TGAACGCACCTTGCGCTCCTTGGTATTCCGAGGAGCATGCCTGTTTGAGTGTCATGGAAT

[                  361                                                    420]
M001              TCTCAACCCACACGTCCTTGTGATGTGGACGGGCTTGGATATTGGAGGTTTCTGCCGGCC
M002              TCTCAACCCACACGTCCTTGTGATGTGGACGGGCTTGGACTTTGGAGGTTTCTGCCGGCC
M003              TCTCAACCCACACGTCCTTGTGATGTGGACGGGCTTGGACTTTGGAGGTTTCTGCCGGCC
M004              TCTCAACCCACACGTCCTTGTGATGTGGACGGGCTTGGACTTTGGAGGTTTCTGCCGGCC
M005              TCTCAACCCACACGTCCTTGTGATGTGGACGGGCTTGGACTTTGGAGGTTTCTGCCGGCC
M006              TCTCAACCCACACGTCCTTGTGATGTGGACGGGCTTGGACTTTGGAGGTTTCTGCCGGCC
M007              TCTCAACCCACACGTCCTTGTGATGTGGACGGGCTTGGATATTGGAGGTTTCTGCCGGCC
M008              TCTCAACCCACACGTCCTTGTGATGTGGACGGGCTTGGATATTGGAGGTTTCTGCCGGCC
M009              TCTCAACCCACACGTCCTTGTGATGTGGACGGGCTTGGATATTGGAGGTTTCTGCCGGCC
M010              TCTCAACCCACACGTCCTTGTGATGTGGACGGGCTTGGACTTTGGAGGTTTCTGCCGGCC
M011              TCTCAACCCACACGTCCTTGTGATGTGGACGGGCTTGGATATTGGAGGTTTCTGCCGGCC
M012              TCTCAACCCACACGTCCTTGTGATGTGGACGGGCTTGGATATTGGAGGTTTCTGCCGGCC
M013              TCTCAACCCACACGTCCTTGTGATGTGGACGGGCTTGGATATTGGAGGTTTCTGCCGGCC
M014              TCTCAACCCACACGTCCTTGTGATGTGGACGGGCTTGGATATTGGAGGTTTCTGCCGGCC
M015              TCTCAACCCACACGTCCTTGTGATGTGGACGGGCTTGGACTTTGGAGGTTTCTGCCGGCC
M016              TCTCAACCCACACATCCTTGTGATGTGGACGGGCTTGGACTTTGGAGGTTTCTGCCGGCC
M017              TCTCAACCCACACATCCTTGTGATGTGGACGGGCTTGGACTTTGGAGGTTTCTGCCGGCC
M018              TCTCAACCCACACATCCTTGTGATGTGGACGGGCTTGGACTTTGGAGGTTTCTGCCGGCC
M019              TCTCAACCCACACGTCCTTGTGACGTGGACGGGCTTGGACTTTGGAGGTTTCTGCCGGCC
M020              TCTCAACCCACACATCCTTGTGATGTGGACGGGCTTGGACTTTGGAGGTTTCTGCCGGCC
M021              TCTCAACCCACACATCCTTGTGACGTGCACGGGCTTGGACTTTGGAGGTTTCTGCCGGCC
M029              TCTCAACCCACACATCCTTGTGATGTGGACGGGCTTGGACTTTGGAGGTTTCTGCCGGCC
M030              TCTCAACCCACACGTCCTTGTGATGTGGACGGGCTTGGACTTTGGAGGTTTCTGCCGGCC
M031              TCTCAACCCACACATCCTTGTGATGTGGACGGGCTTGGACTTTGGAGGTTTCTGCCGGCC
M032              TCTCAACCCACACGTCCTTGTGATGTGGACGGGCTTGGACTTTGGAGGTTTCTGCCGGCC
M033              TCTCAACCCACACATCCTTGTGATGTGGACGGGCTTGGACTTTGGAGGTTTCTGCCGGCC
M034              TCTCAACCCACACGTCCTTGTGATGTGGACGGGCTTGGACTTTGGAGGTTTCTGCCGGCC
M035              TCTCAACCCACACGTCCTTGTGATGTGGACGGGCTTGGATATTGGAGGTTTCTGCCGGCC
M036              TCTCAACCCACACATCCTTGTGATGTGGACGGGCTTGGACTTTGGAGGTTTCTGCCGGCC
M037              TCTCAACCCACACGTCCTTGTGATGTGGACGGGCTTGGACTTTGGAGGTTTCTGCCGGCC
M038              TCTCAACCCACACGTCCTTGTGATGTGGACGGGCTTGGACTTTGGAGGTTTCTGCCGGCC
M039              TCTCAACCCACACGTCCTTGTGATGTGGACGGGCTTGGACTTTGGAGGTTTCTGCCGGCC
M040              TCTCAACCCACACATCCTTGTGATGTGGACGGGCTTGGACTTTGGAGGTTTCTGCCGGCC
WC248             TCTCAACCCACACGTCCTTGTGATGTGGACGGGCTTGGACTTTGGAGGTTTCTGCCGGCC
WC364             TCTCAACCCACACGTCCTTGTGATGTGGACGGGCTTGGACTTTGGAGGTTTCTGCCGGCC
WC367             TCTCAACCCACACGTCCTTGTGATGTGGACGGGCTTGGACATTGGAGGTTTCTGCCGGCC
WC483             TCTCAACCCACACGTCCTTGTGATGTGGACGGGCTTGGACTTTGGAGGTTTCTGCCGGCC
WC493             TCTCAACCCACACATCCTTGTGATGTGGACGGGCTTGGACTTTGGAGGCTCATGCCGGTC
WC555             TCTCAACCCACACATCCTTGTGATGTGGACGGGCTTGGACTTTGGAGGTTTCTGCCGGCC
WC556             TCTCAACCCACACATCCTTGCGATGTGGACGGGCTTGGACTTTGGAGGTTTCTGCCGGCC
WC557             TCTCAACCCACACATCCTTGCGATGTGGACGGGCTTGGACTTTGGAGGTTTCTGCCGGCC
WC581             TCTCAACCCACACGTCCTTGTGATGTGGACGGGCTTGGACTTTGGAGGTTTCTGCCGGCC
WC582             TCTCAACCCACACATCCTTGTGATGTGGACGGGCTTGGACTTTGGAGGTTTCTGCCGGCC
WC583             TCTCAACCCACACGTCCTTGTGATGTGGACGGGCTTGGACTTTGGAGGTTTCTGCCGGCC
WC659             TCTCAACCCACACGTCCTTGTGATGTGGACGGGCTTGGACTTTGGAGGTTTCTGCCGGCC
WC685             TCTCAACCCACACATCCTTGTGATGTGGACGGGCTTGGACTTTGGAGGTTTCTGCCGGCC
WC808             TCTCAACCCACACGTCCTTGTGATGTGGACGGGCTTGGATATTGGAGGTTTCTGCCGGCC
WC828             TCTCAACCCACACATCCTTGTGATGTGGACGGGCTTGGACTTTGGAGGTTTCTGCCGGCC
WC834             TCTCAACCCACACATCCTTGTGATGTGGACGGGCTTGGACTTTGGAGGTTTCTGCCGGCC
WC835             TCTCAACCCACACATCCTTGTGATGTGGACGGGCTTGGACTTTGGAGGTTTCTGCCGGCC
WC836             TCTCAACCCACACATCCTTGTGATGTGGACGGGCTTGGACTTTGGAGGTTTCTGCCGGCC
G_sordulenta_G01  CATCAACCCATATGTCCTTGTGTCG--GATGGGCTTGGA-TTTGGAGGCTTATGCCGGCC

[                  421                                                    480]
M001              CCCCATTCGGGTCGGCTCCTCTGGAATGCATTAGCTCCATCCCTTGCGGATCGGCTCTCG
M002              CCCCATTCGGGTCGGCTCCTCTGGAATGCATTAGCTCCATCCCTTGCGGATCGGCTCTCG
M003              CCCCATTCGGGTCGGCTCCTCTGGAATGCATTAGCTCCATCCCTTGCGGATCGGCTCTCG
M004              CCCCATTCGGGTCGGCTCCTCTGGAATGCATTAGCTCCATCCCTTGCGGATCGGCTCTCG
M005              CCCCATTCGGGTCGGCTCCTCTGGAATGCATTAGCTCCATCCCTTGCGGATCGGCTCTCG
M006              CCCCATTCGGGTCGGCTCCTCTGGAATGCATTAGCTCCATCCCTTGCGGATCGGCTCTCG
```

```
M007               CCCCATTCGGGTCGGCTCCTCTGGAATGCATTAGCTCCATCCCTTGCGGATCGGCTCTCG
M008               CCCCATTCGGGTCGGCTCCTCTGGAATGCATTAGCTCCATCCCTTGCGGATCGGCTCTCG
M009               CCCCATTCGGGTCGGCTCCTCTGGAATGCATTAGCTCCATCCCTTGCGGATCGGCTCTCG
M010               CCCCATTCGGGTCGGCTCCTCTGGAATGCATTAGCTCCATCCCTTGCGGATCGGCTCTCG
M011               CCCCATTCGGGTCGGCTCCTCTGGAATGCATTAGCTCCATCCCTTGCGGATCGGCTCTCG
M012               CCCCATTCGGGTCGGCTCCTCTGGAATGCATTAGCTCCATCCCTTGCGGATCGGCTCTCG
M013               CCCCATTCGGGTCGGCTCCTCTGGAATGCATTAGCTCCATCCCTTGCGGATCGGCTCTCG
M014               CCCCATTCGGGTCGGCTCCTCTGGAATGCATTAGCTCCATCCCTTGCGGATCGGCTCTCG
M015               CCCCATTCGGGTCGGCTCCTCTGGAATGCATTAGCTCCATCCCTTGCGGATCGGCTCTCG
M016               CCCCATTCGGGTCGGCTCCTCTGGAATGCATTAGCTCCATCCCTTGCGGATCGGCTCTCG
M017               CCCCATTCGGGTCGGCTCCTCTGGAATGCATTAGCTCCATCCCTTGCGGATCGGCTCTCG
M018               CCCCATTCGGGTCGGCTCCTCTGGAATGCATTAGCTCCATCCCTTGCGGATCGGCTCTCG
M019               CCCCATTCGGGTCGGCTCCTCTGGAATGCATTAGCTCCATCCCTTGCGGATCGGCTCTCG
M020               CCCCATTCGGGTCGGCTCCTCTGGAATGCATTAGCTCCATCCCTTGCGGATCGGCTCTCG
M021               CCCCATTCGGGTCGGCTCCTCTGGAATGCATTAGCTCCATCCCTTGCGGATCGGCTCTCG
M029               CCCCATTCGGGTCGGCTCCTCTGGAATGCATTAGCTCCATCCCTTGCGGATCGGCTCTCG
M030               CCCCATTCGGGTCGGCTCCTCTGGAATGCATTAGCTCCATCCCTTGCGGATCGGCTCTCG
M031               CCCCATTCGGGTCGGCTCCTCTGGAATGCATTAGCTCCATCCCTTGCGGATCGGCTCTCG
M032               CCCCATTCGGGTCGGCTCCTCTGGAATGCATTAGCTCCATCCCTTGCGGATCGGCTCTCG
M033               CCCCATTCGGGTCGGCTCCTCTGGAATGCATTAGCTCCATCCCTTGCGGATCGGCTCTCG
M034               CCCCATTCGGGTCGGCTCCTCTGGAATGCATTAGCTCCATCCCTTGCGGATCGGCTCTCG
M035               CCCCATTCGGGTCGGCTCCTCTGGAATGCATTAGCTCCATCCCTTGCGGATCGGCTCTCG
M036               CCCCATTCGGGTCGGCTCCTCTGGAATGCATTAGCTCCATCCCTTGCGGATCGGCTCTCG
M037               CCCCATTCGGGTCGGCTCCTCTGGAATGCATTAGCTCCATCCCTTGCGGATCGGCTCTCG
M038               CCCCATTCGGGTCGGCTCCTCTGGAATGCATTAGCTCCATCCCTTGCGGATCGGCTCTCG
M039               CCCCATTCGGGTCGGCTCCTCTGGAATGCATTAGCTCCATCCCTTGCGGATCGGCTCTCG
M040               CCCCATTCGGGTCGGCTCCTCTGGAATGCATTAGCTCCATCCCTTGCGGATCGGCTCTCG
WC248              CCCCATTCGGGTCGGCTCCTCTGGAATGCATTAGCTCCATCCCTTGCGGATCGGCTCTCG
WC364              CCCCATTCGGGTCGGCTCCTCTGGAATGCATTAGCTCCATCCCTTGCGGATCGGCTCTCG
WC367              CCCCATTCGGGTCGGCTCCTCTGGAATGCATTAGCTCCATCCCTTGCGGATCGGCTCTCG
WC483              CCCCATTCGGGTCGGCTCCTCTGGAATGCATTAGCTCCATCCCTTGCGGATCGGCTCTCG
WC493              CCC-ATTCGGGTCGGCTCCTCTGGAATGCATTAGCTCCATCCCTTGCGGATCGGCTCTCG
WC555              CCCCATTCGGGTCGGCTCCTCTGGAATGCATTAGCTCCATCCCTTGCGGATCGGCTCTCG
WC556              CCCCATTCGGGTCGGCTCCTCTGGAATGCATTAGCTCCATCCCTTGCGGATCGGCTCTCG
WC557              CCCCATTCGGGTCGGCTCCTCTGGAATGCATTAGCTCCATCCCTTGCGGATCGGCTCTCG
WC581              CCCCATTCGGGTCGGCTCCTCTGGAATGCATTAGCTCCATCCCTTGCGGATCGGCTCTCG
WC582              CCCCATTCGGGTCGGCTCCTCTGGAATGCATTAGCTCCATCCCTTGCGGATCGGCTCTCG
WC583              CCCCATTCGGGTCGGCTCCTCTGGAATGCATTAGCTCCATCCCTTGCGGATCGGCTCTCG
WC659              CCCCATTCGGGTCGGCTCCTCTGGAATGCATTAGCTCCATCCCTTGCGGATCGGCTCTCG
WC685              CCCCATTCGGGTCGGCTCCTCTGGAATGCATTAGCTCCATCCCTTGCGGATCGGCTCTCG
WC808              CCCCATTCGGGTCGGCTCCTCTGGAATGCATTAGCTCCATCCCTTGCGGATCGGCTCTCG
WC828              CCCCATTCGGGTCGGCTCCTCTGGAATGCATTAGCTCCATCCCTTGCGGATCGGCTCTCG
WC834              CCCCATTCGGGTCGGCTCCTCTGGAATGCATTAGCTCCATCCCTTGCGGATCGGCTCTCG
WC835              CCCCATTCGGGTCGGCTCCTCTGGAATGCATTAGCTCCATCCCTTGCGGATCGGCTCTCG
WC836              CCCCATTCGGGTCGGCTCCTCTGGAATGCATTAGCTCCATCCCTTGCGGATCGGCTCTCG
G_sordulenta_G01   CTC-GTC--GGTCGGCTCCTCTTGAATGCATTAGCTCGATTCCTTGCGGATCGGCTCCCG

[                   481                                                    540]
M001               GTGTGATAATTGTCTACGCCGTGGTCGTTGAAGCCTCAGTCGGGCGAGCTTATAATCGTC
M002               GTGTGATAATTGTCTACGCCGTGGTCGTTGAAGCCTCAGTCGGGCGAGCTTATAATCGTC
M003               GTGTGATAATTGTCTACGCCGTGGTCGTTGAAGCCTCAGTCGGGCGAGCTTATAATCGTC
M004               GTGTGATAATTGTCTACGCCGTGGTCGTTGAAGCCTCAGTCGGGCGAGCTTATAATCGTC
M005               GTGTGATAATTGTCTACGCCGTGGTCGTTGAAGCCTCAGTCGGGCGAGCTTATAATCGTC
M006               GTGTGATAATTGTCTACGCCGTGGTCGTTGAAGCCTCAGTCGGGCGAGCTTATAATCGTC
M007               GTGTGATAATTGTCTACGCCGTGGTCGTTGAAGCCTCAGTCGGGCGAGCTTATAATCGTC
M008               GTGTGATAATTGTCTACGCCGTGGTCGTTGAAGCCTCAGTCGGGCGAGCTTATAATCGTC
M009               GTGTGATAATTGTCTACGCCGTGGTCGTTGAAGCCTCAGTCGGGCGAGCTTATAATCGTC
M010               GTGTGATAATTGTCTACGCCGTGGTCGTTGAAGCCTCAGTCGGGCGAGCTTATAATCGTC
M011               GTGTGATAATTGTCTACGCCGTGGTCGTTGAAGCCTCAGTCGGGCGAGCTTATAATCGTC
M012               GTGTGATAATTGTCTACGCCGTGGTCGTTGAAGCCTCAGTCGGGCGAGCTTATAATCGTC
M013               GTGTGATAATTGTCTACGCCGTGGTCGTTGAAGCCTCAGTCGGGCGAGCTTATAATCGTC
M014               GTGTGATAATTGTCTACGCCGTGGTCGTTGAAGCCTCAGTCGGGCGAGCTTATAATCGTC
M015               GTGTGATAATTGTCTACGCCGTGGTCGTTGAAGCCTCAGTCGGGCGAGCTTATAATCGTC
M016               GTGTGATAATTGTCTACGCCGTGGTCGTTGAAGCCTCAGTTGGGCGAGCTCACAATAGTC
M017               GTGTGATAATTGTCTACGCCGTGGTCGTTGAAGCCTCAGTTGGGCGAGCTCACAATAGTC
M018               GTGTGATAATTGTCTACGCCGTGGTCGTTGAAGCCTCAGTTGGGCGAGCTCACAATAGTC
M019               GTGTGATAATTGTCTACGCCGCGGTCGTTGAAGCCTCGGTCGGGAGAGCTTATAATCGTC
M020               GTGTGATAATTGTCTACGCCGTGGTCGTTGAAGCCTCAGTTGGGCGAGCTCACAATCGTC
```

```
M021                GTGTGATAATTGTCTACGCCGCGGTCGTTGAAGCCTCAGTCGGGAGAGCTCATAATCGTC
M029                GTGTGATAATTGTCTACGCCGTGGTCGTTGAAGCCTCAGTTGGGCGAGCTCACAATCGTC
M030                GTGTGATAATTGTCTACGCCGTGGTCGTTGAAGCCTCAGTTGGGCGAGCTCACAATCGTC
M031                GTGTGATAATTGTCTACGCCGTGGTCGTTGAAGCCTCAGTTGGGCGAGCTCACAATCGTC
M032                GTGTGATAATTGTCTACGCCGTGGTCGTTGAAGCCTCAGTTGGGCGAGCTCACAATCGTC
M033                GTGTGATAATTGTCTACGCCGTGGTCGTTGAAGCCTCAGTTGGGCGAGCTCACAATCGTC
M034                GTGTGATAATTGTCTACGCCGTGGTCGTTGAAGCCTCAGTTGGGCGAGCTCACAATCGTC
M035                GTGTGATAATTGTCTACGCCGTGGTCGTTGAAGCCTCAGTCGGGCGAGCTTATAATCGTC
M036                GTGTGATAATTGTCTACGCCGTGGTCGTTGAAGCCTCAGTTGGGCGAGCTCACAATCGTC
M037                GTGTGATAATTGTCTACGCCGTGGTCGTTGAAGCCTCAGTTGGGCGAGCTCACAATCGTC
M038                GTGTGATAATTGTCTACGCCGTGGTCGTTGAAGCCTCAGTTGGGCGAGCTCACAATCGTC
M039                GTGTGATAATTGTCTACGCCGTGGTCGTTGAAGCCTCAGTTGGGCGAGCTCACAATCGTC
M040                GTGTGATAATTGTCTACGCCGTGGTCGTTGAAGCCTCAGTTGGGCGAGCTCACAATCGTC
WC248               GTGTGATAATTGTCTACGCCGTGGTCGTTGAAGCCTCAGTCGGGCGAGCTTATAATCGTC
WC364               GTGTGATAATTGTCTACGCCGTGGTCGTTGAAGCCTCAGTCGGGCGAGCTTATAATCGTC
WC367               GTGTGATAATTGTCTACGCCGTGGTCGTTGAAGCCTCAGTCGGGCGAGCTTATAATCGTC
WC483               GTGTGATAATTGTCTACGCCGTGGTCGTTGAAGCCTCAGTCGGGCGAGCTTATAATCGTC
WC493               GTGTGATAATTGTCTACGCCGTGGTCGTTGAAGCCTCAGTCGGGCGAGCTTATAATCGTC
WC555               GTGTGATAATTGTCTACGCCGTGGTCGTTGAAGCCTCAGTTGGGCGAGCTCACAATCGTC
WC556               GTGTGATAATTGTCTACGCCGCGGTCGTTGAAGCCTCAGTTGGGAGAGCT-ATAATCGTC
WC557               GTGTGATAATTGTCTACGCCGTGGTCGTTGAAGCCTCAGTTGGGCGAGCTCACAATCGTC
WC581               GTGTGATAATTGTCTACGCCGTGGTCGTTGAAGCCTCAGTTGGGCGAGCTCACAATCGTC
WC582               GTGTGATAATTGTCTACGCCGTGGTCGTTGAAGCCTCAGTTGGGCGAGCTCACAATCGTC
WC583               GTGTGATAATTGTCTACGCCGTGGTCGTTGAAGCCTCAGTTGGGCGAGCTCACAATCGTC
WC659               GTGTGATAATTGTCTACGCCGTGGTCGTTGAAGCCTCAGTTGGGCGAGCTCACAATCGTC
WC685               GTGTGATAATTGTCTACGCCGTGGTCGTTGAAGCCTCAGTTGGGCGAGCTCACAATCGTC
WC808               GTGTGATAATTGTCTACGCCGTGGTCGTTGAAGCCTCAGTCGGGCNAGCTTATAATCGTC
WC828               GTGTGATAATTGTCTACGCCGTGGTCGTTGAAGCCTCAGTTGGGCGAGCTCACAATCGTC
WC834               GTGTGATAATTGTCTACGCCGTGGTCGTTGAAGCCTCAGTTGGGCGAGCTCACAATCGTC
WC835               GTGTGATAATTGTCTACGCCGTGGTCGTTGAAGCCTCAGTTGGGCGAGCTCACAATCGTC
WC836               GTGTGATAATTGTCTACGCCGTGGTCGTTGAAGCCTCAGTTGGGCGAGCTCACAATCGTC
G_sordulenta_G01    GTGTGATAATTGTCTACGCCGTGACCGT-GAAGC----GTTTGGCGAGCTTCGAACCGTC

[                    541                             574]
M001                CCCTCCGGGACAATCGAATATGACATCTGACCTC
M002                CCCTCCGGGACAATCGAATATGACATCTGACCTC
M003                CCCTCCGGGACAATCGAATATGACATCTGACCTC
M004                CCCTCCGGGACAATCGAATATGACATCTGACCTC
M005                CCCTCCGGGACAATCGAATATGACATCTGACCTC
M006                CCCTCCGGGACAATCGAATATGACATCTGACCTC
M007                CCCTCCGGGACAATCGAATATGACATCTGACCTC
M008                CCCTCCGGGACAATCGAATATGACATCTGACCTC
M009                CCCTCCGGGACAATCGAATATGACATCTGACCTC
M010                CCCTCCGGGACAATCGAATATGACATCTGACCTC
M011                CCCTCCGGGACAATCGAATATGACATCTGACCTC
M012                CCCTCCGGGACAATCGAATATGACATCTGACCTC
M013                CCCTCCGGGACAATCGAATATGACATCTGACCTC
M014                CCCTCCGGGACAATCGAATATGACATCTGACCTC
M015                CCCTCCGGGACAATCGAATATGACATCTGACCTC
M016                CCCTACGGGACAATTCAATCTGACATCTGACCTC
M017                CCCTCCGGGACAATTCAATCTGACATCTGACCTC
M018                CCCCACGGGACAATTCAATCTGACATCTGACCTC
M019                CCTTC-GGGACAATCGAATATGACATCTGACCTC
M020                CCCTCCGGGACAATTCAATCTGACATCTGACCTC
M021                CCTTC-GGGACAATCGAATATTACATCTGACCTC
M029                CCCTCCGGGACAATTCAATCTGACATCTGACCTC
M030                CCCTCCGGGACAATTCAATCTGACATCTGACCTC
M031                CCCTCCGGGACAATTCAATCTGACATCTGACCTC
M032                CCCTCCGGGACAATTCAATCTGACATCTGACCTC
M033                CCCTCCGGGACAATTCAATCTGACATCTGACCTC
M034                CCCTCCGGGACAATTCAATCTGACATCTGACCTC
M035                CCCTCCGGGACAATCGAATATGACATCTGACCTC
M036                CCCTCCGGGACAATTCAATCTGACATCTGACCTC
M037                CCCTCCGGGACAATTCAATCTGACATCTGACCTC
M038                CCCTCCGGGACAATTCAATCTGACATCTGACCTC
M039                CCCTCCGGGACAATTCAATCTGACATCTGACCTC
M040                CCCTCCGGGACAATTCAATCTGACATCTGACCTC
WC248               CCCTCCGGGACAATCGAATATGACATCTGACCTC
```

```
WC364             CCCTCCGGGACAATCGAATATGACATCTGACCTC
WC367             CCCTCCGGGACAATCGAATATGACATCTGACCTC
WC483             CCCTCCGGGACAATCGAATA-GACATCTGACCTC
WC493             CCCTCCGGGACAATCGAATATGACATCTGACCTC
WC555             CCCTCCGGGACAATTCAATATGACATCTGACCTC
WC556             CCCCACGGGACAAT-GAATTTGACATCTGACCTC
WC557             CCCCACGGGACAATTCAATATGACATCTGACCTC
WC581             CCCTCCGGGACAATTCAATCTGACATCTGACCTC
WC582             CCCTCCGGGACAATTCAATCTGACATCTGACCTC
WC583             CCCTCCGGGACAATTCAATCTGACATCTGACCTC
WC659             CCCTCCGGGACAATTCAATCTGACATCTGACCTC
WC685             CCCTCCGGGACAATTCAATATGACATCTGACCTC
WC808             CCCTCCGGGACAATCGAATATGACATCTGACCTC
WC828             CCCTCCGGGACAATTCAATCTGACATCTGACCTC
WC834             CCCTCCGGGACAATTCAATCTGACATCTGACCTC
WC835             CCCTCCGGGACAATTCAATCTGACATCTGACCTC
WC836             CCCTCCGGGACAATTCAATCTGACATCTGACCTC
G_sordulenta_G01  CTATGGACAAACTTATATCTTGACATCTGACCTC
```

Appendix B: Sequence alignment of ITS-1, 5.8 S, and ITS-2 from isolates of *Grifola frondosa* and related species

Sequence alignments were performed using the Clustal W algorithm (Higgins *et al.* 1991) in the MegAlign application of the Wisconsin Package (Genetics Computer Group, Madison, WI). Numbers located at the top of each sequence block designate site location within the alignment. The 5.8S region is labeled and underlined for the first isolate (M001). Divergent regions that could not be aligned ambiguously across all isolates are indicated with asterisks.

```
[               1        ********************************                     60]
M004              AGTTCAGAAAAGGGTTGTAGCTGGCCTCAAA-TCCGGG--GCATGTGCACACCCTGCTCA
M009              AGTTCAGAAAAGGGTTGTAGCTGGCCTCAAA-TCCGGG--GCATGTGCACACCCTGCTCA
M030              AGTACAGAAATGGGTTGTCGCTGGCCTCAAAATCCGGG--GCATGTGCACACCCTGCTCA
M037              AGTTCAGAAAAGGGTTGTCGCTGGCCTCAAAATCCGGG--GCATGTGCACACCCTGCTCA
WC493             AGTTCAGAAAAGGGTTGTAGCTGGCCTCAAA-TCCGGG--GCATGTGCACACCCTGCTCA
WC659             AGTTCAGAAAAGGGTTGTCGCTGGCCTCAAAATCCGGG--GCATGTGCACACCCTGCTCA
WC828             AGTTCAGAAAAGGGTTGTCGCTGGCCTCAAAATCCGGG--GCATGTGCACACCCTGCTCA
WC835             AGTTCAGAAAAGGGTTGTCGCTGGCCTCAAAATCCGGG--GCATGTGCACACCCTGCTCA
G_sordulenta_G01  AGTTCTGAAACGGGTTGTAGCTGGCCTTA-----CGAG--GCATGTGCACGCCCTGCTCA
P_umbellatus_G02  AATTCTGACAAGGGTTGTTGCTGGCTGTTTCGTTTGAGCGGCATGTGCACGCCCTGATCA
M_giganteus_G06   ACTCCCAACTTGGGT-GAGGTTG-TTGCTGGCCCTGAGGGGCATGTGCACGTCTCGCTCA

[              61  ************************************************************ 120]
M004              T---CCA-CTCTCACACCTGTGCACTTTCTGTAGGTCGG--TTCG---GGATCTGGTCCC
M009              T---CCA-CTCTCACACCTGTGCACTTTCTGTAGGTCGG--TTCG---GGATCTGGTCCC
M030              T---CCA-CTCTCACACCTGTGCACTTTCTGTAGGTCGG--TTCG---GGATCGGGTCCC
M037              T---CCA-CTCTCACACCTGTGCACTTTCTGTAGGTCGG--TTCG---GGATCTGGTCCC
WC493             T---CCA-CTCTCACACCTGTGCACTTTCTGTAGGTCGG--TTCG---GGATCTGGTCCC
WC659             T---CCA-CTCTCACACCTGTGCACTTTCTGTAGGTCGG--TTCG---GGATCTGGTCCC
WC828             T---CCA-CTCTCACACCTGTGCACTTTCTGTAGGTCGG--TTCG---GGATCTGGTCCC
WC835             T---CCA-CTCTCACACCTGTGCACTTTCTGTAGGTCGG--TTCG---GGATCTGGTCCC
G_sordulenta_G01  T---CCA-CTCT-ACACCTGTGCACCATCTGTAGGTCGG--TTTG---GGTTCGGATGCT
P_umbellatus_G02  TTATCCATCTCACACACCTGTGCACATACTGTAGGTCGGCTTTTGATTGGAGTGGGGTCT
M_giganteus_G06   TTTTATCTCTCACACCCCTGTGCACCTTTCATGGGATGGCTTGCGGCCGTCCGTCGGCCT

[             121 ************************************************************ 180]
M004              TCGCGGGGTCGGGTTCT------GTGCCTTCCTATGTACAATCACAAAC-GCTTCAGTA-
M009              TCGCGGGGTCGGGTTCT------GTGCCTTCCTATGTACAATCACAAAC-GCTTCAGTA-
M030              TCGCGGGGTCGGGTTCT------GCGCCTTCCTATGTACAATCACAAAC-GCTTCAGTA-
M037              TCGCGGGGTCGGGTTCT------GCGCCTTCCTATGTACAATCACAAAC-GCTTCAGTA-
WC493             TCGCGGGGTCGGGTTCT------GTGCCTTCCTATGTACAATCACAAAC-GCTTCAGTA-
WC659             TCGCGGGGTCGGGTTCT------GCGCCTTCCTATGTACAATCACAAAC-GCTTCAGTA-
WC828             TCGCGGGGTCGGGTTCT------GCGCCTTCCTATGTACAATCACAAAC-GCTTCAGTA-
WC835             TCGCGGGGTCGGGTTCT------GCGCCTTCCTATGTACAATCACAAAC-GCTTCAGTA-
G_sordulenta_G01  TCGCGGCGTTCGGGCTC------GGGCCTTCCTATGTACT-TCACACAC-GCTTTAGTA-
P_umbellatus_G02  TCATCGACTCTGCTTTTTAGTTGGGGCCTTCCTATGTTTTATCACACACTACTTCAGTT-
M_giganteus_G06   TTGCGTCGATGGCTCTGCAGCTG------CCTCGTGTTT--TTACAAACCTTTTAATCAG

[             181 ****      ***************************  5.8S→              240]
M004              TTCAGAATGTC-ATTGCGATAATTAAAACGCATCTTA-TACAACTTTCAGCAACGGATCT
M009              TTCAGAATGTC-ATTGCGATAATTAAAACGCATCTTA-TACAACTTTCAGCAACGGATCT
M030              TTCAGAATGTC-ATTGCGATAATTAAAACGCATCTTA-TACAACTTTCAGCAACGGATCT
M037              TTCAGAATGTC-ATTGCGATAATTAAAACGCATCTTA-TACAACTTTCAGCAACGGATCT
WC493             TTCAGAATGTC-ATTGCGATAATTAAAACGCATCTTA-TACAACTTTCAGCAACGGATCT
WC659             TTCAGAATGTC-ATTGCGATAATTAAAACGCATCTTA-TACAACTTTCAGCAACGGATCT
```

```
WC828              TTCAGAATGTC-ATTGCGATAATTAAAACGCATCTTA-TACAACTTTCAGCAACGGATCT
WC835              TTCAGAATGTC-ATTGCGATAATTAAAACGCATCTTA-TACAACTTTCAGCAACGGATCT
G_sordulenta_G01   T-CAGAATGTA-ATTGCGA----TAAAACGCACCTTA-TACAACTTTCAGCAACGGATCT
P_umbellatus_G02   AAAAGAATGTC-CTCTTG-CGTCTAA--CGCATTTAAATACAACTTTCAGCAACGGATCT
M_giganteus_G06    TCTTGAATGTTTATCGCGCGCATGCGCATTTAAATCAATACAACTTTCAGCAACGGATCT

[             241                                                          300]
M004               CTTGGCTCTCGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAA
M009               CTTGGCTCTCGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAA
M030               CTTGGCTCTCGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAA
M037               CTTGGCTCTCGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAA
WC493              CTTGGCTCTCGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAA
WC659              CTTGGCTCTCGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAA
WC828              CTTGGCTCTCGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAA
WC835              CTTGGCTCTCGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAA
G_sordulenta_G01   CTTGGCTCTCGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAA
P_umbellatus_G02   CTTGGCTCTCGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAA
M_giganteus_G06    CTTGGCTCTCGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAA

[             301                                                          360]
M004               TTCAGTGAATCATCGAATCTTTGAACGCACCTTGCGCTCCTTGGTATTCCGAGGAGCATG
M009               TTCAGTGAATCATCGAATCTTTGAACGCACCTTGCGCTCCTTGGTATTCCGAGGAGCATG
M030               TTCAGTGAATCATCGAATCTTTGAACGCACCTTGCGCTCCTTGGTATTCCGAGGAGCATG
M037               TTCAGTGAATCATCGAATCTTTGAACGCACCTTGCGCTCCTTGGTATTCCGAGGAGCATG
WC493              TTCAGTGAATCATCGAATCTTTGAACGCACCTTGCGCTCCTTGGTATTCCGAGGAGCATG
WC659              TTCAGTGAATCATCGAATCTTTGAACGCACCTTGCGCTCCTTGGTATTCCGAGGAGCATG
WC828              TTCAGTGAATCATCGAATCTTTGAACGCACCTTGCGCTCCTTGGTATTCCGAGGAGCATG
WC835              TTCAGTGAATCATCGAATCTTTGAACGCACCTTGCGCTCCTTGGTATTCCGAGGAGCATG
G_sordulenta_G01   TTCAGTGAATCATCGAATCTTTGAACGCACCTTGCGCTCCTTGGTATTCCGAGGAGCATG
P_umbellatus_G02   TTCAGTGAATCATCGAATCTTTGAACGCACCTTGCGCTCCTTGGTATTCCGAGGAGCATG
M_giganteus_G06    TTCAGTGAATCATCGAATCTTTGAACGCACCTTGCGCTCCTTGGTATTCCGAGGAGCATG

[             361              ←5.8S      ********************************** 420]
M004               CCTGTTTGAGTGTCATGGAATTCTCAACCC-ACACGTCCTTGTGATGTGGACGGGCTTGG
M009               CCTGTTTGAGTGTCATGGAATTCTCAACCC-ACACGTCCTTGTGATGTGGACGGGCTTGG
M030               CCTGTTTGAGTGTCATGGAATTCTCAACCC-ACACGTCCTTGTGATGTGGACGGGCTTGG
M037               CCTGTTTGAGTGTCATGGAATTCTCAACCC-ACACGTCCTTGTGATGTGGACGGGCTTGG
WC493              CCTGTTTGAGTGTCATGGAATTCTCAACCC-ACACATCCTTGTGATGTGGACGGGCTTGG
WC659              CCTGTTTGAGTGTCATGGAATTCTCAACCC-ACACGTCCTTGTGATGTGGACGGGCTTGG
WC828              CCTGTTTGAGTGTCATGGAATTCTCAACCC-ACACATCCTTGTGATGTGGACGGGCTTGG
WC835              CCTGTTTGAGTGTCATGGAATTCTCAACCC-ACACATCCTTGTGATGTGGACGGGCTTGG
G_sordulenta_G01   CCTGTTTGAGTGTCATGGAATCATCAACCC-ATATGTCCTTGTGTCG--GATGGGCTTGG
P_umbellatus_G02   CCTGTTTGAGTGTCATGGAATTCTCAACTCTATTTGCCTTTGTGA-ATAGA---GCTTGG
M_giganteus_G06    CCTGTTTGAGTGTCATGGTATTCTCAATTC-GCTC-TCATTTTATTGAGGGCGGCATTGG

[             421 **********************************                       480]
M004                  ACTTTGGAGGTTTCTGCCGGCCCCCCATTCGGGTCGGCTCCTCTGGAATGCATTAGCTCC
M009                  ATATTGGAGGTTTCTGCCGGCCCCCCATTCGGGTCGGCTCCTCTGGAATGCATTAGCTCC
M030                  ACTTTGGAGGTTTCTGCCGGCCCCCCATTCGGGTCGGCTCCTCTGGAATGCATTAGCTCC
M037                  ACTTTGGAGGTTTCTGCCGGCCCCCCATTCGGGTCGGCTCCTCTGGAATGCATTAGCTCC
WC493                 ACTTTGGAGGCTCATGCCGGTCCCC-ATTCGGGTCGGCTCCTCTGGAATGCATTAGCTCC
WC659                 ACTTTGGAGGTTTCTGCCGGCCCCCCATTCGGGTCGGCTCCTCTGGAATGCATTAGCTCC
WC828                 ACTTTGGAGGTTTCTGCCGGCCCCCCATTCGGGTCGGCTCCTCTGGAATGCATTAGCTCC
WC835                 ACTTTGGAGGTTTCTGCCGGCCCCCCATTCGGGTCGGCTCCTCTGGAATGCATTAGCTCC
G_sordulenta_G01      A-TTTGGAGGCTTATGCCGGCCCTC-GTC--GGTCGGCTCCTCTTGAATGCATTAGCTCG
P_umbellatus_G02      ACTTGGAGGTTTATTGCCGGTACCT-GT---GATCGGCTCCTCTTGAATGCATTAGCTCG
M_giganteus_G06       ATGTGGAGGCTCTTTGCTGGCCAT---TTTGTGCCAGCTCCTCTTAAAAATATTAGTGTG

[             481 ******************************************     ******* 540]
M004                  A-TCCCTTGCGGATCGGCTC-TCGGTGTGATAAT-TGTCTACGCCGTGGTCGTTGAAGCC
M009                  A-TCCCTTGCGGATCGGCTC-TCGGTGTGATAAT-TGTCTACGCCGTGGTCGTTGAAGCC
M030                  A-TCCCTTGCGGATCGGCTC-TCGGTGTGATAAT-TGTCTACGCCGTGGTCGTTGAAGCC
M037                  A-TCCCTTGCGGATCGGCTC-TCGGTGTGATAAT-TGTCTACGCCGTGGTCGTTGAAGCC
WC493                 A-TCCCTTGCGGATCGGCTC-TCGGTGTGATAAT-TGTCTACGCCGTGGTCGTTGAAGCC
WC659                 A-TCCCTTGCGGATCGGCTC-TCGGTGTGATAAT-TGTCTACGCCGTGGTCGTTGAAGCC
WC828                 A-TCCCTTGCGGATCGGCTC-TCGGTGTGATAAT-TGTCTACGCCGTGGTCGTTGAAGCC
WC835                 A-TCCCTTGCGGATCGGCTC-TCGGTGTGATAAT-TGTCTACGCCGTGGTCGTTGAAGCC
G_sordulenta_G01      A-TTCCTTGCGGATCGGCTC-CCGGTGTGATAAT-TGTCTACGCCGTGACCGT-GAAGC-
```

```
P_umbellatus_G02  AACCTTTTGTGGATCAGCTTATCGGTGTGATAAAATGTCTACGCCGTTACTGT-GAAGCA
M_giganteus_G06   AATGCTCACTTCAT--GCTCA---GTGTGATAATTATCTTGCATTGTGCTTG--GGTGTG

[              541 **********************************************          600]
M004                 TCAGTCGGGCGAGCTTATAATCGTCCCCTCCGGGACAATCGAATATGACA-TCTGACCTC
M009                 TCAGTCGGGCGAGCTTATAATCGTCCCCTCCGGGACAATCGAATATGACA-TCTGACCTC
M030                 TCAGTTGGGCGAGCTCACAATCGTCCCCTCCGGGACAATTCAATCTGACA-TCTGACCTC
M037                 TCAGTTGGGCGAGCTCACAATCGTCCCCTCCGGGACAATTCAATCTGACA-TCTGACCTC
WC493                TCAGTCGGGCGAGCTTATAATCGTCCCCTCCGGGACAATCGAATATGACA-TCTGACCTC
WC659                TCAGTTGGGCGAGCTCACAATCGTCCCCTCCGGGACAATTCAATCTGACA-TCTGACCTC
WC828                TCAGTTGGGCGAGCTCACAATCGTCCCCTCCGGGACAATTCAATCTGACA-TCTGACCTC
WC835                TCAGTTGGGCGAGCTCACAATCGTCCCCTCCGGGACAATTCAATCTGACA-TCTGACCTC
G_sordulenta_G01  ---GTTTGGCGAGCTTCGAACCGTCCTATGGACAAACTTATATCTTGACA-TCTGACCTC
P_umbellatus_G02  T--ATTATTCG-GCTTCCAATCGTCCTTCACGGGACAATA-ACTTTGACC-TTTGACCTC
M_giganteus_G06   GCGACT--TCATGCTTCTAATCGTC----GCAAGACAACC---TTTGACAATCTGACCTC
```

Appendix C: Blast search result for isolate WC484

>emb|AJ006670.1|SDE6670 Spongipellis delectans 5.8S rRNA gene and internal transcribed
spacers 1 and 2, (ITS1 & ITS2), Sample 26, Length = 650

Score = 1088 bits (549), Expect = 0.0
Identities = 567/573 (98%)
Strand = Plus / Plus

```
WC484:        1   aatttatgacaaggttgtcgctggccctaattgggcatgtgcacgccttgctcattctcc 60
                  ||||||||||||||||||||||| ||||||||||||||||||||||||||||||||||||
S. delectans: 59  aatttatgacaaggttgtcgctggctctaattgggcatgtgcacgccttgctcattctcc 118

WC484:        61  aattcttacacctctgtgcacttttcataggttggttgtggctgtcttcgcggatggttc 120
                  ||||||||||||||||||||||||||||||||||||||||||||||||||||||| |||||
S. delectans: 119 aattcttacacctctgtgcacttttcataggttggttgtggctgtcttcgcggacggttc 178

WC484:        121 agcctgcctatgcttttacaaacgcttcagttatagaatgtatctcgcgtataacgcatt 180
                  ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
S. delectans: 179 agcctgcctatgcttttacaaacgcttcagttatagaatgtatctcgcgtataacgcatt 238

WC484:        181 atatacaactttcagcaacggatctcttggctctcgcatcgatgaagaacgcagcgaaat 240
                  ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
S. delectans: 239 atatacaactttcagcaacggatctcttggctctcgcatcgatgaagaacgcagcgaaat 298

WC484:        241 gcgataagtaatgtgaattgcagaattcagtgaatcatcgaatctttgaacgcaccttgc 300
                  ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
S. delectans: 299 gcgataagtaatgtgaattgcagaattcagtgaatcatcgaatctttgaacgcaccttgc 358

WC484:        301 gccctttggtattccgaagggcatgcctgtttgagtgtcatggtattctcaatactccaa 360
                  |||||||||||||||||||||||||||||||||||||||||||||||||||||| ||||
S. delectans: 359 gccctttggtattccgaagggcatgcctgtttgagtgtcatggtattctcaataccccaa 418

WC484:        361 gtctttgcggataagggtgtattggatttggaggtttatgctggcgtttgtcggctcctc 420
                  |||||||||||| ||||||||||||||||||||||||||||||||||||||||||||||||
S. delectans: 419 gtctttgcggatgagggtgtattggatttggaggtttatgctggcgtttgtcggctcctc 478

WC484:        421 ttaaatgcattagcaaagatgttactgctactcttcagcgtgataattgtctacgctgct 480
                  ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
S. delectans: 479 ttaaatgcattagcaaagatgttactgctactcttcagcgtgataattgtctacgctgcc 538

WC484:        481 gttgtacggtataaataagtctttgcttctaatcgtcttcggacaatttcttgacatctg 540
                  ||||||||||||||||||||||| ||||||||||||||||||||||||||||||||||||
S. delectans: 539 gttgtacggtataaataagtctctgcttctaatcgtcttcggacaatttcttgacatctg 598

WC484:        541 acctcaaatcaggtaggactacccgctgaactt 573
                  |||||||||||||||||||||||||||||||
S. delectans: 599 acctcaaatcaggtaggactacccgctgaactt 631
```

Appendix D: Sequence alignment of partial β-tubulin gene sequences from isolates of *Grifola frondosa* and *G. sordulenta*

Sequence Alignments were performed using the Clustal W algorithm (Higgins *et al.* 1991) in the MegAlign application of the Wisconsin Package (Genetics Computer Group, Madison, WI). Numbers located at the top of each sequence block designate site location within the alignment. Regions of introns are labeled and underlined for the first isolate (M001). Codons are shown in protein coding regions.

```
[            1     Intron 5→                                    ←Intron 5  65]
M001         C GAG GTCTGTG-CATTTCAAATGTCACGTTGGAGAAAATC--TGACGACCGTTGATCGT-AG CCC
M002         C GAG GTCTGTG-CATTTCAAATGTCACGTTGGAGAAAATT--TGACGACCGTTGATCGT-AG CCC
M003         C GAG GTCTGTG-CATTTCAAATGTCACGTTGGAGAAAATC--TGACGACCGTTGATCGT-AG CCC
M004         C GAG GTCTGTG-CATTTCAAATGTCACGTTGGAGAAAATC--TGACGACCGTTGATCGT-AG CCC
M005         C GAG GTCTGTG-CATTTCAAATGTCACGTTGGAGAAAATC--TGACGACCGTTGATCGT-AG CCC
M006         C GAG GTCTGTG-CATTTCAAATGTCACGTTGGAGAAAATC--TGACGACCGTTGATCGT-AG CCC
M007         C GAG GTCTGTG-CATTTCAAATGTCACGTTGGAGAAAATC--TGACGACCGTTGATCGT-AG CCC
M008         C GAG GTCTGTG-CATTTCAAATGTCACGTTGGAGAAAATC--TGACGACCGTTGATCGT-AG CCC
M009         C GAG GTCTGTG-CATTTCAAATGTCACGTTGGAGAAAATC--TGACGACCGTTGATCGT-AG CCC
M010         C GAG GTCTGTG-CATTTCAAATGTCACGTTGGAGAAAATC--TGACGACCGTTGATCGT-AG CCC
M011         C GAG GTCTGTG-CATTTCAAATGTCACGTTGGAGAAAATC--TGACGACCGTTGATCGT-AG CCC
M012         C GAG GTCTGTG-CATTTCAAATGTCACGTTGGAGAAAATC--TGACGACCGTTGATCGT-AG CCC
M013         C GAG GTCTGTG-CATTTCAAATGTCACGTTGGAGAAAATC--TGACGACCGTTGATCGT-AG CCC
M014         C GAG GTCTGCG-CATTTCAAATGTCACGTTGGAGAAAATC--TGACGACCGTTGATCGT-AG CCC
M015         C GAG GTCTGTG-CATTTCAAATGTCACGTTGGAGAAAATC--TGACGACCGTTGATCGT-AG CCC
M016         C GAG GTCTGTG-CATTTCACATGTCACGTTGGATAAAATC--TGATGACCGTTGATCGT-AG CCC
M017         C GAG GTCTGTG-CATTTCACATGTCACGTTGGATAAAATC--TGATGACCGTTGATCAT-AG CCC
M018         C GAG GTCTGTG-CGTTTCACATGTCACGTTGGAGAAAATC--TGANGACCGTTGATCNT-AG CCC
M019         C GAG GTCTGTG-CGTTTCAAATGTTACGTTGGAGAAAATC--TGACGACCGTTGATCAT-AG CCC
M020         C GAG GTCTGTG-CGTTTCAAATGTTACGTTGGAGAAAATC--TGACGACCGTTGATCAT-AG CCC
M021         C GAG GTCTGTG-CGTTTCAAATGTTACGTTGGAGAAAATC--TGACGACCGTTGATCAT-AG CCC
M029         C GAG GTCTGTG-CGTTTCAAATGTCACGTTGGAGAAAATC--TGACGACCGTTGATCAT-AG CCC
M030         C GAG GTCTGTG-CGTTTCAAATGTTACGTTGGAGAAAATC--TGACGACCGTTGATCAT-AG CCC
M031         C GAG GTCTGTG-CGTTTCACATGTCACGTTGGAGAAAATC--TGACGACCGTTGATCAT-AG CCC
M032         C GAG GTCTGTG-CGTTTCAAATGTCACGTTGGAGAAAATC--TGACGACCGTTGATCAT-AG CCC
M033         C GAG GTCTGTG-CGTTTCAAATGTCACGTTGGAGAAAATC--TGACGACCGTTGATCAT-AG CCC
M034         C GAG GTCTGTG-CGTTTCAAATGTCACGTTGGAGAAAATC--TGACGACCGTTGATCAT-AG CCC
M035         C GAG GTCTGCG-CATTTCAAATGTCACGTTGGAGAAAATC--TGACGACCGTTGATCGT-AG CCC
M036         C GAG GTCTGTG-CGTTTCAAATGTCACGTTGGAGAAAATC--TGACGACCGTTGATCAT-AG CCC
M037         C GAG GTCTGTG-CGTTTCAAATGTCACGTTGGAGAAAATC--TGACGACCGTTGATCAT-AG CCC
M038         C GAG GTCTGTG-CGTTTCAAATGTCACGTTGGAGAAAATC--TGACGACCGTTGATCAT-AG CCC
M039         C GAG GTCTGTG-CGTTTCAAATGTCACGTTGGAGAAAATC--TGACGACCGTTGATCAT-AG CCC
M040         C GAG GTCTGTG-CGTTTCAAATGTCACGTTGGAGAAAATC--TGACGACCGTTGATCAT-AG CCC
WC248        C GAG GTCTGTG-CATTTCAAATGTCACGTTGGAGAAAATC--TGACGACCGTTGATCGT-AG CCC
WC364        C GAG GTCTGTG-CATTTCAAATGTCACGTTGGAGAAAATT--TGACGACCGTTGATCGT-AG CCC
WC367        C GAG GTCTGTG-CATTTCAAATGTCACGTTGGAGAAAATC--TGACGACCGTTGATCGT-AG CCC
WC483        C GAG GTCTGTG-CATTTCAAATGTCACGTTGGAGAAAATC--TGACGACCGTTGATCGT-AG CCC
WC493        C GAG GTCTGTG-CATTTCAAATGTTACGTTGGAGAAAATC--TGACGACCGTTGATCAT-AG CCC
WC555        C GAG GTCTGTG-CATTTCACATGTTACGTTGGAGAAAATC--TGACGACCGTTGATCGT-AG CCC
WC556        C GAG GTCTGTG-CGTTTCAAATGTTACGTTGGAGAAAATC--TGACGACCGTTGATCAT-AG CCC
WC557        C GAG GTCTGTG-CGTTTCAAATGTTACGTTGGAGAAAATC--TGACGACCGTTGATCAT-AG CCC
WC581        C GAG GTCTGTG-CGTTTCAAATGTCACGTTGGAGAAAATC--TGACGACCGTTGATCAT-AG CCC
WC582        C GAG GTCTGTG-CGTTTCAAATGTTACGTTGGAGAAAATC--TGACGACCGTTGATCAT-AG CCC
WC583        C GAG GTCTGTG-CGTTTCAAATGTCACGTTGGAGAAAATC--TGACGACCGTTGATCAT-AG CCC
WC659        C GAG GTCTGTG-CGTTTCAAATGTCACGTTGGAGAAAATC--TGACGACCGTTGATCAT-AG CCC
```

```
WC685          C GAG GTCTGTG-CGTTTCAAATGTTACGTTGGAGAAAATC--TGACGACCGTTGATCAT-AG CCC
WC808          C GAG GTCTGTG-CATTTCAAATGTCACGTTGGAGAAAATC--TGACGACCGTTGATCGT-AG CCC
WC828          C GAG GTCTGTG-CGTTTCAAATGTCACGTTGGAGAAAATC--TGACGACCGTTGATCAT-AG CCC
WC834          C GAG GTCTGTG-CGTTTCAAATGTCACGTTGGAGAAAATC--TGACGACCGTTGATCAT-AG CCC
WC835          C GAG GTCTGTG-CGTTTCAAATGTCACGTTGGAGAAAATC--TGACGACCGTTGATCAT-AG CCC
WC836          C GAG GTCTGTG-CGTTTCAAATGTTACGTTGGAGAAAATC--TGACGACCGTTGATCAT-AG CCC
G_sordulenta   C GAG GTGCGTGTCAAAATCAAGACGACGTTGCCCTCCATTAACAGCAGTCGTGACTTGTTAG CCC

[              66                                                              116]
M001           TAC AAC GCA ACC CTC TCC GTG CAC CAA CTG GTC GAG AAC TCT GAT GAG ACT
M002           TAC AAC GCA ACC CTC TCC GTG CAC CAA CTG GTC GAG AAC TCT GAT GAG ACT
M003           TAC AAC GCA ACC CTC TCC GTG CAC CAA CTG GTC GAG AAC TCT GAT GAG ACT
M004           TAC AAC GCA ACC CTC TCC GTG CAC CAA CTG GTC GAG AAC TCT GAT GAG ACT
M005           TAC AAC GCA ACC CTC TCC GTG CAC CAA CTG GTC GAG AAC TCT GAT GAG ACT
M006           TAC AAC GCA ACC CTC TCC GTG CAC CAA CTG GTC GAG AAC TCT GAT GAG ACT
M007           TAC AAC GCA ACC CTC TCC GTG CAC CAA CTG GTC GAG AAC TCT GAT GAG ACT
M008           TAC AAC GCA ACC CTC TCC GTG CAC CAA CTG GTC GAG AAC TCT GAT GAG ACT
M009           TAC AAC GCA ACC CTC TCC GTG CAC CAA CTG GTC GAG AAC TCT GAT GAG ACT
M010           TAC AAC GCA ACC CTC TCC GTG CAC CAA CTG GTC GAG AAC TCT GAT GAG ACT
M011           TAC AAC GCA ACC CTC TCC GTG CAC CAA CTG GTC GAG AAC TCT GAT GAG ACT
M012           TAC AAC GCA ACC CTC TCC GTG CAC CAA CTG GTC GAG AAC TCT GAT GAG ACT
M013           TAC AAC GCA ACC CTC TCC GTG CAC CAA CTG GTC GAG AAC TCT GAT GAG ACT
M014           TAC AAC GCA ACC CTC TCC GTG CAC CAA CTG GTC GAG AAC TCT GAT GAG ACT
M015           TAC AAC GCA ACC CTC TCC GTG CAC CAA CTG GTC GAG AAC TCT GAT GAG ACT
M016           TAC AAC GCA ACC CTC TCC GTG CAT CAA CTG GTC GAG AAC TCT GAT GAG ACT
M017           TAC AAC GCA ACC CTC TCC GTG CAT CAA CTG GTC GAG AAC TCT GAT GAG ACT
M018           TAC AAC GCA ACC CTC TCC GTG CAC CAA CTG GTC GAG AAC TCT GAT GAG ACT
M019           TAC AAC GCA ACC CTC TCC GTG CAT CAA CTG GTC GAG AAC TCT GAT GAG ACT
M020           TAC AAC GCA ACC CTC TCC GTG CAT CAA CTG GTC GAG AAC TCT GAT GAG ACT
M021           TAC AAC GCA ACC CTC TCC GTG CAT CAA CTG GTC GAG AAC TCT GAT GAG ACT
M029           TAC AAC GCA ACC CTC TCC GTG CAT CAA CTG GTC GAG AAC TCT GAT GAG ACT
M030           TAC AAC GCA ACC CTC TCC GTG CAT CAA CTG GTC GAG AAC TCT GAT GAG ACT
M031           TAC AAC GCA ACC CTC TCC GTG CAT CAA CTG GTC GAG AAC TCT GAT GAG ACT
M032           TAC AAC GCA ACC CTC TCC GTG CAT CAA CTG GTC GAG AAC TCT GAT GAG ACT
M033           TAC AAC GCA ACC CTC TCC GTG CAT CAA CTG GTC GAG AAC TCT GAT GAG ACT
M034           TAC AAC GCA ACC CTC TCC GTG CAT CAA CTG GTC GAG AAC TCT GAT GAG ACT
M035           TAC AAC GCA ACC CTC TCC GTG CAC CAA CTG GTC GAG AAC TCT GAT GAG ACT
M036           TAC AAC GCA ACC CTC TCC GTG CAC CAA CTG GTC GAG AAC TCT GAT GAG ACT
M037           TAC AAC GCA ACC CTC TCC GTG CAC CAA CTG GTC GAG AAC TCT GAT GAG ACT
M038           TAC AAC GCA ACC CTC TCC GTG CAT CAA CTG GTC GAG AAC TCT GAT GAG ACT
M039           TAC AAC GCA ACC CTC TCC GTG CAC CAA CTG GTC GAG AAC TCT GAT GAG ACT
M040           TAC AAC GCA ACC CTC TCC GTG CAN CAA CTG GTC GAG AAC TCT GAT GAG ACT
WC248          TAC AAC GCA ACC CTC TCC GTG CAC CAA CTG GTC GAG AAC TCT GAT GAG ACT
WC364          TAC AAC GCA ACC CTC TCC GTG CAC CAA CTG GTC GAG AAC TCT GAT GAG ACT
WC367          TAC AAC GCA ACC CTC TCC GTG CAC CAA CTG GTC GAG AAC TCT GAT GAG ACT
WC483          TAC AAC GCA ACC CTC TCC GTG CAC CAA CTG GTC GAG AAC TCT GAT GAG ACT
WC493          TAC AAC GCA ACC CTC TCC GTG CAC CAA CTG GTC GAG AAC TCT GAT GAG ACT
WC555          TAC AAC GCA ACC CTC TCT GTG CAC CAA CTG GTC GAG AAC TCT GAT GAG ACT
WC556          TAC AAC GCA ACC CTC TCC GTG CAC CAA CTG GTC GAG AAC TCT GAT GAG ACT
WC557          TAC AAC GCA ACC CTC TCC GTG CAC CAA CTG GTC GAG AAC TCT GAT GAG ACT
WC581          TAC AAC GCA ACC CTC TCC GTG CAC CAA CTG GTC GAG AAC TCT GAT GAG ACT
WC582          TAC AAC GCA ACC CTC TCC GTG CAC CAA CTG GTC GAG AAC TCT GAT GAG ACT
WC583          TAC AAC GCA ACC CTC TCC GTG CAT CAA CTG GTC GAG AAC TCT GAT GAG ACT
WC659          TAC AAC GCA ACC CTC TCC GTG CAN CAA CTG GTC GAG AAC TCT GAT GAG ACT
WC685          TAC AAC GCA ACC CTC TCC GTG CAT CAA CTG GTC GAG AAC TCT GAT GAG ACT
WC808          TAC AAC GCA ACC CTC TCC GTG CAC CAA CTG GTC GAG AAC TCT GAT GAG ACT
WC828          TAC AAC GCA ACC CTC TCC GTG CAC CAA CTG GTC GAG AAC TCT GAT GAG ACT
WC834          TAC AAC GCA ACC CTC TCC GTG CAT CAA CTG GTC GAG AAC TCT GAT GAG ACT
WC835          TAC AAC GCA ACC CTC TCC GTG CAC CAA CTG GTC GAG AAC TCT GAT GAG ACT
WC836          TAC AAC GCA ACC CTC TCC GTG CAT CAA CTG GTC GAG AAC TCT GAT GAG ACT
G_sordulenta   TAC AAC GCG ACA CTC TCT GTG CAT CAA CTC GTC GAG AAC TCT GAC CAG ACG

[              117                                                             170]
M001           TTC TGC ATT GAT AAC GAG GCA TTG TAC GAC ATT TGC TTC CGG ACA CTG AAG CTG
M002           TTC TGC ATT GAC AAC GAG GCC TTG TAC GAC ATT TGC TTC CGG ACA CTG AAG CTG
M003           TTC TGC ATT GAT AAC GAG GCC TTG TAC GAC ATT TGC TTC CGG ACA CTG AAG CTG
M004           TTC TGC ATT GAT AAC GAG GCC TTG TAC GAC ATT TGC TTC CGG ACA CTG AAG CTG
M005           TTC TGC ATT GAT AAC GAG GCC TTG TAC GAC ATT TGC TTC CGG ACA CTG AAG CTG
```

```
M006          TTC TGC ATT GAT AAC GAG GCC TTG TAC GAC ATT TGC TTC CGG ACA CTG AAG CTG
M007          TTC TGC ATT GAN AAC GAG GCA CTG TAC GAC ATT TGC TTC CGG ACA CTG AAG CTG
M008          TTC TGC ATT GAT AAC GAG GCC TTG TAC GAC ATT TGC TTC CGG ACA CTG AAG CTG
M009          TTC TGC ATT GAT AAC GAG GCC TTG TAC GAC ATT TGC TTC CGG ACA CTG AAG CTG
M010          TTC TGC ATT GAT AAC GAG GCA CTG TAC GAC ATT TGC TTC CGG ACA CTG AAG CTG
M011          TTC TGC ATT GAT AAC GAG GCC TTG TAC GAC ATT TGC TTC CGG ACA CTG AAG CTG
M012          TTC TGC ATT GAT AAC GAG GCC TTG TAC GAC ATT TGC TTC CGG ACA CTG AAG CTG
M013          TTC TGC ATT GAC AAC GAG GCC TTG TAC GAC ATT TGC TTC CGG ACA CTG AAG CTG
M014          TTC TGC ATT GAT AAC GAG GCC TTG TAC GAC ATT TGC TTC CGG ACA CTG AAG CTG
M015          TTC TGC ATT GAT AAC GAG GCC TTG TAC GAC ATT TGC TTC CGG ACA CTG AAG CTG
M016          TTC TGC ATT GAT AAC GAG GCA CTG TAC GAC ATT TGC TTC CGG ACA CTG AAG CTG
M017          TTC TGC ATT GAT AAN GAG GCA CTG TAC GAC ATT TGC TTC CGG ACA CTG AAG CTG
M018          TTC TGC ATT GAT AAC GAG GCA CTG TAC GAC ATT TGC TTC CGG ACA CTG AAG CTG
M019          TTC TGC ATT GAT AAC GAG GCA CTT TAC GAC ATT TGC TTC CGG ACA CTG AAG CTG
M020          TTC TGC ATT GAT AAC GAG GCA CTT TAC GAC ATT TGC TTC CGG ACA CTG AAG CTG
M021          TTC TGC ATT GAT AAC GAG GCA CTT TAC GAC ATT TGC TTC CGG ACA CTG AAG CTG
M029          TTC TGC ATT GAT AAC GAG GCG CTG TAC GAC ATT TGC TTC CGG ACA CTG AAG CTG
M030          TTC TGC ATT GAT AAC GAG GCA CTG TAC GAC ATT TGC TTC CGG ACA CTG AAG CTG
M031          TTC TGC ATT GAT AAC GAG GCA CTG TAC GAC ATT TGC TTC CGG ACA CTG AAG CTG
M032          TTC TGC ATT GAT AAC GAG GCG CTG TAC GAC ATT TGC TTC CGG ACA CTG AAG CTG
M033          TTC TGC ATT GAT AAC GAG GCG CTG TAC GAC ATT TGC TTC CGG ACA CTG AAG CTG
M034          TTC TGC ATT GAT AAC GAG GCG CTG TAC GAC ATT TGC TTC CGG ACA CTG AAG CTG
M035          TTC TGC ATT GAT AAC GAG GCT TTG TAC GAC ATT TGC TTC CGG ACA CTG AAG CTG
M036          TTC TGC ATT GAT AAC GAG GCN CTG TAC GAC ATT TGC TTC CGG ACA CTG AAG CTG
M037          TTC TGC ATT GAT AAC GAG GCG CTG TAC GAC ATT TGC TTC CGG ACA CTG AAG CTG
M038          TTC TGC ATT GAT AAC GAG GCA CTG TAC GAC ATT TGC TTC CGG ACA CTG AAG CTG
M039          TTC TGC ATT GAT AAC GAG GCA CTG TAC GAC ATT TGC TTC CGG ACA CTG AAG CTG
M040          TTC TGC ATT GAT AAC GAG GCN CTG TAC GAC ATT TGC TTC CGG ACA CTG AAG CTG
WC248         TTC TGC ATT GAT AAC GAG GCA CTG TAC GAC ATT TGC TTC CGG ACA CTG AAG CTG
WC364         TTC TGC ATT GAT AAC GAG GCC TTG TAC GAC ATT TGC TTC CGG ACA CTG AAG CTG
WC367         TTC TGC ATT GAT AAC GAG GCC TTG TAC GAC ATT TGC TTC CGG ACA CTG AAG CTG
WC483         TTC TGC ATT GAT AAC GAG GCC TTG TAC GAC ATT TGC TTC CGG ACA CTG AAG CTG
WC493         TTC TGC ATT GAT AAC GAG GCG CTG TAC GAT ATT TGC TTC CGG ACA CTG AAG CTG
WC555         TTC TGC ATT GAT AAC GAG GCA CTG TAT GAC ATT TGC TTC CGG ACA CTG AAG CTG
WC556         TTC TGC ATT GAT AAC GAG GCG CTG TAC GAC ATT TGC TTC CGG ACA CTG AAG CTG
WC557         TTC TGC ATT GAT AAC GAG GCG CTG TAC GAC ATT TGC TTC CGG ACA CTG AAG CTG
WC581         TTC TGC ATT GAT AAC GAG GCG CTG TAC GAC ATT TGC TTC CGG ACA CTG AAG CTG
WC582         TTC TGC ATT GAT AAC GAG GCG CTG TAC GAC ATT TGC TTC CGG ACA CTG AAG CTG
WC583         TTC TGC ATT GAT AAC GAG GCG CTG TAC GAC ATT TGC TTC CGG ACA CTG AAG CTG
WC659         TTC TGC ATT GAT AAC GAG GCN CTG TAC GAC ATT TGC TTC CGG ACA CTG AAG CTG
WC685         TTC TGC ATT GAT AAC GAG GCN CTG TAC GAC ATT TGC TTC CGG ACA CTG AAG CTG
WC808         TTC TGC ATT GAN AAC GAG GCC TTG TAC GAC ATT TGC TTC CGG ACA CTG AAG CTG
WC828         TTC TGC ATT GAT AAC GAG GCG CTG TAC GAC ATT TGC TTC CGG ACA CTG AAG CTG
WC834         TTC TGC ATT GAT AAC GAG GCG CTG TAC GAC ATT TGC TTC CGG ACA CTG AAG CTG
WC835         TTC TGC ATT GAT AAC GAG GCG CTG TAC GAC ATT TGC TTC CGG ACA CTG AAG CTG
WC836         TTC TGC ATT GAT AAC GAG GCA CTG TAC GAC ATT TGC TTC CGG ACA CTG AAG CTG
G_sordulenta  TTC TGC ATT GAT AAC GAG GCA TTA TAT GAT ATA TGC TTC AGA ACC CTC AAG CTC

[             171                                                                227]
M001          ACG ACA CCG ACA TAC GGC GAN CTG AAT CAC CTC ATT TCC ATC GTC ATG TCC GGT ATT
M002          ACG ACA CCG ACA TAC GGC GAC CTG AAT CAC CTC ATT TCC ATC GTC ATG TCC GGT ATT
M003          ACG ACA CCG ACA TAC GGC GAC CTG AAT CAC CTC ATT TCC ATC GTC ATG TCC GGT ATT
M004          ACG ACA CCG ACA TAC GGC GAC CTG AAT CAC CTC ATT TCC ATC GTC ATG TCC GGT ATT
M005          ACG ACA CCG ACA TAC GGC GAC CTG AAT CAC CTC ATT TCC ATC GTC ATG TCC GGT ATT
M006          ACG ACA CCG ACA TAC GGC GAC CTG AAT CAC CTC ATT TCC ATC GTC ATG TCC GGT ATT
M007          ACG ACA CCG ACA TAC GGC GAT CTG AAT CAC CTC ATT TCC ATC GTC ATG TCC GGT ATT
M008          ACG ACA CCG ACA TAC GGC GAC CTG AAT CAC CTC ATT TCC ATC GTC ATG TCC GGT ATT
M009          ACG ACA CCG ACA TAC GGC GAC CTG AAT CAC CTC ATT TCC ATC GTC ATG TCC GGT ATT
M010          ACG ACA CCG ACA TAC GGC GAT CTG AAT CAC CTC ATT TCC ATC GTC ATG TCC GGT ATT
M011          ACG ACA CCG ACA TAC GGC GAC CTG AAT CAC CTC ATT TCC ATC GTC ATG TCC GGT ATT
M012          ACG ACA CCG ACA TAC GGC GAC CTG AAT CAC CTC ATT TCC ATC GTC ATG TCC GGT ATT
M013          ACG ACA CCG ACA TAC GGC GAC CTG AAT CAC CTC ATT TCC ATC GTC ATG TCC GGT ATT
M014          ACG ACA CCG ACA TAC GGC GAC CTG AAT CAC CTC ATT TCC ATC GTC ATG TCC GGT ATT
M015          ACG ACA CCG ACA TAC GGC GAC CTG AAT CAC CTC ATT TCC ATC GTC ATG TCC GGT ATT
M016          ACG ACA CCG ACA TAC GGC GAT CTG AAC CAC CTC ATT TCC ATC GTC ATG TCC GGT ATT
M017          ACG ACA CCG ACA TAC GGC GAT CTG AAC CAC CTC ATT TCC ATC GTC ATG TCC GGT ATT
M018          ACG ACA CCG ACA TAC GGC GAT CTG AAC CAC CTC ATT TCC ATC GTC ATG TCC GGT ATT
M019          ACG ACA CCG ACA TAC GGC GAT CTG AAC CAC CTC ATC TCC ATC GTC ATG TCC GGT ATT
```

```
M020          ACG ACA CCG ACA TAC GGC GAT CTG AAC CAC CTC ATC TCC ATC GTC ATG TCC GGT ATT
M021          ACG ACA CCG ACA TAC GGC GAT CTG AAC CAC CTC ATC TCC ATC GTC ATG TCC GGT ATT
M029          ACG ACA CCG ACA TAC GGC GAT CTG AAC CAC CTC ATT TCC ATC GTC ATG TCC GGT ATT
M030          ACG ACA CCG ACA TAC GGC GAT CTG AAC CAC CTC ATT TCC ATC GTC ATG TCC GGT ATT
M031          ACG ACA CCG ACA TAC GGC GAT CTG AAC CAC CTC ATT TCC ATC GTC ATG TCC GGT ATT
M032          ACG ACA CCG ACA TAC GGC GAT CTG AAC CAC CTC ATC TCC ATC GTC ATG TCC GGT ATT
M033          ACG ACA CCG ACA TAC GGC GAT CTG AAC CAC CTC ATC TCC ATC GTC ATG TCC GGT ATT
M034          ACG ACA CCG ACA TAC GGC GAT CTG AAC CAC CTC ATT TCC ATC GTC ATG TCC GGT ATT
M035          ACG ACA CCG ACA TAC GGC GAC CTG AAT CAC CTC ATT TCC ATC GTC ATG TCC GGT ATT
M036          ACG ACA CCG ACA TAC GGC GAT CTG AAC CAC CTC ATT TCC ATC GTC ATG TCC GGT ATT
M037          ACG ACA CCG ACA TAC GGC GAT CTG AAC CAC CTC ATT TCC ATC GTC ATG TCC GGT ATT
M038          ACG ACA CCG ACA TAC GGC GAT CTG AAC CAC CTC ATT TCC ATC GTC ATG TCC GGT ATT
M039          ACG ACA CCG ACA TAC GGC GAT CTG AAC CAC CTC ATT TCC ATC GTC ATG TCC GGT ATT
M040          ACG ACA CCG ACA TAC GGC GAT CTG AAC CAC CTC ATT TCC ATC GTC ATG TCC GGT ATT
WC248         ACG ACA CCG ACA TAC GGC GAT CTG AAT CAC CTC ATT TCC ATC GTC ATG TCC GGT ATT
WC364         ACG ACA CCG ACA TAC GGC GAC CTG AAT CAC CTC ATT TCC ATC GTC ATG TCC GGT ATT
WC367         ACG ACA CCG ACA TAC GGC GAC CTG AAT CAC CTC ATT TCC ATC GTC ATG TCC GGT ATT
WC483         ACG ACA CCG ACA TAC GGC GAC CTG AAT CAC CTC ATT TCC ATC GTC ATG TCC GGT ATT
WC493         ACG ACA CCC ACA TAT GGC GAT CTG AAC CAC CTC ATT TCC ATT GTC ATG TCC GGC ATT
WC555         ACG ACA CCG ACA TAC GGC GAT CTG AAC CAC CTC ATT TCC ATC GTC ATG TCC GGT ATT
WC556         ACG ACA CCG ACA TAC GGC GAT CTG AAC CAC CTC ATT TCC ATC GTC ATG TCC GGT ATT
WC557         ACG ACA CCG ACA TAC GGC GAT CTG AAC CAC CTC ATT TCC ATC GTC ATG TCC GGT ATT
WC581         ACG ACA CCG ACA TAC GGC GAT CTG AAC CAC CTC ATT TCC ATC GTC ATG TCC GGT ATT
WC582         ACG ACA CCG ACA TAC GGC GAT CTG AAC CAC CTC ATT TCC ATC GTC ATG TCC GGT ATT
WC583         ACG ACA CCG ACA TAC GGC GAT CTG AAC CAC CTC ATT TCC ATC GTC ATG TCC GGT ATT
WC659         ACG ACA CCG ACA TAC GGC GAT CTG AAC CAC CTC ATN TCC ATC GTC ATG TCC GGT ATT
WC685         ACG ACA CCG ACA TAC GGC GAT CTG AAC CAC CTC ATT TCC ATC GTC ATG TCC GGT ATT
WC808         ACG ACA CCG ACA TAC GGC GAC CTG AAT CAC CTC ATT TCC ATC GTC ATG TCC GGT ATT
WC828         ACG ACA CCG ACA TAC GGC GAT CTG AAC CAC CTC ATT TCC ATC GTC ATG TCC GGT ATT
WC834         ACG ACA CCG ACA TAC GGC GAT CTG AAC CAC CTC ATT TCC ATC GTC ATG TCC GGT ATT
WC835         ACG ACA CCG ACA TAC GGC GAT CTG AAC CAC CTC ATT TCC ATC GTC ATG TCC GGT ATT
WC836         ACG ACA CCG ACA TAC GGC GAT CTG AAC CAC CTC ATT TCC ATC GTC ATG TCC GGT ATT
G_sordulenta  ACT ACA CCA ACT TAT GGT GAC CTT AAC CAC CTT GTA TCG ATT GTC ATG TCC GGT ATC

[             228                                                                   284]
M001          ACA ACT TGT TTG CGT TTC CCT GGT CAG TTG AAC TCC GAT CTC CGG AAG TTG GCT GTC
M002          ACA ACT TGT TTG CGT TTC CCT GGT CAG CTG AAC TCC GAT CTC CGG AAG TTG GCT GTC
M003          ACA ACT TGT TTG CGT TTC CCT GGT CAG CTG AAC TCC GAT CTC CGG AAG TTG GCT GTC
M004          ACA ACT TGT TTG CGT TTC CCT GGT CAG CTG AAC TCC GAT CTC CGG AAG TTG GCT GTC
M005          ACA ACT TGT TTG CGT TTC CCT GGT CAG CTG AAC TCC GAT CTC CGG AAG TTG GCT GTC
M006          ACA ACT TGT TTG CGT TTC CCT GGT CAG CTG AAC TCC GAT CTC CGG AAG TTG GCT GTC
M007          ACA ACT TGT TTG CGT TTC CCT GGT CAG TTG AAC TCC GAT CTC CGG AAG TTG GCT GTC
M008          ACA ACT TGT TTG CGT TTC CCT GGT CAG CTG AAC TCC GAT CTC CGG AAG TTG GCT GTC
M009          ACA ACT TGT TTG CGT TTC CCT GGT CAG CTG AAC TCC GAT CTC CGG AAG TTG GCT GTC
M010          ACA ACT TGT TTG CGT TTC CCT GGT CAG TTG AAC TCC GAT CTC CGG AAG TTG GCT GTC
M011          ACA ACT TGT TTG CGT TTC CCT GGT CAG CTG AAC TCC GAT CTC CGG AAG TTG GCT GTC
M012          ACA ACT TGT TTG CGT TTC CCT GGT CAG CTG AAC TCC GAT CTC CGG AAG TTG GCT GTC
M013          ACA ACT TGT TTG CGT TTC CCT GGT CAG CTG AAC TCC GAT CTC CGG AAG TTG GCT GTC
M014          ACA ACT TGT TTG CGT TTC CCT GGT CAG CTG AAC TCC GAT CTC CGG AAG TTG GCT GTC
M015          ACA ACT TGT TTG CGT TTC CCT GGT CAG CTG AAC TCC GAT CTC CGG AAG TTG GCT GTC
M016          ACA ACT TGC TTG CGT TTC CCT GGT CAG CTG AAC TCC GAT CTC CGA AAG TTG GCT GTC
M017          ACA ACT TGC TTG CGT TTC CCT GGT CAG CTG AAC TCC GAT CTC CGA AAG TTG GCT GTC
M018          ACA ACT TGC TTG CGT TTC CCT GGT CAG CTG AAC TCC GAT CTC CGA AAG TTG GCT GTC
M019          ACA ACT TGC TTG CGT TTC CCT GGT CAG CTG AAC TCC GAT CTC CGA AAG TTG GCT GTC
M020          ACA ACT TGC TTG CGT TTC CCT GGT CAG CTG AAC TCC GAT CTC CGA AAG TTG GCT GTC
M021          ACA ACT TGC TTG CGT TTC CCT GGT CAG CTG AAC TCC GAT CTC CGA AAG TTG GCT GTC
M029          ACA ACT TGC TTG CGT TTC CCT GGT CAG CTG AAC TCC GAT CTC CGA AAG TTG GCT GTC
M030          ACA ACT TGC TTG CGT TTC CCT GGT CAG CTG AAC TCC GAT CTC CGA AAG TTG GCT GTC
M031          ACA ACT TGC TTG CGT TTC CCT GGT CAG CTG AAC TCC GAT CTC CGA AAG TTG GCT GTC
M032          ACA ACT TGC TTG CGT TTC CCT GGT CAG CTG AAC TCC GAT CTC CGA AAG TTG GCT GTC
M033          ACA ACT TGC TTG CGT TTC CCT GGT CAG CTG AAC TCC GAT CTC CGA AAG TTG GCT GTC
M034          ACA ACT TGC TTG CGT TTC CCT GGT CAG CTG AAC TCC GAT CTC CGA AAG TTG GCT GTC
M035          ACA ACT TGT TTG CGT TTC CCT GGT CAG CTG AAC TCC GAT CTC CGG AAG TTG GCT GTC
M036          ACA ACT TGC TTG CGT TTC CCT GGT CAG CTG AAC TCC GAT CTC CGA AAG TTG GCT GTC
M037          ACA ACT TGC TTG CGT TTC CCT GGT CAG CTG AAC TCC GAT CTC CGA AAG TTG GCT GTC
M038          ACA ACT TGC TTG CGT TTC CCT GGT CAG CTG AAC TCC GAT CTC CGA AAG TTG GCT GTC
M039          ACA ACT TGC TTG CGT TTC CCT GGT CAG CTG AAC TCC GAT CTC CGA AAG TTG GCT GTC
M040          ACA ACT TGC TTG CGT TTC CCT GGT CAG CTG AAC TCC GAT CTC CGA AAG TTG GCT GTC
```

```
WC248          ACA ACT TGT TTG CGT TTC CCT GGT CAG TTG AAC TCC GAT CTC CGG AAG TTG GCT GTC
WC364          ACA ACT TGT TTG CGT TTC CCT GGT CAG CTG AAC TCC GAT CTC CGG AAG TTG GCT GTC
WC367          ACA ACT TGT TTG CGT TTC CCT GGT CAG CTG AAC TCC GAT CTC CGG AAG TTG GCT GTC
WC483          ACA ACT TGT TTG CGT TTC CCT GGT CAG CTG AAC TCC GAT CTC CGG AAG TTG GCT GTC
WC493          ACA ACT TGC TTG CGT TTC CCT GGT CAG CTG AAC TCC GAC CTC CGG AAG TTG GCT GTC
WC555          ACA ACT TGC TTG CGT TTC CCT GGT CAG TTG AAC TCC GAT CTC CGA AAG TTG GCT GTC
WC556          ACA ACT TGC TTG CGT TTC CCT GGT CAG CTG AAC TCC GAT CTC CGA AAG TTG GCT GTC
WC557          ACA ACT TGC TTG CGT TTC CCT GGT CAG CTG AAC TCC GAT CTC CGA AAG TTG GCT GTC
WC581          ACA ACT TGC TTG CGT TTC CCT GGT CAG CTG AAC TCC GAT CTC CGA AAG TTG GCT GTC
WC582          ACA ACT TGC TTG CGT TTC CCT GGT CAG CTG AAC TCC GAT CTC CGA AAG TTG GCT GTC
WC583          ACA ACT TGC TTG CGT TTC CCT GGT CAG CTG AAC TCC GAT CTC CGA AAG TTG GCT GTC
WC659          ACA ACT TGC TTG CGT TTC CCT GGT CAG CTG AAC TCC GAT CTC CGA AAG TTG GCT GTC
WC685          ACA ACT TGC TTG CGT TTC CCT GGT CAG CTG AAC TCC GAT CTC CGA AAG TTG GCT GTC
WC808          ACA ACT TGT TTG CGT TTC CCT GGT CAG CTG AAC TCC GAT CTC CGG AAG TTG GCT GTC
WC828          ACA ACT TGC TTG CGT TTC CCT GGT CAG CTG AAC TCC GAT CTC CGA AAG TTG GCT GTC
WC834          ACA ACT TGC TTG CGT TTC CCT GGT CAG CTG AAC TCC GAT CTC CGA AAG TTG GCT GTC
WC835          ACA ACT TGC TTG CGT TTC CCT GGT CAG CTG AAC TCC GAT CTC CGA AAG TTG GCT GTC
WC836          ACA ACT TGC TTG CGT TTC CCT GGT CAG CTG AAC TCC GAT CTC CGA AAG TTG GCT GTC
G_sordulenta   ACG ACT TGC TTG CGT TTC CCT GGT CAG CTG AAT TCT GAC TTG CGG AAG TTG GCT GTC

[              285      Intron 6→                                          ←Intron 6 355]
M001           AAC ATG GGTGA-GTTCTCACTT-GANNCCTTGTGACATGACACTTATCATTGACTGTTAAAAAAT-TA GTT
M002           AAC ATG GGTGA-GTTCTCACTT-GATGCCTTGTGACATGACACTTATCATTGACTGTTAAAAATT-TA GTT
M003           AAC ATG GGTGA-GTTCTCACTT-GACGCCTTGTGACATGACACTTATCATTGACTGTTAAAAAAT-TA GTT
M004           AAC ATG GGTGA-GTTCTCACTT-GACGCCTTGTGACATGACACTTATCATTGACTGTTAAAAAAT-TA GTT
M005           AAC ATG GGTGA-GTTCTCACTT-GATGCCTTGTGACATGACACTTATCATTGACTGTTAAAAATT-TA GTT
M006           AAC ATG GGTGA-GTTCTCACTT-GACGCCTTGTGACATGACACTTATCATTGACTGTTAAAAAAT-TA GTT
M007           AAC ATG GGTGA-GTTCTCACTT-GATTCCTTGTGACATGACACTTATCATTGACTGTTAAAAATT-TA GTT
M008           AAC ATG GGTGA-GTTCTCACTT-GACGCCTTGTGACATGACACTTATCATTGACTGTTAAAAATT-TA GTT
M009           AAC ATG GGTGA-GTTCTCACTT-GATGCCTTGTGACATGACACTTATCATTGACTGTTAAAAATT-TA GTT
M010           AAC ATG GGTGA-GTTCTCACTT-GATTCCTTGTGACATGACACTTATCATTGACTGTTAAAAATT-TA GTT
M011           AAC ATG GGTGA-GTTCTCACTT-GACGCCTTGTGACATGACACTTATCATTGACTGTTAAAAAAT-TA GTT
M012           AAC ATG GGTGA-GTTCTCACTT-GACGCCTTGTGACATGACACTTATCATTGACTGTTAAAAAAT-TA GTT
M013           AAC ATG GGTGA-GTTCTCACTT-GATGCCTTGTGACATGACACTTATCATTGACTGTTAAAAATT-TA GTT
M014           AAC ATG GGTGA-GTTCTCACTT-GATGCCTTGTGACATGACACTTATCATTGACTGTTAAAAATT-TA GTT
M015           AAC ATG GGTGA-GTTCTCACTT-GANGCCTTGTGACATGACACTTATCATTGACTGTTAAAAATT-TA GTT
M016           AAC ATG GGTGA-GTTCTCACTT-GATTCCTTGTGATATGACACTTATGATTGACTGTTGAAATTT-TA GTC
M017           AAC ATG GGTGA-GTTCTCACTT-GATTCCTTGTGATATGACACTTTATGATTGACTGTTGAAATTT-TA GTC
M018           AAC ATG GGTGA-GTTCTCACTT-GATTCCTTGTGATATGACACTTATGATTGACTGTTAAAATTT-TA GTC
M019           AAC ATG GGTGA-GTTCTCACTT-GATTCCTTGTGATATGACACTTATGATTGACTGTTGAAATTT-TA GTC
M020           AAC ATG GGTGA-GTTCTCACTT-GATTCCTTGTGATATGACACTTATGATTGACTGTTGAAATTT-TA GTC
M021           AAC ATG GGTGA-GTTCTCACTT-GATTCCTTGTGATATGACACTTATGATTGACTGTTGAAATTT-TA GTC
M029           AAC ATG GGTGA-GTTCTCACTT-GATTCCTTGTGATATGACACTTATGATTGACTGTTGAAATTT-TA GTC
M030           AAC ATG GGTGA-GTTCTCACTT-GATTCCTTGTGATATGACACTTATGATTGACTGTTGAAATTT-TA GTC
M031           AAC ATG GGTGA-GTTCTCACTT-GATTCCTTGTGATATGACACTTATGATTGACTGTTGAAATTT-TA GTC
M032           AAC ATG GGTGA-GTTCTCACTT-GATTCCTTGTGATATGACACTTATGATTGACTGTTGAAATTT-TA GTC
M033           AAC ATG GGTAA-GTTCTCACTT-GATTCCTTGTGATATGACACTTATGATTGACTGTTAAAATTT-TA GTC
M034           AAC ATG GGTGA-GTTCTCACTT-GATTCCTTGTGATATGACACTTATGATTGACTGTTGAAATTT-TA GTC
M035           AAC ATG GGTGA-GTTCTCACTT-GATGCCTTGTGACATGACACTTATCATTGACTGTTAAAAATT-TA GTT
M036           AAC ATG GGTGA-GTTCTCACTT-GATTCCTTGTGATATAACACTTATGATTGACTGTTAAAATTT-TA GTC
M037           AAC ATG GGTGA-GTTCTCACTT-GATTCCTTGTGATATAACACTTATGATTGACTGTTAAAATTT-TA GTC
M038           AAC ATG GGTGA-GTTCTCACTT-GATTCCTTGTGATATGACACTTATGATTGACTGTTGAAATTT-TA GTC
M039           AAC ATG GGTGA-GTTCTCACTT-GATTCCTTGTGATATGACACTTATGATTGACTGTTAAAATTT-TA GTC
M040           AAC ATG GGTGA-GTTCTCACTT-GATTCCTTGTGATATGACACTTATGATTGACTGTTGAAATTT-TA GTC
WC248          AAC ATG GGTGA-GTTCTCACTT-GATTCCTTGTGACATGACACTTATCATTGACTGTTAAAAATT-TA GTT
WC364          AAC ATG GGTGA-GTTCTCACTT-GATGCCTTGTGACATGACACTTATCATTGACTGTTAAAAATT-TA GTT
WC367          AAC ATG GGTGA-GTTCTCACTT-GATGCCTTGTGACATGACACTTATCATTGACTGTTAAAAATT-TA GTT
WC483          AAC ATG GGTGA-GTTCTCACTT-GATGCCTTGTGACATGACACTTATCATTGACTGTTAAAAATT-TA GTT
WC493          AAC ATG GGTGA-GTTTTCACTT-GATTCCTTGTGATATGGCACTTATGATTGACTGTTGAAATTC-TA GTC
WC555          AAC ATG GGTGA-GTTCTCACTT-GATTCCTTGTGATATGACACTTATGATTGACTGTTGAAATTT-TA GTC
WC556          AAC ATG GGTGA-GTTCTCACTT-GATTCCTTGTGATATGACACTTATGATTGACTGTCAAAATTT-TA GTC
WC557          AAC ATG GGTGA-GTTCTCACTT-GATTCCTTGTGATATGACACTTATGATTGACTGTCAAAATTT-TA GTC
WC581          AAC ATG GGTGA-GTTCTCACTT-GATTCCTTGTGATATAACACTTATGATTGACTGTTAAAATTT-TA GTC
WC582          AAC ATG GGTGA-GTTCTCACTT-GATTCCTTGTGATATGACACTTATGATTGACTGTCAAAATTT-TA GTC
WC583          AAC ATG GGTGA-GTTCTCACTT-GATTCCTTGTGATATGACACTTATGATTGACTGTTGAAATTT-TA GTC
WC659          AAC ATG GGTAA-GTTCTCACTT-GATTCCTTGTGATATAACACTTATGATTGACTGTTAAAATTT-TA GTC
WC685          AAC ATG GGTGA-GTTCTCACTT-GATTCCTTGTGATATGACACTTATGATTGACTGTTGAAATTT-TA GTC
WC808          AAC ATG GGTGA-GTTCTCACTT-GACGCCTTGTGACATGACACTTATCATTGACTGTTAAAAAAT-TA GTT
```

```
WC828          AAC ATG GGTAA-GTTCTCACTT-GATTCCTTGTGATATAACACTTATGATTGACTGTTAAAATTT-TA GTC
WC834          AAC ATG GGTGA-GTTCTCACTT-GATTCCTTGTGATATGACACTTATGATTGACTGTTGAAATTT-TA GTC
WC835          AAC ATG GGTAA-GTTCTCACTT-GATTCCTTGTGATATAACACTTATGATTGACTGTTAAAATTT-TA GTC
WC836          AAC ATG GGTAA-GTTCTCACTT-GATTCCTTGTGATATAACACTTATGATTGACTGTTGAAATTT-TA GTC
G_sordulenta   AAC ATG GGTAATGCTTTCCTTCAGACTGGCCTAGATGCGTT-TTTCTCATTCGATGTTTTCTTTTGCA GTT

[              356                                                                 406]
M001           CCC TTC CCC CGT CTC CAC TTC TTC ATG ACC GGT TTC GCG CCC TTG ACT GCG
M002           CCC TTC CCC CGT CTC CAC TTC TTC ATG ACC GGT TTC GCG CCC TTG ACT GCG
M003           CCC TTC CCC CGT CTC CAC TTC TTC ATG ACC GGT TTC GCG CCC TTG ACT GCG
M004           CCC TTC CCC CGT CTC CAC TTC TTC ATG ACC GGT TTC GCG CCC TTG ACT GCG
M005           CCC TTC CCC CGT CTC CAC TTC TTC ATG ACC GGT TTC GCG CCC TTG ACT GCG
M006           CCC TTC CCC CGT CTC CAC TTC TTC ATG ACC GGT TTC GCG CCC TTG ACT GCG
M007           CCC TTC CCC CGT CTC CAC TTC TTC ATG ACC GGT TTC GCG CCC TTG ACT GCG
M008           CCC TTC CCC CGT CTC CAC TTC TTC ATG ACC GGT TTC GCG CCC TTG ACT GCG
M009           CCC TTC CCC CGT CTC CAC TTC TTC ATG ACT GGT TTC GCG CCC TTG ACT GCG
M010           CCC TTC CCC CGT CTC CAC TTC TTC ATG ACC GGT TTC GCG CCC TTG ACT GCG
M011           CCC TTC CCC CGT CTC CAC TTC TTC ATG ACC GGT TTC GCG CCC TTG ACT GCG
M012           CCC TTC CCC CGT CTC CAC TTC TTC ATG ACC GGT TTC GCG CCC TTG ACT GCG
M013           CCC TTC CCC CGT CTC CAC TTC TTC ATG ACT GGT TTC GCG CCC TTG ACT GCG
M014           CCC TTC CCC CGT CTC CAC TTC TTC ATG ACC GGT TTC GCG CCC TTG ACT GCG
M015           CCC TTC CCC CGT CTC CAC TTC TTC ATG ACC GGT TTC GCG CCC TTG ACT GCG
M016           CCC TTC CCC CGT CTC CAC TTC TTC ATG ACT GGT TTC GCG CCC TTG ACT GCG
M017           CCC TTC CCC CGT CTC CAC TTC TTC ATG ACT GGT TTC GCG CCC TTG ACT GCG
M018           CCC TTC CCC CGT CTC CAC TTC TTC ATG ACT GGT TTC GCG CCC TTG ACT GCG
M019           CCC TTC CCC CGT CTC CAC TTC TTC ATG ACT GGT TTC GCG CCC TTG ACT GCG
M020           CCC TTC CCC CGT CTC CAC TTC TTC ATG ACT GGT TTC GCG CCC TTG ACT GCG
M021           CCC TTC CCC CGT CTC CAC TTC TTC ATG ACT GGT TTC GCG CCC TTG ACT GCG
M029           CCC TTC CCC CGT CTC CAC TTC TTC ATG ACT GGT TTC GCG CCC TTG ACT GCG
M030           CCC TTC CCC CGT CTC CAC TTC TTC ATG ACT GGT TTC GCG CCC TTG ACT GCG
M031           CCC TTC CCC CGT CTC CAC TTC TTC ATG ACT GGT TTC GCG CCC TTG ACT GCG
M032           CCC TTC CCC CGT CTC CAC TTC TTC ATG ACT GGT TTC GCG CCC TTG ACT GCG
M033           CCC TTC CCC CGT CTC CAC TTC TTC ATG ACT GGT TTC GCG CCC TTG ACT GCG
M034           CCC TTC CCC CGT CTC CAC TTC TTC ATG ACT GGT TTC GCG CCC TTG ACT GCG
M035           CCC TTC CCC CGT CTC CAC TTC TTC ATG ACC GGT TTC GCG CCC TTG ACT GCG
M036           CCC TTC CCC CGT CTC CAC TTC TTC ATG ACT GGT TTC GCG CCC TTG ACT GCG
M037           CCC TTC CCC CGT CTC CAC TTC TTC ATG ACT GGT TTC GCG CCC TTG ACT GCG
M038           CCC TTC CCC CGT CTC CAC TTC TTC ATG ACT GGT TTC GCG CCC TTG ACT GCG
M039           CCC TTC CCC CGT CTC CAC TTC TTC ATG ACT GGT TTC GCG CCC TTG ACT GCG
M040           CCC TTC CCC CGT CTC CAC TTC TTC ATG ACT GGT TTC GCG CCC TTG ACT GCG
WC248          CCC TTC CCC CGT CTC CAC TTC TTC ATG ACC GGT TTC GCG CCC TTG ACT GCG
WC364          CCC TTC CCC CGT CTC CAC TTC TTC ATG ACC GGT TTC GCG CCC TTG ACT GCG
WC367          CCC TTC CCC CGT CTC CAC TTN TTC ATG ACC GGT TTC GCG CCC TTG ACT GCG
WC483          CCC TTC CCC CGT CTC CAC TTC TTC ATG ACC GGT TTC GCG CCC TTG ACT GCG
WC493          CCC TTC CCC CGT CTC CAC TTC TTC ATG ACT GGT TTC GCG CCC TTG ACT GCG
WC555          CCC TTC CCC CGT CTC CAC TTC TTC ATG ACT GGT TTC GCG CCC TTG ACT GCG
WC556          CCC TTC CCC CGT CTC CAC TTC TTC ATG ACT GGT TTC GCG CCC TTG ACT GCG
WC557          CCC TTC CCC CGT CTC CAC TTC TTC ATG ACT GGT TTC GCG CCC TTG ACT GCG
WC581          CCC TTC CCC CGT CTC CAC TTC TTC ATG ACT GGT TTC GCG CCC TTG ACT GCG
WC582          CCC TTC CCC CGT CTC CAC TTC TTC ATG ACT GGT TTC GCG CCC TTG ACT GCG
WC583          CCC TTC CCC CGT CTC CAC TTC TTC ATG ACT GGT TTC GCG CCC TTG ACT GCG
WC659          CCC TTC CCC CGT CTC CAC TTC TTC ATG ACT GGT TTC GCG CCC TTG ACT GCG
WC685          CCC TTC CCC CGT CTC CAC TTC TTC ATG ACT GGT TTC GCG CCC TTG ACT GCG
WC808          CCC TTC CCC CGT CTC CAC TTC TTC ATG ACC GGT TTC GCG CCC TTG ACT GCG
WC828          CCC TTC CCC CGT CTC CAC TTC TTC ATG ACT GGT TTC GCG CCC TTG ACT GCG
WC834          CCC TTC CCC CGT CTC CAC TTC TTC ATG ACT GGT TTC GCG CCC TTG ACT GCG
WC835          CCC TTC CCC CGT CTC CAC TTC TTC ATG ACT GGT TTC GCG CCC TTG ACT GCG
WC836          CCC TTC CCC CGT CTC CAC TTC TTC ATG ACT GGT TTC GCG CCC TTG ACT GCG
G_sordulenta   CCC TTC CCT CGT CTT CAT TTC TTC ATG ACC GGC TTC GCG CCT TTG ACC GCT

[              407                                                                 460]
M001           CGC GGC AGC CAG CAG TAC CGT GCT GTC ACT GTA CCC GAG CTG ACT CAA CAG ATG
M002           CGC GGC AGC CAG CAG TAC CGT GCT GTC ACT GTA CCC GAG CTG ACT CAA CAG ATG
M003           CGC GGC AGC CAG CAG TAC CGT GCT GTC ACT GTA CCC GAG CTG ACT CAA CAG ATG
M004           CGC GGC AGC CAG CAG TAC CGT GCT GTC ACT GTA CCC GAG CTG ACT CAA CAG ATG
M005           CGC GGC AGC CAG CAG TAC CGT GCT GTC ACT GTA CCC GAG CTG ACT CAA CAG ATG
M006           CGC GGC AGC CAG CAG TAC CGT GCT GTC ACT GTA CCC GAG CTG ACT CAA CAG ATG
```

```
M007          CGC GGC AGC CAG CAG TAC CGT GCT GTC ACT GTA CCC GAG CTG ACT CAA CAG ATG
M008          CGC GGC AGC CAG CAG TAC CGT GCT GTC ACT GTA CCC GAG CTG ACT CAA CAG ATG
M009          CGC GGC AGC CAG CAG TAC CGT GCT GTC ACT GTA CCC GAG CTG ACT CAA CAG ATG
M010          CGC GGC AGC CAG CAG TAC CGT GCT GTC ACT GTA CCC GAG CTG ACT CAA CAG ATG
M011          CGC GGC AGC CAG CAG TAC CGT GCT GTC ACT GTA CCC GAG CTG ACT CAA CAG ATG
M012          CGC GGC AGC CAG CAG TAC CGT GCT GTC ACT GTA CCC GAG CTG ACT CAA CAG ATG
M013          CGC GGC AGC CAG CAG TAC CGT GCT GTC ACT GTA CCC GAG CTG ACT CAA CAG ATG
M014          CGC GGC AGC CAG CAG TAC CGT GCT GTC ACT GTA CCC GAG CTG ACT CAA CAG ATG
M015          CGC GGC AGC CAG CAG TAC CGT GCT GTC ACT GTA CCC GAG CTG ACT CAA CAG ATG
M016          CGC GGC AGC CAG CAG TAC CGT GCT GTC ACT GTA CCC GAG CTG ACT CAA CAG ATG
M017          CGC GGC AGC CAG CAG TAC CGT GCT GTC ACT GTA CCC GAG CTG ACT CAA CAG ATG
M018          CGC GGC AGC CAG CAG TAC CGT GCT GTC ACT GTA CCC GAG CTG ACT CAA CAG ATG
M019          CGC GGC AGC CAG CAG TAC CGT GCT GTC ACT GTA CCC GAG CTG ACT CAA CAG ATG
M020          CGC GGC AGC CAG CAG TAC CGT GCT GTC ACT GTA CCC GAG CTG ACT CAA CAG ATG
M021          CGC GGC AGC CAG CAG TAC CGT GCT GTC ACT GTA CCC GAG CTG ACT CAA CAG ATG
M029          CGC GGC AGC CAG CAG TAC CGT GCT GTC ACT GTA CCC GAG CTG ACT CAA CAG ATG
M030          CGC GGC AGC CAG CAG TAC CGT GCT GTC ACT GTA CCC GAG CTG ACT CAA CAG ATG
M031          CGC GGC AGC CAG CAG TAC CGT GCT GTC ACT GTA CCC GAG CTG ACT CAA CAG ATG
M032          CGC GGC AGC CAG CAG TAC CGT GCT GTC ACT GTA CCC GAG CTG ACT CAA CAG ATG
M033          CGC GGC AGC CAG CAG TAC CGT GCT GTC ACT GTA CCC GAG CTG ACT CAA CAG ATG
M034          CGC GGC AGC CAG CAG TAC CGT GCT GTC ACT GTA CCC GAG CTG ACT CAA CAG ATG
M035          CGC GGC AGC CAG CAG TAC CGT GCT GTC ACT GTA CCC GAG CTG ACT CAA CAG ATG
M036          CGC GGC AGC CAG CAG TAC CGT GCT GTC ACT GTA CCC GAG CTG ACT CAA CAG ATG
M037          CGC GGC AGC CAG CAG TAC CGT GCT GTC ACT GTA CCC GAG CTG ACT CAA CAG ATG
M038          CGC GGC AGC CAG CAG TAC CGT GCT GTC ACT GTA CCC GAG CTG ACT CAA CAG ATG
M039          CGC GGC AGC CAG CAG TAC CGT GCT GTC ACT GTA CCC GAG CTG ACT CAA CAG ATG
M040          CGC GGC AGC CAG CAG TAC CGT GCT GTC ACT GTA CCC GAG CTG ACT CAA CAG ATG
WC248         CGC GGC AGC CAG CAG TAC CGT GCT GTC ACT GTA CCC GAG CTG ACT CAA CAG ATG
WC364         CGC GGC AGC CAG CAG TAC CGT GCT GTC ACT GTA CCC GAG CTG ACT CAA CAG ATG
WC367         CGC GGC AGC CAA CAG TAC CGT GCT GTC ACT GTA CCC GAG CTG ACT CAA CAG ATG
WC483         CGC GGC AGC CAA CAG TAC CGT GCT GTC ACT GTA CCC GAG CTG ACT CAA CAG ATG
WC493         CGC GGC AGC CAG CAG TAT CGT GCT GTC ACT GTA CCC GAG CTG ACT CAA CAG ATG
WC555         CGC GGC AGC CAG CAG TAC CGT GCT GTC ACT GTA CCC GAG CTG ACT CAA CAG ATG
WC556         CGC GGC AGC CAG CAG TAC CGT GCT GTC ACT GTA CCC GAG CTG ACT CAA CAG ATG
WC557         CGC GGC AGC CAG CAG TAC CGT GCT GTC ACT GTA CCC GAG CTG ACT CAA CAG ATG
WC581         CGC GGC AGC CAG CAG TAC CGT GCT GTC ACT GTA CCC GAG CTG ACT CAA CAG ATG
WC582         CGC GGC AGC CAG CAG TAC CGT GCT GTC ACT GTA CCC GAG CTG ACT CAA CAG ATG
WC583         CGC GGC AGC CAG CAG TAC CGT GCT GTC ACT GTA CCC GAG CTG ACT CAA CAG ATG
WC659         CGC GGC AGC CAG CAG TAC CGT GCT GTC ACT GTA CCC GAG CTG ACT CAA CAG ATG
WC685         CGC GGC AGC CAG CAG TAC CGT GCT GTC ACT GTA CCC GAG CTG ACT CAA CAG ATG
WC808         CGC GGC AGC CAG CAG TAC CGT GCT GTC ACT GTA CCC GAG CTG ACT CAA CAG ATG
WC828         CGC GGC AGC CAG CAG TAC CGT GCT GTC ACT GTA CCC GAG CTG ACT CAA CAG ATG
WC834         CGC GGC AGC CAG CAG TAC CGT GCT GTC ACT GTA CCC GAG CTG ACT CAA CAG ATG
WC835         CGC GGC AGC CAG CAG TAC CGT GCT GTC ACT GTA CCC GAG CTG ACT CAA CAG ATG
WC836         CGC GGC AGC CAG CAG TAC CGT GCT GTC ACT GTA CCC GAG CTG ACT CAA CAG ATG
G_sordulenta  CGG GGT AGC CAG CAA TAC CGC GCG GTC ACC GTC CCT GAG CTG ACG CAG CAA ATG

[             461                                                               514]
M001          TTC GAT GCC AAG AAC ATG ATG GCT GCG TCC GAC CCC CGG CAT GGC CGA TAC CTC
M002          TTC GAT GCC AAG AAC ATG ATG GCT GCG TCC GAC CCC CGG CAT GGC CGA TAC CTC
M003          TTC GAT GCC AAG AAC ATG ATG GCT GCG TCC GAC CCC CGG CAT GGC CGA TAC CTC
M004          TTC GAT GCC AAG AAC ATG ATG GCT GCG TCC GAC CCC CGG CAT GGC CGA TAC CTC
M005          TTC GAT GCC AAG AAC ATG ATG GCT GCG TCC GAC CCC CGG CAT GGC CGA TAC CTC
M006          TTC GAT GCC AAG AAC ATG ATG GCT GCG TCC GAC CCC CGG CAT GGC CGA TAC CTC
M007          TTC GAT GCC AAG AAC ATG ATG GCT GCG TCC GAC CCC CGG CAT GGC CGA TAC CTC
M008          TTC GAT GCC AAG AAC ATG ATG GCT GCG TCC GAC CCC CGG CAT GGC CGA TAC CTC
M009          TTC GAT GCC AAG AAC ATG ATG GCT GCG TCC GAC CCC CGG CAT GGC CGA TAC CTC
M010          TTC GAT GCC AAG AAC ATG ATG GCT GCG TCC GAC CCC CGG CAT GGC CGA TAC CTC
M011          TTC GAT GCC AAG AAC ATG ATG GCT GCG TCC GAC CCC CGG CAT GGC CGA TAC CTC
M012          TTC GAT GCC AAG AAC ATG ATG GCT GCG TCC GAC CCC CGG CAT GGC CGA TAC CTC
M013          TTC GAT GCC AAG AAC ATG ATG GCT GCG TCC GAC CCC CGG CAT GGC CGA TAC CTC
M014          TTC GAT GCC AAG AAC ATG ATG GCT GCG TCC GAC CCC CGG CAT GGC CGA TAC CTC
M015          TTC GAT GCC AAG AAC ATG ATG GCT GCG TCC GAC CCC CGG CAT GGC CGA TAC CTC
M016          TTC GAT GCC AAG AAC ATG ATG GCT GCG TCN GAC CCC CGG CAT GGG CGA TAC CTG
M017          TTC GAT GCC AAG AAC ATG ATG GCT GCG TCC GAC CCC CGG CAT GGG CGA TAC CTG
M018          TTC GAT GCC AAG AAC ATG ATG GCT GCG TCC GAC CCC CGG CAT GGG CGA TAC CTG
M019          TTC GAT GCC AAG AAC ATG ATG GCT GCG TCC GAC CCC CGG CAT GGG CGA TAC CTG
M020          TTC GAT GCC AAG AAC ATG ATG GCT GCG TCC GAC CCC CGG CAT GGG CGA TAC CTG
```

```
M021          TTC GAT GCC AAG AAC ATG ATG GCT GCG TCC GAC CCC CGG CAT GGG CGA TAC CTG
M029          TTC GAT GCC AAG AAC ATG ATG GCT GCG TCC GAC CCC CGG CAT GGG CGA TAC CTG
M030          TTC GAT GCC AAG AAC ATG ATG GCT GCG TCC GAC CCC CGG CAT GGG CGA TAC CTG
M031          TTC GAT GCC AAG AAC ATG ATG GCT GCG TCC GAC CCC CGG CAT GGG CGA TAC CTG
M032          TTC GAT GCC AAG AAC ATG ATG GCT GCG TCC GAC CCC CGG CAT GGG CGA TAC CTG
M033          TTC GAT GCC AAG AAC ATG ATG GCT GCG TCC GAC CCC CGG CAT GGG CGA TAC CTG
M034          TTC GAT GCC AAG AAC ATG ATG GCT GCG TCC GAT CCC CGG CAT GGG CGA TAC CTG
M035          TTC GAT GCC AAG AAC ATG ATG GCT GCG TCC GAC CCC CGG CAT GGC CGA TAC CTC
M036          TTC GAT GCC AAG AAC ATG ATG GCT GCG TCC GAC CCC CGG CAT GGG CGA TAC CTG
M037          TTC GAT GCC AAG AAC ATG ATG GCT GCG TCC GAC CCC CGG CAT GGG CGA TAC CTG
M038          TTC GAT GCC AAG AAC ATG ATG GCT GCG TCC GAC CCC CGG CAT GGG CGA TAC CTG
M039          TTC GAT GCC AAG AAC ATG ATG GCT GCG TCC GAC CCC CGG CAT GGG CGA TAC CTG
M040          TTC GAT GCC AAG AAC ATG ATG GCT GCG TCC GAC CCC CGG CAT GGG CGA TAC CTG
WC248         TTC GAT GCC AAG AAC ATG ATG GCT GCG TCC GAC CCC CGG CAT GGC CGA TAC CTC
WC364         TTC GAT GCC AAG AAC ATG ATG GCT GCG TCC GAC CCC CGG CAT GGC CGA TAC CTC
WC367         TTC GAT GCC AAG AAC ATG ATG GNT GCG TCC GAC CCC CGG CAT GGC CGA TAC CTC
WC483         TTC GAT GCC AAG AAC ATG ATG GCT GCG TCC GAC CCC CGG CAT GGC CGA TAC CTC
WC493         TTC GAT GCG AAG AAC ATG ATG GCT GCG TCC GAC CCC CGG CAT GGG CGA TAC CTG
WC555         TTC GAT GCC AAG AAC ATG ATG GCT GCG TCC GAC CCC CGG CAT GGG CGA TAC CTG
WC556         TTC GAT GCC AAG AAC ATG ATG GCT GCG TCC GAC CCC CGG CAT GGG CGA TAC CTG
WC557         TTC GAT GCC AAG AAC ATG ATG GCT GCG TCC GAC CCC CGG CAT GGG CGA TAC CTG
WC581         TTC GAT GCC AAG AAC ATG ATG GCT GCG TCC GAC CCC CGG CAT GGG CGA TAC CTG
WC582         TTC GAT GCC AAG AAC ATG ATG GCT GCG TCC GAC CCC CGG CAT GGG CGA TAC CTG
WC583         TTC GAT GCC AAG AAC ATG ATG GCT GCG TCC GAT CCC CGG CAT GGG CGA TAC CTG
WC659         TTC GAT GCC AAG AAC ATG ATG GCT GCG TCC GAC CCC CGG CAT GGG CGA TAC CTG
WC685         TTC GAT GCC AAG AAC ATG ATG GCT GCG TCC GAC CCC CGG CAT GGG CGA TAC CTG
WC808         TTC GAT GCC AAG AAC ATG ATG GCT GCG TCC GAC CCC CGG CAT GGC CGA TAC CTC
WC828         TTC GAT GCC AAG AAC ATG ATG GCT GCG TCC GAT CCC CGG CAT GGG CGA TAC CTG
WC834         TTC GAT GCC AAG AAC ATG ATG GCT GCG TCC GAT CCC CGG CAT GGG CGA TAC CTG
WC835         TTC GAT GCC AAG AAC ATG ATG GCT GCG TCC GAT CCC CGG CAT GGG CGA TAC CTG
WC836         TTC GAT GCC AAG AAC ATG ATG GCT GCG TCC GAC CCC CGG CAT GGG CGA TAC CTG
G_sordulenta  TTC GAT GCC AAG AAC ATG ATG GCT GCG TCC GAC CCC AGG CAT GGC CGC TAC CTC

[             515 Intron 7→                                          ←Intron 7       587]
M001          ACT GTATGCAACATCC--TAGCACA-GAGCGTACCAATCTGATGTATCTGTTGCCTATTTTATAG GTT GCC GC
M002          ACT GTATGCAACATCC--TAGCACA-GAGCGTACCAATCTGATGTATCTGTTGCCTATTTTATAG GTT GCC GC
M003          ACT GTATGCAACATCC--TAGCACA-GAGCGTACCAATCTGATGTATCTGTTGCCTATTTTATAG GTT GCC GC
M004          ACT GTATGCAACATCC--TAGCACA-GAGCGTACCAATCTGATGTATCTGTTGCCTATTTTATAG GTT GCC GC
M005          ACT GTATGCAACATCC--TAGCACA-GAGCGTACCAATCTGATGTATCTGTTGCT--TTTTATAG GTT GCC GC
M006          ACT GTATGCAACATCC--TAGCACA-GAGCGTACCAATCTGATGTATCTGTTGCCTATTTTATAG GTT GCC GC
M007          ACT GTATGCAACATCC--TAGCACA-GAGCGTACCAATCTGATGTATCTGTTGCCTATTTTATAG GTT GCT GC
M008          ACT GTATGCAACATCC--TAGCACA-GAGCGTACCAATCTGATGTATCTGTTGCCTATTTTATAG GTT GCC GC
M009          ACT GTATGCAACATCC--TAGCACA-GAGCGTACCAATCTGATGTATCTGTTGCCTATTATATAG GTT GCC GC
M010          ACT GTATGCAACATCC--TAGCACA-GAGCGTACCAATCTGATGTATCTGTTGCCTATTTTATAG GTT GCT GC
M011          ACT GTATGCAACATCC--TAGCACA-GAGCGTACCAATCTGATGTATCTGTTGCCTATTTTATAG GTT GCC GC
M012          ACT GTATGCAACATCC--TAGCACA-GAGCGTACCAATCTGATGTATCTGTTGCCTATTTTATAG GTT GCC GC
M013          ACT GTATGCAACATCC--TAGCACAAGAGCGTACCAATCTGAAGTATCTGTTGCCTATTATAAAG GTT GCC GC
M014          ACT GTATGCAACATCC--TAGCACA-GAGCGTACCAATCTGATGTATCTGTTGCCTATTTTATAG GTT GCC GC
M015          ACT GTATGCAACATCC--TAGCACA-GAGCGTACCAATCTGATGTATCTGTTGCCTATTATATAG GTT GCC GC
M016          ACC GTATGCGGCTCCCCCTATTACG-GAGCGTACCAATCTGATCTATTTGTTACGTTTTTCATAG GTT GCC GC
M017          ACC GTATGCGGCTCCCCCTATTACG-GAGCGTACCAATCTGATCTATTTGTTACATTTTTCATAG GTT GCC GC
M018          ACC GTATGCGGCTCCCCCTATTACG-GAGCGTACCAATCTGATCTATTTGTTACNTTTTTCATAG GTT GCC GC
M019          ACC GTATGCGGCTCCCCCTATTACG-GAGCGTACCAATCTGATCTATTTGTTACATTTTTCATAG GTT GCC GC
M020          ACC GTATGCGGCTCCCCCTATTACG-GAGCGTACCAATCTGATCTATTTGTTACATTTTTCATAG GTT GCC GC
M021          ACC GTATGCGGCTCCCCCTATTACG-GAGCGTACCAATCTGATCTATTTGTTACATTTTTCATAG GTT GCC GC
M029          ACC GTATGCGGCTCCCCTTATTACG-GAGCGTACCAATCTGATCTATTTGTTACGTTTTTCATAG GTT GCC GC
M030          ACC GTATGCGGCTCTCCTTATTACG-GAGCGTACCAATCTGATCTATTTGTTACATTTTTCATAG GTT GCC GC
M031          ACC GTATGCGGCTCCCCCTATTACG-GAGCGTACCAATCTGATCTATTTGTTACATTTTTCATAG GTT GCC GC
M032          ACC GTATGCGGCTCTCCTTATTACG-GAGCGTACCAATCTGATCTATTTGTTACGTTTTTCATAG GTT GCC GC
M033          ACC GTATGCGGCTCCCCTTATTACG-GAGCGTACCAATCTGATCTATTTGTTACGTTTTTCATAG GTT GCC GC
M034          ACC GTATGCGGCTCTCCTTATTACG-GAGCGTACCAATCTGATCTATTTGTTACGTTTTTCATAG GTT GCC GC
M035          ACT GTATGCAACATCC--TAGCACA-GAGCGTACCAATCTGATGTATCTGTTGCCTATTTTATAG GTT GCC GC
M036          ACC GTATGCGGCTCCCCTTATTACG-GAGCGTACCAATCTGATCTATTTGTTACATTTTTCATAG GTT GCC GC
M037          ACC GTATGCGGCTCCCCTTATTACG-GAGCGTACCAATCTGATCTATTTGTTACATTTTTCATAG GTT GCC GC
M038          ACC GTATGCGGCTCTCCTTATTACG-GAGCGTACCAATCTGATCTATTTGTTACGTTTTTCATAG GTT GCC GC
M039          ACC GTATGCGGCTCTCCTTATTACG-GAGCGTACCAATCTGATCTATTTGTTACGTTTTTCATAG GTT GCC GC
M040          ACC GTATGCGGCTCNCCTTATTACG-GAGCGTACCAATCTGATCTATTTGTTACNTTTTTCATAG GTT GCC GC
WC248         ACT GTATGCAACATCC--TAGCACA-GAGCGTACCAATCTGATGTATCTGTTGCCTATTTTATAG GTT GCT GC
```

```
WC364          ACT GTATGCAACATCC--TAGCACA-GAGCGTACCAATCTGATGTATCTGTTGCCTATTATATAG GTT GCC GC
WC367          ACT GTATGCAACATCC--TAGCACA-GAGCGTACCAATCTGATGTATCTGTTGCT--TTTTATAG GTT GCC GC
WC483          ACT GTATGCAACATCC--TAGCACA-GAGCGTACCAATCTGATGTATCTGTTGCT--TTTTATAG GTT GCC GC
WC493          ACC GTATGCGGCTCCCCCTATCACG-GAGCGTATCAATCTGATCTATTTGTTNCNTTTTTCATAG GTT GCC GC
WC555          ACC GTATGCGGCTCCCCTTATTACG-GAGCGTACCAATCTGATCTATTTGTTACATTTTTCATAG GTT GCC GC
WC556          ACC GTATGCGGCTCCCCTTATTACG-GAGCGTACCAATCTGATCTATTTGTTACGTTTTTCATAG GTT GCC GC
WC557          ACC GTATGCGGCTCCCCTTATTACG-GAGCGTACCAATCTGATCTATTTGTTACGTTTTTCATAG GTT GCC GC
WC581          ACC GTATGCGGCTCTCCTTATTACG-GAGCGTACCAATCTGATCTATTTGTTACGTTTTTCATAG GTT GCC GC
WC582          ACC GTATGCGGCTCCCCTTATTACG-GAGTGTACCAATCTGATCTATTTGTTACGTTTTTCATAG GTT GCC GC
WC583          ACC GTATGCGGCTCTCCTTATTACG-GAGCGTACCAATCTGATCTATTTGTTACATTTTTCATAG GTT GCC GC
WC659          ACC GTATGCGGCTCCCCTTATTACG-GAGCGTACCAATCTGATCTATTTGTTACNTTTTTCATAG GTT GCC GC
WC685          ACC GTATGCGGCTCTCCTTATTACG-GAGCGTACCAATCTGATCTATTTGTTACGTTTTTCATAG GTT GCC GC
WC808          ACT GTATGCAACATCC--TAGCACA-GAGCGTACCAATCTGATGTATCTGTTGCCTATTTTATAG GTT GCC GC
WC828          ACC GTATGCGGCTCTCCTTATTACG-GAGCGTACCAATCTGATCTATTTGTTACGTTTTTCATAG GTT GCC GC
WC834          ACC GTATGCGGCTCTCCTTATTACG-GAGCGTACCAATCTGATCTATTTGTTACATTTTTCATAG GTT GCC GC
WC835          ACC GTATGCGGCTCTCCTTATTACG-GAGCGTACCAATCTGATCTATTTGTTACATTTTTCATAG GTT GCC GC
WC836          ACC GTATGCGGCTCCCCNTATTACG-GAGCGTACCAATCTGATCTATTTGTTACATTTTTCATAG GTT GCC GC
G_sordulenta   ACT GTAGGTGTTAATGTTTCTTCT----GTGTTCCG-------TCATCTGAAACCTGTTCCATAG GTT GCT GC
```

Appendix E: Sequence alignment of partial β-tubulin gene sequences from isolates of *Grifola frondosa* and related species

Sequence alignments were performed using the Clustal W algorithm (Higgins *et al.* 1991) in the MegAlign application of the Wisconsin Package (Genetics Computer Group, Madison, WI). Numbers at the top of each sequence block designate site location within the sequence alignment. Regions of exons are labeled and underlined for the first isolate (M001). Codons are shown in protein coding regions.

```
[                 1  exon 6→                                                    53]
M004              AG CCC TAC AAC GCA ACC CTC TCC GTG CAC CAA CTG GTC GAG AAC TCT GAT GAG
M009              AG CCC TAC AAC GCA ACC CTC TCC GTG CAC CAA CTG GTC GAG AAC TCT GAT GAG
M030              AG CCC TAC AAC GCA ACC CTC TCC GTG CAT CAA CTG GTC GAG AAC TCT GAT GAG
M037              AG CCC TAC AAC GCA ACC CTC TCC GTG CAC CAA CTG GTC GAG AAC TCT GAT GAG
WC493             AG CCC TAC AAC GCA ACC CTC TCC GTG CAC CAA CTG GTC GAG AAC TCT GAT GAG
WC659             AG CCC TAC AAC GCA ACC CTC TCC GTG CAN CAA CTG GTC GAG AAC TCT GAT GAG
WC828             AG CCC TAC AAC GCA ACC CTC TCC GTG CAC CAA CTG GTC GAG AAC TCT GAT GAG
WC835             AG CCC TAC AAC GCA ACC CTC TCC GTG CAC CAA CTG GTC GAG AAC TCT GAT GAG
G_sordulenta      AG CCC TAC AAC GCG ACA CTC TCT GTG CAT CAA CTC GTC GAG AAC TCT GAC CAG
P_umbellatus_G02  AG CCC TAC AAC GCG ACG CTC TCG GTC CAC CAG CTC GTT GAA AAC TCG GAT GAG
G_lucidium        AG CCG TAC AAC GCC ACC CTC TCC GTT CAT CAG CTC GTT GAG AAC TCG GAC CAG

[                 54                                                            107]
M004              ACT TTC TGC ATT GAT AAC GAG GCC TTG TAC GAC ATT TGC TTC CGG ACA CTG AAG
M009              ACT TTC TGC ATT GAT AAC GAG GCC TTG TAC GAC ATT TGC TTC CGG ACA CTG AAG
M030              ACT TTC TGC ATT GAT AAC GAG GCA CTG TAC GAC ATT TGC TTC CGG ACA CTG AAG
M037              ACT TTC TGC ATT GAT AAC GAG GCG CTG TAC GAC ATT TGC TTC CGG ACA CTG AAG
WC493             ACT TTC TGC ATT GAT AAC GAG GCG CTG TAC GAT ATT TGC TTC CGG ACA CTG AAG
WC659             ACT TTC TGC ATT GAT AAC GAG GCN CTG TAC GAC ATT TGC TTC CGG ACA CTG AAG
WC828             ACT TTC TGC ATT GAT AAC GAG GCG CTG TAC GAC ATT TGC TTC CGG ACA CTG AAG
WC835             ACT TTC TGC ATT GAT AAC GAG GCG CTG TAC GAC ATT TGC TTC CGG ACA CTG AAG
G_sordulenta      ACG TTC TGC ATT GAT AAC GAG GCA TTA TAT GAT ATA TGC TTC AGA ACC CTC AAG
P_umbellatus_G02  ACC TTC TGT ATT GAC AAC GAG GCG CTG TAC GAC ATC TGC TTC CGG ACA CTG AAG
G_lucidium        ACG TTC TGT ATC GAC AAC GAG GCG CTC TAC GAC ATC TGC TTC AGG ACG CTC AAG

[                 108                                                           161]
M004              CTG ACG ACA CCG ACA TAC GGC GAC CTG AAT CAC CTC ATT TCC ATC GTC ATG TCC
M009              CTG ACG ACA CCG ACA TAC GGC GAC CTG AAT CAC CTC ATT TCC ATC GTC ATG TCC
M030              CTG ACG ACA CCG ACA TAC GGC GAT CTG AAC CAC CTC ATT TCC ATC GTC ATG TCC
M037              CTG ACG ACA CCG ACA TAC GGC GAT CTG AAC CAC CTC ATT TCC ATC GTC ATG TCC
WC493             CTG ACG ACA CCC ACA TAT GGC GAT CTG AAC CAC CTC ATT TCC ATT GTC ATG TCC
WC659             CTG ACG ACA CCG ACA TAC GGC GAT CTG AAC CAC CTC ATN TCC ATC GTC ATG TCC
WC828             CTG ACG ACA CCG ACA TAC GGC GAT CTG AAC CAC CTC ATT TCC ATC GTC ATG TCC
WC835             CTG ACG ACA CCG ACA TAC GGC GAT CTG AAC CAC CTC ATT TCC ATC GTC ATG TCC
G_sordulenta      CTC ACT ACA CCA ACT TAT GGT GAC CTT AAC CAC CTT GTA TCG ATT GTC ATG TCC
P_umbellatus_G02  CTG TCG ACA CCG ACA TAC GGG GAT CTC AAC CAC CTC GTT TCC ATT GTC ATG TCC
G_lucidium        CTT ACC ACG CCC ACA TAT GGT GAT CTC AAC CAC CTC GTC TCT ATT GTC ATG TCG

[                 162                                                           215]
M004              GGT ATT ACA ACT TGT TTG CGT TTC CCT GGT CAG CTG AAC TCC GAT CTC CGG AAG
M009              GGT ATT ACA ACT TGT TTG CGT TTC CCT GGT CAG CTG AAC TCC GAT CTC CGG AAG
M030              GGT ATT ACA ACT TGC TTG CGT TTC CCT GGT CAG CTG AAC TCC GAT CTC CGA AAG
M037              GGT ATT ACA ACT TGC TTG CGT TTC CCT GGT CAG CTG AAC TCC GAT CTC CGA AAG
WC493             GGC ATT ACA ACT TGC TTG CGT TTC CCT GGT CAG CTG AAC TCC GAC CTC CGG AAG
WC659             GGT ATT ACA ACT TGC TTG CGT TTC CCT GGT CAG CTG AAC TCC GAT CTC CGA AAG
```

```
WC828             GGT ATT ACA ACT TGC TTG CGT TTC CCT GGT CAG CTG AAC TCC GAT CTC CGA AAG
WC835             GGT ATT ACA ACT TGC TTG CGT TTC CCT GGT CAG CTG AAC TCC GAT CTC CGA AAG
G_sordulenta      GGT ATC ACG ACT TGC TTG CGT TTC CCT GGT CAG CTG AAT TCT GAC TTG CGG AAG
P_umbellatus_G02  GGT ATC ACG ACA TGC TTG CGT TTC CCT GGT CAG CTT AAC TCT GAC CTG CGT AAG
G_lucidium        GGT ATC ACG ACT TGC CTG CGT TTC CCT GGT CAG CTC AAC TCG GAC CTC AGG AAG

[                  216          ←exon 6      exon 7→                                 267]
M004              TTG GCT GTC AAC ATG G GTA GTT CCC TTC CCC CGT CTC CAC TTC TTC ATG ACC
M009              TTG GCT GTC AAC ATG G GTA GTT CCC TTC CCC CGT CTC CAC TTC TTC ATG ACT
M030              TTG GCT GTC AAC ATG G GTA GTC CCC TTC CCC CGT CTC CAC TTC TTC ATG ACT
M037              TTG GCT GTC AAC ATG G GTA GTC CCC TTC CCC CGT CTC CAC TTC TTC ATG ACT
WC493             TTG GCT GTC AAC ATG G GTA GTC CCC TTC CCC CGT CTC CAC TTC TTC ATG ACT
WC659             TTG GCT GTC AAC ATG G GTA GTC CCC TTC CCC CGT CTC CAC TTC TTC ATG ACT
WC828             TTG GCT GTC AAC ATG G GTA GTC CCC TTC CCC CGT CTC CAC TTC TTC ATG ACT
WC835             TTG GCT GTC AAC ATG G GTA GTC CCC TTC CCC CGT CTC CAC TTC TTC ATG ACT
G_sordulenta      TTG GCT GTC AAC ATG G GTA GTT CCC TTC CCT CGT CTT CAT TTC TTC ATG ACC
P_umbellatus_G02  CTC GCT GTC AAC ATG G GTA GTT CCC TTC CCT CGT CTC CAT TTC TTC ATG ACC
G_lucidium        TTG GCT GTC AAC ATG G GTA GTT CCC TTC CCT CGT CTT CAC TTC TTC ATG ACC

[                  268                                                              321]
M004              GGT TTC GCG CCC TTG ACT GCG CGC GGC AGC CAG CAG TAC CGT GCT GTC ACT GTA
M009              GGT TTC GCG CCC TTG ACT GCG CGC GGC AGC CAG CAG TAC CGT GCT GTC ACT GTA
M030              GGT TTC GCG CCC TTG ACT GCG CGC GGC AGC CAG CAG TAC CGT GCT GTC ACT GTA
M037              GGT TTC GCG CCC TTG ACT GCG CGC GGC AGC CAG CAG TAC CGT GCT GTC ACT GTA
WC493             GGT TTC GCG CCC TTG ACT GCG CGC GGC AGC CAG CAG TAT CGT GCT GTC ACT GTA
WC659             GGT TTC GCG CCC TTG ACT GCG CGC GGC AGC CAG CAG TAC CGT GCT GTC ACT GTA
WC828             GGT TTC GCG CCC TTG ACT GCG CGC GGC AGC CAG CAG TAC CGT GCT GTC ACT GTA
WC835             GGT TTC GCG CCC TTG ACT GCG CGC GGC AGC CAG CAG TAC CGT GCT GTC ACT GTA
G_sordulenta      GGC TTC GCG CCT TTG ACC GCT CGG GGT AGC CAG CAA TAC CGC GCG GTC ACC GTC
P_umbellatus_G02  GGT TTC GCC CCT TTG ACT GCA CGT GGC AGC CAA CAA TAC CGT GCA GTC ACT GTG
G_lucidium        GGT TTT GCT CCC CTG ACC GCC CGC GGC AGC CAG CAG TAC CGT GCT GTC ACT GTT

[                  322                                                              375]
M004              CCC GAG CTG ACT CAA CAG ATG TTC GAT GCC AAG AAC ATG ATG GCT GCG TCC GAC
M009              CCC GAG CTG ACT CAA CAG ATG TTC GAT GCC AAG AAC ATG ATG GCT GCG TCC GAC
M030              CCC GAG CTG ACT CAA CAG ATG TTC GAT GCC AAG AAC ATG ATG GCT GCG TCC GAC
M037              CCC GAG CTG ACT CAA CAG ATG TTC GAT GCC AAG AAC ATG ATG GCT GCG TCC GAC
WC493             CCC GAG CTG ACT CAA CAG ATG TTC GAT GCG AAG AAC ATG ATG GCT GCG TCC GAC
WC659             CCC GAG CTG ACT CAA CAG ATG TTC GAT GCC AAG AAC ATG ATG GCT GCG TCC GAC
WC828             CCC GAG CTG ACT CAA CAG ATG TTC GAT GCC AAG AAC ATG ATG GCT GCG TCC GAT
WC835             CCC GAG CTG ACT CAA CAG ATG TTC GAT GCC AAG AAC ATG ATG GCT GCG TCC GAT
G_sordulenta      CCT GAG CTG ACG CAG CAA ATG TTC GAT GCC AAG AAC ATG ATG GCT GCG TCC GAC
P_umbellatus_G02  CCC GAG CTC ACG CAG CAG ATG TTC GAC GCG AAG AAC ATG ATG GCT GCG TCT GAC
G_lucidium        CCC GAG CTC ACG CAG CAG ATG TTC GAT GCT AAG AAC ATG ATG GCC GCC TCG GAC

[                  376                    ←exon 7 401]
M004              CCC CGG CAT GGC CGA TAC CTC ACT GT
M009              CCC CGG CAT GGC CGA TAC CTC ACT GT
M030              CCC CGG CAT GGG CGA TAC CTG ACC GT
M037              CCC CGG CAT GGG CGA TAC CTG ACC GT
WC493             CCC CGG CAT GGG CGA TAC CTG ACC GT
WC659             CCC CGG CAT GGG CGA TAC CTG ACC GT
WC828             CCC CGG CAT GGG CGA TAC CTG ACC GT
WC835             CCC CGG CAT GGG CGA TAC CTG ACC GT
G_sordulenta      CCC AGG CAT GGC CGC TAC CTC ACT GT
P_umbellatus_G02  CCC CGT CAC GGT CGC TAC CTG ACG GT
G_lucidium        CCG AGG CAC GGT CGT TAC CTC ACT GT
```

Appendix F: Results of genotype and nutrient experiments descripted in Chapter 4

Table 4.13. Crop cycles time (wk) showing spawn run time and primordia and fruitbody development of 23 isolates of *Grifola frondosa* grown on sawdust substrate supplemented with 20% nutrient at The Mushroom Research Center (Experiment #1 - #4). For a graphic summary of this data see Figure 4.6.

A. Experiment #1					
	Time (weeks) to complete stage of development				
Isolates	Spawn run	Primordia	Fruitbody	Total crop cycle	number of replicates [a]
WC828	5.0	3.0	2.0	10.0	15
WC835	6.5	2.5	2.0	11.0	15
WC836	6.0	3.0	2.0	11.0	15
WC659	16.0	- [b]	-	16.0	0
WC834	5.0	11.0	-	16.0	0
B. Experiment #2					
Isolates	Spawn run	Primordia	Fruitbody	Total crop cycle	number of replicates
M040	6.0	3.0	2.0	11.0	15
M014	16.0	-	-	16.0	0
M007	16.0	-	-	16.0	0
M004	7.0	1.0	2.0	10.0	3
WC493	7.0	2.0	1.0	10.0	3
WC582	16.0	-	-	16.0	0
WC248	16.0	-	-	16.0	0
C. Experiment #3					
Isolates	Spawn run	Primordia	Fruitbody	Total crop cycle	number of replicates
M036	4.0	3.0	2.0	9.0	15
M019	6.5	9.5	-	16.0	0
M013	10.0	2.0	2.0	14.0	5
M011	16.0	-	-	16.0	0
M009	7.0	2.0	2.0	11.0	2
M002	6.0	10.0	-	16.0	0
WC483	9.0	2.0	1.0	12.0	3
D. Experiment #4					
Isolates	Spawn run	Primordia	Fruitbody	Total crop cycle	number of replicates
WC828	5.0	3.0	2.0	10.0	15
M015	5.0	3.0	3.0	11.0	15
M036	5.0	2.0	2.0	9.0	15
M037	4.0	3.0	3.0	10.0	15
M038	4.0	2.5	2.0	9.5	15
M039	4.0	2.0	2.0	8.0	15
M040	6.0	4.0	2.0	12.0	15

[a] Number of replicates (bags) producing mushrooms.
[b] - = Primordia and/or fruitbodies did not develop.

Table 4.14. Percentage biological efficiency (%BE) and quality among 10 *Grifola frondosa* isolates grown on sawdust substrate supplemented with 20% nutrient evaluated in crops I and II of Experiment #1 and #4 at the Mushroom Research Center. For a graphic summary of this data see Figure 4.7 and 4.8.

A. Experiment #1						
	Crop I		Crop II		Combined (Crop I & II)	
Isolates	BE (%)	Quality	BE (%)	Quality [a]	BE (%)	Quality
WC828	35.4 a [b]	1.3 a	33.5 a	1.4 a	34.5 a	1.4 a
WC835	22.1 b	2.9 b	21.2 b	3.0 b	21.7 b	3.0 b
WC836	21.4 b	1.2 a	24.8 ab	1.1 a	23.1 b	1.2 a
B. Experiment #4						
	Crop I		Crop II		Mean (Crop I & II)	
Isolates	BE (%)	Quality	BE (%)	Quality	BE (%)	Quality
WC828	36.5 ab	1.3 a	40.5 a	1.1 a	38.5	1.2
M015	33.0 ab	3.4 c	30.1 b	3.0 b	31.6	3.2
M036	36.4 ab	1.3 a	42.6 a	1.2 a	39.5	1.3
M037	33.0 ab	1.4 a	38.5 a	1.3 a	35.8	1.4
M038	31.1 b	1.2 a	32.0 b	1.1 a	31.6	1.2
M039	34.9 ab	2.0 b	39.7 a	1.5 a	37.3	1.8
M040	37.6 a	1.2 a	40.2 a	1.1 a	38.9	1.2
Total Mean	34.6	1.7	37.7	1.5		

[a] Quality rating based on scale of 1-4 where 1 is highest quality and 4 is lowest quality.
[b] Means in the same experiment in the same column followed by the same letter are not significantly different at the P=0.05 level according to Tukey-Kramer HSD.

Table 4.15. Effect of selected nutrients (20% total), added alone or in combination to oak sawdust, on crop cycle time (weeks) for *Grifola frondosa* (WC828) grown at the Mushroom Research Center (Experiment #5). For a graphic summary of this data see Figure 4.9.

	Selected nutrient supplements (%)				Crop cycle time (weeks)			
Treatment	Wheat Bran	Millet	Rye	Corn meal	Spawn run	Primordia development	Fruitbody development	Total crop cycle
1	20	0	0	0	5.5	3.5	2.0	11.0
2	0	20	0	0	16.0	- [a]	-	16.0
3	0	0	20	0	6.0	3.0	2.0	11.0
4	0	0	0	20	16.0	-	-	16.0
5	10	10	0	0	7.0	3.0	2.0	12.0
6	10	0	10	0	4.0	3.5	2.5	10.0
7	10	0	0	10	11.0	2.0	2.0	15.0
8	0	10	10	0	11.0	2.0	2.0	15.0
9	0	10	0	10	16.0	-	-	16.0
10	0	0	10	10	10.0	3.0	2.0	15.0
11	6.7	6.7	6.7	0	6.5	3.0	2.0	11.5
12	6.7	6.7	0	6.7	8.0	2.5	2.0	12.5
13	6.7	0	6.7	6.7	7.0	3.5	2.0	12.5
14	0	6.7	6.7	6.7	12.0	2.0	2.0	16.0
15	5	5	5	5	8.0	3.5	2.0	13.5

[a] - = Primordia and/or fruitbodies did not develop.

Table 4.16. The effect of selected nutrients (20% total) on percentage biological efficiency (%BE) and quality for isolate WC828 for crops I and II grown at the Mushroom Research Center (Experiment #5). For a graphic summary of this data see Figure 4.10.

	Nutrient supplements (%)				Crop I		Crop II		Combined (Crop I & II)	
Treatment	Wheat Bran	Millet	Rye	Corn meal	BE (%)	Quality [x]	BE (%)	Quality	BE (%)	Quality
1	20	0	0	0	26.8 bc [y]	1.5 ab	20.2 c	1.3 a	23.5 b	1.4 a
2	0	20	0	0	0 [z]	-	0	-	0	-
3	0	0	20	0	27.6 bc	1.7 abc	30.1 ab	1.7 ab	28.9 b	1.7 ab
4	0	0	0	20	0	-	0	-	0	-
5	10	10	0	0	32.3 ab	1.6 abc	35.6 ab	1.3 a	34.0 a	1.4 a
6	10	0	10	0	40.5 a	1.2 a	38.7 a	1.1 a	39.6 a	1.1 a
7	10	0	0	10	28.3 bc	2.7 cd	25.8 bc	3.0 c	27.1 b	2.8 b
8	0	10	10	0	12.3 d	3.0 d	22.9 c	2.3 bc	17.6 c	2.7 b
9	0	10	0	10	0	-	0	-	0	-
10	0	0	10	10	28.9 bc	2.6 cd	30.0 ab	2.4 bc	29.4 b	2.5 b
11	6.7	6.7	6.7	0	34.0 ab	1.3 ab	40.1 a	1.2 a	37.1 a	1.3 a
12	6.7	6.7	0	6.7	20.1 cd	2.8 d	18.3 c	3.1 c	19.2 c	3.0 b
13	6.7	0	6.7	6.7	26.4 bc	1.7 abc	27.1 bc	1.5 ab	26.7 b	1.6 ab
14	0	6.7	6.7	6.7	14.0 d	2.6 cd	15.8 c	2.9 c	14.9 c	2.8 b
15	5	5	5	5	27.5 bc	2.2 cd	25.2 bc	2.3 bc	26.3 b	2.3 b

[x] Quality rating based on scale of 1-4 where 1 is highest quality and 4 is lowest quality.
[y] Means in the same column followed by the same letter are not significantly different at the P=0.05 level according to Tukey-Kramer HSD.
[z] Treatments where no fruiting occurred (0) were eliminated from the analysis of variance.

Table 4.17. Effect of various levels of selected nutrients (wheat bran and millet) added to a sawdust (oak) substrate on crop cycle time (weeks) for *Grifola frondosa* (WC828) grown at the Mushroom Research Center (Experiment #7 and 8). For a graphic summary of this data see Figure 4.11.

A. Experiment #7 (20% total nutrient level).

	Selected nutrient supplements (%)		Crop cycle time (weeks)			
Treatment	Wheat bran	Millet	Spawn run	Primordia development	Fruitbody development	Total crop cycle
1	0	20	16.0	- [a]	-	16.0
2	5	15	16.0	-	-	16.0
3	10	10	8.0	2.0	3.0	13.0
4	15	5	5.5	3.0	3.0	11.5
5	20	0	5.0	4.0	2.0	11.0

B. Experiment #8 (30% total nutrient level).

	Selected nutrient supplements (%)		Crop cycle time (weeks)			
Treatment	Wheat bran	Millet	Spawn run	Primordia development	Fruitbody development	Total crop cycle
1	0	30	16.0	-	-	16.0
2	7.5	22.5	4.0	5.0	3.0	12.0
3	15	15	3.0	5.0	2.0	10.0
4	22.5	7.5	4.0	3.0	3.0	10.0
5	30	0	4.0	2.5	2.5	9.0

[a] - = Primordia and/or fruitbodies did not develop.

Table 4.18. Effect of various levels of selected nutrients (wheat bran and rye) added to a sawdust (oak) substrate on crop cycle time (weeks) for *Grifola frondosa* (WC828) grown at the Mushroom Research Center (Experiment #10 and 11). For a graphic summary of this data see Figure 4.12.

A. Experiment #10 (20% total nutrient level)

Treatment	Selected nutrient supplements (%)		Crop cycle time (weeks)			
	Wheat bran	Rye	Spawn run	Primordia development	Fruitbody development	Total crop cycle
1	0	20	6.0	3.0	2.0	11.0
2	5	15	4.0	4.0	2.0	10.0
3	10	10	4.0	3.0	3.0	10.0
4	15	5	3.0	5.0	2.0	10.0
5	20	0	4.0	4.0	3.0	11.0

B. Experiment #11 (30% total nutrient level)

Treatment	Selected nutrient supplements (%)		Crop cycle time (weeks)			
	Wheat bran	Rye	Spawn run	Primordia development	Fruitbody development	Total crop cycle
1	0	30	10.0	2.0	2.0	14.0
2	7.5	22.5	3.0	1.0	6.0	10.0
3	15	15	5.0	3.0	2.0	10.0
4	22.5	7.5	3.0	2.0	3.0	8.0
5	30	0	4.0	2.0	3.0	9.0

Table 4.19. Effect of various levels of selected nutrients (wheat bran, millet and rye) added to a sawdust (oak) substrate on crop cycle time (weeks) for *Grifola frondosa* (WC828) grown at the Mushroom Research Center (Experiment #12 and 13). For a graphic summary of this data see Figure 4.13.

A. Experiment #12 (20% total nutrient level)							
	Selected nutrient supplements (%)			Crop cycle time (weeks)			
Treatment	Wheat bran	Millet	Rye	Spawn run	Primordia development	Fruitbody development	Total crop cycle
1	0	0	20	7.0	2.0	2.0	11.0
2	0	6.7	13.3	16.0	- [a]	-	16.0
3	0	13.3	6.7	16.0	-	-	16.0
4	0	20	0	16.0	-	-	16.0
5	6.7	0	13.3	5.0	3.0	2.0	10.0
6	6.7	6.7	6.7	6.5	3.0	2.0	11.5
7	6.7	13.3	0	16.0	-	-	16.0
8	13.3	0	6.7	6.0	2.0	2.0	10.0
9	13.3	6.7	0	6.0	3.0	2.0	11.0
10	20	0	0	5.0	4.0	2.0	11.0
B. Experiment #13 (30% total nutrient level)							
	Selected nutrient supplements (%)			Crop cycle time (weeks)			
Treatment	Wheat bran	Millet	Rye	Spawn run	Primordia development	Fruitbody development	Total crop cycle
1	0	0	30	8.0	3.0	2.0	13.0
2	0	10	20	8.0	3.0	2.0	13.0
3	0	20	10	8.0	4.0	3.0	15.0
4	0	30	0	16.0	-	-	16.0
5	10	0	20	5.0	2.0	3.0	10.0
6	10	10	10	6.0	2.0	4.0	12.0
7	10	20	0	7.0	3.0	2.0	12.0
8	20	0	10	4.0	2.0	3.0	9.0
9	20	10	0	5.0	2.0	3.0	10.0
10	30	0	0	4.0	2.0	3.0	9.0

[a] - = Primordia and/or fruitbodies did not develop.

Table 4.20. Percentage biological efficiency (%BE) and quality for *Grifola frondosa* (WC828) grown on substrates containing various levels of selected nutrients (wheat bran and millet at 20% or 30% total) at the Mushroom Research Center (Experiment #7 and 8). For a graphic summary of this data see Figure 4.14.

A. Experiment #7 (20% total nutrient level)				
	Selected nutrient supplements (%)			
Treatment	Wheat bran	Millet	BE (%)	Quality [x]
1	0	20	0.0	-
2	5	15	0.0	-
3	10	10	30.3NS [y]	1.6NS
4	15	5	30.1NS	1.9NS
5	20	0	23.4NS	1.8NS
B. Experiment #8 (30% nutrient level)				
	Selected nutrient supplements (%)			
Treatment	Wheat bran	Millet	BE (%)	Quality
1	0	30	0.0	-
2	7.5	22.5	29.7b [z]	2.9b
3	15	15	40.2a	1.8a
4	22.5	7.5	30.2b	2.1a
5	30	0	20.6c	2.4ab

[x] Quality rating based on scale of 1-4 when 1 is highest quality and 4 is lowest quality.
[y] NS = nonsignificant at P=0.05.
[z] Means in the same experiment in the same column followed by the same letter are not significantly different at the P=0.05 level according to Tukey-Kramer HSD.

Table 4.21. Percentage biological efficiency (%BE) and quality for 5 treatments evaluated in two experiments (#10 and 11) for the effect of various levels of selected nutrients (wheat bran and rye at 20% or 30% total) for *Grifola frondosa* (WC828) grown at the Mushroom Research Center. For a graphic summary of this data see Figure 4.15.

A. Experiment #10 (20% total nutrient level)				
	Selected nutrient supplements (%)			
Treatment	Wheat bran	Rye	BE (%)	Quality [x]
1	0	20	30.3b [y]	1.8NS [z]
2	5	15	39.7a	1.9NS
3	10	10	41.5a	1.3NS
4	15	5	39.0a	1.5NS
5	20	0	24.1b	1.7NS
B. Experiment #11 (30% total nutrient level)				
	Selected nutrient supplements (%)			
Treatment	Wheat bran	Rye	BE (%)	Quality
1	0	30	38.9a	2.0NS
2	7.5	22.5	40.8a	2.1NS
3	15	15	45.6a	2.0NS
4	22.5	7.5	36.5a	1.7NS
5	30	0	22.3b	2.5NS

[x] Quality rating based on scale of 1-4 when 1 is highest quality and 4 is lowest quality.
[y] Means in the same experiment in the same column followed by the same letter are not significantly different at the P=0.05 level according to Tukey-Kramer HSD.
[z] NS = nonsignificant.

Table 4.22. Percentage biological efficiency (%BE) and quality for *Grifola frondosa* (WC828) grown on substrate supplemented with various levels of selected nutrients (wheat bran, millet and rye at 20% total) at the Mushroom Research Center (Experiment #12). For a graphic summary of this data see Figure 4.16.

	Selected nutrient supplements (%)			Crop I		Crop II		Mean (Crop I & II)	
Treatment	Wheat Bran	Millet	Rye	BE (%)	Quality [a]	BE (%)	Quality	BE (%)	Quality
1	0	0	20	29.2 b [b]	1.8 NS [d]	25.3 b	1.9 NS	27.3	1.85
2	0	6.7	13.3	0.0 [c]	-	0.0	-	0.0	-
3	0	13.3	6.7	0.0	-	0.0	-	0.0	-
4	0	20	0	0.0	-	0.0	-	0.0	-
5	6.7	0	13.3	36.1 a	1.6 NS	36.6 a	1.7 NS	36.4	1.65
6	6.7	6.7	6.7	38.5 a	1.4 NS	36.1 a	1.4 NS	0.0	-
7	6.7	13.3	0	0.0	-	0.0	-	37.3	1.4
8	13.3	0	6.7	42.0 a	1.3 NS	38.3 a	1.2 NS	40.1	1.25
9	13.3	6.7	0	32.9 ab	1.8 NS	30.2 ab	1.7 NS	31.5	1.75
10	20	0	0	24.4 b	1.6 NS	20.7 b	1.9 NS	22.6	1.75
	Total Mean			33.9		31.2			

[a] Quality rating based on scale of 1-4 when 1 is highest quality and 4 is lowest quality.
[b] Means in the same experiment in the same column followed by the same letter are not significantly different at the P=0.05 level according to Tukey-Kramer HSD.
[c] Treatments where no fruiting occurred (0.0) were eliminated from the analysis of variance.
[d] NS = nonsignificant.

Table 4.23. Percentage biological efficiency (%BE) and quality for *Grifola frondosa* (WC828) grown on substrate supplemented with various levels of selected nutrients (wheat bran, millet and rye at 30% total) at the Mushroom Research Center (Experiment #13). For a graphic summary of this data see Figure 4.17.

	Selected nutrient supplements (%)			Crop I		Crop II		Mean (Crop I & II)	
Treatment	Wheat Bran	Millet	Rye	BE (%)	Quality [a]	BE (%)	Quality	BE (%)	Quality
1	0	0	30	39.0 b [b]	2.0 ab	30.8 bc	2.0 ab	34.9	2.0 a
2	0	10	20	31.6 b	1.9 ab	28.5 bc	1.6 ab	30.1	1.8 a
3	0	20	10	33.4 b	1.6 a	27.9 bc	1.4 a	30.7	1.5 a
4	0	30	0	0 [c]	-	0	-	0	-
5	10	0	20	44.1 ab	1.7 a	43.8 a	1.6 ab	44.0	1.7 a
6	10	10	10	48.9 a	1.7 a	45.2 a	1.8 ab	47.1	1.8 a
7	10	20	0	34.1 b	2.0 ab	30.9 bc	1.9 ab	32.5	2.0 a
8	20	0	10	39.2 b	1.7 a	34.2 ab	1.8 ab	36.7	1.8 a
9	20	10	0	36.9 b	2.0 ab	36.1 ab	1.8 ab	36.5	1.9 a
10	30	0	0	22.2 c	2.5 b	22.8 c	2.3 b	22.5	2.4 b
	Total mean			36.6		33.4			

[a] Quality rating based on scale of 1-4 when 1 is highest quality and 4 is lowest quality.

[b] Means in the same experiment in the same column followed by the same letter are not significantly different at the P=0.05 level according to Tukey-Kramer HSD.

[c] Treatments where no fruiting occurred (0) were eliminated from the analysis of variance.

Literature Cited

Adachi, K., H. Nanba, M. Otsuka, and H. Kuroda. 1988. Blood pressure-lowering activity present in the fruit body of *Grifola frondosa* (maitake). Chem. Pharmaceut. Bull. (Tokyo) 36:1000-1006.

Alexopoulos, C.J., C.W. Mims and M. Blackwell. 1996. *Introductory Mycology.* 4th ed. John Wiley & Sons, New York.

Altschul, S.F., T.L. Madden, A.A. Schäffer, J. Zhang, Z. Zhang, W. Miller, and D.J. Lipman. 1997. Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucleic Acids Res. 25:3389-3402.

Anonymous. 1983. Production of medicinal *Grifola* polysaccharides. Nippon Beet Sugar MFG Co. Ltd. Japanese Kokai Tokkyo Koho Japanese Patent 58/36395 A2 [83/36395], 3 Mar. 1983, 6pp.

Anonymous. 1985. Production of the antitumor agent GF-1 from *Grifola frondosa tokachiana*. Nippon Beet Sugar MFG Co. Ltd. Japanese Kokai Tokkyo Koho Japanese Patent 60/58925 A2 [85/58925], 5 Mar. 1985, 8pp.

Arakawa, N., K. Enomoto, H. Mukohyama, K. Nakajima, O. Tanabe and C. Inagaki. 1977. Effect of basidiomycetes on plasma cholesterol in rats. Eiyo To Shokuryo 30:29-33.

Arora, D. 1986. *Mushrooms Demystified: A Comprehensive Guide to the Fleshy Fungi*. 2nd ed. Ten Speed Press, Berkeley.

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

Bessette, A.E., A.R. Bessette and D.W. Fischer. 1997. *Mushrooms of Northeastern North America.* 1st ed. Syracuse University Press, Syracuse, N.Y.

Breitenbach, J. and F. Kränzlin. 1991. Fungi of Switzerland: a contribution to the knowledge of the fungal flora of Switzerland. Lucerne : Verlag Mykologia, Switzerland.

Bremer, K. 1994. Branch support and tree stability. Cladistics 10:295-304.

Brown, D., Wensink, P. and Jordan, E. 1972. A comparison of the ribosomal DNAs of *Xenopus laevis* and *Xenopus mulleri*: Evolution of tandem genes. J. Mol. Biol. 63:57-73.

Bruns, T.D., T.J. White and J.W. Taylor. 1991. Fungal molecular systematics. Ann. Rev. Ecol. Syst. 22:525-564.

Chang, S.T. 1999. World production of cultivated edible and medicinal mushroom in 1997 with emphasis on *Lentinus edodes* (Berk.) Sing. in China. Internat. J. Medicinal Mushrooms 1:291-300.

Chang, S.T., J.A. Buswell and S. Chiu. 1993. *Mushroom Biology and Mushroom Products.* Chinese Univ. Press, Hong Kong.

Chen, X., C.P. Romaine, M.D. Ospina-Giraldo and D.J. Royse. 1999. A polymerase chain reaction-based test for the identification of *Trichoderma harzianum* biotypes 2 and 4, responsible for the worldwide green mold epidemic in cultivated *Agaricus bisporus*. Appl. Microbio. Biotech. 52:246-250.

Cooley, R.N., R.F. M. van Gorcom, C.A.M.J.J. van den Hondel and C.E.Caten. 1991. Isolation of a benomyl-resistant allele of the β-tubulin gene from

Septoria nodorum and its use as dominant selectable marker. J. Gen. Microbiol. 137:2085-2091.

Corner, E.J.H. 1989. *Ad Polyporaceas V.* Nova Hedwigia Heft 96.

Dickson, J. 1785. *Fasciculus Plantarum Crytogamicarum Britanniae.* Londini: Nicol.

Donaldson, G.C., L.A. Ball, P.E. Axelrood and N.L. Glass. 1995. Primer sets developed to amplify conserved genes from filamentous ascomycetes are useful in differentiating *Fusarium* species associated with conifers. Appl. Environ. Microbiol. 61:1331-40.

Donk, M.A. 1974. *Check List of European Polypores*. Verhand kon. Nederl. Akad. Wetsch. Afd. Nat. II, 62.

Douglas, D.A. and D.J. Royse. 1986. Shiitake cultivation on sawdust: evaluation of selected genotypes for biological efficiency and mushrrom size. Mycologia 78:929-933.

Edlind, T.D., J. Li, G.S. Visvesvara, M.H. Vodkin, G.L. McLaughlin and S.L. Katiyar. 1996. Phylogenetic analysis of β-tubulin sequences from amitochondrial protozoa. Molec. Phylogenet Evol. 5:359-367.

Farr, D.F., G.F. Bills, G.P. Chamuris and A.Y. Rossman. 1989. *Fungi on Plants and Plant Products in The United States*. APS Press, St. Paul, Minn., USA.

Farris, J. S., M. Källersjö, A. G. Kluge, and C. Bult. 1995. Testing significance of incongruence. Cladistics 10:315-391.

Felsenstein, J. 1985. Confidence limits on phylogenies: an approach using the bootstrap. Evolution 39:783-791.

Fries, E. 1821. *Systema Mycologicum: Sistens Fungorum Ordines, Genera et Species, Huc U.S.que Cognitas, Quas ad Normam Methodi Naturalis Determinavit, Disposuit Atque Descripsit*. v.1-4. Lundae : ex officina Berlingiana.

Geiser, D.M., F.M. Harbinski, and J.W. Taylor. 2000. Molecular and analytical tools for characterizing *Aspergillus* and *Penicillium* species at the intra- and interspecific levels. *In*: Samson, R.A. and J.I. Pitt. (ed.) *Proceedings of the Third Aspergillus and Penicillium Workshop*. Harwood Academic Press, Amsterdam.

Geiser, D.M., J.C. Friscad and J.W. Taylor. 1998a. Evolutionary relationships in *Aspergillus* section *Fumigati* inferred from partial β-tubulin and hydrophobin DNA sequences. Mycologia 90:8321-845.

Geiser, D.M., J.I. Pitt and J.W. Taylor. 1998b. Cryptic speciation and recombination in the aflatoxin-producing fungus *Aspergillus flavus*. Proc. Natl. Acad. Sci. 95:388-393.

Gilbertson, R.L. and L. Ryvarden. 1987. *North American Polypores.* Fungiflora, Oslo, Norway.

Goldman, G. H., W. Temmernan, D. Jacobs, R. Contreras, M. VanMontagu and A. Herrera-Estrella. 1993. A nucleotide substitution in one of the β-tubulin genes of *Trichoderma viride* confers resistance to the antimiotic drug methyl benzimidazole-2-yl-carbamate. Mol. Gen. Genet. 73:73-80.

Gray, S.F. 1821. *A Natural Arrangement of British Plants, According to Their Relations to Each Other As Pointed Out By Jussieu, De Candolle, Brown, &C Including Those Cultivated For Use. With An Introduction to Botany, in Which*

the Terms Newly Introduced Are Explained. Baldwin, Cradock and Joy, London.

Hasegawa, M., H. Kishino and T. Yano. 1985. Dating of the human-ape splitting by a molecular clock of mitochondrial DNA. J. Molec. Evol. 22:160-174.

Hawksworth, D. L., Kirk, P. M., Sutton, B. C. and Pegler, D. N. 1995. *Ainsworth & Bisby's Dictionary of the Fungi.* 8th ed. CAB Internat.

Heim, P. 1954. Observation sur le noyau des Basidiomycetes. Rev. Mycol. (Paris) 19:201-249.

Hibbett, D.S., L.B. Gilbert and M.J. Donoghue. 2000. Evolutionary instability of ectomycorrhizal symbioses in basidiomycetes. Nature 407:506-508.

Hibbett, D.S., E.M. Pine, E. Langer, G. Langer, and M.J. Donoghue. 1997. Evolution of gilled mushrooms and puffballs inferred from ribosomal DNA sequences. Proc. Natl. Acad. Sci. USA. 94:12002-12006.

Hibbett, D.S., Y. Fukumasa-Nakai, A. Tsuneda, and M.J. Donoghue. 1995. Phylogenetic diversity in shiitake inferred from nuclear ribosomal DNA sequences. Mycologia 87:618-638.

Hibbett, D.S. and R. Vilgalys. 1993. Phylogenetic relationships of the basidiomycete genus *Lentinus* inferred from molecular and morphological characters. Syst. Bot. 18:407-433.

Hibbett, D.S. 1992. Towards a phylogenetic classification for shiitake: taxonomic history and molecular perspectives. Rept. Tottori Mycol. Inst. 30:30-42.

Higgins, D. G., A. J. Bleasby and R. Fuchs. 1991. CLUSTAL W: improved software for multiple sequence alignment. CABIOS 8:189-191.

Hills, D.M. and J.J. Bull. 1993. An empirical test of bootstrapping as a method for assessing confidence in phylogenetic analysis. Syst. Biol. 42:182-192.

Hobbs, C. 1996. *Medicinal Mushrooms : An Exploration of Tradition, Healing & Culture.* Botanica Press, Santa Cruz, CA.

Horinouchi, S., Y. Saitou and E. Takahashi. 1999. Gene encoding recombination type trehalose phosphorylase, vector containing the gene, transformant transformed with the gene and production of recombination type trehalose phosphorylase with the transformant. Patent: JP 1998276785-A 20-Oct.-1998; Kureha Chem. Ind. Co. ltd.

Huelsenbeck, J. P., J.J.Bull, and C.W. Cunningham. 1996. Combining data in phylogenetic analysis. Trends Ecol. Evol. 11:152-158.

Imazeki, R and T. Hong. 1989. *Colored Illustrations of Mushroom of Japan. Vol I and II.* Hoikusha, Tokyo.

Jodon, M.H. and D.J. Royse. 1979. Care and handling of cultures of the cultivated mushroom. The Pennsylvania Agricultural Experiment Station Bulletin No. 258. Pennsylvania State University, University Park, PA.

Jong, S.C. and J.M. Birmingham. 1990. The medicinal value of the mushroom *Grifola* Maitake. World J. Microbiol. Biotechnol. 6:227-235.

Jong, S.C., J.M. Birmingham and S.H. Pai. 1991. Immunomodulatory substances of fungal orgin EOS-J. Immunol. Immunopharmacol. 11:115-122.

Jülich, W. 1981. *Higher Taxa of Basidiomycetes.* Bibliotheca Mycologica 85, J. Cramer.

Kabir, Y., M. Yamaguchi and S. Kimura. 1987. Effect of shiitake (*Lentinus edodes*) and maitake (*Grifola frondosa*) mushrooms on blood pressure and plasma lipids of spontaneously hypertensive rats. J. Nutr. Sci. Vitaminol. 33:341-346.

Kawagishi, H. 1990. Isolation and characterization of a lectin from *Grifola frondosa* fruiting bodies. Biochimica et Biophysica acta : Internat. J. Biochem. Biophysi. 1034:247-252.

Kawagishi, H. 1995. Mushroom lectins. Fd. Rev. Internat. 11:63-68.

Kim, B.G. and Magae, Y. 1999. Development of a transformation system in *Pleurotus ostreatus*. (Unpublished). Direct submitted sequence, NCBI AF132911.

Kirchhoff, B. 1996. Investigations of genotypes and substrates for the fruitbody production of *Grifola frondosa* (Dicks.:Fr.). p437-441. *In*: Royse, D.J. (ed.) *Mushroom Biology and Mushroom Products: Proceedings of the 2nd International Conference*. Pennsylvania State University.

Koenraadt, H., S.C. Somerville and A.L. Jones. 1992. Characterization of mutations in the beta-tubulin gene of benomyl-resistant field isolates of *Venturia inaequalis* and other plant pathogenic fungi. Phytopathology 82:1348-1354.

Kunitomo, Y. 1992. Sawdust-based cultivation of maitake. *In* Nishi, S. *et al*. (ed.) *Cultivation and Breeding of Mushroom*. [in Japanese] p229-239. Nougyo tosho Co. Ltd., Tokyo, Japan.

Kubo, K. and H. Nanba. 1997 Anti-hyperliposis effect of maitake fruit body (*Grifola frondosa*). I. Biol. Pharmaceuti. Bull. 20:781-785.

Lee, E. 1994. Production of shiitake, oyster and maitake mushrooms in Connecticut. Mushroom News (3):11-14.

Li, W. 1997. *Molecular Evolution*. Sinauer Associates, Inc., Sunderland, MA.

Li, W. and D. Graur. 1991. *Fundamentals of Molecular Evolution*. Sinauer Associates, Inc., Sunderland, MA.

Lincoff, G. 1981a. *Guide to Mushrooms.* Simon and Schuster, New York.

Lincoff, G. 1981b. *The Audubon Society Field Guide to North American Mushrooms*. Alfred A. Knopf, New York.

May, G. S., M.L.-S. Tsang, H. Smith, S. Fidel and N. R. Morris. 1987. *Aspergillus nidulans* beta-tubulin genes are unusually divergent. Gene 55:231-243.

Matsumoto, T. and I. Ohira. 1982. Some factors affecting the mycelial growth of *Grifola frondosa* in culture. Rep. Tottori Mycol. Inst. 20:140-147.

Matsuo, T, and Y. Yamamoto, H. Muraguchi and T. Kamada. 1999. Effects of amino-acid substitutions in beta tubulin on benomyl sensitivity and micotubule functions in *Coprinus cinereus*. Mycoscience 40:241-249. Direct submitted sequence. NCBI AB000116.

Mayuzumi, Y. and T. Mizuno. 1997. Cultivation methods of maitake (*Grifola frondosa*). Fd. Rev. Internat. 13:357-364.

Miyazaki, T., N. Oikawa, H. Yamada and T. Yadomae. 1978. Studies on fungal polysaccharides-XXII. Structural examination of antitumor, water-soluable glucans from *Grifola umbellata* by use of four types of glucanase. Carbohyd. Res. 65:235-243.

Mizuno, T., H. Saito, T. Nishitoba and H. Kawagishi. 1995. Antitumor-active substances from mushrooms. Fd. Rev. Internat. 11:23-61.

Mizuno, T. and C. Zhuang. 1995. Maitake, *Grifola frondosa*: pharmacological effects. Fd. Rev. Internat. 11:135-149.

Mizuno, T. and B-K. Kim. 1996. *Ganoderma lucidum*. Il-Yang Pharm. Co. Ltd., Japan.

Nanba, H. 1993. Maitake mushrooms- the king of mushrooms. Mushroom News (2):22-25.

Neff, N.F., J.H. Thomas, P. Grisafi and D. Botstein. 1983. Isolation of the β-tubulin gene from yeast and demonstration of its essential function *in vitro*. Cell 3:211-219.

Ni, M. 1987. *Molecular Evolutionary Genetics.* Columbia University Press, New York.

Nobles, M.K. 1965. Identification of cultures of wood-inhabiting hymenomycetes. Canad. J. Bot. 43:1097-1139.

O'Donnell, K., H.C. Kistler, E. Cigelnik and R.C. Ploetz. 1998. Multiple evolutionary origins of the fungus causing Panama disease of banana: Concordant evidence from nuclear and mitochondrial gene genealogies. Proc. Natl. Acad. Sci. 95:2044-2049.

Ohmasa, M. 1994. *Mushroom production in Japan.* Forestry and Forest Products Research Institute, Danchi-Nai, Ibaraki, Japan.

Ohnishi,Y., H. Yamazaki, K. Saito and S. Horinouchi. 1998. Direct Submission. Submitted (05-JAN-1998) to the DDBJ/EMBL/GenBank databases. Accession AB010105.

Oikawa, S., Y. Sato, T. Yadomae, N. Ono, M. Osawa and Y. Susuki. 1987. Antitumor Grifolan-N and its production by *Grifola frondosa tokachiana*. Japanese Kokai Tokkyo Koho Japanese Patent 62/209091 A2 [87/209091].

Orbach, M.J., E.B. Porro and C. Yanofski. 1986. Cloning and characterization of the gene for beta-tubulin from a benomyl-resistant mutant of *Neurospora crassa* and its use as a dominant selectable marker. Mol. Cell. Biol. 6:2452-2461.

Panaccione, D.G. and R.M. Hanau. 1990. Characterization of two divergent beta-tubulin genes from *Colletotrichum graminicola.* Gene 86:163-170.

Rajchenberg, M. and A. Greslebin. 1995. Cultural characters, compatibility tests and taxonomic remarks of selected polypores of the patagonian Andes forests of Argentina. Mycotaxon 56:325-346.

Royse, D.J. 1985. Effect of spawn run time and substrate nutrient on yield and size of the shiitake mushroom. Mycologia 77:756-762.

Royse, D.J. 1992. Recycling of spent shiitake substrate for production of the oyster mushroom, *Pleurotus sajor-caju.* Appl. Microbiol. Biotech. 38:179-182.

Royse, D.J. 1997. Specialty mushrooms and their cultivation. Hort. Rev.19:59-97.

Royse, D.J. and B.D. Bahler. 1988. The effect of alfalfa hay and delayed-release nutrient bon biological efficiency of *Pleurotus sajor-caju.* Mush. J. Tropics.8:59-65.

Royse D.J., B.D. Bahler and C.C. Bahler. 1990. Enhanced yield of shiitake by saccharide amendment of the synthetic substate. Appl. Environ. Microbiol. 56:479-482.

Russo, P., J.T. Juuti and M. Raudaskoski. 1992. Cloning, sequence and expression of a β-tubulin-encoding gene in the homobasidiomycete *Schizophyllum commune*. Gene 119:175-182.

Saccardo, P.A. and J.B. Traverso. 1882. *Sylloge fungorum omnium hucusque cognitorum*. *Vol. XX*. Patavii: Sumptibus auctoris.

Saito, K. 1998. Production of trehalose synthase from a basidiomycete, *Grifola frondosa*, in *Escherichia coli*. Appl. Microbiol. Biotech. 50:193-198.

SAS Institute. 1997. *SAS User's Guide: Statistics.* SAS Institute Statistical Analysis System, Cary, NC.

Singer, R. 1969. Mycoflora Australis. Lehre: Cramer.

Stamets, P. 2000. *Growing Gourmet and Medicinal Mushrooms*, 3rd ed. Ten Speed Press, Berkeley, CA.

Sullivan, K.F. 1988. Structure and utilization of tubulin isotypes. Annu. Rev. Cell Biol. 4:687-716.

Swofford, D. L. 2000. PAUP*. Phylogenetic Analysis Using Parsimony (*and Other Methods), 4.0b4a. Sinauer Associates, Sunderland, MA.

Takama, F., S. Ninomiya, R. Yoda, H. Ishii, and S. Muraki. 1981. Parenchyma cells, chemical components of maitake mushroom (*Grifola frondosa* S.F. gray) cultured artificially, and their changes by storage and boiling. Mushroom Sci. 11:767-779.

Takeyama, T., I. Suzuki, N. Ohno, S. Oikawa, K. Sato, M. Ohsawa and T. Yadomae. 1987. Host-mediated antitumor effect of grifolan NMF-5N, a polysaccharides obtained from *Grifola frondosa*. J. Pharmacobio-Dynamics 10:644-651.

Thon, M.R. 1998. Evolutionary relationships among members of the genus *Lentinula* and other selected Basidiomycetes based on beta-tubulin and ribosomal DNA sequences. PhD Thesis, The Pennsylvania State University.

Thon, M.R. and D.J Royse. 1999a. Partial beta-tubulin gene sequences for evolutionary studies in the Basidiomycotina. Mycologia 91:468-474.

Thon, M.R. and D.J. Royse. 1999b. Evidence for two independent lineages of shiitake of the Americas (*Lentinula boryana*) based on rDNA and β-tubulin genes sequences. Mol. Phylogen. Evol. 13:520-524.

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

Vilgalys, R. and B.L. Sun. 1994. Ancient and recent patterns of geographic speciation in the oyster mushroom *Pleurotus* revealed by phylogenetic analysis of ribosomal DNA sequences. Proc. Natl. Acad. Sci. USA. 91:4599-4603.

Webster, J. 1980. *Introduction to Fungi.* Cambridge University Press, Cambridge.

White, T., T. Bruns, S. Lee and J. Taylor. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. p315-322. *In* Innis *et al.*, (ed.) *PCR Protocols: A Guide to Methods and Applications.* Academic Press, New York.

Wu, P. 1955. *Shen Nong's Herbal.* (200BC - 200 AD). Xing-Yan Sun and Feng-Yi Sun. ed. (Qing Dynasty). Shang Wu Press, Shang Hai.

Yagishita, K., H. Jinnouchi, H. Yamamoto and T. Miyakawa. 1978. Effect of *Grifola frondosa* (maitake), *Coriolus versicolor* (Kawaratake), and *Lentinus edodes* (shiitake) on cholesterol metabolism in rats-II. Nibon Daigaku Nojuigakubu Gakujutsu Kenkyu Hokoko 35:28-40.

Yagishita, K., T. Miyakawa, H. Jinnouchi and H. Yamamoto. 1977. Effect of *Grifola frondosa*, *Coriolus versicolor*, and *Lentinus edodes* on cholesterol metabolism in rats-I. Nibon Daigaku Nojuigakubu Gakujutsu Kenkyu Hokoko 34:1-13.

Yamanaka, K. 1997. Production of cultivated edible mushrooms. Fd. Rev. Internat. 13:327-333.

Yarden, O. and T. Katan. 1993. Mutations leading to substitutions at amino acids 198 and 200 of beta-tubulin that correlate with benomyl-resistance phenotypes of field isolates of *Botrytis cinera.* Phytopathology 83:1478-1483.

Yao,Y., D.N. Pegler, and M.W. Chase. 1998. Application of ITS (nrDNA) sequences in the phylogenetic study of *Tyromyces* s. l. (*Coriolaceae*). Unpublished.

Ying, J., X. Mao, Q. Ma, Y. Zong and H. Wen. 1987. *Icones of Amedicinal Fungi from China.* Translated by Y. Xu. Science press, Beijing.

Yoshizawa, N., T. Itoh, J. Takemura, S. Yokota and T. Idei. 1997. Mushroom cultivation using maitake [*Grifola frondosa* (Fr.) S.F.Gray] cultural wastes. Bull. Utsunomiya Univ. Forests 33:109-116.

Wang, H., T-B Ng and V.E.C Ooi. 1998. Lectins from mushrooms. Mycol. Res. 102:897-906.

White, T., T. Bruns, S. Lee and J. Taylor 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics**.** Pp 315-322. In: PCR Protocols. M. Innis, D. Gelfand, J. Sninsky and T. White (eds.) San Diego, Academic Press.

Zhao, Ji-Ding and X. Zhang. 1992. *The Polypores of China.* Bibliotheca Mycologica. J. Cramer, Berlin.

Zhao, Z.G., X.L. Yang, and Z.M. Li. 1983. The cultivation of *Polyporus frondosa*. Edible Fungi 5:8-9.

Vita

Qing Shen

EDUCATION

June 1998-Present. Ph.D. Candidate. Department of Plant Pathology, The Pennsylvania State University, University Park, PA.

August 1991-July 1994. M.S. Microbiology. Department of Microbiology, Nankai University, Tianjin, P.R. China.

August 1987-July 1991. B.S. Microbioloy. Department of Biology, Nankai University, Tianjin, P.R. China.

AWARDS AND MEMBERSHIP

James P. Roberts Scholarship, 2000. The Pennsylvania State University.

Wang Ke-Chang and Guang Hua Scholarships, 1990-1993. Nankai University

Mycological Society of America. 1998-present.

World Society for Mushroom Biology and Mushroom Products, 2000-present.

EXPERIENCE

Research Assistant. May 1998-May 2001. Department of Plant Pathology, The Pennsylvania State University, PA

Teaching Assistant. August 2000-December 2000. Department of Plant Pathology, The Pennsylvania State University, PA

Research Assistant. July 1994-May 1998. Tianjin Research Center for Agricultural Biotechnology, Tianjin, China.

Teaching Assistant. August 1992-January 1993. Department of Microbiology, Nankai University, Tianjin, China.

PUBLICATIONS

Shen, Q. and D.J. Royse. 2000. Effects of germplasm on mushroom growth, biological efficiency, and yield of maitake. The 2000 Mycological Society of America Annual Meeting. Burlington, VT. (Abstr.)

Shen, Q. and D.J. Royse. 2000. Molecular phylogenetic analysis of *Grifola* based on internal transcribed spacer ribosomal DNA sequences. The 2000 Mycological Society of America Annual Meeting. Burlington, VT. (Abstr.)

Chen, X.W., Q. Shen, and H. Fan. 1998. Study on selection of spore high-yielding strain of *Ganoderma lucidum* and its spore-releasing pattern. Acta Scientiarum Naturalium Universitatis Nankaiensis 31:109-112.

PRESENTATIONS

Shen, Q. 2000. Maitake germplasm and the effects of selected nutrient supplements on mushroom yield. 10th Specialty Mushroom Workshop, University Park, PA.

Shen, Q. 2000. Cultivation and molecular systematics of *Lentinula edodes*. The Pennsylvania State University, University Park, PA.

Shen, Q. 1999. *Ganoderma* spp. - pharmacology, phytopathology and systematics. The Pennsylvania State University, University Park, PA.