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STUDIES IN AMATOXIN-PRODUCING GENERA OF FUNGI: PHYLOGENETICS AND TOXIN DISTRIBUTION

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ABSTRACT

STUDIES IN AMATOXIN-PRODUCING GENERA OF FUNGI:
PHYLOGENETICS AND TOXICOLOGY

By

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The distribution of cyclic peptide amatoxins and the related phallotoxins were examined using HPLC and FAB mass spectrometry in three of the four genera that produce amatoxins. Amatoxins are responsible for 90% of fatal mushroom poisonings in humans. In a study of South African *Amanita* species, both types of toxins were found in members of *Amanita* section *Phalloideae*, and were not found in any other section of *Amanita*. Amatoxins and phallotoxins were detected in *Amanita reidii* for the first time. Phallotoxins were detected in the lawn mushroom *Conocybe lactea* although amatoxins were absent. This is the first report of phallotoxins outside the genus *Amanita*.

The phylogenetic relationships within *Amanita* and *Conocybe* were examined using PCR-RFLPs and DNA sequencing of the 5.8S, 28S and ITS regions of the nuclear rDNA, and the large subunit of the mitochondrial rDNA. In *Amanita,* amatoxin producers (*Amanita* section *Phalloideae*) formed a monophyletic clade in all analyses. Earlier reports of monophyly in *Amanita* sections *Amanita, Caesareae* and *Vaginata* based on 28S rDNA sequence data were supported by the ITS and mitochondrial large rDNA sequence data. Analysis of an extended 28S rDNA sequence dataset placed three *Amanita* species basal to the outgroup genus *Limacella*.

RFLPs and ITS sequence data from specimens of *Amanita* infected by the ascomycete mycoparasite *Hypomyces* hyalinus were compared to reference data from healthy *Amanita* species to identify the parasitized specimens. Hosts were identified as *Amanita rubescens* sensu lato, *A. flavoconia* and *A. brunnescens* in *Amanita* section *Validae*. Two parasitized specimens yielded DNA sequence matching that of mycorrhizal fungi outside of *Amanita*. Reports of parasitism of *A. bisporigera* and *A. muscaria,* both toxic, based on proximity to non-parasitized basidiocarps were not confirmed in this study.

Systematic studies indicated that *Conocybe lactea* is not closely related to the amatoxin producer *C. filaris*, suggesting that toxin production has arisen independently in the two taxa. North American specimens of *C. lactea* were found to be indistinguishable on the basis of DNA sequence from North American specimens of *C. crispa*. The European *C.* crispa was revealed to be a different species than North American *C. crispa* and *C. lactea*. *Gastrocybe lateritia* was placed in the genus *Conocybe*, closely related to North American *C. lactea*.

To identify the gene for amatoxin synthesis, *Galerina marginata* was examined using PCR with degenerate primers designed to detect cyclic peptide synthetase (CPS) gene fragments. Attempts to amplify and sequence CPS genes were unsuccessful, due possibly to the use of ascomycete sequences to develop primers for use in a basidiomycete. Attempts to isolate amatoxin synthetase using ATP/pyrophosphate exchange assays are underway.

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INTRODUCTION

Mushroom poisoning - an overview

Mushroom poisoning, or mycetism, has occurred throughout human history. I follow Benjamin (1995) in defining mushroom poisoning as a state of intoxication that proceeds as a natural consequence of eating a fleshy macrofungus that produces a compound inherently toxic to a majority of humans upon consumption. This definition excludes idiosyncratic reactions, food allergies, poisoning by mycotoxins, pesticide or heavy metal poisoning resulting from contaminated mushrooms, and food poisoning resulting from spoilage.

Mushroom poisoning cases reported to American Poison Control centers between 1989 and 2000 averaged 9,467 cases per year. Poison Control Center data are inflated due to the large number of reports of children eating unknown mushrooms. These are treated as poisoning cases until the identity of the mushroom can be established, even in the absence of symptoms. Fifty-three percent of the cases reported for the years 1991 - 2000 (for which a breakdown by toxin type is given) involved either known nontoxic or unidentified mushrooms producing no symptoms (data from the American Association of Poison Control Centers' Toxic Exposure Surveillance System, <http://www.aapcc.org/annual.htm>). Of 9,208 mushroom poisoning cases reported to Poison Control Centers in 1989, the fungus in 8,355, or 90.5% of the

total, was classified as "unknown if toxic" (Trestrail 1991). In 6,046 of these cases the patient experienced no effect.

An additional assessment of the mushroom poisoning situation in the United States can be obtained from the North American Mycological Association's (NAMA's) Mushroom Poisoning Case Registry. The Case Registry has the advantage that only cases resulting in adverse symptoms are reported; however, reporting is voluntary, and many people who encounter, diagnose and treat mushroom poisoning may not be aware of the Case Registry. One thousand eight hundred and eighty one cases of mushroom poisoning had been reported to the NAMA Case Registry between its initiation in 1984 and 2000, with a minimum of 44 cases reported in 1989, and a maximum of 174 in 1991 (Trestrail 1998; Cochran 1999; Cochran 2000; Cochran 2001). The majority of cases (approximately 73%) were in adults.

Approximately 4% of known mushroom species are poisonous, 1.8% are popular edibles, and another 18% are "probably edible" (neither sought after nor likely to do harm) (Benjamin 1995). The remaining 75% of mushroom species are considered inedible; not dangerous but too woody, small, slimy, powdery, hairy, or otherwise undesirable. Of the poisonous mushroom species, the majority cause gastrointestinal distress within less than one hour of ingestion of the mushroom. Symptoms range from nausea to vomiting and/or diarrhea in varying degrees of severity. Hospitalization may be required to combat dehydration and pain in severe cases, but the poisoning is self-limiting and symptoms rapidly resolve once the offending mushroom has left the body. Several mushrooms

elicit more severe symptoms, and some can be deadly. Fifteen potentially deadly species occur in Michigan, out of an estimated 2,500 species overall (Hallen & Adams 2002).

Delayed-action poisoning syndromes

Most serious are the delayed-action poisoning syndromes caused by amatoxins, *Cortinarius* toxins and monomethylhydrazine (MMH). MMH poisoning occurs each spring when people gather and eat the toxic false morels, ascomycete fungi in the genus *Gyromitra*. These fungi contain gyromitrin, a hydrazine that is rapidly converted in the human body to monomethylhydrazine (MMH), a principle component of rocket fuel. Hydrazines interfere with enzyme systems that require a pyridoxine cofactor, leading to decreased GABA concentrations among other potential problems (Trestrail 1994; Michelot & Toth 1991). The metabolism of gyromitrin to form a series of different hydrazines, many of which are unstable and highly reactive, also contributes to toxicity. A hit-and-miss component of false morel toxicity is attributable to a wide range of variables. The amount of toxin present varies considerably between individual mushrooms, with samples from the western United States being considered less toxic than those from the eastern US or Europe (Benjamin 1995). The susceptibility of individual humans to the toxins varies as much as the quantity of the toxins themselves. The toxin is heat-labile, and can be largely removed by cooking, though care must be taken not to inhale any cooking vapors. The LD_{50}

of gyromitrin is 20 - 50 mg/kg body weight in adults, and 10 - 30 mg/kg body weight in children, comparable to 1 - 5 cups of fresh mushrooms (Benjamin 1995).

Cortinarius toxins are present in certain mushrooms in the genus *Cortinarius*, subgenus *Leprocybe*. There have been two competing theories about the toxic principle, one holding that the chemical is a bipyridal with the trivial name orellanine, and the other favoring the cyclic peptide cortinarin (also spelled "cortinarine") (Benjamin 1995). The preponderance of research supports orellanine as the toxic principle (Bresinsky & Besl 1990; Danel, Saviuc & Garon 2001). *Cortinarius* poisoning is particularly insidious in that a minimum of two days passes between ingestion of the mushroom and presentation of symptoms, and presentation can be delayed by up to three weeks (Danel, Saviuc & Garon 2001). Orellanine has a specific affinity for the kidneys. Animal experiments and biopsies of poisoned human kidneys have consistently shown damage to the epithelium of both the proximal and distal tubules. The glomeruli are not involved (Benjamin 1995). It has been suggested that orellanine inhibits protein synthesis in poisoned cells (Benjamin 1995). The human LD_{50} is unknown; oral LD_{50} in mice is 90 mg/kg body weight (12.5 mg/kg administered by intraperitoneal injection) (Danel, Saviuc & Garon 2001). The toxicity appears to be lower than that of the amatoxins; 3-10 mushroom caps "may be sufficient to produce irreversible kidney damage in an adult" (Benjamin 1995, p. 251), compared with one cap of the comparably-sized *Amanita phalloides*. No verified cases of *Cortinarius* poisoning are on record for North America, despite the occurrence of

orellanine-producing mushrooms (Keller-Dilitz et al. 1985). More than 200 cases have been reported in Europe since Grzymala's first report of *Cortinarius* poisoning, in 1965 (Danel and colleagues examined 245 cases in their 2001 review).

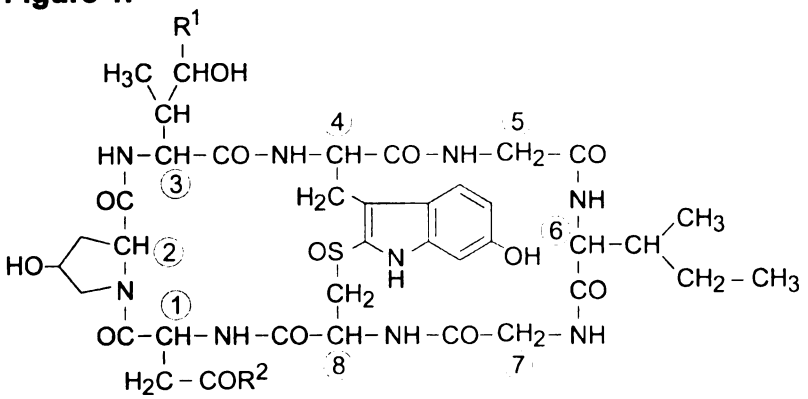
Amatoxins

The most common fungal toxins involved in serious to fatal human poisoning cases are the amatoxins. Four hundred and fifty-two cases of amatoxin poisoning were reported to American Poison Control Centers between 1991 and 2000. Three hundred and twenty of these cases were treated in a health care facility. In 134 cases, the poisoning was classified as moderate or major, and eight deaths resulted (<http://www.aapcc.org/annual.htm>). The relatively low death rate may be attributable to prompt treatment; emesis or lavage administered within two hours post ingestion can remove the majority of the toxin (Benjamin 1995). Amatoxins are bicyclic octapeptides (Fig. 1). Poisoning is characterized by a (6)12 - 18(36) hour delay before symptoms present. The initial symptoms are moderate to severe gastrointestinal distress accompanied by vomiting and diarrhea. This phase frequently requires hospitalization due to the severity of the symptoms. Within 12 - 36 hours symptoms subside (but see Wieland 1969 for a mention of death at the gastritis stage). There follows a remission period of 12 - 36 hours during which the patient feels better and in some cases may be discharged from the hospital. The third and final stage is

characterized by liver failure and, rarely, additional organ damage. The mortality rate for amatoxin poisoning in humans is 10 - 30 %. Mortality rates from the 1970s and earlier of 50 - 90 % have been reduced by a more widespread recognition of the poisoning syndrome, aggressive treatment and the advent of liver transplantation.

Liver failure is the common culmination of amatoxin poisoning and, indeed, liver cells actively import amatoxins (Kröncke et al. 1986). However, all eukaryotic cells are susceptible to amatoxins and the perceived sensitivity to the liver is due to its function as a detoxifying organ and the consequent high exposure to amatoxins (Benjamin 1995). The gastrointestinal tract encounters amatoxins before the liver does, and it is noteworthy that the first symptoms of poisoning are abdominal pain, vomiting and diarrhea. Severe poisoning cases exhibit kidney damage, and the heart may be affected in rare cases (Benjamin 1995).

Figure 1.



Structural formula for amatoxins. R^1 = CH_2OH in α- and β-amanitin and CH_3 in γ-amanitin. R^2 = NH_2 in α- and γ-amanitin and OH in β-amanitin. By convention, amino acids are numbered in a clockwise fashion, starting with asparagine.

There is no antidote for amatoxin poisoning. The only mushroom toxin to possess an antidote is muscarine, which occurs in *Inocybe* and *Clitocybe*, and can be treated with atropine (Benjamin 1995). Due to some unfortunate early history - muscarine is named for *Amanita muscaria*, in which it occurs in insignificant quantities - atropine has been used extensively in treating mushroom poisoning in general, and particularly *Amanita* poisoning. This practice is dangerous and has been discredited for all but muscarine poisoning.

Despite over a century of research, amatoxin poisoning, and most other types of mushroom poisoning, must be treated symptomatically. Attempts to raise antibodies to amatoxins and thus produce an antiserum were thwarted by the fact that the conjugation of amatoxin to an antibody leads to a ten- to fifty-times increase in toxicity (Cessi & Fiume 1969; Faulstich, Kirchner & Derenzini 1988). Silibinin and penicillin G both block amatoxin uptake by hepatic cells in an experimental system, and show therapeutic promise (Jahn, Faulstich & Wieland 1980). Silibinin and the related silymarin, derivatives of the milk thistle *Silybum marianum*, appear to be both safer and more effective than penicillin G (Faulstich & Zilker 1994; Benjamin 1995), but the effective intravenous form has not been approved for use in the United States. The primary form of treatment involves removing the toxins from the patient. Emesis or lavage are not effective by the time symptoms present, six hours or more post ingestion. Hemoperfusion and hemodialysis may be used, but are rarely effective by the time symptoms present. The most effective means of removing toxins at this stage is the

administration of activated charcoal, which binds to the toxins, interrupting their enterohepatic circulation (Faulstich & Zilker 1994).

Amatoxins were first characterized in the genus *Amanita*, from which they get their name (Wieland & Hallermayer, 1941). *Amanita* species have been known for their toxicity for at least 2000 years. In arguably the most celebrated case of mushroom poisoning, extracts from the death cap, *Amanita phalloides,* were used to poison the Roman Emperor Claudius (see Benjamin 1995, pp. 33-34). *Amanita* species have been studied intensively for the production of toxic compounds since the 1860s (Wieland 1969). In addition to amatoxins, *Amanita* species produce muscimol (Benjamin 1995), muscarine in trace quantities (Wieland 1969; 1986), bufotenine (Seeger & Stijve 1980) and phallotoxins (Lynen & Wieland 1938; Wieland 1987).

Amatoxins are poisonous in very low doses. The human LD_{50} is estimated at 0.1 mg toxin / kg body weight, or approximately 7 mg for an adult male. This is similar to the LD_{50} values for dogs and guinea pigs (Wieland 1986). One average sized fruiting body of *Amanita phalloides* can be estimated to contain 10 - 12 mg of amatoxins (Wieland 1986), more than a lethal dose. Amatoxins are poisonous in varying degrees to all eukaryotic organisms. The mode of action is the specific inhibition of RNA polymerase II (pol II; RNA polymerase B) (Lindell et al. 1970). At high levels of amatoxins, RNA polymerase III may be inhibited (Horgen, Vaisius & Ammirati 1978); however, the levels at which this occurs are many times the mammalian lethal dose.

Cochet-Meilhac and Chambon (1974) found a K_D of 6.4×10^{-9} M for an α-amanitin-pol II complex at physiological temperatures, similar to the inhibition constant, K_i, of 1.0×10^{-8} M. Pol II is the primary transcription enzyme and is responsible for messenger RNA (mRNA) synthesis. Amatoxins do not prevent DNA or dNTP binding, or release of the nascent RNA chain. Rather, the toxins cause a dramatic slowing in the translocation of the polymerase along the DNA template (Wieland 1986; Chafin, Guo & Price 1995). This mode of action accounts for the delay observed in all cases of amatoxin poisoning. Transcription comes to a halt in poisoned cells, followed by the cessation of translation as the pool of existing mRNA is used up. The ultimate result is cell death, caused when the supply of essential proteins has been exhausted.

Sensitivity to amatoxins is correlated with taxonomic position. All eukaryotes possess some sensitivity to amatoxins. Mammals possess the highest observed sensitivities, although not all mammals are sensitive to ingested amatoxins. Ingested amatoxins have no effect on mice, although α-amanitin has an LD_{50} of 0.4 - 0.8 mg/kg body weight when the toxin is injected (Wieland 1986). The same is true for many rodents, although not the guinea pig, which appears to be as sensitive as humans to oral amatoxins. Reptiles are less sensitive than mammals, and insects are less sensitive than reptiles. Plants are less sensitive than insects, and fungi are less sensitive than plants. At the bottom of the list are the amatoxin-producing fungi. Nuclei from *Amanita phalloides* experienced no inhibition of pol II when exposed to amatoxins at a concentration of 25 μg/ml, and showed 31% inhibition at 75 μg/ml. By comparison, pol II from

Agaricus bisporus showed 8% inhibition at 25 μg/ml amatoxin, and rabbit brain pol II showed 63% inhibition at 0.25 μg/ml (Horgen, Vaisius & Ammirati 1978). Prokaryotic RNA polymerases are wholly insensitive to amatoxins.

Amatoxins exist as a family of related chemicals, differing in the degree of hydroxylation of the individual amino acids. α-, β- and γ-amanitins are the prevalent amatoxins in mushrooms, and are the primary chemicals responsible for poisonings. ε-amanitin and amaninamide are other pol II-inhibiting members of the amatoxin family, while amanullin, pro-amanullin and amanin are nontoxic.

The ability of amatoxins to bind to pol II is based on structure. The OH-group in 4-*trans*-hydroxy-L-proline appears to be necessary, as its removal causes a great decline in toxicity (Wieland 1986). Some hydroxylation of the position 3 isoleucine is required for toxicity (Wieland 1980). α- and β-amanitin have dihydroxyisoleucine, while γ- and ε-amanitin possess hydroxyisoleucine, at position 3. Amanullin, a naturally occurring amatoxin lacking any hydroxylation of this isoleucine, is nontoxic, as are synthetic analogues. If the ring formed by the cysteine-to-tryptophan bridge is disrupted, toxicity is lost (Wieland 1986).

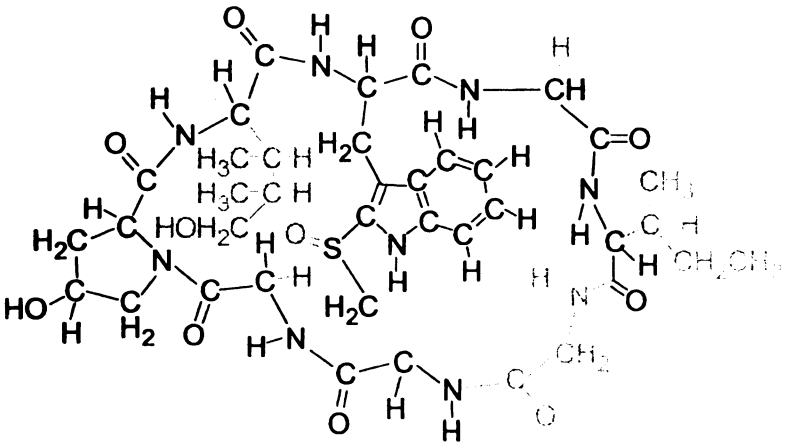
The role of amatoxins as transcription inhibitors has led to their use as biochemical tools. Amatoxins have been used in elucidating biosynthetic pathways in which the involvement of mRNA transcription is suspected (Wieland 1986). They have been used in virus research to determine whether eukaryotic or viral RNA polymerases are being utilized; the viral polymerases are insensitive to amatoxins (Wieland 1986). The effects of a transcription inhibitor on rapidly

growing and dividing cells has led to the use of amatoxins in cancer research (Wieland & Faulstich 1991).

Phallotoxins

Structurally similar to amatoxins are phallotoxins, bicyclic heptapeptides (Figure 2). Phallotoxins were first isolated from *Amanita phalloides*, from which they receive their name. Like amatoxins, phallotoxins form a family of related chemicals. The first-described phallotoxin, phalloidin, was isolated and purified three years prior to the first amatoxin, α-amanitin (Lynen & Wieland 1938; Wieland 1986). For many years, phallotoxins were thought to be responsible for the initial, gastrointestinal phase of *Amanita* poisoning, with amatoxins contributing the terminal liver pathology. This belief has appeared in a review article as recently as 1993 (Köppel 1993). Now we know that phallotoxins are not absorbed by the digestive tract (Wieland & Faulstich 1978) and that gastrointestinal cells are insensitive to these toxins. There is no evidence for phallotoxin involvement in *Amanita* poisoning, and all symptoms can be explained by amatoxins alone (Benjamin 1995). Phallotoxins given parenterally to experimental animals cause liver necrosis and death. The toxins are readily taken into the liver, where they bind to and stabilize actin in the filamentous F-actin form (Wieland 1977; Wieland 1987).

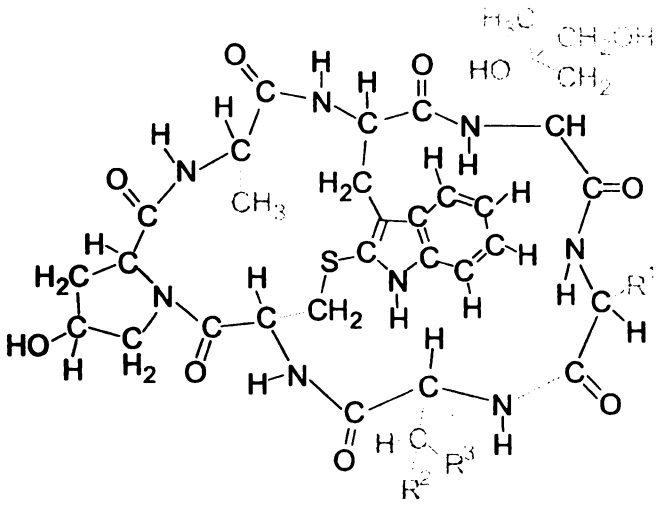
Figure 2.



Amatoxins

α-Amanitin R= $CH_2C(=O)NH_2$

β-Amanitin R= $CH_2C(=O)OH$



Phallotoxins

Phalloidin $R^1 = CH_3$, $R^2 = CH_3$, $R^3 = OH$

Phallacidin $R^1 = CH(CH_3)_2$, $R^2 = OH$, $R^3 = CO_2H$

Structural comparison of principle amatoxins and phallotoxins. Atoms in black are identical in both toxin families, while those in gray differ.

Detection of amatoxins and phallotoxins

Several methods for detecting amatoxins and phallotoxins have been developed over the past fifty years. The so-called Meixner test is a crude colorimetric method. A raw extract of a mushroom is applied to a lignin-rich paper, such as newsprint. Once the fungal extract has dried, a drop of concentrated hydrochloric acid is applied. A blue color developing within several minutes indicates the presence of 5-substituted tryptamines, as are present in amatoxins (Meixner 1979; Beutler & Der Marderosian 1981). However, Meixner false positives are common because the minor hallucinogen bufotenine, present in *Amanita citrina*, and other, unidentified fungal compounds also contain 5-substituted tryptamines (Beutler 1980; Beutler & Vergeer 1980). Despite its limitations, the Meixner test is the quickest and easiest available test for amatoxins, and it is therefore of considerable value in a clinical situation. Other methods that have been used include the cinnamaldehyde method of paper chromatography that detects amatoxins (Block et al. 1955); thin-layer chromatography that measures amatoxins and phallotoxins (Palyza & Kulhánek 1970); and high performance thin-layer chromatography measuring amatoxins (Stijve & Seeger 1979). Additionally, Enjalbert et al. (1992) have developed a high performance liquid chromatography (HPLC) method that can distinguish between and quantify four amatoxins and four phallotoxins. The aforementioned methods have been developed for the detection of toxins in mushrooms. A

further area of interest is the detection of toxins in biological fluids from patients. Dorizzi et al. (1992) present a thorough review.

While chromatographic methods predominate, radioimmunoassay (RIA) has been frequently used to detect toxins both in mushrooms (Faulstich & Cochet-Meilhac 1976) and in serum and bodily fluids, in the case of poisoning. The inhibition of RNA polymerase II, particularly in calf thymus, can serve as an amatoxin assay (Preston et al. 1982). Fast atom bombardment mass spectroscopy (FAB-mass spec) may be used in conjunction with chromatography or other methods to provide a positive identification.

Taxonomy of amatoxin-producing fungi

Amatoxins are produced by certain species in four unrelated genera of basidiomycete fungi: *Amanita* (family *Amanitaceae*, order *Agaricales*); *Lepiota* (family *Agaricaceae*, order *Agaricales*); *Conocybe* (family *Bolbitiaceae*, order *Agaricales*) and *Galerina* (family *Cortinariaceae*, order *Cortinariales*). Toxin production is limited to a discrete, closely related group of species within each genus: section *Phalloidae* in *Amanita,* section *Ovisporae* in *Lepiota*, section *Naucoriopsis* in *Galerina*, and the single species *Conocybe filaris* in *Conocybe*. Reports of amatoxins occurring in a wide range of mushrooms, including edible species (Faulstich & Cochet-Meilhac 1976; Preston et al. 1982) have been made based on detection of toxins near the detection limit of the procedures being employed (Wieland 1986). These have not been substantiated when the same

species were tested using more sensitive HPLC methods (Enjalbert et al. 1993; Hallen, unpublished results).

Within a species amatoxin production is disjunct, with individual mushrooms varying with regard to toxin production. Tyler and colleagues (1966) found individuals of the destroying angels *Amanita virosa* and *A. verna* that lacked amatoxin. Beutler (1980) identified one *A. phalloides* specimen, out of 205, that lacked detectable amatoxins and phallotoxins when evaluated with the Meixner test and thin-layer chromatography. The specimen was fresh, which suggests that the toxin was constitutively absent. Yocum and Simons (1976) detected no amatoxins or phallotoxins in three out of four specimens of *A. verna*. Beutler and Der Marderosian (1981) used thin-layer chromatography to split *A. virosa* into two chemotaxonomic types: Type A, which possesses both amatoxins and phallotoxins, and type B, which possesses phallotoxins but no detectable amatoxins.

The "destroying angels", white *Amanita* species in section *Phalloidae* containing amatoxins, are in a state of taxonomic flux. The number of species is not agreed upon. Species have been delimited on the bases of spore size, spore length-to-breadth ratio and number of spores per basidium, all of which are plastic characters. The accurate identification of white *Amanita*s in section *Phalloidae* is thus quite difficult (Jenkins 1986). It is uncertain whether *Amanita verna* is a distinct taxon, or whether it is simply a white form of *A. phalloides*. *Amanita virosa* sensu auct. amer. may be the same as the European taxon by that name, or may be a four-spored variant of *A. bisporigera*. *Amanita bisporigera*

has the highest toxin levels of North American *Amanita* species (Tyler et al. 1966), with little variation in toxin content between different fruit bodies, whereas approximately half of *A. virosa* specimens test negative for amatoxins. The identities of these mushrooms are therefore of more than academic concern.

Two major phylogenetic studies of *Amanita* have been conducted in the past five years. Weiss, Yang and Oberwinkler (1998) and Drehmel, Moncalvo and Vilgalys (1999) conducted independent studies of *Amanita* using a portion of the 28S nuclear ribosomal DNA (rDNA) operon. In both studies, the toxin-producing members of section *Phalloidae* form a monophyletic clade, but only a few (6) toxin-producing species are examined. The placement and circumscription of other sections in *Amanita*, and the monophyly of *Amanita* subgenus *Amanita*, differ between the two studies, due, in part, to the inclusion of different taxa. Further work by Moncalvo and colleagues (2000) demonstrates that, in the 28S region, *Amanita* shows much greater divergence than any other known basidiomycete genus, leading to ambiguities in alignment of DNA sequence.

Overview of the following chapters

The remainder of this thesis details several studies on amatoxin-producing fungi and related species. Chapters one, two and three cover research on *Amanita*. In chapter one, I use molecular phylogenetics to determine whether amatoxin production has arisen once or multiple times in *Amanita*. The

relationships within the morphologically similar "destroying angel" complex are examined and North American and European specimens called A. virosa are compared. The ITS1-5.8S-ITS2 rDNA (ITS region), a portion of the mitochondrial large rDNA, and a portion of the 28S rDNA were sequenced for phylogenetic analysis. Amatoxin-producing *Amanita* species formed a well-supported, derived, monophyletic clade in all analyses. This result supports the hypothesis of a single origin of amatoxin synthesis within the genus. In chapter two, restriction fragment length polymorphisms (RFLPs) and sequence of PCR products of the ITS region are used to identify *Amanita* species aborted by the ascomycete parasite *Hypomyces hyalinus*. Chapter three presents an HPLC study on amatoxin and phallotoxin production in indigenous and introduced South African *Amanita* species, and contains the first report of amatoxins in the introduced species *A. reidii*.

Chapter four covers taxonomic and toxicological studies of *Conocybe*, primarily section *Candidae*. It includes the discovery of phallotoxins in *Conocybe lactea*, the first report of these toxins outside of the genus *Amanita*. Chapter 5 covers the potential of *Galerina marginata*, which produces amatoxins in culture, to serve as a model organism for amatoxin synthesis, and non-ribosomal peptide synthesis in basidiomycetes. Future directions and a brief summary conclude the chapter and the body of the thesis.

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CHAPTER 1

MOLECULAR PHYLOGENETICS OF *AMANITA*, WITH A FOCUS ON SECTION *PHALLOIDEAE*

Abstract

Portions of the nuclear large, internal transcribed spacers, and mitochondrial large rDNA were sequenced and used in parsimony and likelihood analyses of the genus *Amanita*. The validity of the circumscription of the sections of *Amanita* was examined. The mitochondrial large rDNA provided little resolution, but distinguished four sections of *Amanita* and will be of utility in identifying mycorrhizae. Sections *Amanita* and *Caesareae* were upheld as monophyletic in all analyses. Amatoxin-producing species in section *Phalloideae* also formed a monophyletic cluster in all analyses, suggesting a single gain of the capacity to produce these toxins. A parsimony analysis of nuclear large rDNA from 112 specimens showed the basal members of *Amanita* forming a polyphyletic group with the genus *Limacella*.

Introduction

Amanita Pers. is a common mycorrhizal inhabitant of temperate forests worldwide. The genus consists of at least 200 species (Hawksworth et al. 1995)

and is readily recognizable. *Amanita* species are medium to large mushrooms possessing white-to-light-colored free gills with white-to-light-colored spores and a universal veil. The universal veil envelops the entire immature fruit body, rupturing as the fruit body expands. Portions of the universal veil may remain on the pileus as patches or warts, and a volva at the base of the stipe formed of universal veil tissue is common. Most species possess a partial veil in addition to the universal veil. The partial veil covers and protects the developing gills, and remains after pileus expansion as an annulus surrounding the stipe. The only other agarics to form both universal and partial veils are in the genus *Limacella* Earle which belongs with *Amanita* in the family *Amanitaceae* R. Heim ex Pouzar (Hawksworth et al. 1995). *Limacella* has been separated from *Amanita* because it possesses a universal veil that is gelatinous rather than dry. *Amanita* section *Vaginatae* (Fr.) Quél possesses a universal veil only, in the form of a membranous, saccate volva at the base of the stipe. Section *Vaginatae* was at one time considered a separate genus, *Amanitopsis* (Roze) Konr. & Maubl., but microscopic characters permitted placement in *Amanita* (Bas 1969). This placement has been supported by molecular phylogenetics (Weiß, Yang & Oberwinkler 1998; Drehmel, Moncalvo & Vilgalys 1999; Oda, Tanaka & Tsuda 1999).

Delimitation of the species and circumscription of subgeneric taxa are matters of debate (Bas 1969; Jenkins 1986; Singer 1986; Drehmel, Moncalvo & Vilgalys 1999). When Bas (1969) wrote his monograph, more than 50 sections of *Amanita* had been proposed based on morphology. Corner & Bas (1962; see

also Bas 1969) circumscribe the genus into two subgenera and six sections. Subgenus *Lepidella* (Gilbert) Vesely possesses spores that stain blue-black in iodine (amyloid), while subgenus *Amanita* has non-staining (inamyloid) spores. Subgenus *Lepidella* contains sections *Amidella* (Gilbert) Konr. & Maubl., *Lepidella, Phalloideae* (Fr.) Quél. and *Validae* (Fr.) Quél. Subgenus *Amanita* contains sections *Amanita* and *Vaginatae*. The sections are delimited largely on the basis of universal and partial veil characters. For a key to the subgenera and sections of *Amanita* see Bas (1969). Singer's (1986) classification differs from Corner & Bas (1962) by the addition of three sections and the renaming of a fourth. Singer (1986) splits section *Phalloideae* into sections *Phalloideae* and *Mappae* Gilbert, and renames section *Lepidella* as section *Roanokensis* Sing. He also transfers some taxa from sections *Amanita* and *Vaginatae* into section *Ovigerae* Sing., and further splits section *Vaginatae* into sections *Vaginatae* and *Caesareae* Sing.

Several recent papers have addressed the subgeneric delimitation of *Amanita* using molecular phylogenies of the nuclear large ribosomal RNA gene (28S region; Weiß, Yang & Oberwinkler 1998; Drehmel, Moncalvo & Vilgalys 1999) and the ITS 1 - 5.8S - ITS 2 regions of the nuclear ribosomal RNA operon (ITS region; Oda, Tanaka & Tsuda 1999). These studies have upheld sections *Amidella, Caesareae* and *Vaginatae* as distinct monophyletic groups. If section *Ovigerae* is discounted, section *Amanita* is monophyletic (each study includes one member of section *Ovigerae*, which falls within section *Amanita* in two studies (Weiß, Yang & Oberwinkler 1998; Oda, Tanaka & Tsuda 1999), and

basal to section *Amanita* in the third (Drehmel, Moncalvo & Vilgalys 1999)). Sections *Mappae*, *Phalloideae* and *Validae* are problematic. *Phalloideae* is not monophyletic in any treatment. *Phalloideae* sensu Corner & Bas (1962) is polyphyletic, forming at least three distinct groups in the analysis of Weiß, Yang & Oberwinkler (1998): amatoxin-producing taxa, section *Mappae* sensu Singer (1986), and *A. manginiana* Har. & Pat. and *A. pseudoporphyria* Hongo. *A. manginiana* and *A. pseudoporphyria* are basal to the other *Phalloideae*, but their placement is otherwise unresolved. Section *Mappae* forms a sister group to section *Validae*. Weiß, Yang & Oberwinkler (1998) and Oda, Tanaka & Tsuda (1999) treat *Mappae* as part of a monophyletic section *Validae*. Drehmel, Moncalvo & Vilgalys (1999) treat *Mappae* as section *Phalloideae*, subsection *Validae*, series *Mappae* (sister to series *Validae*). Molecular phylogenetics have weakly supported section *Lepidella* and have been inconclusive in the placement of section *Lepidella* in relation to the other sections. Drehmel, Moncalvo & Vilgalys (1999) and Oda, Tanaka & Tsuda (1999) support the monophyly of the subgenera *Amanita* and *Lepidella*. Weiß, Yang & Oberwinkler (1998) found a paraphyletic subgenus *Lepidella*, but with low bootstrap support.

Each of the three recent phylogenetic studies has taken a broad overview of the genus *Amanita*, sampling broadly across all sections. Our studies concentrate on section *Phalloideae* with more extensive sampling. Section *Phalloideae* contains the amatoxin-producing species that are responsible for the vast majority of serious mushroom poisonings in humans, in particular *Amanita phalloides* (Fr.:Fr.) Link and the "deadly white" or "destroying angel" complex

centered around *A. bisporigera* Atk. *A. phalloides* is distinct, but many of the destroying angels cannot be readily distinguished on the basis of morphology. Jenkins (1986) states, "(t)he difficulty of macroscopic differentiation of the several 'white' Amanitas is common knowledge among those professionals and amateurs who have made attempts at identification. Color, size, and texture are so similar that an accurate identification cannot be made using these features. ... The only taxa which appear to be 'relatively' distinct are *A. verna, A. virosa, A. bisporigera* and *A. tenuifolia*. As for the rest, the only difference appears to be spore size." Several species of deadly white *Amanita*s have been circumscribed on the bases of spore size, spore length-to-breadth ratio, and the number of spores per basidium. The delimitation of these species requires reevaluation because individual specimens may exhibit variations or gradations in these characters.

By examining the phylogenetic relationships in section *Phalloideae*, we were able to develop an evolutionary hypothesis for amatoxin production in *Amanita*. In this study, DNA sequencing and phylogenetic analyses of the ITS and partial 28S regions were used to clarify relationships among members of the *A. bisporigera* complex and circumscribe the section *Phalloideae*. Mitochondrial large rDNA sequence was examined for its utility in assessing the phylogeny of *Amanita*. Finally, the relationship between *Amanita* and its sister group, *Limacella*, the only two genera in the *Amanitaceae,* was examined.

Materials and Methods

Specimens used are shown in Tables 1 & 2. Specimens denoted "PH-10" or "WH-#" (Table 1) were identified by and obtained from Dr. Rodham Tulloss, who has conducted extensive morphological studies on the genus *Amanita*.

DNA extraction, amplification and sequencing

DNA was extracted from gill and pileus tissue of *Amanita* following Raeder & Broda (1985).

Approximately 1-20 ng of the total genomic DNA was used per 25 μl reaction mixture for polymerase chain reaction (PCR) amplification. Various brands of prepackaged buffers and polymerases were used for PCR amplification. Primer ITS 1F (Gardes & Bruns 1993) or ITS F (Glen et al. 2001) was used in combination with primer ITS 4B (Gardes & Bruns 1993) or ITS 4 (White et al. 1990) to amplify the ITS 1-5.8S-ITS 2 regions of the nuclear rDNA (ITS region). Primers CTB6 (Bruns & Li; cited in Hughey et al. 2000) and TW13 (White et al. 1990) were used to amplify approximately 600 bases of the 28S nuclear rDNA. Primers MLin 3 and CML 7.5 (Bruns & Li; cited in Hughey et al. 2000) were used to amplify approximately 500 bases of the mitochondrial large rDNA.

Table 1. Specimens from which DNA was extracted and sequenced.

Taxon	Section	Locale	Accession #[a]
Amanita sp. SA 22	*Lepidella*	South Africa	PRUM 3611
Amanita sp. T27 (WH-30)	*Phalloidae*	Texas, USA	RET 1-30-89-AWR
Amanita arochae (WH-65)	*Phalloidae*	Costa Rica	RET 6-25-95-K
Amanita bisporigera	*Phalloidae*	Michigan, USA	MSC 380551
Amanita bisporigera f. *tetraspora*	*Phalloidae*	Mexico	F1118140
Amanita brunnescens	*Validae*	Maine, USA	MSC 380552
Amanita citrina f. *lavendula*	*Validae*	Michigan, USA	MSC 380550
Amanita citrina var. citrina sensu auct. amer.	*Validae*	Michigan, USA	MSC 380559
Amanita cylindrispora (WH-28)	*Amidella*	New Jersey, USA	RET S. Tulloss 8-11-96-B
Amanita flavoconia	*Validae*	Vermont, USA	MSC 380548
Amanita fulva sensu auct. amer.	*Vaginatae*	Michigan, USA	MSC 380554
Amanita gilbertii (WH-2)	*Amidella*	France	RET Massart 97013
Amanita magnivelaris (WH-1)	*Phalloidae*	Florida	RET Kuechmann s.n.
Amanita marmorata ssp. *myrtacearum*	*Phalloidae*	Hawaii, USA	DED5845
Amanita muscaria orange	*Amanita*	Michigan, USA	MSC 380556
Amanita muscaria var. *alba*	*Amanita*	Michigan, USA	MSC 380555
Amanita muscaria var. *guessowii*	*Amanita*	Micihgan, USA	MSC 380549
Amanita ocreata (WH-8)	*Phalloidae*	California, USA	RET NAMA 98 s.n.
Amanita phalloides	*Phalloidae*	Australia	MEL 2028861
Amanita phalloides	*Phalloidae*	California, USA	MSC 380564
Amanita phalloides (PH-10)	*Phalloidae*	Norway	O Gulden 49/94
Amanita phalloides f. *umbrina*	*Phalloidae*	South Africa	PREM 48618
Amanita phalloides var. *alba* (WH-22)	*Phalloidae*	France	RET Massart 90041
Amanita pleropus	*Lepidella*	South Africa	PREM 47480
Amanita reidii	*Phalloidae*	South Africa	PRUM 4306
Amanita cf. *subphalloides*	*Validae*	Indiana, USA	F1116789
Amanita thiersii	*Lepidella*	Illinois, USA	F1127062
Amanita vaginata sensu auct. amer.	*Vaginatae*	Minnesota, USA	MIN 839788
Amanita virosa China	*Phalloidae*	China	F1121430
Amanita virosa France (WH-21)	*Phalloidae*	France	RET Massart 98025
Amanita virosa sensu auct. amer. (WH-44)	*Phalloidae*	New Jersey, USA	RET Carlson 7-24-96-K
Amanita cf. *virosa* sensu auct. mexic. (WH-24)	Unknown	Mexico	RET Montoya E. 1558

Table 1. Specimens from which DNA was extracted and sequenced. [a]DED = herbarium of Dennis Desjardin, San Francisco State University, San Francisco, CA, USA; F = Field Museum of Natural History, Chicago, USA; MIN = University of Minnesota Herbarium, St. Paul, MN, USA; MSC = Beal Darlington Herbarium, Michigan State University, East Lansing, MI, USA; PREM = National Herbarium, National Botanical Institute, Pretoria, South Africa; PRUM = RET = herbarium of Rodham E. Tulloss, Roosevelt, NJ, USA. Specimens were collected by Heather Hallen (all MSC specimens except *A. phalloides* from California), Gro Gulden (*A. phalloides* from Norway); Sarah E. K. Tulloss and Rodham E. Tulloss (*A. cylindrispora*); the Texas Mycological Society (*A.* sp. T27); Roy E. Halling (*A. arocheae*); F. Massart (*A. gilbertii, A. phalloides* f. *alba*, *A. virosa* from France); Bruce Kuechmann (*A. magnivelaris*); Dennis E. Desjardin (*A. marmorata* ssp. *Myrtacearum*); the North American Mycological Association (*A. ocreata*); Fred Stevens (*A. phalloides* from California); Pat Leacock (*A. vaginata*); Britt Carlson & Rodham E. Tulloss (*A. virosa* sensu auct. Amer.); A. Montoya Esquival & Rodham E. Tulloss (*A.* cf. *virosa* sensu auct. Mexic.)

Table 2. Data from GenBank used in the 28S extended database.

Taxon	Accession #	Reference
Amanita angustilamellata	AF024440	Weiss, Yang & Oberwinkler 1998
Amanita armillariiformis	AF261437	Moncalvo et al. 2002
Amanita armillariiformis	AF261436	Moncalvo et al. 2002
Amanita avellaneosquamosa	AF024441	Weiss, Yang & Oberwinkler 1998
Amanita bisporigera	AF097384	Drehmel, Moncalvo & Vilgalys 1999
Amanita bisporigera	AF097385	Drehmel, Moncalvo & Vilgalys 1999
Amanita brunneofuliginea	AF024442	Weiss, Yang & Oberwinkler 1998
Amanita brunnescens	AF097379	Drehmel, Moncalvo & Vilgalys 1999
Amanita caesarea	AF024443	Weiss, Yang & Oberwinkler 1998
Amanita calyptrata	AD001545	Bruns et al. 1998
Amanita ceciliae	AF097372	Drehmel, Moncalvo & Vilgalys 1999
Amanita ceciliae	AF024444	Weiss, Yang & Oberwinkler 1998
Amanita chepangiana	AF024445	Weiss, Yang & Oberwinkler 1998
Amanita citrina	AF097377	Drehmel, Moncalvo & Vilgalys 1999
Amanita citrina	AF097378	Drehmel, Moncalvo & Vilgalys 1999
Amanita citrina	AF024446	Weiss, Yang & Oberwinkler 1998
Amanita citrina grisea	AF024447	Weiss, Yang & Oberwinkler 1998
Amanita clarisquamosa	AF024448	Weiss, Yang & Oberwinkler 1998
Amanita cokeri	AF097395	Drehmel, Moncalvo & Vilgalys 1999
Amanita cokeri	AF097398	Drehmel, Moncalvo & Vilgalys 1999
Amanita excelsa	AF024449	Weiss, Yang & Oberwinkler 1998
Amanita farinosa	AF024450	Weiss, Yang & Oberwinkler 1998
Amanita farinosa	AF097370	Drehmel, Moncalvo & Vilgalys 1999
Amanita flavipes	AF024451	Weiss, Yang & Oberwinkler 1998
Amanita flavoconia	AF042609	Drehmel, Moncalvo & Vilgalys 1999
Amanita flavorubescens	AF097380	Drehmel, Moncalvo & Vilgalys 1999
Amanita franchetii	AF097381	Drehmel, Moncalvo & Vilgalys 1999
Amanita franchetii	AF156915	Taylor & Bruns 1999
Amanita fritillaria	AF024452	Weiss, Yang & Oberwinkler 1998
Amanita frostiana	AF024453	Weiss, Yang & Oberwinkler 1998
Amanita fuliginea	AF024454	Weiss, Yang & Oberwinkler 1998
Amanita fulva	AF097373	Drehmel, Moncalvo & Vilgalys 1999
Amanita fulva	AF024455	Weiss, Yang & Oberwinkler 1998
Amanita aff fulva	AF024456	Weiss, Yang & Oberwinkler 1998
Amanita gemmata	AD001547	Bruns et al. 1998
Amanita gemmata	AF024457	Weiss, Yang & Oberwinkler 1998
Amanita gemmata	AF097371	Drehmel, Moncalvo & Vilgalys 1999
Amanita gemmata	AF335440	Berbee, Inderbitzen & Zhang. Unpublished.
Amanita hemibapha ocracea	AF024458	Weiss, Yang & Oberwinkler 1998
Amanita incarnatifolia	AF024459	Weiss, Yang & Oberwinkler 1998
Amanita jacksonii	AF097376	Drehmel, Moncalvo & Vilgalys 1999
Amanita japonica	AF024460	Weiss, Yang & Oberwinkler 1998
Amanita lignitincta	AF024461	Weiss, Yang & Oberwinkler 1998
Amanita longistriata	AF024462	Weiss, Yang & Oberwinkler 1998
Amanita magniverrucata	AD001548	Bruns et al. 1998
Amanita manginiana	AF024463	Weiss, Yang & Oberwinkler 1998

Table 2, cont.

Amanita mira	AF024464	Weiss, Yang & Oberwinkler 1998
Amanita muscaria	AD001549	Bruns et al. 1998
Amanita muscaria	AF042643	Drehmel, Moncalvo & Vilgalys 1999
Amanita muscaria	AF097368	Drehmel, Moncalvo & Vilgalys 1999
Amanita muscaria	AF024465	Weiss, Yang & Oberwinkler 1998
Amanita muscaria	AJ406558	Langer. Unpublished.
Amanita muscaria var. persicina	AF097367	Drehmel, Moncalvo & Vilgalys 1999
Amanita nivalis	AF024466	Weiss, Yang & Oberwinkler 1998
Amanita pachycolea	AD001550	Bruns et al. 1998
Amanita pantherina	AD001551	Bruns et al. 1998
Amanita pantherina	AF024467	Weiss, Yang & Oberwinkler 1998
Amanita pantherina lutea	AF024468	Weiss, Yang & Oberwinkler 1998
Amanita peckiana	AF042608	Drehmel, Moncalvo & Vilgalys 1999
Amanita peckiana	AF097387	Drehmel, Moncalvo & Vilgalys 1999
Amanita phalloides	AD001548	Bruns et al. 1998
Amanita phalloides	AF024469	Weiss, Yang & Oberwinkler 1998
Amanita phalloides	AF261435	Moncalvo et al. 2002
Amanita pilosella	AF024470	Weiss, Yang & Oberwinkler 1998
Amanita pseudoporphyria	AF024471	Weiss, Yang & Oberwinkler 1998
Amanita pseudovaginata	AF024472	Weiss, Yang & Oberwinkler 1998
Amanita rhoadsii	AF097391	Drehmel, Moncalvo & Vilgalys 1999
Amanita rhopalopus	AF097393	Drehmel, Moncalvo & Vilgalys 1999
Amanita roseitincta	AF097369	Drehmel, Moncalvo & Vilgalys 1999
Amanita rubescens	AF042607	Drehmel, Moncalvo & Vilgalys 1999
Amanita rubescens	AF097382	Drehmel, Moncalvo & Vilgalys 1999
Amanita rubescens	AF097383	Drehmel, Moncalvo & Vilgalys 1999
Amanita rubrovolvata	AF024473	Weiss, Yang & Oberwinkler 1998
Amanita silvicola	AD001553	Bruns et al. 1998
Amanita sinensis	AF024474	Weiss, Yang & Oberwinkler 1998
Amanita solitaria	AF024475	Weiss, Yang & Oberwinkler 1998
Amanita solitariiformis	AF097389	Drehmel, Moncalvo & Vilgalys 1999
Amanita solitariiformis	AF097390	Drehmel, Moncalvo & Vilgalys 1999
Amanita sp Lepidella	AF097392	Drehmel, Moncalvo & Vilgalys 1999
Amanita strobiliformis	AF024476	Weiss, Yang & Oberwinkler 1998
Amanita subfrostiana	AF024477	Weiss, Yang & Oberwinkler 1998
Amanita subglobosa	AF024478	Weiss, Yang & Oberwinkler 1998
Amanita subjunquillea alba	AF024479	Weiss, Yang & Oberwinkler 1998
Amanita sychnopyramis	AF024480	Weiss, Yang & Oberwinkler 1998
Amanita umbrinolutea	AF024481	Weiss, Yang & Oberwinkler 1998
Amanita vaginata	AF097375	Drehmel, Moncalvo & Vilgalys 1999
Amanita vaginata	AF024482	Weiss, Yang & Oberwinkler 1998
Amanita verrucosivolva	AF024483	Weiss, Yang & Oberwinkler 1998
Amanita virgineoides	AF024484	Weiss, Yang & Oberwinkler 1998
Amanita virosa	AF097386	Drehmel, Moncalvo & Vilgalys 1999
Amanita virosa	AF159086	Moncalvo, Drehmel & Vilgalys 2000
Amanita cf virosa	AF024486	Weiss, Yang & Oberwinkler 1998
Amanita volvata	AF024485	Weiss, Yang & Oberwinkler 1998
Amanita volvata	AF097388	Drehmel, Moncalvo & Vilgalys 1999

Table 2, cont.

Amanita aff volvata	AF024487	Weiss, Yang & Oberwinkler 1998
Amanita yuaniana	AF024488	Weiss, Yang & Oberwinkler 1998
Limacella glioderma	AF024489	Weiss, Yang & Oberwinkler 1998
Limacella glishra	U85301	Drehmel, Moncalvo & Vilgalys 1999
Tricholoma flavovirens	AD001652	Bruns et al. 1998

The cycling reactions were performed in a DNA thermal cycler (Perkin-Elmer, Norwalk, CT, USA) with an annealing temperature of 55°C following Tank & Sang (2001). Alternately, a 60°C to 45°C touchdown protocol was used on some templates that did not amplify with the 55°C annealing temperature. The amplification ended with an additional 10 min extension at 72°C, and storage at 4°C. PCR amplification products were separated, and purified following Hughey et al. (2000). Alternatively, products were gel purified and cloned using TOPO® TA (Invitrogen, Carlsbad, CA, USA) or pGEM® (Promega, Madison, WI, USA) cloning kits. Sequencing was performed by the Michigan State University Genomics Technology Support Facility, using dye terminator capillary electrophoresis on an ABI Prism® 3700 DNA Analyzer (Applied Biosystems, Foster City, CA, USA).

Phylogenetic analyses

Sequences were aligned using Clustal W (Thompson, Higgins & Gibson 1994), and were further aligned by eye. Phylogenetic analyses were performed using PAUP* 4.0b10 (Swofford 2002). Maximum parsimony was used with a heuristic search algorithm. A consensus tree was built from all equally most parsimonious trees. Five hundred bootstrap replications (maxtrees=300) were

run to attain bootstrap values (Hedges 1992). Maximum likelihood analyses were performed in PAUP* using likelihood settings determined by Modeltest 3.06 (Posada & Crandall 1998). Trees that had the shortest length and the greatest ln likelihood were displayed using TreeView version 1.6.6 (Page 1996).

Results

Approximately 100 bp from the 5' end of ITS 1 were not alignable between sections of *Amanita*, and were excluded from analyses. The dataset was of 25 taxa and consisted of 571 aligned nucleotides including gaps introduced during alignment (261 informative sites). A heuristic search produced nine equally most parsimonious trees of 894 steps and consistency index (CI) of 0.688, retention index (RI) of 0.726, and rescaled consistency index (RC) of 0.499. Likelihood settings from the best-fit model (HKY+I+G) were selected by hierarchical likelihood rates testing in Modeltest (Posada & Crandall 1998). The tree with the greatest likelihood value, ln likelihood of -4518.01, is shown in Fig. 3. Toxin-producing members of section *Phalloideae* form a monophyletic clade with 100% bootstrap support.

(Text continues on page 41)

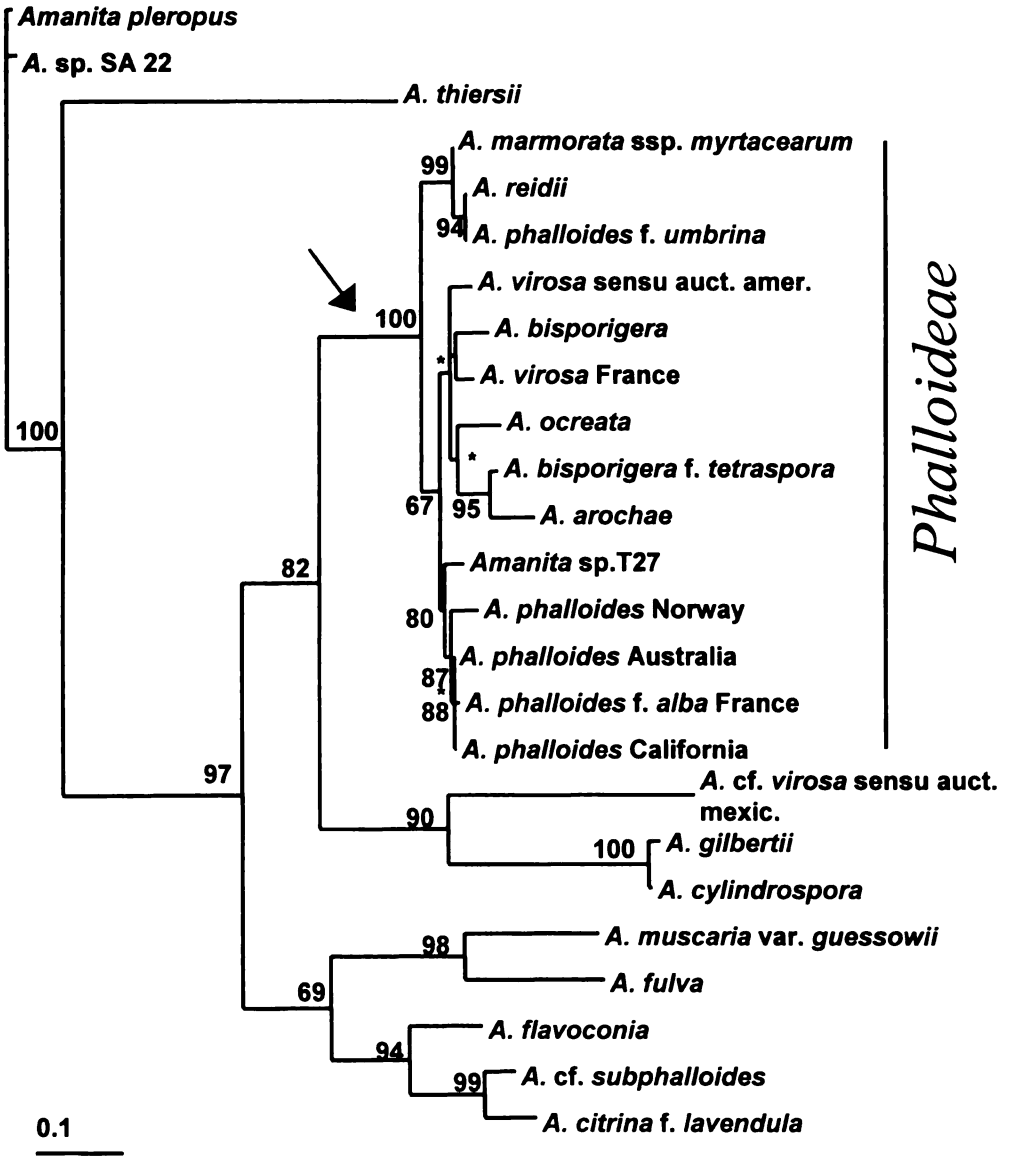

Fig. 3. Cladogram produced by maximum likelihood analysis of the ITS 1, 5.8S and ITS 2 regions of the nuclear ribosomal DNA operon.Topology corresponds to one of nine equally most parsimonious trees (894 steps). Branch lengths correspond to genetic distance (expected nucleotide substitutions per site). Numbers at nodes are bootstrap indices of support (%). Arrow indicates node leading to amatoxin-producing taxa. * indicates branches that collapse on the strict consensus tree.

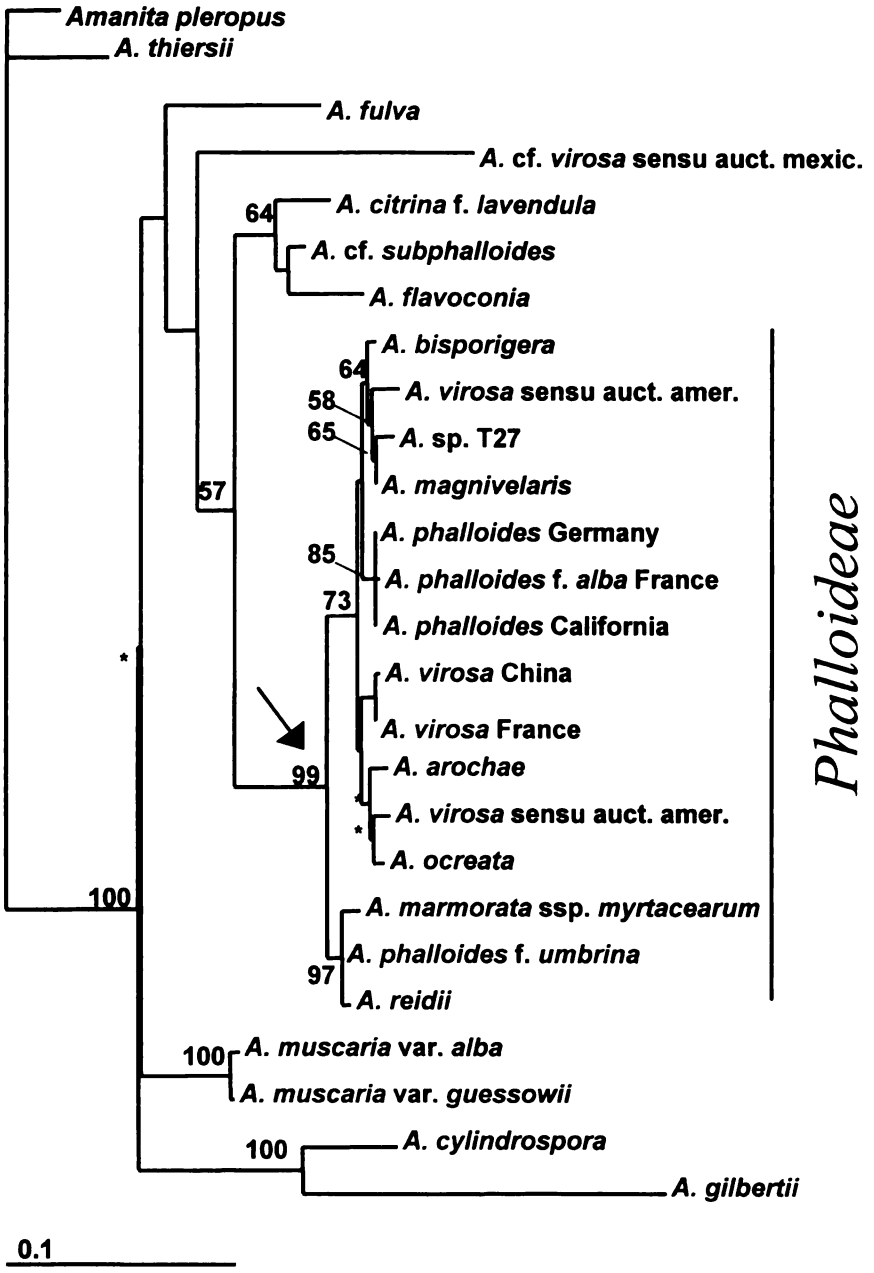

Fig. 4. Cladogram produced by maximum likelihood analysis of the 28S region of the nuclear ribosomal DNA operon.Topology corresponds to one of 132 equally most parsimonious trees (361 steps). Branch lengths correspond to genetic distance (expected nucleotide substitutions per site). Numbers at nodes are bootstrap indices of support (%). Arrow indicates node leading to amatoxin-producing taxa. * indicates branches that collapse on the strict consensus tree.

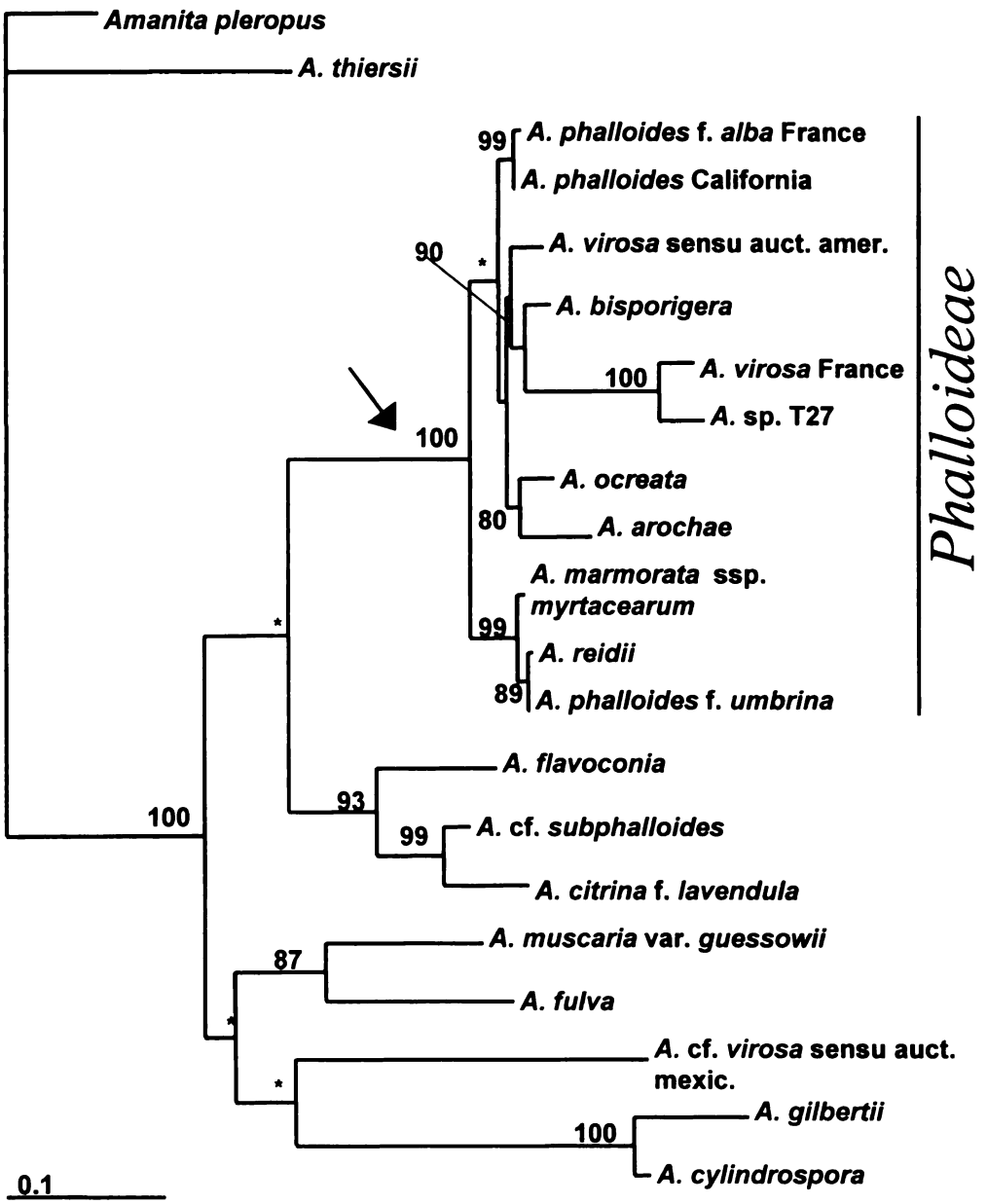

Fig. 5. Cladogram produced by maximum likelihood analysis of the combined ITS and 28S regions of the nuclear ribosomal DNA operon.Topology corresponds to one of four equally most parsimonious trees (1338 steps). Branch lengths correspond to genetic distance (expected nucleotide substitutions per site). Numbers at nodes are bootstrap indices of support (%). Arrow indicates node leading to amatoxin-producing taxa. * indicates branches that collapse on the strict consensus tree.

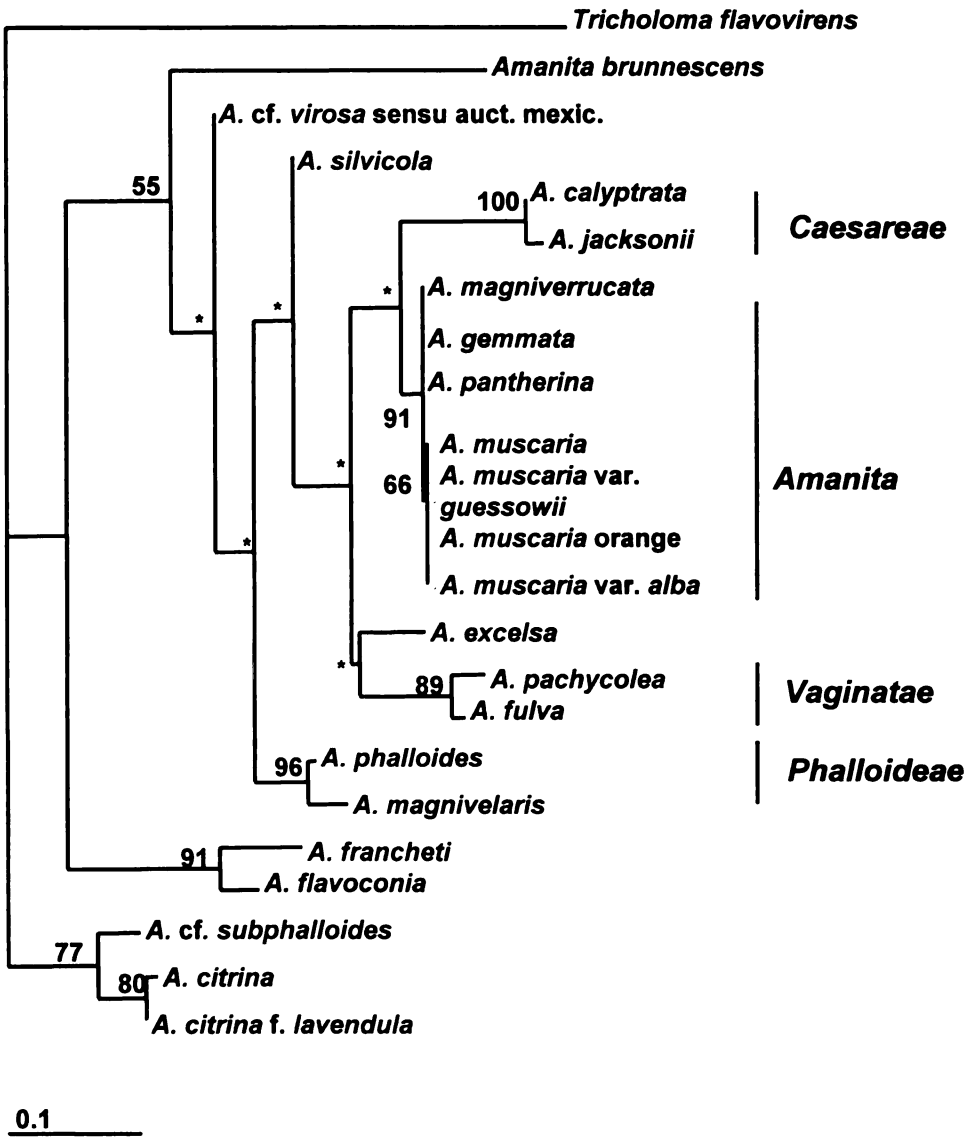

Fig. 6. Cladogram produced by maximum likelihood analysis of the mitochondrial large ribosomal DNA operon.Topology corresponds to one of 7 equally most parsimonious trees (156 steps). Branch lengths correspond to genetic distance (expected nucleotide substitutions per site). Numbers at nodes are bootstrap indices of support (%). * indicates branches that collapse on the strict consensus tree.

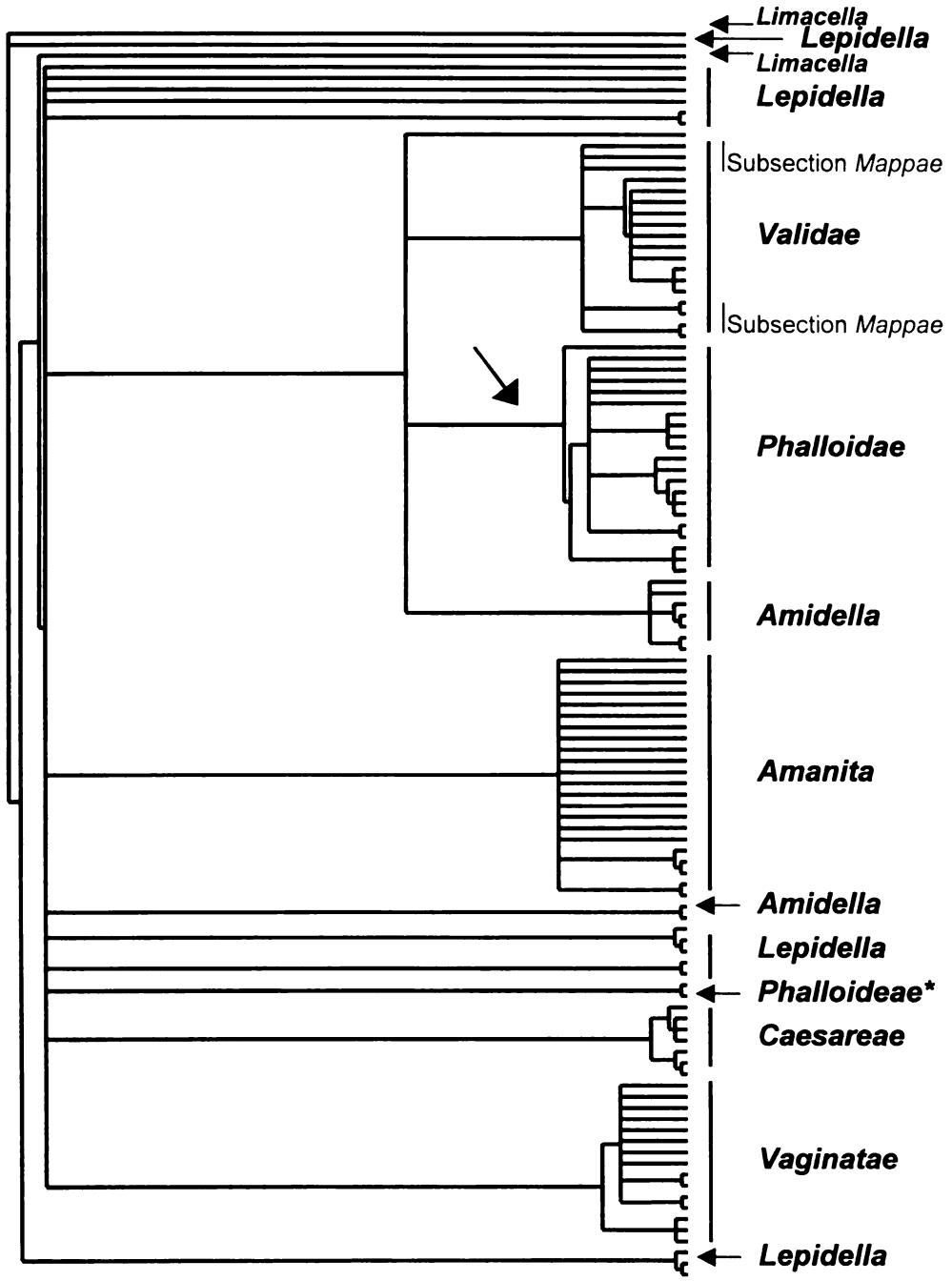

Fig. 7a. Consensus based on 300 equally parsimonious trees (length 1275) of the 28S region of the nuclear ribosomal DNA subunit. Names of sections in genus *Amanita* are given. *Limacella* (top) is the other genus in the *Amanitaceae* and was used as an outgroup. Arrow indicates node leading to amatoxin-producing taxa. * indicates *Amanita manginiana* and *A. pseudoporphyria*, traditionally placed in section *Phalloideae*.

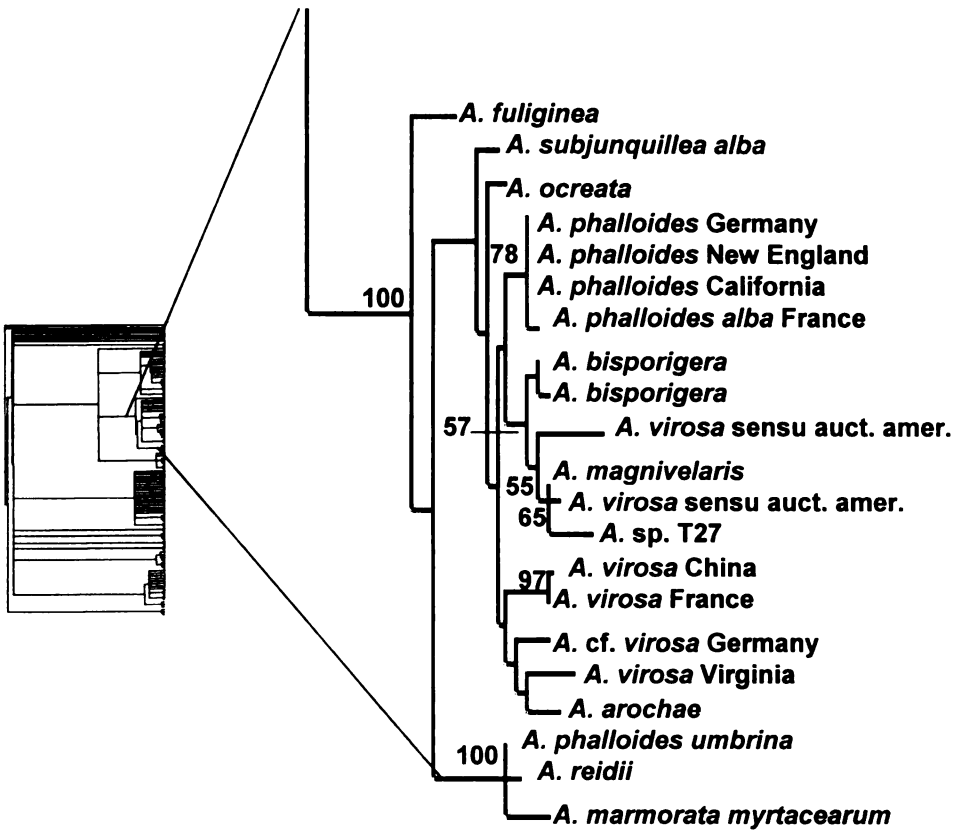

Fig. 7b. Amatoxin-producing members of section *Phalloideae* from the parsimony analysis of the extended 28S dataset. Numbers at nodes are bootstrap indices of support.

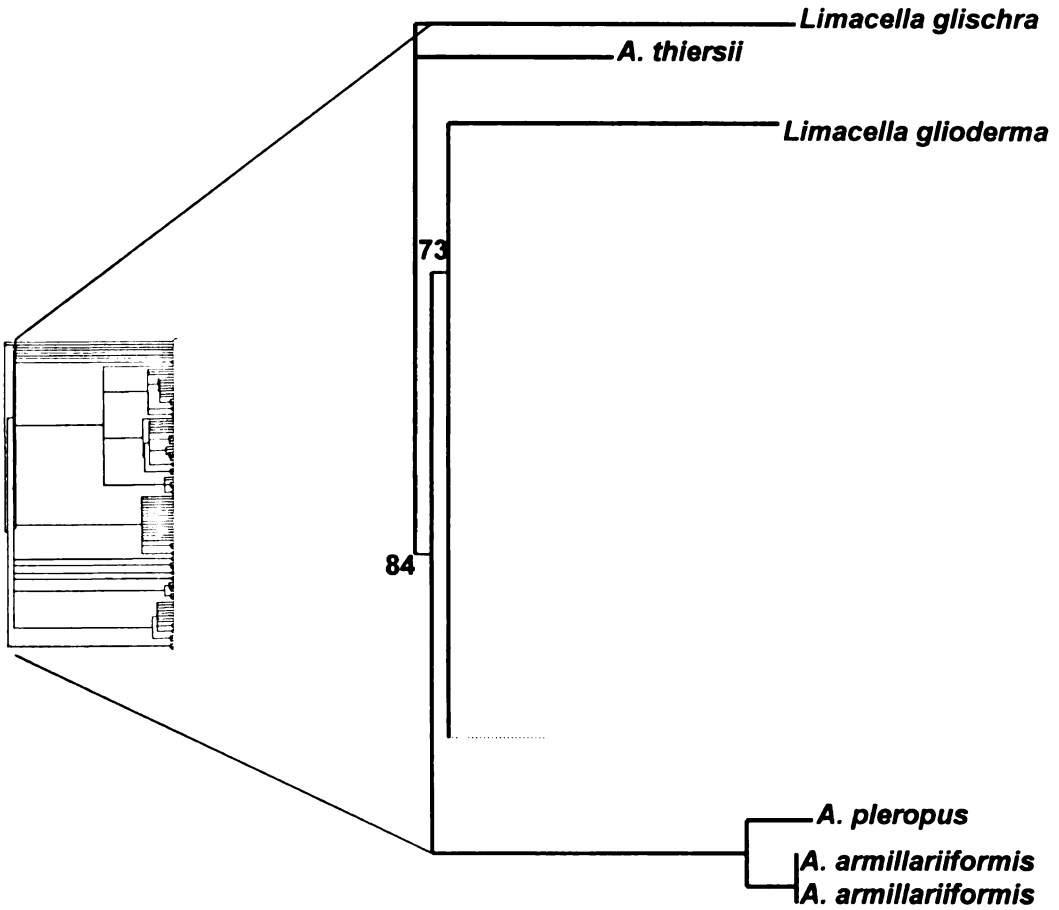

Fig. 7c. Basal taxa from the parsimony analysis of the extended 28S dataset. Numbers at nodes are bootstrap indices of support.

The 28S sequence dataset was of 26 taxa and 526 aligned nucleotides (118 informative sites). A heuristic search produced 132 equally most parsimonious trees of 361 steps, CI = 0.673, RI = 0.722, RC = 0.486. Likelihood settings from the best-fit model (TrN+G) were selected by hierarchical likelihood rate testing in Modeltest (Posada & Crandall 1998). One of the three trees with the greatest likelihood value, ln likelihood of -2497.75, is shown in Fig. 4. Amatoxin-producing members of section *Phalloideae* form a monophyletic clade with 99% bootstrap support.

The combined ITS and 28S dataset was of 21 taxa and 1089 aligned nucleotides (378 informative sites). A heuristic search produced four equally most parsimonious trees of 1338 steps, CI = 0.661, RI = 0.663 and RC = 0.438. Likelihood settings from the best-fit model (TrN+I+G) were selected by hierarchical likelihood rates testing in Modeltest (Posada & Crandall 1998). The tree with the greatest likelihood value, ln likelihood of -7418.964, is shown in Fig. 5. Amatoxin-producing members of section *Phalloideae* form a monophyletic clade with 100% bootstrap support.

The mitochondrial large dataset was of 24 taxa: 23 *Amanita* specimens and *Tricholoma flavovirens*, which was included as an outgroup. The dataset was of 310 aligned nucleotides (46 informative sites). A heuristic search produced seven equally parsimonious trees of 156 steps, CI = 0.641, RI = 0.726, RC = 0.463. Likelihood settings from the best-fit model (F81+I+G) were selected by hierarchical likelihood rates testing in Modeltest (Posada & Crandall 1998). The tree with the greatest likelihood value, ln likelihood = - 1142.43, is shown in Fig.

6. Sections *Amanita, Caesareae, Phalloideae* and *Vaginata* formed monophyletic clades, with bootstrap support of 89% and above.

An extended 28S dataset was prepared by combining the sequences of 26 taxa with an additional 86 sequences from GenBank to yield a total sequences. The dataset was of 542 aligned nucleotides (202 informative sites). A heuristic search produced 300 equally most parsimonious trees of 1275 steps, CI = 0.355, RI = 0.761 and RC = 0.271, a strict consensus of which is shown in Fig. 7a. Sections *Amidella* and *Lepidella* were both polyphyletic. Amatoxin-producing members of section *Phalloideae* formed a monophyletic clade with 100% bootstrap support (Fig. 7b). The genus *Amanita* itself may be polyphyletic, as *Limacella glioderma* was placed within *Amanita* with 84% bootstrap support (Fig. 7c).

With the exception of the taxon identified as *A.* cf. *virosa* sensu auct. mexic. (according to Mexican authors), all purportedly amatoxin-producing members of section *Phalloideae* formed a monophyletic clade in all analyses. Section *Validae* formed a monophyletic clade, with a monophyletic subsection *Mappae* nesting within, in the ITS and 28S analyses, but was found paraphyletic in the mitochondrial large analysis.

Discussion

Among taxa that produce amatoxins there is little resolution. *A. phalloides* f. *umbrina* Ferry, *A. reidii* Eicker & van Greuning and *A. marmorata* ssp.

myrtacearum O.K. Miller, D. Hemmes & G. Wong consistently form a clade with high (96% and above) bootstrap support. This supports the belief that these taxa are synonymous (Eicker, van Greuning & Reid 1993; Hallen, Adams & Eicker *in press*; RE Tulloss, pers. comm.) The white *A. phalloides* f. *alba* Britzelm, to which the name *A. verna* has sometimes been misapplied, forms a monophyletic clade with *A. phalloides* f. *phalloides* specimens from Europe, Australia and North America. The name *A. verna* (Bull.:Fr.) Lamarck has frequently been misapplied to white morphs of *A. phalloides*, and is of uncertain validity. Type material would need to be examined to determine whether *A. verna* is a valid taxon. *Amanita virosa* in the sense of American authors (sensu auct. amer.) does not cluster with Old World *A. virosa* Lamarck specimens; nor does it cluster with *A. bisporigera* or the four-spored *A. bisporigera* var. *tetraspora* as morphology would suggest (Tulloss et al. 1995).

The amatoxin-producing *Phalloideae* form a well-supported clade (96-100% bootstrap support in all analyses, suggesting a single gain of the ability to produce amatoxins in an ancestral member of this section. This affirms the validity of amatoxin presence as a chemotaxonomic character (Beutler 1980). The fact that *Phalloideae* is a derived clade suggests that amatoxin gain in the genus *Amanita* occurred separately from amatoxin gain in other genera.

There was uncertainty with regard to the identification of the species denoted *Amanita* cf. *virosa* sensu auct. mexic. This taxon is placed well apart from other *A. virosa* specimens and section *Phalloideae* in all analyses, and the likeliest hypothesis is that the specimen was misidentified. The phylogenetic

placement of this taxon is uncertain as it varies depending on the dataset. In all cases it is basal to sections *Phalloideae* and *Validae*.

Some species that have been placed in section *Phalloideae*, such as *A. manginiana* and *A. pseudoporphyria*, are placed well apart from the amatoxin-producing *Phalloideae* in the extended 28S dataset. This placement was also evident in the analyses of Weiß, Yang & Oberwinkler (1998), and it is clear that the classification of these species requires some consideration.

Sections *Amanita, Caesareae, Phalloideae* and *Vaginata* formed monophyletic clades in analyses of the mitochondrial large rDNA. Section *Validae* and *Validae* subsection *Mappae* were polyphyletic. The portion of the mitochondrial large rDNA examined is highly conserved and is not always of utility in distinguishing between species or closely related genera (Bruns et al. 1998). The evident distinctiveness of sections *Amanita, Caesareae, Phalloideae* and *Vaginata* on the basis of this data is therefore unexpected. The ability to identify *Amanita* species to section may prove very useful in mycorrhizal research. However, the relatively low number of informative sites (46 out of 312) for this gene renders it of little utility in formulating evolutionary hypotheses.

A. pleropus (Kalchbr. & MacOwan) D. Reid and *A. thiersii* Bas (section *Lepidella*) have long been considered "primitive" basal taxa on the basis of their morphology (Bas 1969). This is confirmed by the phylogenetic analyses. In the parsimony analysis of the extended 28S dataset, these species, together with *A. armilariiformis* Trueblood & Jenkins, were basal to *Limacella glioderma*. *A. armillariiformis, A. pleropus* and *A. thiersii* possess dry, membranous universal

veils whereas *Limacella* possesses a gelatinous veil. It can be inferred based on the 28S analysis that the character of a gelatinous veil might have been given inappropriate weight in differentiating genera. The placement of a *Limacella* species within the basal *Amanita*s renders both genera polyphyletic. Further *Limacella* and basal *Amanita* taxa will need to be evaluated, and additional genes will need to be sequenced in *Limacella* before we can judge the validity of the generic circumscriptions.

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CHAPTER 2

IDENTIFICATION OF *AMANITA* SPECIES PARASITIZED BY *HYPOMYCES HYALINUS*

Abstract

RFLPs and DNA sequencing of PCR products of the ITS-5.8S regions of *Amanita* specimens infected with the parasite *Hypomyces hyalinus* were used to identify the host species. Twenty two uninfected *Amanita* taxa were utilized as reference standards for comparisons of RFLP and sequence homology. Phylogenetic analyses of sequence enhanced identification of several of the hosts. Parasitized *Amanita*s were in *Amanita* Section *Validae*. Several parasitized specimens were identified as taxa allied with *Amanita rubescens*, supporting earlier reports based on proximity to uninfected fruiting bodies and ecology. The parasitized taxa included *A. brunnescens, A. flavoconia, A. novinupta* and *A. orsonii*. Two amorphous basidiocarps parasitized by *H. hyalinus* and possessing fertile perithecia contained DNA sequence from a *Russula* and a *Thelephora* species.

Introduction

Species of *Amanita* Pers. are subjected to parasitism by *Hypomyces hyalinus* (Schw.:Fr.) Tul. (Hypocreales, Pyrenomycetes). The perithecia of *H.*

hyalinus form over the entire host surface, aborting the basidiocarp and rendering it unidentifiable (Fig. 8).

Figure 8. *Amanita* basidiocarps parasitized by *Hypomyces hyalinus*.

Infected Amanitas may grow to approximately full height but the pileus does not expand. No recognizable lamellae are present, and basidiospores are not produced. Infected specimens frequently grow intermingled with identifiable, uninfected specimens. The proximity of uninfected fruiting bodies and the tendency of some parasitized specimens to stain reddish upon bruising (rubescent nature) has led to the assumption that the host species is predominantly *A. rubescens* sensu auct. amer. (Bessette, Bessette & Fischer, 1997). Lincoff (1981; quoted by Phillips, 1991) describes the habitat of *H. hyalinus* as "on *Amanita rubescens, Amanita flavorubescens, Amanita frostiana,* and possibly *Amanita bisporigera*." Rogerson and Samuels (1996) include *A.*

muscaria (L.:Fr.) Pers. in the list of parasitized species, but go on to state, "*Hypomyces hyalinus* is presumed only to occur on *Amanita* species because the only healthy basidiocarps associated with the *Hypomyces* are species of *Amanita*. The parasitism of *H. hyalinus* is so complete and the host so distorted, that in the absence of nearby healthy basidiocarps it is not possible to determine the host." *A. rubescens* and related taxa in *Amanita* Section *Validae* are edible, and in some parts of the country parasitized *Amanita*s are collected and eaten (TJ Volk, pers. comm., 28 August 2001). *A. bisporigera* Atk. is deadly poisonous and *A. muscaria* and A. *frostiana* (Peck) Sacc. contain the central nervous system toxin ibotenic acid. The identity of the species of parasitized *Amanita* thus becomes of importance. Reports of *H. hyalinus* on *A. frostiana* may be based on misidentification; *A. flavoconia* in section *Validae* is frequently misidentified *as A. frostiana* (Jenkins 1977; Tulloss 1998). The type collection of *A. frostiana* includes material of *A. flavoconia* (Jenkins 1977). We have undertaken this study to begin to address the question of which species of *Amanita* are being parasitized.

The field of mycorrhizal research has given rise to several molecular techniques for identifying basidiomycete fungi in the absence of a recognizable fruit body. The principle methods used are restriction fragment length polymorphisms (RFLPs) of PCR products (Gardes et al. 1991; Kårén et al. 1997) and direct sequencing of PCR products (Glen et al. 2001; Bruns 1996). The use of both techniques is limited by the requirement for data on fungi of known identity. RFLPs are further limited by the existence of sequence variation within a

fungal species, which may result in differing restriction patterns (Kårén et al. 1997). This, however, may be overcome by subjecting multiple individuals of the same species to restriction digests (Bruns et al. 1998).

DNA sequencing is the most reliable method for identifying an unknown fungus unidentifiable by morphological characters (Bruns et al. 1998). Large amounts of sequence data for both the ITS1-5.8S-ITS2 region of the nuclear ribosomal RNA operon (ITS region) and the mitochondrial large subunit rRNA gene are available on GenBank, operated by the National Center for Biotechnology Information (<http://www.ncbi.nlm.nih.gov/>). Sequence from the mitochondrial large region can place a hymenomycete at the familial or subfamilial level with a high level of confidence (Bruns et al. 1998), but is inadequate for resolving identity within a genus or between closely related genera. The ITS region is superior at determining relationships between closely related fungi (Bruns 1996). Furthermore, basidiomycete-specific ITS primers exist (Gardes & Bruns 1993; Glen et al. 2001) which permit amplification of *Amanita* without co-amplification of the ascomycete parasite. Our purpose was to use both RFLPs and sequence data from the ITS region to identify the host species in parasitized specimens of *Amanita*.

Materials and Methods

Specimens used are outlined in Table 3. RFLP patterns and ITS sequences were obtained from reference species from both subgenera and

Table 3. Parasitized and non-parasitized *Amanita* specimens examined

Taxon	Section[a]	Locale	Accession #[b]
Amanita PA 01		Vermont, USA	MSC 380560
Amanita PA 03		Vermont, USA	MSC 380560
Amanita PA 05		Vermont, USA	MSC 380560
Amanita PA 13		Michigan, USA	MSC 380561
Amanita PA 17		Michigan, USA	MSC 380561
Amanita PA 19		Michigan, USA	MSC 380561
Amanita PA 22		Michigan, USA	MSC 380561
Amanita PA 33		Michigan, USA	MSC 380561
Amanita PA 43		Wisconsin, USA	MSC 380562
Amanita PA 8		Michigan, USA	MSC 380561
Hypomyces hyalinus		Wisconsin, USA	MSC 380559
Amanita muscaria orange	*Amanita*	Michigan, USA	MSC 380556
Amanita muscaria var. *alba*	*Amanita*	Michigan, USA	MSC 380555
Amanita muscaria var. *guessowii*	*Amanita*	Micihgan, USA	MSC 380549
Amanita cylindrispora	*Amidella*	New Jersey, USA	RET S. Tulloss 8-11-96-B
Amanita hemibapha var. *hemibapha*[c]	*Caesarae*	Japan	AB015699
Amanita thiersii	*Lepidella*	Illinois, USA	F1127062
Amanita bisporigera	*Phalloidae*	Michigan, USA	MSC 380551
Amanita fulva ss auct. amer.	*Vaginatae*	Indiana, USA	MSC 380554
Amanita sinicoflava	*Vaginatae*	Minnesota, USA	MIN 838924
Amanita vaginata ss auct. amer.	*Vaginatae*	Minnesota, USA	MIN 839788
Amanita brunnescens	*Validae*	Maine, USA	MSC 380552
Amanita cf. *subphalloides*	*Validae*	Indiana, USA	F1116789
Amanita citrina f. *lavendula*	*Validae*	Michigan, USA	MSC 380550
Amanita citrina var. *citrina*	*Validae*	Japan	AB015679
Amanita citrina var. *grisea*	*Validae*	Japan	AB015680
Amanita excelsa sensu D. Reid & Eicker	*Validae*	Belfast, Mpumalanga, South Africa	MSC 375639
Amanita flavipes	*Validae*	Japan	AB015696
Amanita flavoconia	*Validae*	Minnesota, USA	KH94
Amanita flavoconia	*Validae*	Vermont, USA	MSC 380548
Amanita flavoconia (white-stiped form)	*Validae*	New Jersey, USA	RET 9-8-99-J
Amanita novinupta	*Validae*	Oregon, USA	RET 4-14-92-JEL1
Amanita porphyria	*Validae*	Minnesota, USA	DJM 1148
Amanita porphyria	*Validae*	Japan	AB015677
Amanita rubescens[d]	*Validae*	Japan	AB015682
Amanita rubescens ss auct. amer.	*Validae*	Michigan, USA	MSC 380557
Amanita rubescens ss auct. amer.	*Validae*	Michigan, USA	MSC 380558
Amanita rubescens var. *alba*	*Validae*	South Carolina, USA	RET 7-19-86-B
Amanita rubescens var. *congolensis*	*Validae*	Zambia	RET Arora 00-384
Amanita rubescens var. *congolensis*	*Validae*	Zimbabwe	RET Arora 00-443

Table 3. Parasitized and non-parasitized *Amanita* specimens examined.

[a]Section for the parasitized specimens was unknown at the beginning of the study and is not listed.

[b]Numbers beginning "AB" are GenBank accession numbers and refer to sequences from Oda, Taneka & Tsuda (1999). "MSC" = Beal Darlington Herbarium, Michigan State University, East Lansing, MI, USA All MSC specimens were collected by H. Hallen. "MIN" = University of Minnesota herbarium, St. Paul, MN, USA. *A. sinicoflava* and *A. vaginata* were collected by P. Leacock. "DJM" (collected by D.J. McLaughlin) and "KH" (collected by K. Harris) are unaccessioned collections at MIN. "F" = Field Museum of Natural History, Chicago, IL, USA. *A.* cf. *subphalloides* was collected by G. Wesley, det. I. Morrar. *A. thiersii* was collected by H.L. Monoson. "RET" = herbarium of Rodham Tulloss, Roosevelt, NJ, USA. Both specimens of *A.* rubescens var. congolensis were collected by D. Arora. A. *cylindrispora* was collected by S. Tulloss. *A. flavoconia* (white-stiped variety), *A. novinupta* and *A. rubescens* var. alba were collected by R. Tulloss.

[c]The morphology of Japanese specimens denoted *"A. rubescens"* suggests that they are most likely *A. orsonii* (R. E. Tulloss, pers. comm., July 30, 2002)

several sections of the genus *Amanita*, with a special emphasis on section *Validae*.

DNA extraction, amplification and sequencing

Parasitized *Amanita* specimens were collected in the summer of 2000 from Michigan, Vermont and Wisconsin. Herbarium specimens of parasitized *Amanita* were also procured from the University of Michigan. DNA was extracted using a cetyltrimethyl-ammonium bromide (CTAB) method (Scott and Playford 1996). Dried basidiocarps were rehydrated in the respective extraction buffer before grinding. *Amanita* DNA was selectively favored by using sections from the interior of the fruiting body, avoiding the outer layer of perithecia. Tissue was ground with a pestle and 0.2 g sand in 4 mL of the extraction buffer. The extract was then filtered through 1 layer of Miracloth (Calbiochem-Novobiochem Corp., La Jolla, California). The filtrate was purified with phenol:chloroform:isoamyl alcohol (24:24:1) extractions and was centrifuged to remove solids. The water-soluble fraction was precipitated with isopropanol and centrifugation. The precipitate was air-dried under vacuum then resuspended in 50 μl water.

DNA was extracted from mature, non-parasitized *Amanita* fruiting bodies and from *Hypomyces* perithecia following Raeder & Broda (1985).

Approximately 1-20 ng of the total genomic DNA was used per 25 μl reaction mixture for polymerase chain reaction (PCR) amplification. Various brands of prepackaged buffers and polymerases were used for PCR

amplification. The fungal-specific primer ITS 1F (CTTGGTCATTTAGAGGAAGTAA; Gardes & Bruns 1993) was used in combination with basidiomycete-specific ITS 4B (CAGGAGACTTGTACACGGTCCAG; Gardes & Bruns 1993) to selectively amplify the *Amanita* host and to amplify the reference *Amanita* species. Additionally, the basidiomycete-specific primer combination of ITS F (CCCTRTTGCTGAGAAXYTGRTC; Glen et al. 2001) and ITS 4B was tested. ITS 1F was used with the universal primer ITS 4 (TCCTCCGCTTATTGATATGC; White et al. 1990) to amplify the *Hypomyces*.

PCR was performed in a DNA thermal cycler (Perkin-Elmer, Norwalk, CT, USA), using the protocol: 4 min at 70°C (1 cycle); 1.5 min at 94°C, 1 min at 52°C, 1.5 min at 72°C (4 cycles); 1 min at 94°C, 1 min at 55°C, 1.5 min at 72°C (29 cycles). The amplification ended with an additional 10 min extension at 72°C, and storage at 4°C. Annealing temperatures of 50°C (4 cycles)/52°C (29 cycles) and 55°C (4 cycles)/57°C (29 cycles) were also tested. PCR amplification products were separated, and purified following Hughey et al. (2000). Alternatively, products were gel purified and cloned using TOPO® TA (Invitrogen, Carlsbad, CA, USA) or pGEM® (Promega, Madison, WI, USA) cloning kits. Sequencing was performed by the Michigan State University Genomics Technology Support Facility, using dye terminated capillary electrophoresis on an ABI Prism® 3700 DNA Analyzer (Applied Biosystems, Foster City, CA, USA).

Restriction fragment length polymorphisms

MapDraw version 4.05 (DNASTAR, Inc., Madison, WI, USA) was used to evaluate ITS 1F:ITS 4B sequences of 22 reference *Amanita* taxa in order to determine restriction endonucleases that would allow for easy identification. *Alu* I and *Fnu* 4HI were selected. PCR products of both parasitized and non-parasitized *Amanita* specimens were separately digested for 2 - 4 h at 37 C with each restriction endonuclease. The reaction mixture consisted of 1.0 μl of the appropriate restriction buffer (supplied by the manufacturer), 4.8 μl water, 0.2 μl restriction endonuclease and 4 μl PCR product. The restriction products were run for 45 min on a 2% agarose gel at 70 V on an electrophoresis system (Fisher Scientific, Pittsburgh, PA, USA), stained with ethidium bromide and visualized using AlphaImager Version 3.2 (Alpha Innotech Corporation, San Leandro, CA, USA).

Phylogenetic analysis

Sequences were aligned using Clustal W (Thompson, Higgins & Gibson 1994), and were further aligned by eye. The ITS region, covering a portion of the 18S, all of the ITS1, 5.8S and ITS2, and the beginning of the 28S nuclear ribosomal operons, was analyzed for the parasitized *Amanita* specimens and the reference taxa in *Amanita* section *Validae*. *Amanita bisporigera* (section

Phalloidae) was used as an outgroup. Alignment difficulties precluded selecting an outgroup from outside the genus. The alignment was 951 positions, including gaps. Additionally, 340 bp (113 bp from ITS 1, the entire 5.8S region, and 65 bp from ITS 2) were analyzed for parasitized specimens plus representatives from each section of *Amanita*.

As we wished to determine probable identity, and not to formulate evolutionary hypotheses, a distance method was chosen for the analyses. Neighbor joining trees based on Kimura 2-parameter distances were generated using PAUP* 4.0b10 (Swofford 2002). 1000 hundred bootstrap replications were run using Kimura 2-parameter distance as the optimality criterion to attain bootstrap values. Bootstrap values are printed beside the branches; values less than 50 are not shown.

Results

Annealing temperatures between 52°C and 57°C were tested in the PCR reactions. A 52°C annealing temperature resulted in multiple bands for parasitized *Amanita* specimens due to lowered stringency, while 57°C resulted in less product (a weaker band) than 55°C. An annealing temperature of 55°C was optimal. Primer ITS F, a basidiomycete-specific substitute for ITS 1F, was evaluated but yielded multiple bands upon amplification, even at 57°C, possibly due to internal primer recognition sites.

Out of 46 parasitized *Amanita* specimens from which DNA was extracted, fifteen yielded discernible PCR products when amplified with ITS 1F:ITS 4B. Herbarium specimens approximately 30 years old did not yield PCR products. RFLP patterns (Figs. 9-12) were able to provide positive identifications of three parasitized *Amanita* specimens. Specimens PA 13 and PA 33 matched *Alu* I and *Fnu* 4HI digests of *A. novinupta* Tulloss & J. Lindgren. Specimen PA 43 matched reference material of *A. brunnescens* Atk. and *A. citrina* sensu auct. amer., which differed from *A. rubescens* sensu auct. amer. by the presence of a faint band of approximately 350 bp in the *Fnu* 4HI digest. Specimen PA 01 was identified as either *A. rubescens* sensu auct. amer. or *A. flavoconia* Atk. Specimens PA 03, PA 08, PA 19 and PA 22 did not match any of the reference material in the *Fnu* 4HI digest.

Figure 10. Alu 1 digest of parasitized *Amanita* specimens. Shapes indicate matches to the reference gel (Fig. 9)

Figure 9. Alu 1 digest of reference *Amanita* specimens. Shapes indicate reference to the parasitized *Amanita* gel (Fig. 10).

Figure 12. Fnu 4H1 digest of parasitized *Amanita* specimens. Stars indicate matches to the reference gel. (Fig. 11)

Figure 11. Fnu 4H1 digest of reference *Amanita* specimens. Stars indicate reference to the parasitized *Amanita* gel (Fig. 12).

We were able to obtain sequence for nine parasitized specimens. Based on the neighbor joining trees (Figs. 13-14) specimens PA 13, PA 22 and PA 33 were identified as *Amanita novinupta*. PA 43 was identified as *A. brunnescens*. PA 1 was identified as *A.* aff. *flavoconia*. These findings supported the tentative identifications of parasitized *Amanita* specimens made on the basis of the RFLPs. PA 08 and PA 19 grouped with the *A.* "*rubescens*" from the study of Oda, Taneka & Tsuda (1999), which is most likely *A. orsonii* Kumar & Lakanpal (R. E. Tulloss, pers. comm., 30 July 2002). We were unable to obtain reference RFLP patterns from *A. orsonii*. The sequence obtained for PA 17 showed 93% homology with members of the family *Thelephoraceae* over the entire 657 bp sequence when subjected to a BLAST search. The DNA extraction, PCR reaction, purification, cloning and sequencing of PA 17 were repeated, and the same result was obtained. The sequence obtained from PA 03 showed 87-90% sequence homology with *Russula* and *Lactarius* species over the 18S, 5.8S and ITS 2 regions and a portion of the ITS 1 region. No significant homology with species in any Section of the genus *Amanita* was found for PA 03 or 17, which could not be aligned with the other *Amanita* sequences.

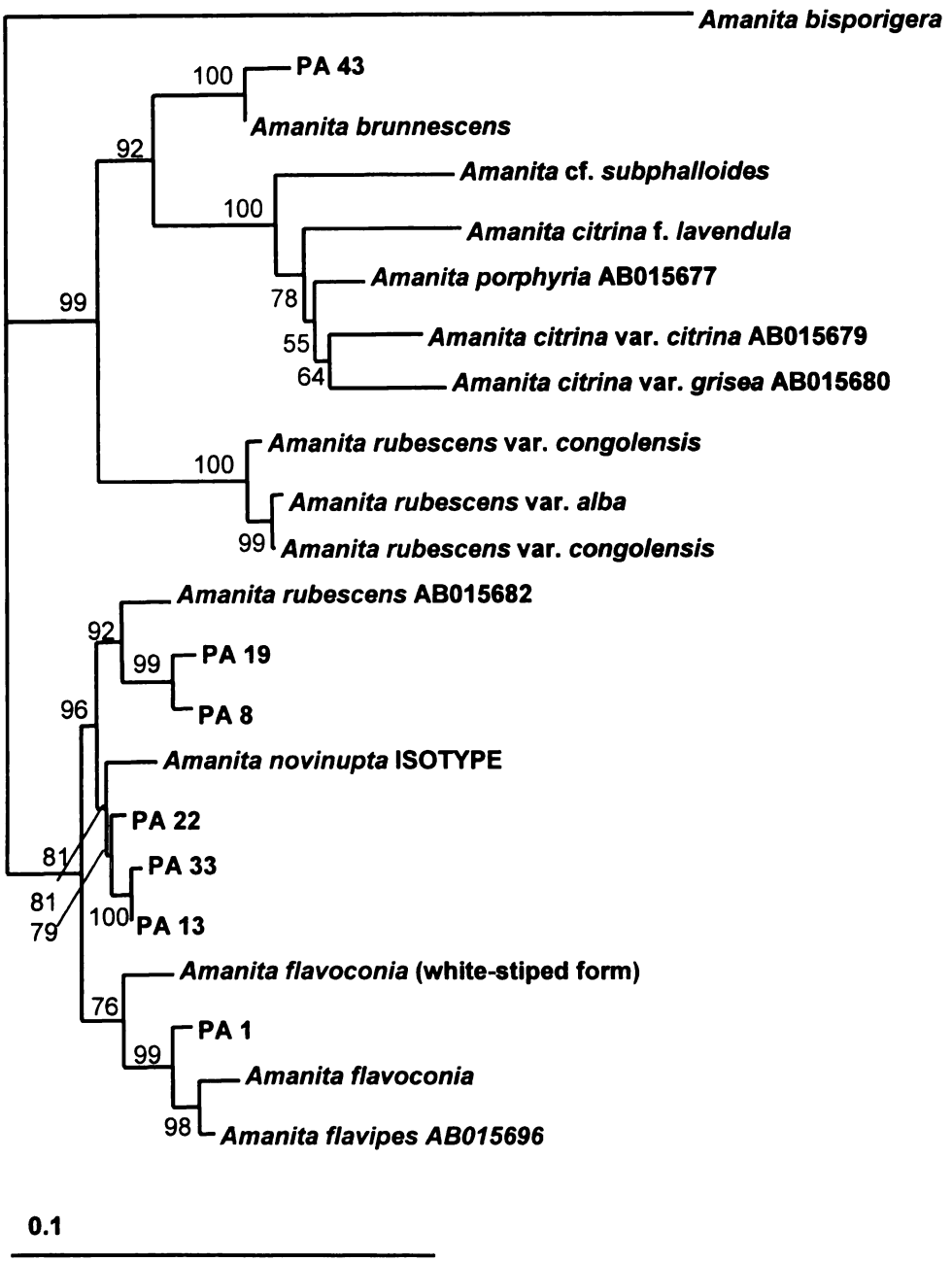

Fig. 13. Neighbor-joining tree of the ITS region of parasitized *Amanita* specimens and *Amanita* Section *Validae*. Numbers at nodes are bootstrap indices of support (%). Branch lengths correspond to genetic distance (expected number of nucleotide substitutions per site).

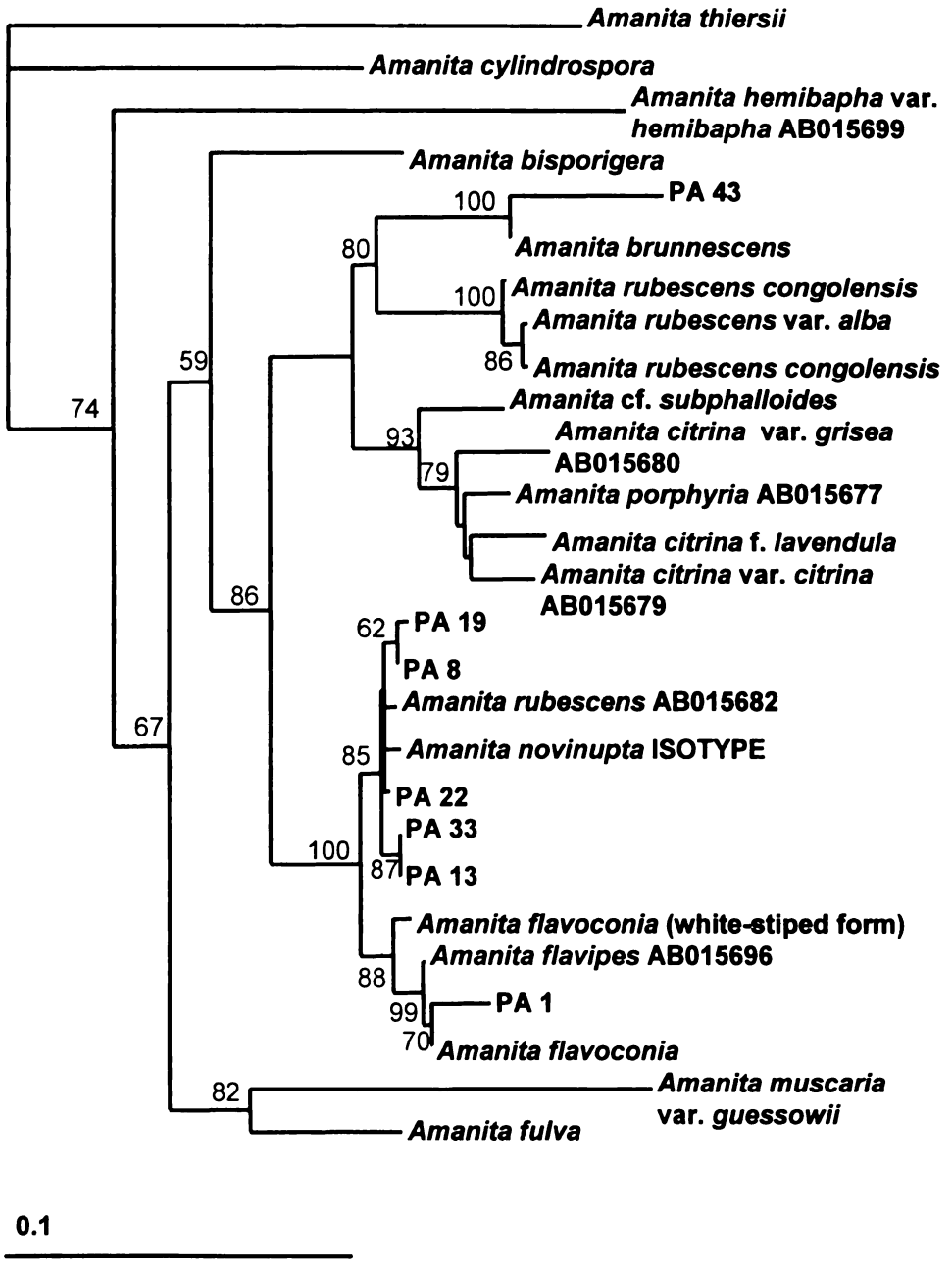

Fig. 14. Neighbor-joining tree of the 5.8S and partial ITS 1 and ITS 2 regions of parasitized *Amanita* specimens and representative *Amanita* species from other sections in the genus. Numbers at nodes are bootstrap indices of support (%). Branch lengths correspond to genetic distance (expected number of nucleotide substitutions per site).

Discussion

RFLPs were sufficient in three of the nine specimens examined to identify the parasitized *Amanita*. The cases in which RFLPs did not yield an identification were due to lack of the appropriate reference taxon (PA 08 and 19), amplification of basidiomycete contaminants (PA 03 and 17), and insufficient resolution of reference taxa by the restriction endonucleases used (PA 01 and 22). We based our restriction enzyme selection on the cut sites generated on one member of each species. The inability of restriction digests to match PA 01 conclusively with *A. flavoconia* and PA 22 with *A. novinupta* shows that restriction endonuclease choice was suboptimal. Kårén and colleagues (1997) observed sufficient intraspecific variation in *Mbo* I digests of ITS PCR products to enable them to accurately identify 27 species of ectomycorrhizal fungi, including several *Amanita* species. Expanding the collection of restriction digests of reference taxa, and possibly adding *Mbo* I digests, would improve the chances of accurately matching a parasitized specimen to a known species. Sequencing of the ITS region is more time-consuming than RFLP analyses, but provides superior resolution. When possible, we would recommend sequencing over RFLPs for identifying unknown fungi, due to the possibility of multiple taxa sharing RFLP banding patterns.

A. rubescens (Pers.:Fr.) Pers. is an Old World taxon and is not known from North America (Tulloss & Lindgren 1994). *A. novinupta* and *A. orsonii* are rubescent taxa phenetically closely allied to *A. rubescens* (Pers.:Fr.) Pers. Both

taxa have been called *A. rubescens* by western North American authors and southern and eastern Asian authors, respectively. The identification of five parasitized *Amanita* specimens as *A. novinupta* and *A. orsonii* is therefore in agreement with North American reports of *A. rubescens* sensu lato as a primary host for *H. hyalinus*. *A. rubescens* is an Old World taxon, and is not known from North America or southern Asia (Tulloss & Lindgren 1994; Tulloss et al. 2001). On the other hand, the groupings with *A. orsonii* and *A. novinupta* suggest that there is insufficient resolution provided between taxa, since neither taxon is known to occur in Michigan based on current literature.

Singer (1986) placed *A. brunnescens* in *Amanita* section *Mappae,* separate from section *Validae*. However, Singer's distinction was based on the presence of bufotenine in *A. citrina* and *A. brunnescens*. Recent phylogenetic analyses (Weiß, Yang & Oberwinkler 1998; Drehmel, Moncalvo & Vilgalys 1999; Oda, Tanaka & Tsuda 1999) suggest that this chemotaxonomic character is insufficient to merit the separation of *Mappae* and *Validae*. Bufotenine is a hallucinogenic compound frequently found on the skin of toads. While bufotenine is not active in humans when ingested (Benjamin 1995), *A. brunnescens* is considered mildly poisonous and should not be eaten. The fact that *A. brunnescens* can be parasitized by *H. hyalinus* provides reason for warning that parasitized *Amanita*s should not be used as food.

Clearly, several species of *Amanita* are being parasitized by *Hypomyces hyalinus*. All parasitized species identified to date have been members of section *Validae*, if they could be identified as *Amanita* species, but the small sample size

does not preclude the possibility of other sections being susceptible. While we did not confirm reports based on morphology and proximity of *A. bisporigera* as a host (Lincoff 1981), Section *Phalloidae* is the sister group to section *Validae* (Weiß, Yang & Oberwinkler 1998; Drehmel, Moncalvo & Vilgalys 1999; Oda, Tanaka & Tsuda 1999), and, as such, might be susceptible to parasitism.

PA 03, from which *Russulaceae* sequence was obtained, and PA 17, from which *Thelephoraceae* sequence was obtained, merit discussion. Members of the genera *Russula* and *Lactarius* are parasitized by *Hypomyces lactifluorum* (Schweinitz:Fr.) Tulasne. The *Hypomyces* entirely covers the *Russula* or *Lactarius* fruiting body, producing a sterile, distorted mushroom. However, the morphology of *Russulaceae* infected by *H. lactiflorum* differs significantly from that of *Amanita* infected by *H. hyalinus*. An infected *Amanita* is club-shaped, with minimal expansion of the pileus, while infected *Russulaceae* show a broad, funnel-shaped pileus with folds visible where the lamellae were initiated. *H. hyalinus* is pink to white and does not discolor in KOH, while *H. lactifluorum* is bright orange-red and immediately stains purple in 4% KOH (Rogerson & Samuels 1996). *H. lactifluorum* produces equally two-celled ascospores with prominent apices, while the ascospores of *H. hyalinus* are divided into two unequal cells and the apices are not prominent. These characters have enabled us to rule out the possibility of misidentification of the *Hypomyces* species. The parasite of PA 03 was consistent with *Hyopmyces hyalinus* on the bases of ascospore characters and KOH reaction, Fruiting bodies of the *Russulaceae* possess characteristic swollen cells (sphaerocysts), which are visible in infected

specimens, while *Amanita* lacks sphaerocysts. We found no sphaerocysts in PA 03. Therefore, the fruiting body is not one of a *Russula* and the parasite is not one known to infect the *Russulaceae*.

Members of the *Russulaceae* and *Thelephoraceae* are ectomycorrhizal, as are *Amanita* species. Recent works have shown that members of the ectomycorrhizal community are in intimate association with one another, enabling minerals and nutrients to flow not only from plant to fungus, but from fungus to fungus and from plant to plant, via fungal intermediaries (Simard et al. 1997; Halling 2001). We hypothesize that hyphae from the other mycorrhizal fungi were able to invade the *Amanita* specimens, possibly as a consequence of the *Hypomyces* infection.

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CHAPTER 3

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AMATOXINS AND PHALLOTOXINS IN INDIGENOUS AND INTRODUCED SOUTH AFRICAN *AMANITA* SPECIES

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Abstract

The production of lethal amatoxins and phallotoxins in species of *Amanita* from South Africa was investigated by HPLC analyses. The indigenous mushrooms *Amanita foetidissima* and *A. pleropus* tested negative for production of these toxins. Several introduced species were analyzed; of these, *A. phalloides* var. *phalloides*, *A. phalloides* var. *alba* and *A. reidii* contained amatoxins and phallotoxins. Despite reports of rapid degradation of phallotoxins upon drying, phallotoxins and amatoxins were both readily detectable in dried herbarium specimens up to 17 years old. Previous reports of phallotoxins in *A. rubescens* were not substantiated.

Introduction

Members of the genus *Amanita* Pers., with their characteristic white spores, free gills, and the presence of both a universal and a partial veil, are among the most readily recognized fleshy fungi. This genus has been a subject of intensive study over the past century (Corner & Bas 1962; Bas 1969; Jenkins, 1977; Wieland 1986; Reid & Eicker 1991; Yang 1997).

Several species of *Amanita* occur in South Africa (Reid & Eicker 1991; van der Westhuizen & Eicker 1994). Many species, such as *A. excelsa* (Fr.) Kummer, *A. muscaria* (L.:Fr.) Pers., *A. pantherina* (DC.:Fr.) Krombh., *A. phalloides* (Fr.:Fr.) Link and *A. rubescens* (Pers.:Fr.) Pers., are believed to have been introduced from Europe on trees as mycorrhizal associates (Reid & Eicker 1991). *Amanita reidii* Eicker & van Greuning was described from a South African specimen but occurs only in association with *Eucalyptus* species and may have been introduced from Australia. *Amanita pleropus* (Kalchbr. & MacOwan) Reid and *A. foetidissima* Reid & Eicker are believed to be indigenous.

Amanita is a large genus, with several hundred species (Hawksworth et al. 1995) divided between two subgenera and several sections. Subgenus *Lepidella* is characterized by the blackening of the spores in iodine (amyloid reaction), while subgenus *Amanita* has inamyloid spores. Subgenus *Lepidella* contains sections *Amidella, Lepidella, Phalloideae* and *Validae*. Subgenus *Amanita* contains sections *Amanita* and *Vaginatae*. Sections are sensu Corner & Bas

(1962), and are further distinguished on the basis of universal and partial veil characters (Corner & Bas, 1962, Jenkins 1986).

The genus has been subject to particular scrutiny due to the production of toxins by several species (Wieland 1986). Toxins produced by *Amanita* species include the central nervous system toxin ibotenic acid, produced by certain species of *Amanita* section *Amanita* (e.g., *A. muscaria* and *A. pantherina*) and the hallucinogen bufotenine, produced by *A. citrina* (Schaeff.) Pers and *A. brunnescens* Atk. Most important are the two families of cyclic peptide toxins, amatoxins and phallotoxins, that are produced by several species of *Amanita* section *Phalloideae* (e.g., *A. phalloides, A. virosa* Lamarck, *A. verna* (Bull.:Fr.) Lamarck, and others). *Amanita phalloides* is known to produce relatively high quantities of α-, β- and γ–amatoxins, the phallotoxins phalloidin and phallacidin, and smaller quantities of related chemicals (Wieland 1986). Amatoxins tend to be localized in the lamellae and annulus, while the area of highest phallotoxin concentration is usually the volva (Enjalbert, Bourrier & Andary 1989; Enjalbert, Cassanas & Andary 1989; Enjalbert et al. 1993). In species that produce amatoxins and phallotoxins, both types of toxins are detectable in lamellar tissue (Enjalbert et al. 1992; Hallen, unpublished results). Phallotoxins have only been reported in the genus *Amanita*. Amatoxins are found in three other genera: *Conocybe* Fayod, *Galerina* Earle and *Lepiota* (Pers.) Gray (Benjamin 1995).

Amatoxins and ibotenic acid have both been implicated in fatal human and animal poisonings (Wieland 1986; Benjamin 1995; Naudé & Berry 1997). The isoxazole toxins, ibotenic acid and its metabolite muscimol, will rarely kill an

adult; most fatal outcomes are in child or animal poisonings (Benjamin 1995, Naudé & Berry 1997). Amatoxins are frequently lethal, and are responsible for 90% of fatal human mushroom poisonings worldwide (Benjamin 1995). Amatoxins are potent inhibitors of RNA polymerase II (RNA polymerase B), indirectly halting protein synthesis (Wieland 1986). The human LD_{50} is 0.1 mg kg^{-1} body weight. This is approximately 7 mg toxin for an adult male, or approximately 1 cm^3 of tissue from *A. phalloides*. Phallotoxins are structurally similar to amatoxins and are hypothesized to share a common biosynthetic pathway. Phallotoxins have not been implicated in human poisonings because they are not absorbed from the gastrointestinal tract (Benjamin, 1995).

In this study, high-performance liquid chromatography (HPLC) has been used to evaluate a number of mushrooms from South Africa for presence of two amatoxins, α- and β-amanitin, and two phallotoxins, phalloidin and phallacidin. We utilized an HPLC protocol that has been proven sensitive enough to detect toxins in nanogram quantities (Enjalbert et al. 1992). We further confirmed the presence of the toxins by mass spectrometry. While detailed toxicological studies of many of the northern hemisphere species of *Amanita* have been conducted (Malak 1974; Beutler 1980; Wieland 1986), this is the first report of evaluations of endemic and introduced species collected in South Africa.

Materials and methods

The specimens evaluated are detailed in Table 4.

Table 4: Analysis of amatoxins and phallotoxins in *Amanita* species.

Taxon	Section	Provenance	Year collected	Accession #[a]	α[b]	β[b]	C[b]	H[b]
A. "capensis"[c,e]	Unknown	Mpumalanga	1992	PRU 3356	-	-	-	-
A. excelsa	*Validae*	Belfast, Mpumalanga	1998	MSC 375639	-	-	-	-
A. excelsa[d]	*Validae*	Belfast, Mpumalanga	1999	MSC 375640	-	-	-	-
A. foetidissima	*Lepidella*	Pretoria	1992	PRU 3505	-	-	-	-
A. foetidissima	*Lepidella*	Pretoria	1993	PRU 3498	-	-	-	-
A. foetidissima	*Lepidella*	Pretoria	1994	PRU 4168	-	-	-	-
A. muscaria	*Amanita*	Pretoria	1998	MSC 377980	-	-	-	-
A. nauseosa	*Lepidella*	LC deVilliers sports ground	1989	PRU 2703	-	-	-	-
A. pantherina	*Amanita*	Pretoria	1991	PRU 3156	-	-	-	-
A. pantherina	*Amanita*	Mpumalanga	1993	PRU 3667	-	-	-	-
A. pantherina	*Amanita*	Sabie, Mpumalanga	1998	MSC 375641	-	-	-	-
A. pantherina	*Amanita*	Pretoria	1999	MSC 375642	-	-	-	-
A. pantherina	*Amanita*	Belfast, Mpumalanga	1999	MSC 375643	-	-	-	-
A. phalloides var. *phalloides*	*Phalloidae*	Pretoria	1994	PRU 3959	+	+	+	+
A. phalloides var. *phalloides*	*Phalloidae*	unknown	1994	PRU 4258	+	+	+	+
A. phalloides var. *phalloides*[d]	*Phalloidae*	Pretoria	1998	MSC 375644	+	+	-	+
A. phalloides var. *phalloides*	*Phalloidae*	Saasveld, George Cape	1983	PRE 47293	+	+	+	+
A. phalloides var. *alba*	*Phalloidae*	Bergvliet State Forest, Sabie	1985	PRE 48659	+	+	+	+
A. phalloides f. *umbrina*	*Phalloidae*	Bergvliet State Forest, Sabie	1985	PRE 48654	+	+	+	+
A. phalloides f. *umbrina*[d,e]	*Phalloidae*	Bergvliet State Forest, Sabie	1984	PRE 48618	+	+	+	+
A. pleropus[d]	*Lepidella*	Brummesia National Research Institute Gardens, Pretoria	1984	PRE 47480	-	-	-	-
A. reidii[d,e]	*Phalloidae*	Hide-away, Melkrivier, Northern Province	1990	PRU 4306	+	+	+	+
A. rubescens	*Validae*	Belfast, Mpumalanga	1998	MSC 375645	-	-	-	-
A. rubescens	*Validae*	Belfast, Mpumalanga	1998	MSC 375646	-	-	-	-
A. rubescens[d]	*Validae*	Belfast, Mpumalanga	1999	MSC 375647	-	-	-	-
A. rubescens	*Validae*	Pretoria	1999	MSC 375930	-	-	-	-
A. species[e]	*Lepidella*	Lynnwood Glen Nature Reserve	1993	PRU 3611	-	-	-	-
A. species[e]	Unknown	Darow, Cape Province	1996	PRU 4149	-	-	-	-

Table 4. Analysis of amatoxins and phallotoxins in *Amanita* species.
[a]MSC = Beal-Darlington Herbarium, Michigan State University, East Lansing, MI, USA 48824-1312. PRU = H. G. W. J. Schweickerdt Herbarium, Botany Department, University of Pretoria, Pretoria 0002, Gauteng Province, South Africa. PRE = National Herbarium, National Botanical Institute, Private Bag X101, Pretoria 0001, Gauteng Province, South Africa.
[b]α = α-amanitin; β = β-amanitin; C = phallacidin; H = phalloidin. – indicates no toxin was detectable; + indicates that toxin was detected.
[c]*Amanita capensis* lacks a type specimen and has never been validly published, so the identification and taxonomic affinities of this taxon are uncertain. Quotation marks are added to indicate its uncertain affinities.
[d]Multiple specimens from this collection were assayed, results were the same for all specimens.
[e]Specimen is being further examined by DNA sequence analysis for species determination (Hallen, unpublished results).

Fungi were evaluated for toxins using a modification of the method of Enjalbert et al. (1992). Dried specimens were rehydrated in KOH, then rinsed thoroughly with distilled water. Excess water was blotted from the specimens and specimens were then diced and weighed. Eight to 200 mg of the tissues were suspended in 1.5 ml extraction medium containing methanol:distilled water:0.01M HCl (5:4:1) g^{-1} tissue., Suspended tissues were incubated at 4°C for 12 h. Methanol was HPLC grade (J.T. Baker, Phillipsburg, New Jersey, USA). Samples were then centrifuged at 1000 x g and 4°C for 10 min, and the supernatant was collected. The pellets were resuspended in 0.6 ml extraction medium g^{-1} tissue, incubated at 4°C for an additional 12 h and centrifuged. The supernatants from the first and second centrifugation were pooled. Extractions were from lamellar tissue for all samples except *A. rubescens*. Both the lamellae and the volva were used in *A. rubescens* to facilitate testing for phallotoxins which have been reported in this species (Malak, 1974).

HPLC analysis of amatoxins and phallotoxins was performed on a Model 114 HPLC apparatus (Beckman Instruments, Inc., Fullerton, California, USA) with detection at 295 nm. Amatoxins and phallotoxins were separated using a 0711-0231 C-18 column (Perkin-Elmer Corporation, Norwalk, Connecticut, USA) and a 30 min gradient of solution A to solution B. Solution A was 0.2 M ammonium acetate, adjusted to pH 5 with glacial acetic acid, and solution B was acetonitrile. Flow rate was 1 ml min^{-1}. Samples were maintained at a temperature of 4°C until injection. Twenty μl of each sample were injected.

Standards were purified α-amanitin, β-amanitin, phallacidin and phalloidin (Sigma Chemical, St. Louis, Missouri, USA). Each toxin was at a concentration of 100 μg ml^{-1}, which is comparable to the concentration of toxins naturally occurring in *A. phalloides* (Enjalbert et al., 1992).

Peaks eluted at approximately 70 - 80 % acetonitrile (Figure 21). Putative toxin peaks were identified by comparison with the toxin standards, and eluted fractions were manually collected from the HPLC apparatus. Eluted fractions were subjected to fast atom bombardment (FAB) mass spectrometry, at the Mass Spectrometry Facility at Michigan State University, to confirm identity. FAB mass spectra were obtained using a model HX-110 double-focusing mass spectrometer (JEOL USA, Peabody, Massachusetts, USA) operating in the positive ion mode. Ions were produced by bombardment with a beam of xenon atoms (6 kV). The accelerating voltage was 10 kV and the resolution was set at 1000. The instrument scanned from *m/z* (mass to charge ratio) 50 to 1500.

Results and Discussion

The results of the analyses are shown in Table 4. It was found that only species in *Amanita* section *Phalloideae* showed presence of amatoxins or phallotoxins. These species included *A. reidii, A. phalloides* var. *phalloides*, *A. phalloides* var. *alba* Gillet (= *A. phalloides* f. *alba* Britzelm), and *"A. phalloides* f. *umbrina"* (use of quotation marks is explained below). Each of these species showed HPLC peaks that agreed with the standards of α- and β-amanitin,

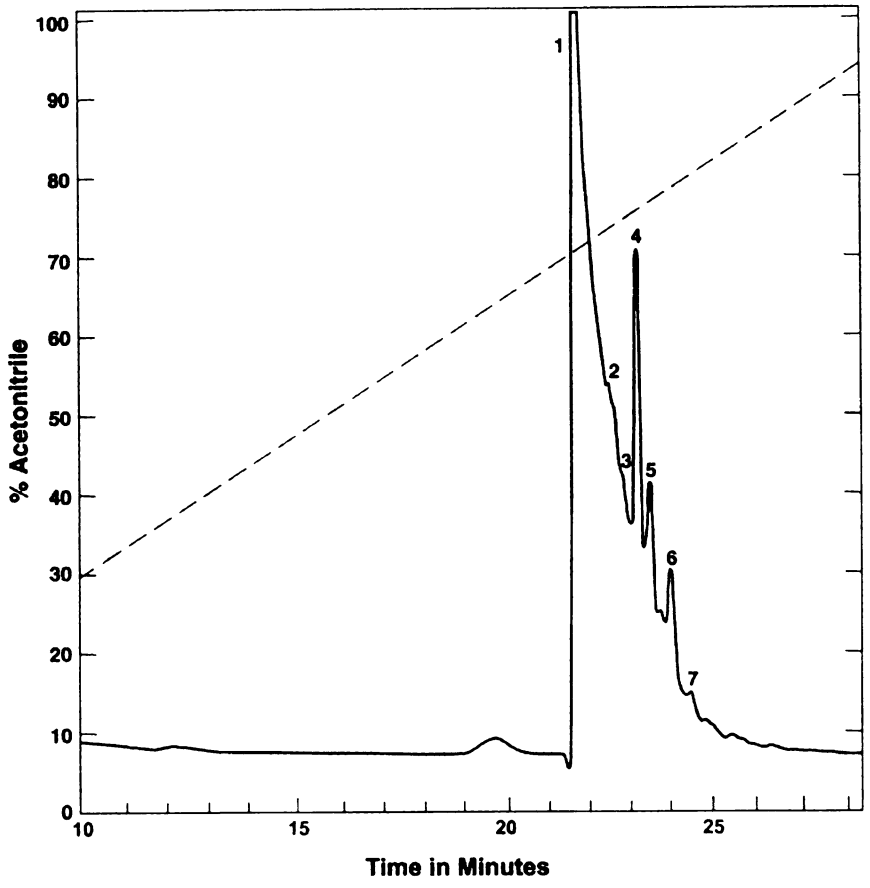

Figure 15. HPLC results for *Amanita phalloides* f. *umbrina* (= *A. reidii*) PREM 48654. The dashed line indicates the percent acetonitrile. Solid line shows absorbance at 295 nm. Peak #1 represents β-amanitin, 3 α-amanitin, 5 phallacidin and 6 phalloidin. Peak 4 is likely γ-amanitin; this could not be confirmed due to the lack of a γ-amanitin standard.

phalloidin and phallacidin. Other amatoxins and phallotoxins, for which standards were not available, may have been present. Peaks identified by HPLC (Figure 15) were confirmed by FAB mass spectroscopy of the eluted fractions in *A. reidii* and "*A. phalloides* f. *umbrina*".

The duration between collection of the mushrooms and HPLC analysis ranged from less than one month to 17 years. Despite reports of rapid degradation of phallotoxins upon drying (Stijve & Seeger 1979; Klán & Baudišová 1993), both phallotoxins and amatoxins were readily detectable in dried

herbarium specimens of "*A. phalloides* f. *umbrina*" up to 17 years old. We have detected both toxins in *Amanita* species up to 21 years old, but there is a diminution in peak strength with increasing sample age (Hallen, unpublished results). Apparently, following a sharp decrease in the concentration of the heat-labile phallotoxins during drying, there is little degradation over time of the remaining phallotoxins.

The distribution of amatoxins in mushrooms has long been a subject of controversy. Faulstich and Cochet-Meilhac (1976) reported the presence of trace quantities of amatoxins in all mushroom species tested, including the common edible species *Agaricus bisporus* (J. E. Lange) Pilát using radioimmunoassay (RIA). Preston et al. (1982) also detected trace quantities of amatoxins in edible mushrooms, using *in vitro* inhibition of RNA polymerase II activity. Collectively, these findings were taken to indicate that all basidiomycetes produce amatoxins. This was rapidly promulgated through the literature (e.g., Wieland & Faulstich 1978; Horgen et al. 1978) but these findings were later refuted (Enjalbert et al. 1993) because of methodological considerations. The levels of toxin detected by Faulstich & Cochet-Meilhac were at the limits of detection for the RIA procedure. These levels could be accounted for by contamination. When Faulstich repeated the assay in a different laboratory using new glassware, no toxins were detected in edible fungi (Wieland 1986). Similarly, Preston and colleagues based their evaluations solely on inhibition of calf thymus RNA polymerase II, without any further assays. The levels of putative toxin detected in nontoxic species, including *Amanita* species such as *A. brunnescens*,

were near the limits of detection for this methodology. No toxins have been detected in these species following extensive testing using more sensitive HPLC procedures (Enjalbert et al. 1992, 1993; Hallen, unpublished results).

The edible species *A. rubescens* did not contain detectable toxins in our studies. Neither phallotoxins nor amatoxins were detected in either lamellar or volval preparations of *A. rubescens*. This contradicts the report of the detection of phallotoxins in *A. rubescens* using thin layer chromatography (Malak, 1974). The edible *A. excelsa,* and the poisonous *A. pantherina* and *A. muscaria,* contained no detectable amatoxins or phallotoxins using our analytical techniques. However, our methods do not detect other toxins, notably ibotenic acid or muscimol. Neither amatoxins nor phallotoxins were detected in the indigenous species *A. foetidissima, A. pleropus*, the species of uncertain identity *A. "capensis"*, or the unidentified species PRU 3611 and PRU 4149. Tests for other fungal toxins need to be performed, and more specimens need to be examined, before these species are considered safe to eat.

Despite the fact that *A. phalloides* f. *umbrina* was originally used to describe aged specimens of *A. phalloides* (Ferry, 1911), the name came into colloquial use in South Africa in referring to the streaked, gray-brown mushroom now known as *A. reidii* (Eicker, van Greuning & Reid 1993). Thus the colloquial usage (which we have denoted by quotation marks: *"A. phalloides* f. *umbrina*") may be considered synonymous with *A. reidii,* as in van der Westhuizen & Eicker (1994), while the original sense is not taxonomically valid (Eicker, van Greuning & Reid 1993). *Amanita reidii* may be synonymous with the Australian *A.*

marmorata ssp. *marmorata* Cleland & Gilbert and the Hawaiian *A. marmorata* ssp. *myrtacearum* O.K. Miller, D. Hemmes & G. Wong (R. Tulloss, personal communication). These taxa are all mycorrhizal on *Eucalyptus*, and appear to have accompanied their host plant from Australia. *Amanita reidii* was placed in section *Phalloideae* based on morphological characters, and has been presumed toxic due to its affinities. This study is the first direct analysis of amatoxins and phallotoxins in this taxon, and confirms the presence of the toxins.

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CHAPTER 4

TAXONOMY AND TOXICITY OF *CONOCYBE LACTEA* AND RELATED SPECIES

Abstract

Conocybe lactea was examined as part of a larger study on the distribution of amatoxins and phallotoxins in fungi, and the taxonomic relationships between these fungi. Because amatoxins are present in a congener, *C. filaris*, the locally abundant *C. lactea* was examined using HPLC and mass spectroscopy. Amatoxins were not found, but the related phallotoxins were present in small quantities. *C. lactea* was the first fungus outside of the genus *Amanita* in which phallotoxins have been detected. Despite the presence of a related toxin, *C. lactea* was found not to be taxonomically close to *C. filaris*. Phylogenetic analyses using nuclear ribosomal RNA genes indicated that North American specimens of *C. lactea* were conspecific with North American specimens of *C. crispa* in *Conocybe* Section *Candidae*. European *C. crispa* was a distinct taxon. The implications of Hausknecht's use of the name *Conocybe albipes* for these taxa are discussed. Nucleotide data confirmed placement of the sequestrate taxon *Gastrocybe lateritia* in Section *Candidae*, but as a distinct taxon. It is hypothesized that the unique sequestrate morphology of *G. lateritia* may be caused by a bacterial infection.

Introduction

The genus *Conocybe* Fayod (*Bolbitiaceae, Agaricales*), with 70 species, is the largest genus in the *Bolbitiaceae* (Hawksworth et al. 1995). *Conocybe* species occur worldwide. A variety of secondary metabolites are produced within the genus. *Conocybe cyanopus* (Atkinson) Kühner and *C. smithii* Watling, like many blue-staining agarics, contain the hallucinogenic compound psilocybin (Benedict et al. 1962; Benedict, Tyler & Watling 1967). The cyclic peptide amatoxins are present in North American collections of *C. filaris* (Brady et al. 1975), but have not been found in European collections (Benjamin 1995). *C. lactea* (Lange) Métrod produces an unidentified nematicidal compound in culture (Hutchison, Madzia & Barron 1995).

Conocybe lactea is a common mushroom on cultivated lawns and meadows in North America (Arora 1986; Bessette, Bessette & Fischer 1997), the UK (Watling 1982), Europe, Asia and northern Africa (Breitenbach & Kränzlin 1995). In the central and northern United States and Canada, *C. lactea* fruits between early June and early September (Kauffman 1918; Hallen, pers. obs.) The mushrooms are ephemeral, with the buttons first visible around 8:00 p.m. Caps expand during the course of the night, but spores do not normally mature until 9:00 a.m. the following morning. Spore discharge occurs between 9:00 a.m. and 11:00 a.m., by which time the fungi normally appear shriveled and

deliquescent to desiccated. Collapse of the fruit bodies usually follows (Hallen, pers. obs.).

Conocybe lactea is distinguished by its conical, white to buff pileus. The spores are smooth, ochre-brown and possess a large germ pore. There are four spores per basidium and distinctive lecythiform marginal cystidia are present. The pileus color, spore type, cystidial type, and deliquescent nature of the fungus are the defining features of *Conocybe* section *Candidae*. The Section is composed of *C. lactea*, *C. crispa* (Longyear) Singer, *C. crispella* (Murrill) Singer and *C. subcrispa* (Murrill) Singer (Singer 1986). *C. crispella* and *C. subcrispa* are small, subtropical taxa of limited distribution and are rarely collected. *C. crispa* differs from *C. lactea* in the possession of two-spored basidia and "crisped", or wavy, lamellae. *C. crispa* shares the distribution of *C. lactea*, but is less common. Ecological characteristics of these species are identical. Some authors consider *C. crispa* to be a form of *C. lactea* (Watling 1982; Breitenbach & Kränzlin 1995). The number of spores per basidium can vary considerably within a single fruiting body of either species (Breitenbach & Kränzlin 1995) and intermediate forms, possessing both crisped and straight lamellae, have been observed (D. Malloch, pers. comm., 10 Feb. 2001).

Gastrocybe lateritia Watling is known primarily from northeastern North America (Bessette, Bessette & Fischer 1997). Like *C. lactea* and *C. crispa*, it is a grass-inhabiting species forming ephemeral fruiting bodies between dusk and mid-morning the following day, from June to September. Fruiting bodies possess, on average, four spores per basidium. Watling, in the type description, describes

the pileus as "ellipsoid-campanulate or conic, hardly or never expanding, greasy to viscid rapidly becoming reduced to a gelatinous mass," (Watling 1968). The pileus is normally ochre-brown upon maturity due to the coloration of the spores showing through the translucent pilear tissue. In immature specimens pileus color resembles that of *C. lactea*. Forcible spore discharge is lacking, due to the gelatinous-deliquescent nature of the pileus (Watling 1968; Weber 1989; Hallen, pers. obs.) The stipe is frequently longer in proportion to the pileus size than that of *C. lactea* or *C. crispa*. At maturity, the fragile, elongate stipe can no longer support the weight of the gelatinous pileus and the mushroom collapses (Weber 1989).

G. lateritia shares the ecology of *C. lactea* and *C. crispa*. The habitat, season, fruiting time and duration are identical. The similarities between these taxa are noted by Watling: "It is characteristic of members of the *C. lactea* group ... for the pileus to become tacky and soft when mature and this same group parallels [*G. lateritia*] in that the pileus is often long and slender, cylindrical and only slightly expanding; the stipe is also white or hyaline," (Watling 1968). Despite these similarities, the genus *Gastrocybe* was created to accommodate this taxon solely on the basis of its unusual sequestrate nature. Intermediate forms occur between *G. lateritia* and *C. lactea* and, rarely, between *G. lateritia* and *C. crispa*. In these forms, the surface of the pileus may remain dry or only slightly tacky while the gills deliquesce before completing development. Alternately, the ordinary *G. lateritia* morphology complete with fully gelatinous

pileus may develop, but the coloration remains white to buff as in *Gastrocybe* buttons or mature *C. lactea* and *C. crispa* specimens.

In this paper, we investigate the relationships between *C. lactea, C. crispa* and *G. lateritia* using sequence data from the ITS1, ITS 2, 5.8 S and a portion of the 28S regions of the nuclear ribosomal RNA operon. These regions have been shown to be of good utility in examining relationships between closely related fungi (White et al. 1990). Representative species from each section of *Conocybe*, as well as *Bolbitius* and *Agrocybe* species, have been sequenced to provide resolution. Additionally, several sequestrate taxa with presumed affinities to the *Bolbitiaceae* have been sequenced.

Because certain specimens of *C. filaris* produce the potentially deadly amatoxins, HPLC was used to evaluate the locally abundant *C. lactea* and allied taxa for both amatoxins and the related phallotoxins as part of a larger study of the distribution of these toxins. Mass spectrometry was used as a complementary procedure.

Materials and methods

The specimens examined are detailed in Table 5.

Table 5. Specimens of *Conocybe* and related genera examined.

Taxon	Locale[c]	Year collected	Accession #
Agrocybe praecox	Ingham Co., Michigan	2000	MSC 378486
Agrocybe semiorbicularis	Ingham Co., Michigan	2000	MSC 378490
Bolbitius lacteus	Ingham Co., Michigan	2000	MSC 378485
Bolbitius tener		1945	MICH: W. H. Long 11121
Bolbitius variicolor	Ingham Co., Michigan	2000	MSC 378488
Bolbitius vitellinus	Ingham Co., Michigan	2000	MSC 378484
Conocybe coprophila	Custer Co., Idaho	1962	MICH: A. H. Smith 65641
Conocybe crispa[a]	Ingham Co., Michigan	2000	MSC 378491
Conocybe crispa[b]	Ingham Co., Michigan	2001	MSC 378493
Conocybe crispa	Yorkshire, United Kingdom	1961	E: G137
Conocybe filaris	Marin Co., California	1998	MSC 378482
Conocybe huijsmannii var. *conica*	Kepong, Kuala Lumpur, Malaysia	1992	E: Wat 24446
Conocybe lactea	Yorkshire, United Kingdom	1990	E: Wat.22175
Conocybe lactea[a]	Ingham Co., Michigan	1998	MSC 378481
Conocybe lactea[a,b]	Ingham Co., Michigan	1999	MSC 378483
Conocybe lactea[a]	Ingham Co., Michigan	2000	MSC 378487
Conocybe lactea[a,b]	Ingham Co., Michigan	2001	MSC 378492
Conocybe lactea	Benton Co., Oregon	2002	MSC 380513
Conocybe lactea	Lane Co., Oregon	2002	MSC 380514
Conocybe lactea	Laramie Co., Wyoming	2002	MSC 380515
Conocybe lactea	Laramie Co., Wyoming	2002	MSC 380516
Conocybe rickenii	Indian Gap, Great Smokies National Park	1952	MICH: Hessler 20421
Conocybe subcrispa	Alameda Co., California	1933	MICH: E. Morse
Conocybe subnuda	Multnoma Co., Oregon	1995	L. L. Norvell 1950623-01
Conocybe tenera	Portneuf Co., Quebec, Canada	1967	MICH: R. L. Shaffer 5892
Cyttarophyllum besseyi	Santa Fe Co., New Mexico	1967	MICH: K. A. Harrison 6825
Galeropsis desertorum	Moravia	1930	PR 154181
Gastrocybe "yellow"	Sierra Co., California	2001	D. E. Desjardin 7326
Gastrocybe deceptiva[c]	Rooks Co., Kansas	1896	FH: E. Bartholomew 2239
Gastrocybe lateritia[c]	Ithaca, Michigan	1947	MICH: V. Potter 3654
Gastrocybe lateritia[a]	Ingham Co., Michigan	1999	MSC 380542
Gastrocybe lateritia[a]	Ingham Co., Michigan	2000	MSC 380543
Gastrocybe lateritia	Ingham Co., Michigan	2001	MSC 378494

Table 5, cont.

Intermediate 1[d]	Ingham Co., Michigan	2000	MSC 380544
Intermediate 2[d]	Ingham Co., Michigan	2000	MSC 380545
Intermediate 3[e]	Ingham Co., Michigan	2001	MSC 380546
Lactea-crispa intermediate	Ingham Co., Michigan	2001	MSC 380547
Weraroa cucullata	Sierra Co., California	2001	D. E. Desjardin

Specimens examined in this study. All specimens were subject to DNA extraction and sequencing except *C. lactea* 1998 and 2000, and *G. lateritia* 2000.
[a]Specimen(s) subjected to HPLC analysis.
[b]More than one specimen was examined.
[c]Type material.
[d]Intermediate form between *C. lactea* and *G. lateritia* in which the cap was dry and white but never expanded.
[e]Intermediate form between *C. lactea* and *G. lateritia* in which the cap expanded but was gelatinous and ochre-brown at maturity.

HPLC and mass spectrometry

Fungi were evaluated for the presence of toxins using a modification of the method of Enjalbert et al. (1992). Evaluations were performed only upon fresh specimens, as phallotoxins degrade considerably upon drying (Klán & Baudisová 1993). The toxins were extracted from 0.3 to 1.0 mg of tissue from the lamellae and pileus using 1.5 ml extraction medium containing methanol:distilled water:0.01M HCl (5:4:1) g^{-1} tissue. Methanol was HPLC grade (J.T. Baker, Phillipsburg, New Jersey, USA). Suspended tissues were incubated at 4°C for 12 h. Samples were then centrifuged at 1000 x g and 4°C for 10 min, and the supernatant was collected. The pellets were resuspended in 0.6 ml extraction

medium g^{-1} tissue, incubated at 4°C for an additional 12 h and centrifuged. The supernatants from the first and second centrifugation were pooled.

HPLC analysis of amatoxins and phallotoxins was performed on a Model 114 HPLC apparatus (Beckman Instruments, Inc., Fullerton, California, USA) with detection at 295 nm. Amatoxins and phallotoxins were separated using a reverse-phase C-18 column (Aquapore OD-300, 7μm, 200x4.6 mm; Perkin-Elmer Corporation, Norwalk, Connecticut, USA) and a 30 min gradient of solution A to solution B. Solution A was 0.2 M ammonium acetate, adjusted to pH 5 with glacial acetic acid, and solution B was acetonitrile. Flow rate was 1 ml min^{-1}. Samples were maintained at a temperature of 4°C until injection. 20 - 200 μl of each sample were injected. Standards were purified α-amanitin, β-amanitin, phallacidin and phalloidin (Sigma Chemical Company, St. Louis, Missouri, USA), each used at a concentration of 100 μg ml^{-1}. *Amanita bisporigera* Atk. *in* Lewis samples of known toxicity were run as additional controls.

Peaks eluted at approximately 70 - 80 % acetonitrile. Putative toxin peaks were identified by comparison with the toxin standards, and eluted fractions were manually collected from the HPLC apparatus. Eluted fractions were subjected to fast atom bombardment (FAB) mass spectrometry, at the Mass Spectrometry Facility at Michigan State University, to confirm identity. FAB mass spectra of *C. lactea* and a phallotoxin standard were obtained using a model HX-110 double-focusing mass spectrometer (JEOL USA, Peabody, Massachusetts, USA) operating in the positive ion mode. Ions were produced by bombardment with a beam of xenon atoms (6 kV). The accelerating voltage was 10 kV and the

resolution was set at 1000. The instrument scanned from *m/z* (mass to charge ratio) 50 to 1500.

DNA extraction, amplification and sequencing

DNA was extracted from gill and pileus tissue from dried basidiocarps (10 mg). Fungal tissue was placed in a microfuge tube, frozen in liquid nitrogen and macerated. One ml cetyltrimethylammonium bromide (CTAB) mixture (5% w/v CTAB, 1.4 M NaCl, 20 mM EDTA pH 8.0, 100 mM Tris-HCl pH 8.0, 1% w/v polyvinylpyrrolidone (PVP-360)) and 2 μl β-mercaptoethanol were then added. The tube was incubated at 65°C for 1 h. The mixture was extracted once with phenol:chloroform:isoamyl alcohol (24:24:1) and once with chloroform and centrifuged to remove solids. The water-soluble fraction was precipitated with two volumes absolute ethanol and centrifugation, followed by a rinse with 70% ethanol and a second centrifugation. The precipitate was air-dried under vacuum then resuspended in 50 μl water.

Approximately 1-20 ng of the total genomic DNA was used per 25 μl reaction mixture for polymerase chain reaction (PCR) amplification. Various brands of prepackaged buffers and polymerases were used for PCR amplification. Primer combinations used were ITS 1F:ITS 4B (Gardes & Bruns 1993) and ITS F (Glen et al. 2001):ITS 4 (White et al. 1990) for the ITS1/5.8S/ITS2 region; and CTB6 (Bruns & Li; cited in Hughey et al. 2000):TW13 (White et al. 1990) for the nuclear large subunit (28S) ribosomal DNA.

The cycling reactions were performed in a DNA thermal cycler (Perkin-Elmer) following Tank & Sang (2001). Alternately, a 60°C to 45°C touchdown protocol was used on some templates that did not amplify with the Tank & Sang protocol. The amplification ended with an additional 10 min extension at 72°C, and storage at 4°C. PCR amplification products were separated, and purified following Hughey et al. (2000). Alternatively, products were purified using TOPO® TA (Invitrogen, Carlsbad, CA, USA) or pGEM® (Promega, Madison, WI, USA) cloning kits. Sequencing was performed by the Michigan State University Genomics Technology Support Facility, using dye terminated capillary electrophoresis on an ABI Prism® 3700 DNA Analyzer (Applied Biosystems, Foster City, CA, USA).

Phylogenetic analysis

Sequences were aligned using Clustal W (Thompson, Higgins & Gibson 1994), and were further aligned by eye. The ITS region covered a portion of the 18S, all of the ITS1, 5.8S and ITS2, and the beginning of the 28S nuclear ribosomal operons. The alignment was 930 positions, including gaps. Gaps were coded following Simmons and Ochoterena (2000). The alignment for the 28S region was 537 bases. The ITS and 28S regions were analyzed independently and in combination. A total of 141 positions where alignment was ambiguous were excluded from analyses of the ITS and combined datasets.

Phylogenetic analyses were performed using PAUP* 4.0b10 (Swofford 2002). *Weraroa cucullata* (Seaver & Shope) Thiers and Watling was used as an outgroup. Maximum parsimony was used with a heuristic search algorithm. A consensus tree was built from all equally parsimonious trees. Three hundred bootstrap replications (maxtrees=300) were run to attain bootstrap values. Bootstrap values are printed above the branches; values less than 50 are not shown. Modeltest 3.06 (Posada & Crandall 1998) was used to determine likelihood settings, which were used to run maximum likelihood analyses in PAUP*. HKY+G was selected as the optimum likelihood model for all datasets. Parsimony analyses of the combined dataset were run in which 1) *C. lactea* was constrained to be monophyletic, 2) *C. crispa* was constrained to monophyly, and 3) *Gastrocybe* and *Galeropsis* were constrained to monophyly.

Culture

Wedges of lamellar tissue from *C. lactea*, *C. crispa* and *G. lateritia* were surface sterilized with 70 % ethanol and were placed on PDA with 10 ppm benomyl and 200 ppm streptomycin. *G. lateritia* samples were additionally grown on PDA with 10 ppm benomyl, 500 ppm streptomycin and 200 ppm tetracycline. A bacterium was present on all *G. lateritia* samples and was identified by the Michigan State University Plant Diagnostic Clinic using BioLog (BioLog, Hayward, CA, USA).

RESULTS

HPLC and mass spectrometry

Eighteen fresh fruiting bodies of *C. lactea* were subjected to HPLC analysis. Putative phalloidin peaks were identified in 11 *C. lactea* samples. The identity of these peaks was confirmed by mass spectrometry (Fig. 16). Based on comparison with the toxin standard, the quantity of phallotoxins present in *C. lactea* was estimated at approximately 3 ng per g, less than one tenth the concentration found in *Amanita bisporigera*. No traces of amatoxins were found in any of the *C. lactea* fruiting bodies analyzed, while both amatoxins and phallotoxins were readily detectable in *Amanita bisporigera* samples used as controls.

Three fresh fruiting bodies of *C. crispa* and five of *G. lateritia* were also analyzed using HPLC. In none of these cases was any phallotoxin detectable.

Phylogenetic analyses

North American samples of *C. lactea* and *C. crispa* formed one clade in all phylogenetic analyses (Fig. 17-22), with European *C. crispa* segregating as a distinct taxon outside of Section *Candidae*. The European *C. lactea* was indistinguishable from North American specimens on the basis of the partial 28 S sequence, but possessed two distinct ITS sequences, both placing the European

taxon within Section *Candidae*, but separate from North American *C. lactea* specimens. When North American *C. lactea* was constrained to monophyly the *C. lactea* clade nested within a paraphyletic *C. crispa*. Likewise, a monophyletic *C. crispa* nested within a paraphyletic *C. lactea*. Inclusion of the European *C. crispa* specimen in a monophyletic *C. crispa* clade resulted in a tree 71 steps longer than the most parsimonious trees. *Gastrocybe lateritia* formed a sister group to the *C. lactea/C. crispa* clade and clearly belongs in *Conocybe* Section *Candidae*. *G. deceptiva* and an unidentified *Gastrocybe*-like fungus from the Sierra Nevadas (*Gastrocybe* "yellow") are placed in the same clade as *Bolbitius* species. Constraining *G. lateritia* to monophyly with *Galeropsis desertorum*, in keeping with Moreno et al.'s (1989) placement of *Gastrocybe* in *Galeropsis*, resulted in a tree 40 steps longer than the most parsimonious trees.

Bacterial identification

Bacteria were isolated from *G. lateritia* whenever attempts were made to culture the latter. One type that was consistently present proved to be resistant to 500 ppm streptomycin and 200 ppm tetracycline. Metabolic testing (BioLog) was used to identify this type as a member of the *Chryseobacterium gleum/indologenes* group.

(Text resumes on page 107)

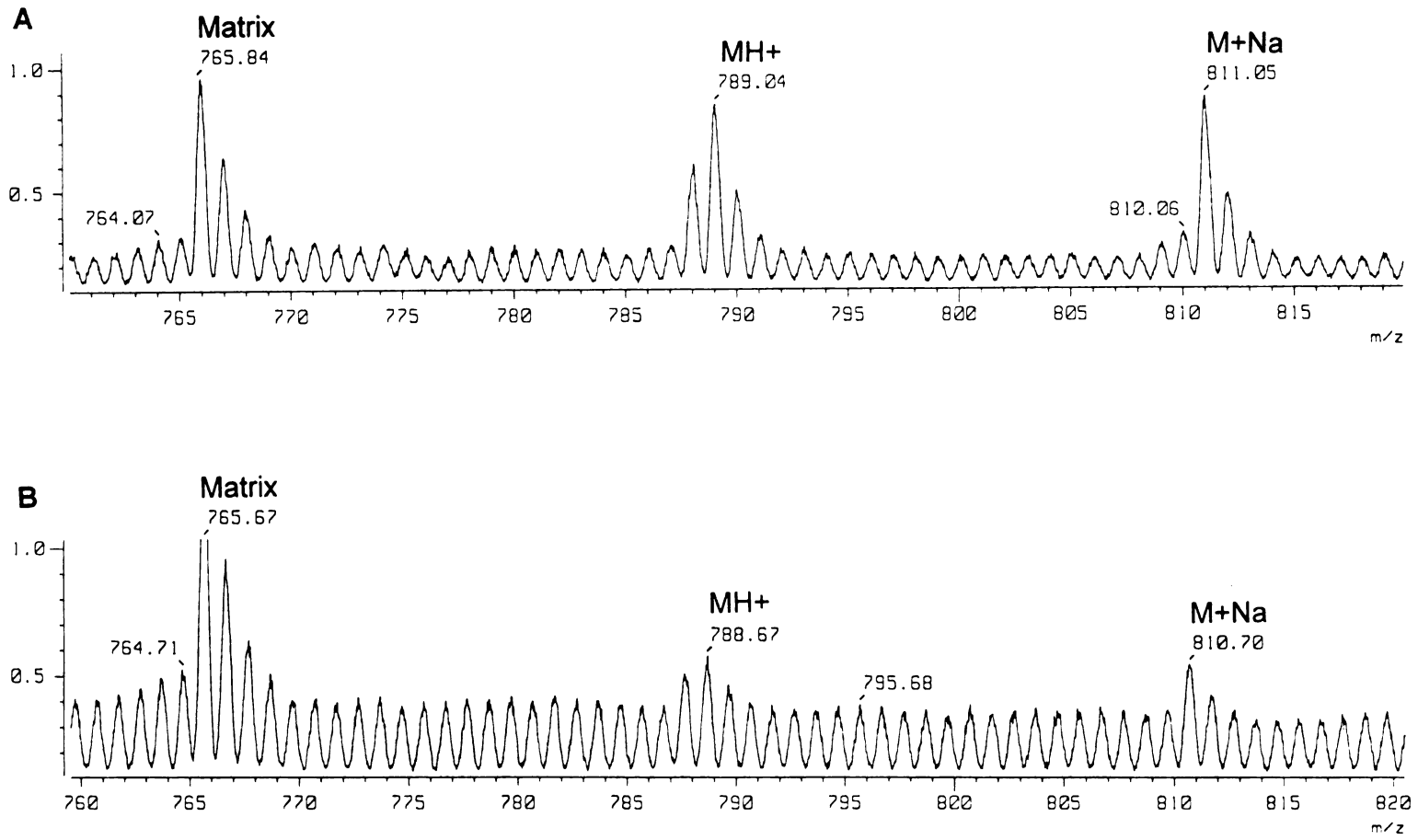

Figure 16. FAB mass spectra. Matrix = nitrobenzyl alcohol matrix. MH+ and M+Na = phalloidin + proton and phalloidin + sodium, respectively. **A** Phalloidin standard. **B** *Conocybe lactea*.

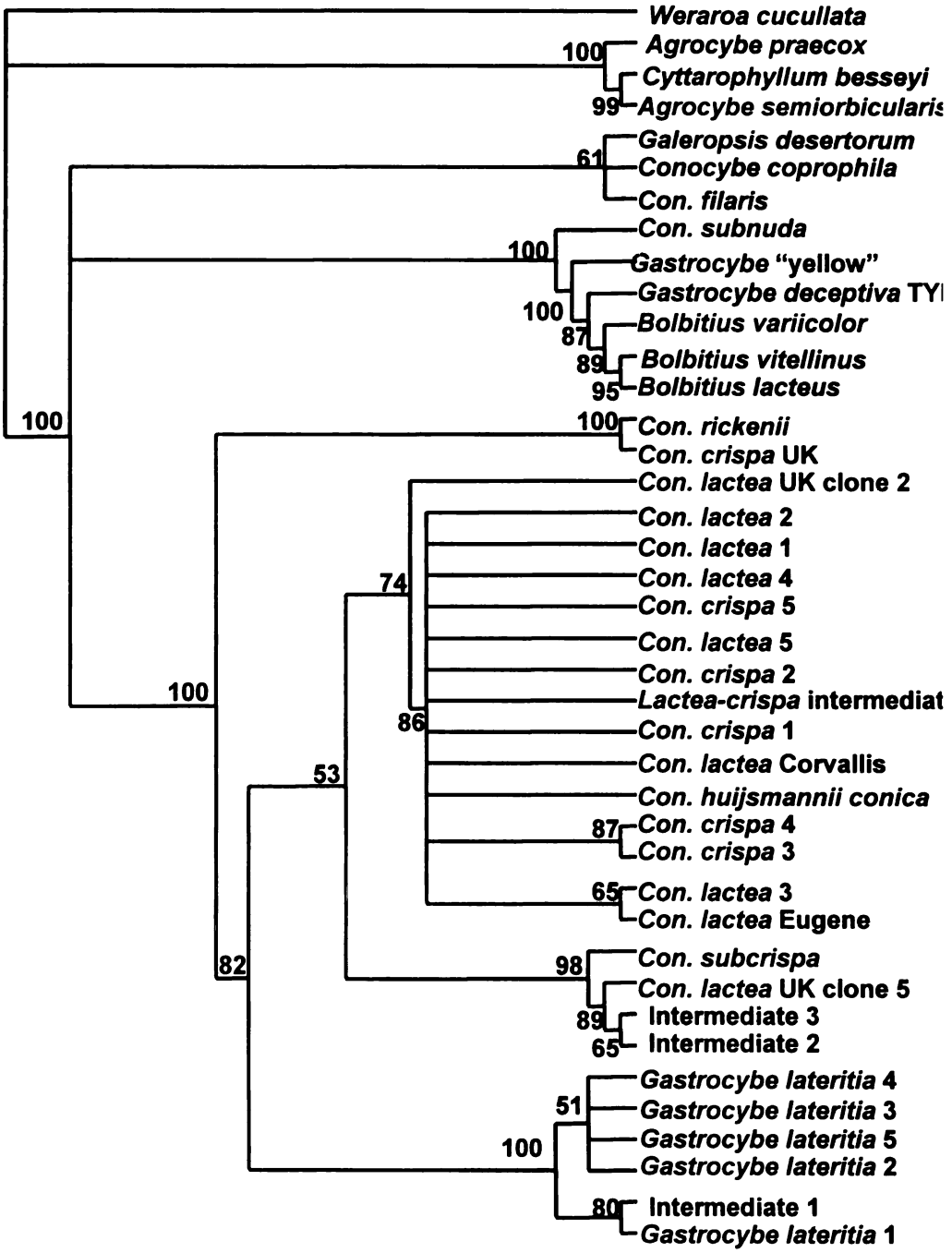

Fig. 17. Consensus based on 300 equally parsimonious trees (length 1090) of the ITS 1, 5.8S and ITS 2 regions of the nuclear ribosomal DNA subunit. Numbers at nodes are bootstrap indices of support (%).

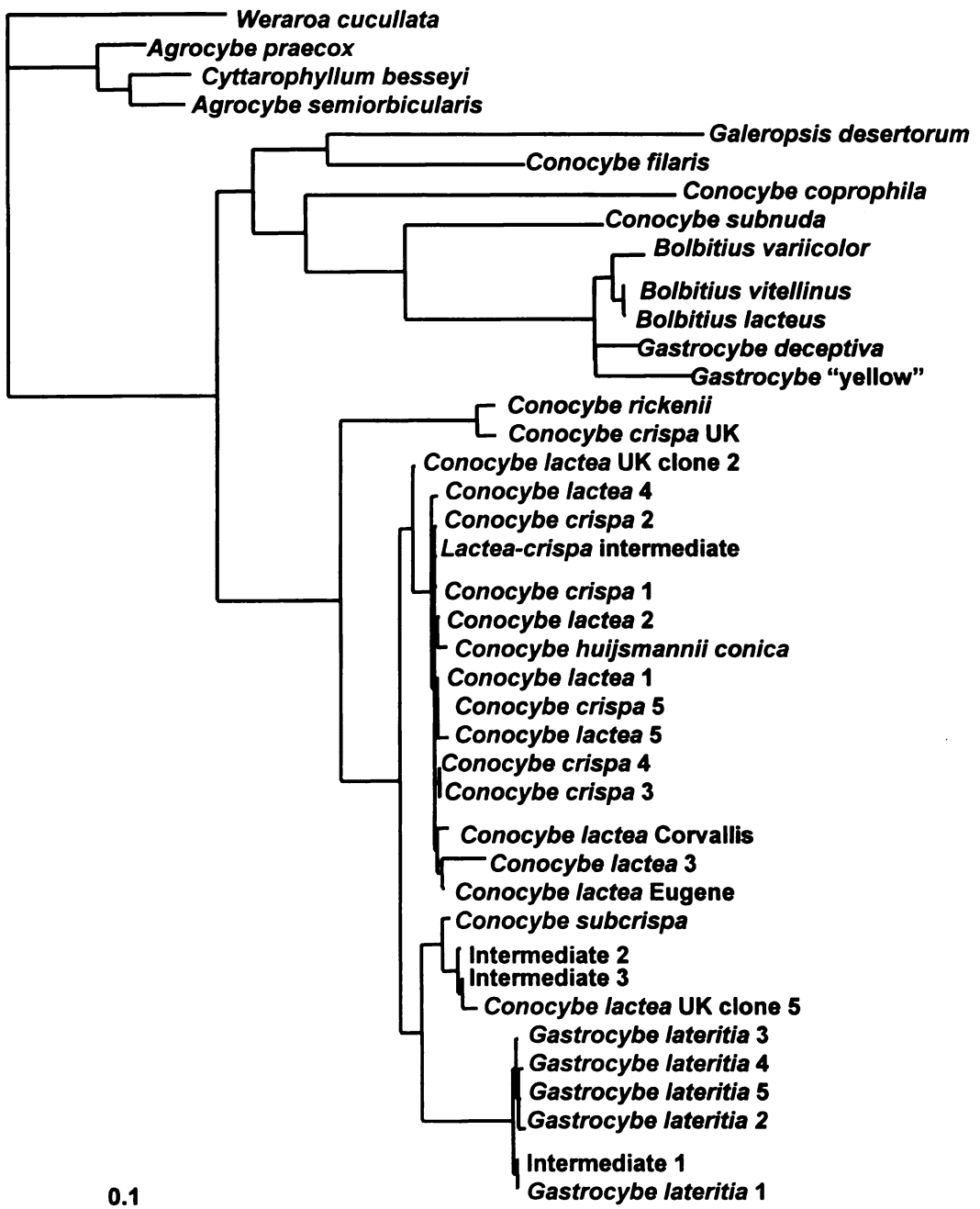

Fig. 18. Cladogram of the maximum likelihood analysis of the ITS 1, 5.8S and ITS 2 regions of the nuclear ribosomal DNA subunit. One of three trees with ln likelihood = -5938.207. Branch lengths correspond to genetic distance (expected nucleotide substitutions per site).

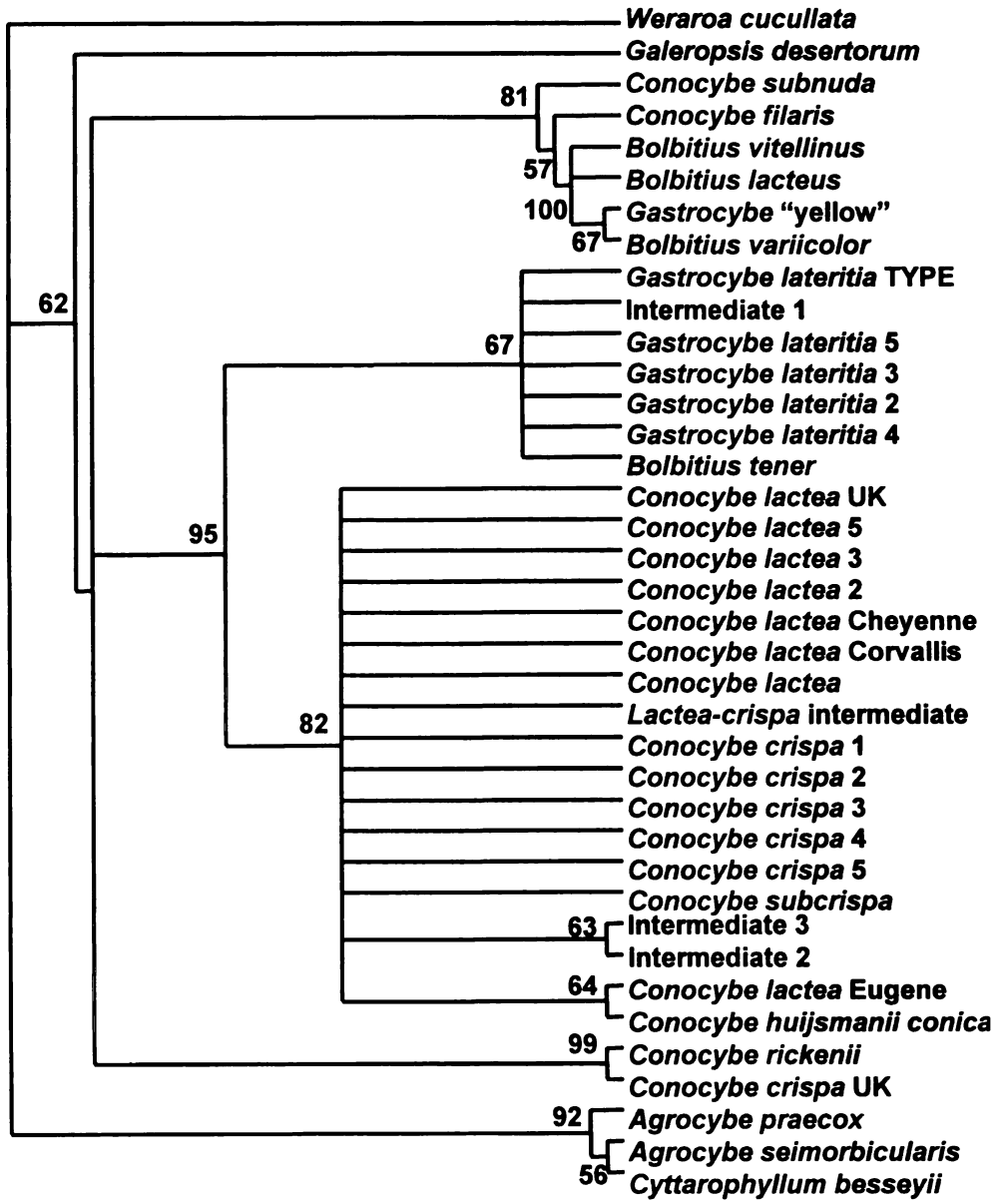

Fig. 19. Consensus based on 70 equally parsimonious trees (length 171) of the partial 28S ribosomal DNA subunit. Numbers at nodes are bootstrap indices of support (%).

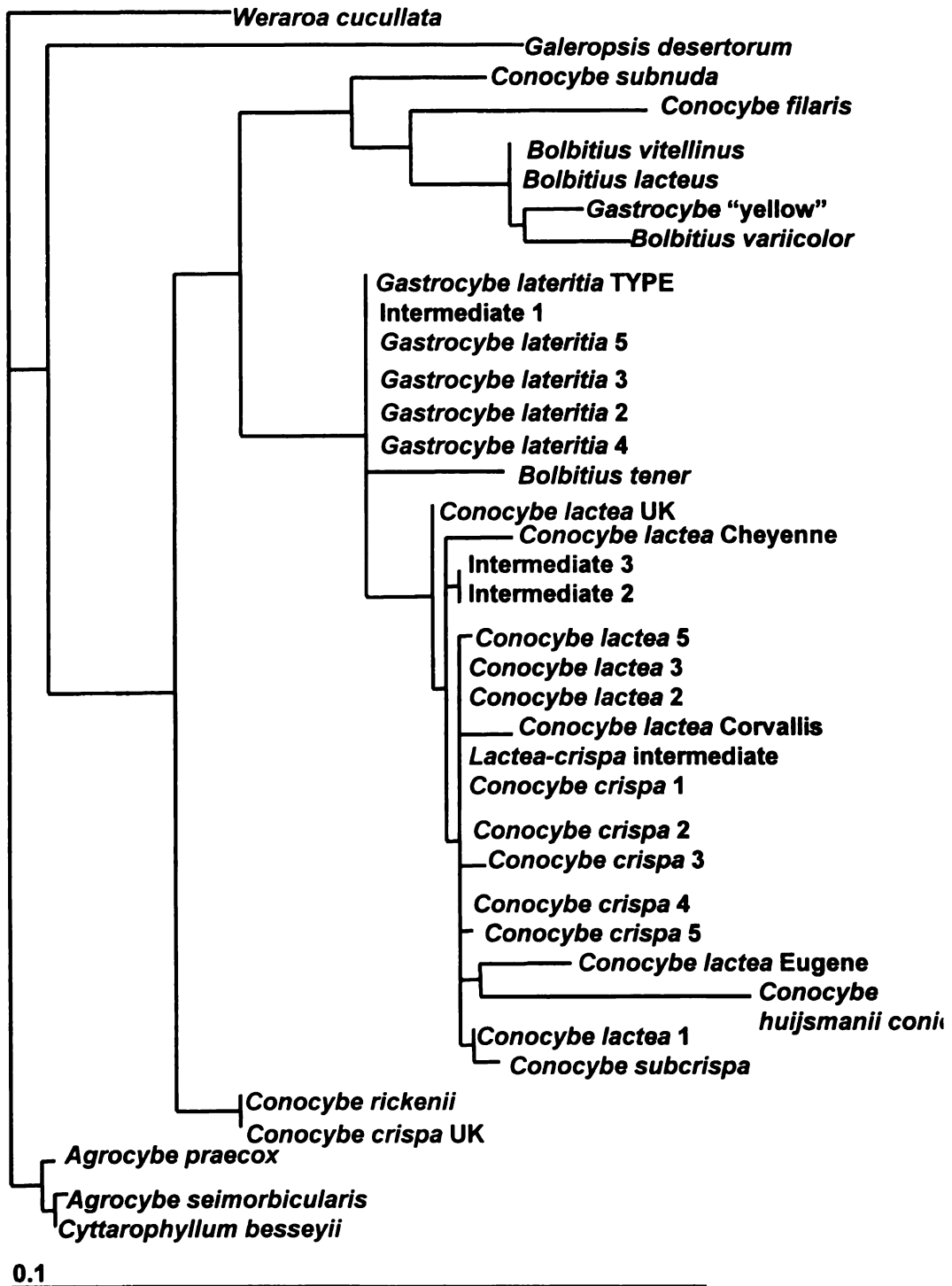

Fig. 20. Cladogram of the maximum likelihood analysis of the partial 28S ribosomal DNA subunit. One of two trees with ln likelihood = -1833.145. Branch lengths correspond to genetic distance (expected nucleotide substitutions per site).

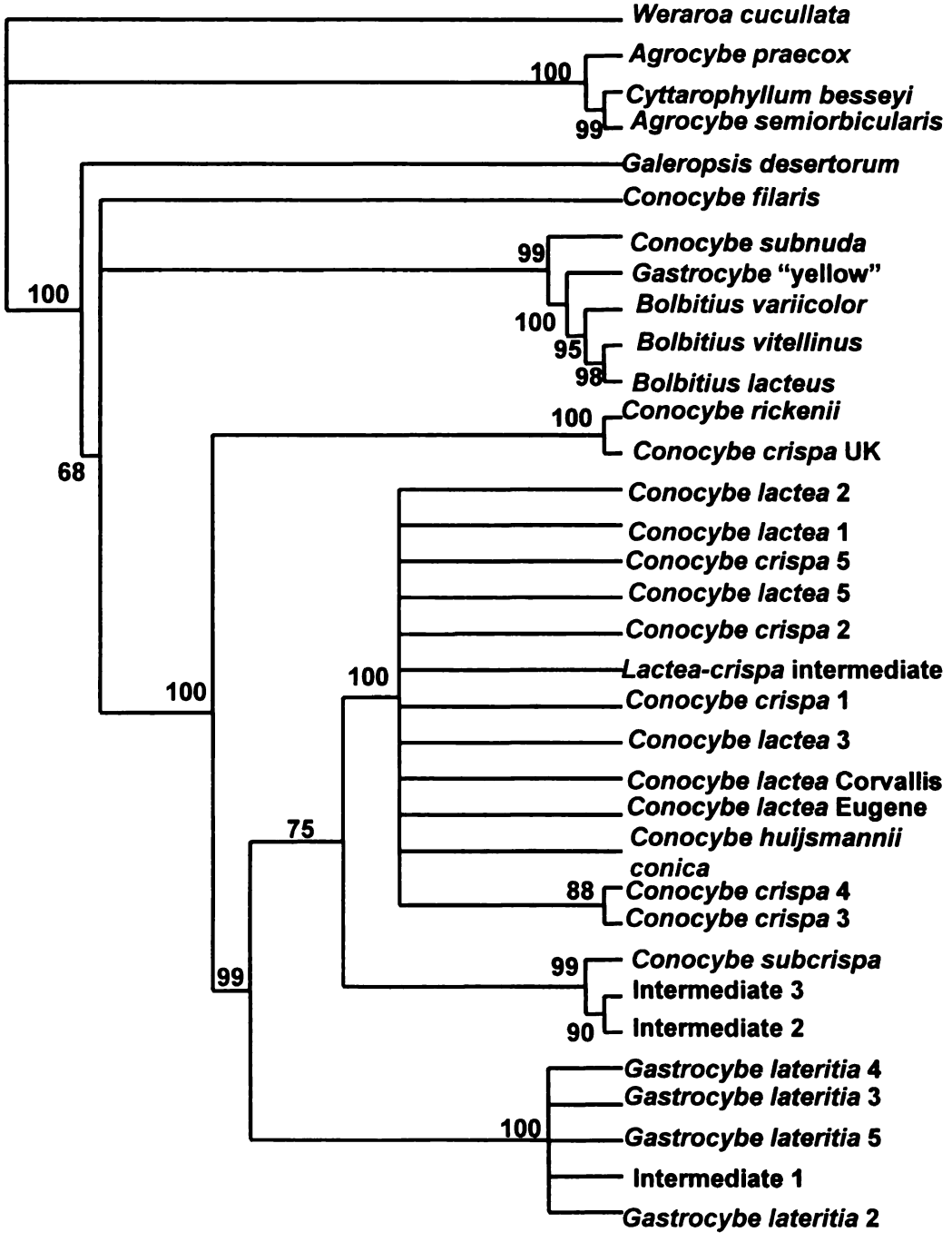

Fig. 21. Consensus based on 300 equally parsimonious trees (length 1122) of the combined ITS and partial 28S regions. Numbers at nodes are bootstrap indices of support (%).

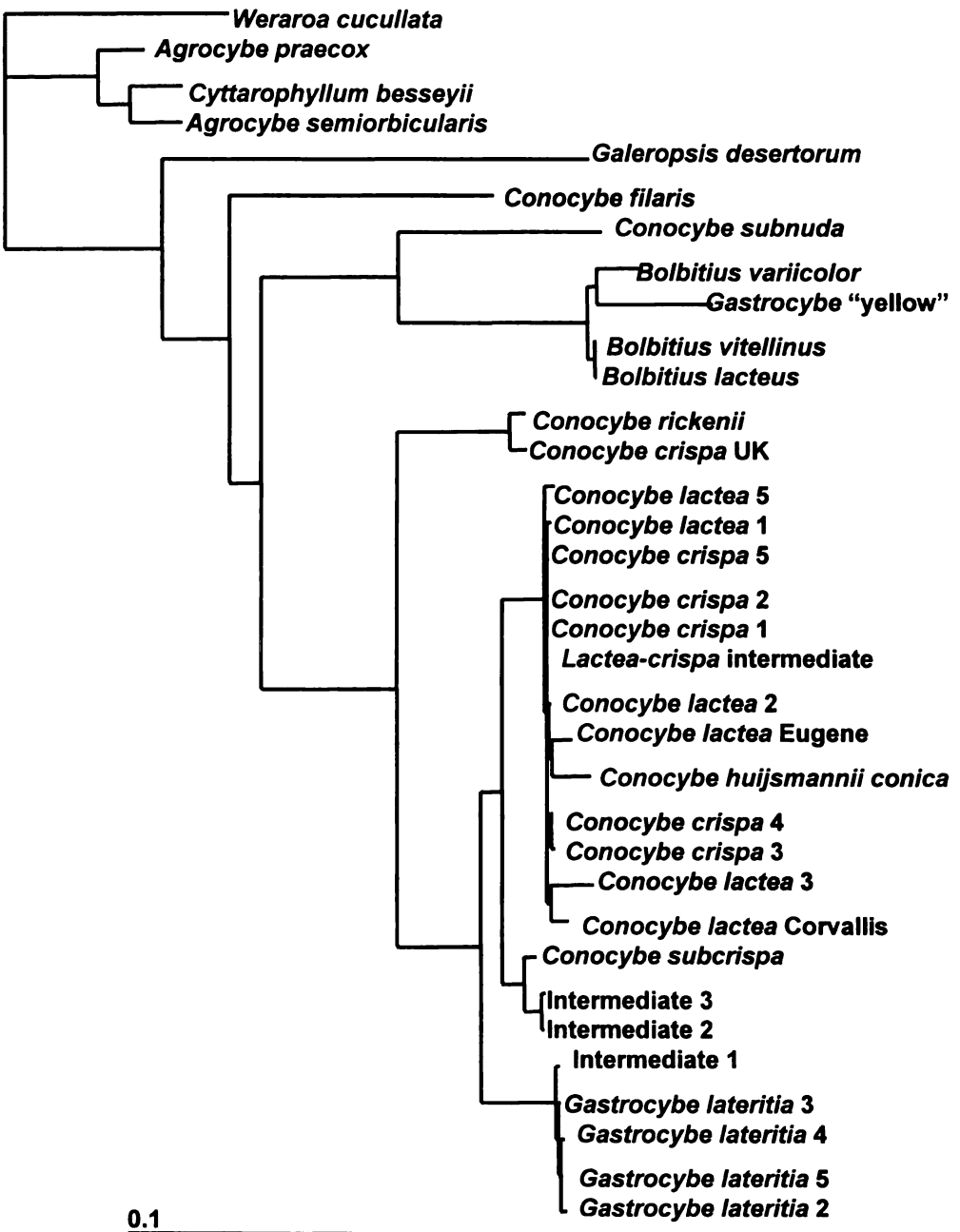

Fig. 22. Cladogram of the maximum likelihood analysis of the combined ITS and partial 28S regions. ln likelihood = -7263.045. Branch lengths correspond to genetic distance (expected nucleotide substitutions per site).

Discussion

HPLC and mass spectrometry

This is the first report of phallotoxins outside of the genus *Amanita*, and the first report of phallotoxins consistently being present in the absence of amatoxins. Phallotoxins are destroyed by heat and are not absorbed by the mammalian digestive system (Benjamin 1995), and thus have not been implicated in any human poisonings. However, the LD_{50} of 0.2 mg/kg body weight in mice for phallotoxins given in interperitoneal injection, and the possibility of a shared biosynthetic pathway for phallotoxins and amatoxins, suggest that *C. lactea* should not be considered edible, despite reports to the contrary (Bessette, Bessette & Fischer 1997).

The inability of our methods to detect phallotoxins in 39% of fresh specimens examined may mean either that phallotoxins were present below the level of detection, or that the toxins were not present in all specimens. Several fungal cyclic peptides are known to occur sporadically. Yocum and Simons (1977) reported no evidence of either amatoxins or phallotoxins in three out of four specimens of *Amanita verna* examined, a mushroom capable of producing both. Sporadic phallotoxin distribution in *Amanita* has also been reported by Beutler (1980).

No phallotoxins were found in *C. crispa* or in *G. lateritia*. The latter is not particularly surprising, as the phylogenetic evidence suggest that *G. lateritia* is

indeed a separate taxon from *C. lactea* and *C. crispa*. The lack of phallotoxins in *C. crispa* is less readily explained; however, *C. crispa* is less common than *C. lactea* and few samples were examined.

Systematics

Despite the presence of cyclic peptide toxins in both species, phylogenetic analyses suggest that *C. lactea* is not closely related to the amatoxin-producer, *C. filaris*. No phallotoxin has been detected in *C. filaris*, and it is probable that the biosynthetic pathways for the different toxins arose separately in the *C. filaris* and *C. lactea* lineages.

Hausknecht (1998), working with European material, renames *Conocybe lactea* as *Conocybe albipes* (Otth) Hausknecht, because the basionym *Bolbitius albipes* Otth 1871 predates *Galera lactea* J. E. Lange 1940, on which *C. lactea* is based. His analyses are morphological, and are based primarily upon European specimens, although one specimen apiece from Egypt and Mexico, and three from New Zealand, are examined as well.

Two differing ITS sequences were obtained from one fruiting body of the European *C. lactea*, rendering proper phylogenetic placement of the organism difficult. However, the European taxon was clearly closely related to the North American *C. lactea*. Divergent ITS sequences have been reported in other fungi (O'Donnell 1992). *C. huijsmanii* var. *conica* Watling, which Hausknecht treats also as *C. albipes*, fell within the North American *C. lactea-C. crispa* clade. We

will need to examine further European specimens before a definitive placement is possible. Whether the North American taxon belongs, with the European specimens, in *C. albipes* var. *albipes*, or whether it would be better treated as a subspecies, remains unresolved.

Molecular phylogenetic analyses were consistently unable to distinguish between *C. lactea* and *C. crispa*, suggesting a very recent time of divergence. Alternatively, the wavy pileus and crisped gills of *C. crispa* may be an environmentally-determined variant of the more common *C. lactea* form. This hypothesis receives support from the ecology of the fungi, and the plasticity of such features as gill morphology and number of spores per basidium. The placement of the European *C. crispa* specimen in a clade separate from North American *C. lactea* and *C. crispa* specimens, and the nomenclatural implications thereof, is discussed below.

Conocybe crispa was described from North American material (Longyear 1899). The holotype of *C. crispa* was at MSC, but was misplaced and appears to have been accidentally destroyed in 1973 by someone who did not know its value (Watling & Gregory 1981). The European *C. crispa* is rare, and is separated from the North American taxon by Hausknecht (1998), who reduces it to *C. albipes* var. *pseudocrispa* Hausknecht, while the North American *C. crispa* is transferred to *C. albipes* var. *crispa* Hausknecht. Given the evident differences in DNA sequence (see Figs. 17-22), separating the two taxa at the species level would appear to be warranted. This raises potential nomenclatural problems as

the European *C. crispa* is the taxon that is clearly distinct from the *C. albipes* group.

Until further molecular comparisons can be made between European and North American specimens, we encourage the adoption of *C. albipes* to refer to North American specimens that have been referred to *C. lactea*, and *C. albipes* var. *crispa* for North American *C. crispa* specimens. The name "*Conocybe lactea*" is problematic (Hausknecht 1998; Watling, pers. comm., 3 April 2002) and ought not be used.

Our analyses clearly distinguished the sequestrate taxon *G. lateritia* from *C. lactea* and *C. crispa*, but placed *G. lateritia* as sister to these taxa within *Conocybe* Section *Candidae*. *G. deceptiva* Baroni and the *Gastrocybe*-like specimen denoted *Gastrocybe* "yellow" both formed a clade with *Bolbitius vitellinus* and allies. While *Gastrocybe* thus was not monophyletic, Watling's original placement of *Gastrocybe* within the *Bolbitiaceae* (Watling 1968) was supported. Moreno et al. (1989) synonymized *Gastrocybe* Watling with *Galeropsis* Velenovský & Dvorak on the basis of morphological features of the type collections, while retaining both taxa in the *Bolbitiaceae*. *Gastrocybe* "yellow" is morphologically similar to *Bolbitius elegans* E. Horak, G. Moreno, A. Ortega & Esteve-Rav., a gasteroid-like fungus that Horak and colleagues (2002) have nevertheless placed in *Bolbitius*. Some authorities (e.g. Hawksworth et al. 1995) recognize the *Galeropsidaceae* Singer as the appropriate place for sequestrate forms of *Bolbitiaceae* and *Strophariaceae*.

The development of secotioid, or sequestrate, forms has been observed repeatedly in the *Agaricales, Boletales* and *Cortinariales* (Singer 1958; Thiers 1984). Secotioid fungi appear intermediate between agarics and gastromycetes: a differentiated pileus and stipe are present, but the pileus never expands and forcible spore discharge is lost. The lamellae are usually recognizable when the fungus is sectioned, but are contorted and frequently anastomosed. A close and direct relationship is frequently seen between secotioid and agaricoid taxa. After decades of debate, the consensus is that the direction of evolution is from the agaricoid morphology to the secotioid (Thiers 1984; Baura, Szaro & Bruns 1992; Hibbett et al. 1997).

G. lateritia, with its moist, gelatinous nature, is unusual among secotioids, the majority of which are dry and frequently appear in arid habitats. While certain other taxa (*Bolbitius* Fr., *Coprinus* Pers., *Coprinellus* P. Karst., and *Coprinopsis* P. Karst.) deliquesce at maturity, these do so following spore discharge, not prior to discharge as in *G. lateritia*. Combined with the elongation of the stipe, the deliquescence of *G. lateritia* produces a fungus which looks sick, not secotioid. The consistent presence of the bacterium, coupled with the sick morphology and the existence of intermediate forms, has led us to hypothesize that *G. lateritia* may in fact represent a diseased *Conocybe* species from Section *Candidae,* but of unknown identity.

Acknowledgements

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CHAPTER 5

NON-RIBOSOMAL PEPTIDE SYNTHETASES AND *GALERINA MARGINATA*

Abstract

Amatoxins are cyclic peptides, and are believed to be biosynthesized by an enzymatic (non-ribosomal) system. The putative enzyme, amatoxin synthetase, is expected to be encoded by a ca. 30 kb gene. The gene is expected to share conserved sequence motifs found in other fungal cyclic peptide synthetases. The wood decay mushroom *Galerina marginata* has been selected as a model organism for elucidating the pathway of amatoxin biosynthesis because it produces amatoxins in culture and grows more readily in culture than *Amanita* species. Fourteen primers, six of which were newly designed and eight taken from the literature, were used in various combinations to amplify amatoxin synthetase gene fragments. These attempts were unsuccessful, due to low primer specificity and possibly to flaws in the primer design approach. Therefore, attempts to use pyrophosphate exchange assay to isolate the synthetase enzyme were initiated. Placing amatoxin synthetase in an evolutionary framework using future sequence data has the potential to resolve many questions about the nature and ecological role of amatoxin synthetase, and cyclic peptide synthetases in general.

Introduction

Cyclic peptides are biosynthesized in a unique process. While these peptides are composed of amino acids, an enzymatic mode of synthesis is employed, as opposed to the ribosomal synthesis employed in the synthesis of typical proteins. The enzymes involved, cyclic peptide synthetases (CPSs), are large proteins encoded by some of the longest known open reading frames (Weber et al. 1994), and much remains unknown about their synthesis, ecological function and evolutionary history. The enzyme obtains, activates, bonds and cyclizes the cyclic peptide end product (Kleinkauf & von Döhren 1996). Each cyclic peptide amino acid requires a separate domain of several hundred amino acids in the synthetase enzyme (Panaccione, 1996). Each domain contains certain conserved regions, which allow for the development of degenerate primers for use in the polymerase chain reaction (PCR) (Panaccione, 1996; see Figure 23 and Tables 6-8).

Amatoxins are a family of cyclic octapeptides that are responsible for more than 90 % of fatal mushroom poisonings in humans. Amatoxins are produced in four genera of mushrooms, *Amanita, Galerina, Lepiota* and *Conocybe*. α- and β-amanitin are the prevalent amatoxins in the death cap mushroom, *Amanita phalloides*, while α- and γ-amanitin prevail in *Galerina* and *Lepiota* (Benedict & Brady 1967; Haines, Lichstein & Glickerman 1985; Faulstich & Zilker 1994). *Conocybe* contains only very low levels of any given amatoxin (Brady et al. 1975).

Table 6. Conserved regions in fungal cyclic peptide synthetases

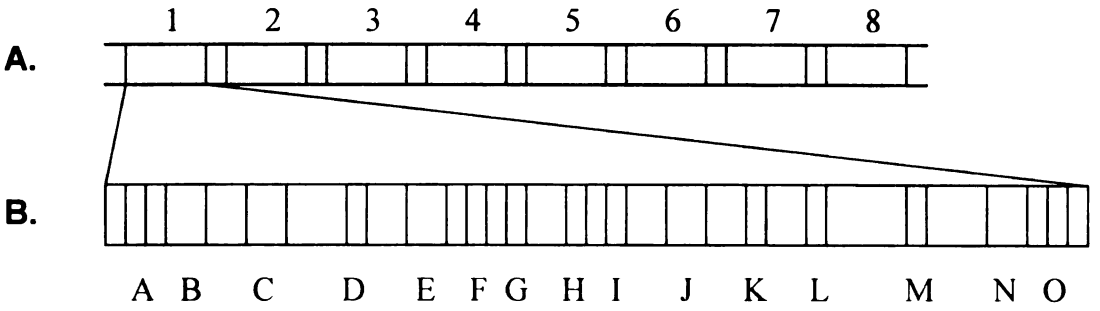
		C		D	E	F	G	H
AM:		FTSGSTGDPKG	LIFGGTICVP	NGFGPTETA	PFGAVGELVVEGT	RIYKTGDLV	KIRGNRVDLGEIE	KLPAYMVP
AM:		FTSGSTGLPKG	LMHGGCVCIP	NGYGPAECS	PIGGVGELLIEGA	RLYKTGDIV	KIHGQRIELGEIE	SLPAYMIP
AM:		FTSGSTGEPKG	LMSGGCICIP	NCYGPAEAA	PIGAVGELLIEGS	PEIYKYDED	KIRGQRTELGEIE	RLPLYMIP
HTS1:	AILKAGGV	FTSGSTGVPKC	LFYGGTLCIP	NVYGPAENT	PVGSIGELLIESG	RFYRTGDLV	KLAGQRVELGDVE	RLPSYMVP
HTS1:		FTSGSTGVPKG	LAVGGCLCIP	NMYGPTEAT	PIGAVGELIIEGS	TLYKTGDLV	KMNGQRFELGEVE	VLPLFMVP
HTS1:		YTSGTSGKPKG	LYAGGCLFIP	NSYGPTEAC	PIGAVGELCIEAP	RVYRTGDLV	KLRGQRIELGEIE	VLPHYMVP
HTS1:		FTSGSTGKPKG	LVYGGCLCIP	AGYGPTETC	PYGATGELYIQPT	RAYKTGDLV	KIRGQRVELAEIE	
TrichV:	GILKAGGV	FTSGSTGKPKG	LIAGATVCVP	NGYGPAEAC	PTGCVGELLLQGS	RFYKTGDLV	KVRGQRIELGEIE	
TrichH:	GILKAGGA	YTSGSTGKPKG	LLVGATLCVP	NIYGPAEIC	PIGCIGELFIDGH	KVYRSGDLA	KLRGQRIELAEIE	
Clav 1:		FTSGTTGTPKG	LDCGGCLCIP	NSYGPCECT	PVGSIGELMLEGP	RLYKTGDLV	KFHGQRIELSEIE	
Clav 2:		??????GKPKG	LRMGGCVCVP	QGYGPAECS	PPGAIGELVLEGP	RLYKTGDL?		
Ust:		WTSGTTGLPKG		NLYGPAEAT	IKGAVGELVLGGP	RVYRTGDLV	KINGIRIELLEIN	SLPSYMVP
Ust:	AILKAGHA	YTSGSTGKPKG		NAYGPSEAT	LRGEPGELYIGGE	QIURTGDMV		RLPQYMVP
Ust:	AIWKAGKA	FTSGSTGKPKA		NAWGPTEAT	PSGAIGELAVGGP	RVYRTGDLG		RLATYMVP

	J	K
AM:	FFALGGDSIAA	IEDVYACTPLQEGLLAL
AM:	FIRLGGDSIAA	IEDVYPCTPLQEGLLSL
AM:	FFRLGGDSIAA	VEDVYPCTPMQRGLMSL
HTS1:	FIHAGGDSITA	
HTS1:		IEDVFPCTPMQEALVAL
HTS1:	FIHAGGDSITA	IEDVYPCTPLQESLMAA
HTS1:	FFSLGGSSISA	
TrichV:	FLGIGGDSIAA	
TrichH:	FYSLGGDSIRI	
Ust:	FTSVGLDSIRA	IQSILPCTPLQEGMLAE
Ust:	LIMAGLDSLTV	IETALPCVPIQVALVSQ
Ust:	FHRLGIDSISA	LVSVLPCTPLQSGMIAQ
Ust:	LSQLGLDSISA	

Table 6. Conserved regions in cyclic peptide synthetases

Conserved regions in fungal cyclic peptide synthetases. Cyclic peptide sequences were obtained from NCBI GenBank. AM = AM-toxin synthetase from *Alternaria alternata* AF184074, Clav1 and Clav2 are ergopeptine synthetases from *Claviceps purpurea* AF022911 and U30621, HTS1 = HC-toxin synthetase from *Cochliobolus carbonum* M98024, TrichH = peptide synthetase from *Trichoderma harzianum* AF304355, TrichV = peptide synthetase from *Trichoderma virens* AF351825, UST = ferrochrome siderophore from *Ustilago maydis* AAB93493. Headings C, D, E, F, G, H, J and K refer to conserved motifs as outlined in Kleinkauf and von Doehren (1996) and shown in Figure 1.

Figure 23.



Structure of a typical cyclic peptide synthetase. **A** The entire synthetase for an eight amino acid cyclic peptide, as predicted for amatoxin synthetase. Shaded areas represent domains for the separate amino acids. Each domain shares conserved sequence motifs. **B** Enlargement of one domain, showing roughly where various activities are encoded (shaded areas). A-I encode adenylation functions, J encodes the acyl carrier, and K-O function in condensation. Some CPSs contain additional sequences P-Q, which function in epimerization, N-methylation and other modifications. After Kleinkauf & von Döhren (1996) and Panaccione (1996).

The ecology of amatoxins is unknown. Most known enzymatically-produced peptides are toxic; examples include the antibiotics gramicidin S and penicillin, the host-specific phytotoxin HC-toxin, and the eukaryotic RNA polymerase II inhibitor amanitin. However, the role of amatoxins in the organism and in nature is unclear. Unlike penicillin, which is secreted by *Penicillium* species into the substrate and can thus discourage potential competitors, amatoxins are not secreted (Murayoka & Shinozawa, 2000). While plants have been experimentally shown to be sensitive to amatoxins (Faulstich, 1980), the lack of secretion by the fungus means that plants are not exposed to these toxins in nature. Indeed, *Amanita* species are obligate mycorrhizal associates of trees, and cannot afford to poison their hosts. *Galerina* species are decomposers of dead wood, while *Conocybe* and *Lepiota* species are grass saprophytes; the lack

of any toxin secretion would eliminate any potential use of the toxin in substrate colonization. Due to the mode of action, amatoxin poisoning exhibits an inevitable delay of several hours. An animal is not going to learn to avoid a particular mushroom if sickness does not occur until several hours later. Prokaryotic RNA polymerases are not affected by amatoxins (Wieland & Faulstich, 1991).

The genera of mushrooms that produce amatoxins are distributed through four families in two orders of fungi. The lack of any close relationship between amatoxin-producing genera raises questions about the evolutionary history of amatoxins. Why are amatoxins produced by *Amanita* and *Lepiota*, but not by *Limacella*, which falls between the two in phylogenetic analyses? Why by some *Amanita* species in Section *Phalloidae* and not others? Why by some *A. virosa* individuals and not others? Are amatoxins produced in many additional basidiomycetes at levels below detection? Is a nonfunctional amatoxin synthetase gene present in non-producers? Has amatoxin synthesis arisen multiple times independently, or is it a case of horizontal transfer?

The hypothesis that amatoxins are produced routinely in basidiomycetes was put forward by Faulstich and Cochet-Meilhac (1976) and elaborated by Preston et al. (1982), who posited a regulatory role. The support for this hypothesis was the low levels of amatoxin the authors found in analyses of several mushroom species, including edible ones. However, in both studies, the toxins in nontoxic species were near the limits of detection for the protocols

employed, and more sensitive HPLC analyses have consistently failed to detect amatoxins in these species (Enjalbert et al. 1992; Hallen, unpublished results).

Cyclic peptide synthetases are encoded by large genes. A 15.7-kb open reading frame (ORF) encodes HC-toxin synthetase, which produces a four amino acid peptide (Scott-Craig et al. 1992). Cyclosporin synthetase is encoded by a 45.8-kb ORF and synthesizes an eleven amino acid product (Weber et al. 1994). The synthetase for the octapeptide amatoxins, by extrapolation, might be expected to require a 30 kb ORF. The likelihood of a gene this size evolving independently on four occasions is presumably low. The hypothesis that the gene arose once, in an ancestral agaric, and that multiple independent point mutations have rendered the gene nonfunctional in most species cannot be so readily dismissed.

A fourth possibility is horizontal transfer (Walton 2000). Peptide synthetases are widely distributed in prokaryotes. Eukaryotic peptide synthetase genes possess certain prokaryotic gene features, notably the lack of introns. Additionally, transposon-like sequences have been observed in HC-toxin regulatory loci (Panaccione et al. 1996). Horizontal transfer of a mobile gene could explain the disjunct distribution at both the inter- and intraspecific levels observed in the case of amatoxins.

None of the potential explanations of amatoxin distribution can be satisfactorily addressed by the traditional methods of evaluating fungi for amatoxins. A molecular genetic approach is needed. Once the amatoxin synthetase gene has been isolated and sequenced from one organism, it can be

used in evaluating other organisms. Is a mutated gene present in the nontoxic morph of *Amanita verna*? Is the gene wholly absent, as is the case in *Cochliobolus carbonum* mutants that lack HC-toxin (Panaccione et al. 1992)? What is the degree of relationship between amatoxin synthetase genes in the different genera of amatoxin producers? These questions could be addressed by the use of DNA-DNA hybridization or the development of amatoxin synthetase-specific PCR primers. Finally, a gene tree could be constructed and compared with existing species evolution data for these fungi. Extensive work has been done to develop molecular phylogenies of *Amanita* (Weiß, Yang & Oberwinkler 1998; Drehmel, Moncalvo & Vilgalys 1999; Hallen, unpublished) and *Galerina* (Gulden, Dunham & Stockman 2001).

Despite the fame (or infamy) of poisonous *Amanita* species, we have chosen to begin our search for amatoxin synthetase in *Galerina marginata*. *G. marginata* contains 0.1 - 0.8 mg amatoxin per g dry weight (Bresinsky & Besl 1990). These are lower toxin levels than in *Amanita* but still sufficient to cause harm. Occasional poisonings occur among people seeking hallucinogenic mushrooms, many of which are superficially similar to *Galerina* species. *Galerina* is the only amatoxin-producing genus that will produce the toxins while growing in culture (Benedict & Brady, 1967). In the other three genera, toxin is only produced in the mature mushroom, which usually cannot be produced in the lab. The woodrotting *Galerina marginata* is a relatively more tractable organism than mycorrhizal *Amanita* species. *Amanita* species grow very slowly in culture and contain a number of inhibitory compounds that interfere with DNA extraction and

the use of molecular biology techniques (Hallen, pers. obs.). Additionally, *Amanita* species produce several other cyclic peptides that would interfere with the isolation and identification of the targeted toxin.

Materials and Methods

Culture and HPLC

Galerina cultures were obtained from the USDA Forest Products Laboratory, Madison, WI. Cultures were *Galerina autumnalis* HHB-11959-sp (dikaryon), *G. autumnalis* HHB-11959 55-1 (monokaryon), *G. heterocystis* CBS (Wy-4228) (dikaryon), *G. marginata* RLG-8365-sp (dikaryon) and *G. stylifera* HHB-12845-sp (dikaryon). Thirty ml liquid shake cultures were grown in HSV for biomass growth, and transferred to 30 ml carbon-starved GFV liquid medium (Muraoka & Shinozawa 2000). Cultures were grown for an additional 15 - 20 days, filtered through miracloth and blotted to remove excess liquid. Additionally, cultures were grown on HSV broth only. Cultures grown on HSV broth only were transferred every 15 - 20 days.

Cultures were evaluated for toxins using a modification of the method of Enjalbert et al. (1992). Additionally, dried fruiting bodies of *G. marginata* were rehydrated and subjected to HPLC. Eight to 200 mg of the tissues were suspended in 1.5 ml extraction medium containing methanol:distilled water:0.01M HCl (5:4:1) g^{-1} tissue. Methanol was HPLC grade (J.T. Baker,

Phillipsburg, New Jersey, USA). Suspended tissues were incubated at 4°C for 12 h. Samples were then centrifuged at 1000 x g and 4°C for 10 min, and the supernatant was collected. The pellets were resuspended in 0.6 ml extraction medium g^{-1} tissue, incubated at 4°C for an additional 12 h and centrifuged. The supernatants from the first and second centrifugation were pooled.

HPLC analysis of amatoxins was performed on a Model 114 HPLC apparatus (Beckman Instruments, Inc., Fullerton, California, USA) with detection at 295 nm. Amatoxins were separated using a reverse-phase C-18 column (Aquapore OD-300, 7μm, 200x4.6 mm; Perkin-Elmer Corporation, Norwalk, Connecticut, USA) and a 30 min gradient of solution A to solution B. Solution A was 0.2 M ammonium acetate, adjusted to pH 5 with glacial acetic acid, and solution B was acetonitrile. Flow rate was 1 ml min^{-1}. Samples were maintained at a temperature of 4°C until injection. Twenty μl of each sample were injected.

Standards were purified α-amanitin and β-amanitin (Sigma Chemical Company, St. Louis, Missouri, USA). Twenty μl of a toxin standard solution, containing each toxin at a concentration of 100 μg ml^{-1}, were injected

Peaks eluted at approximately 70 - 80 % acetonitrile. Putative toxin peaks were identified by comparison with the toxin standards. In the case of γ-amanitin, for which a standard was not commercially available, peaks could be tentatively identified by comparison with published HPLC data (Enjalbert et al. 1992).

Primer development and PCR

Primers were taken from the literature or designed based on the conserved sequence motifs given in Table 6. Primers and the combinations in which they were used are listed in Tables 7 and 8.

Table 7. Primers used in this study.

Primer	Sequence (5' - 3')	Amplifies	Source
TGD	AWIGARKSICCICCIRRSIMRAARAA	YRTGD L/R R (f)	Turgay & Marahiel 199
G	TCTAGAGGNAARCCNAARGG	RGKPKG (f)	Panaccione 1996
JA 1	CARGARGGIYTIATGGC	QEGLMA (f)	Wiest (pers. comm.)
JA 4F	TTYACITCIGGITCIACIGG	FTSGSTG (f)	Wiest (pers. comm.)
ELGEIE	GARYTNGSNGARATHGA	EL G/A EIE (f)	Hallen
YGP	TAYGGNCCNACNGA	YGPTE (f)	Hallen
YRT	TAYMGIACIGGIGAYYTIGT	YRTGDLV (f)	Hallen
LGG	TWYCGIACIGGIGAYYKIGKICG	LLXLGGXS (r)	Turgay & Marahiel 199
Y	ARRTCNCCNGTYTTRTATCTAGA	YKTGDL (r)	Panaccione 1996
JA 2	CCIGAIAYIGTIGYICCRAA	FG T/A T V/I SG (r)	Wiest (pers. comm.)
JA 5	GGIACYTGITGRTCYTT	KDTQVK (r)	Wiest (pers. comm.)
DVY	GTKCANGSRWANACRTCYTC	EDV Y/F A/P CT (r)	Hallen
GGDS	GCNGYDATNSWRTCNCCNCC	GGDSI A/T A (r)	Hallen
PCTPLQ	TGIARIGGIGTRCAIGG	PCTPLQ (r)	Hallen

[a](f) = forward primer, (r) = reverse primer.

Table 8. Estimated product size (bp) for each primer combination

Primer	LGG	Y	JA2	JA5	DVY	GGD	PCT
TGD	630	3500*	1690	50	780	630	780
G	1290	750	2360	820	1510	1290	1510
JA 1	3060	2430	800	2510	3500*	3060	3500*
JA 4F	1290	750	2360	820	1510	1290	1510
ELGEIE	520	3450	1570	3500*	780	520	780
YGP	740	210	1950	270	1130	730	1130
YRT	630	3500*	1690	50	780	630	780

Estimates are derived from sequence of *Cochliobolus carbonum*.
* Primers are partially complementary. An approximately 3500 bp product could result from primers binding to analogous sites in different domains.

DNA was extracted from 250 mg of a fruiting body collected in 1996 that had tested positive for amatoxins. Approximately 10 mg of gill tissue apiece was

placed in each of 20 microfuge tubes, frozen in liquid nitrogen and macerated. Then, 1 ml cetyltrimethyammonium bromide (CTAB) mixture (5% w/v CTAB, 1.4 M NaCl, 20 mM EDTA pH 8.0, 100 mM Tris-HCl pH 8.0, 1% w/v polyvinylpyrrolidone (PVP-360)) and 2 μl β-mercaptoethanol were added to each tube. The tube was incubated at 65°C for 1 h. The mixture was purified with phenol:chloroform:isoamyl alcohol (24:24:1) and chloroform extractions, then centrifuged to remove solids. The water soluble fraction was precipitated with absolute ethanol and centrifugation, followed by a rinse with 70% ethanol and a second centrifugation. The precipitate was air-dried under vacuum then resuspended in 50 μl water.

Approximately 1-20 ng of the total genomic DNA was used per 25 μl reaction mixture for polymerase chain reaction (PCR) amplification. Various brands of prepackaged buffers and polymerases were used for PCR amplification. Primers were used in the combinations given in Table 2. Additionally, primers were used individually as controls. An HC-toxin producing strain of *Cochliobolus carbonum* was used as a positive control.

The cycling reactions were performed in a DNA thermal cycler, model PTC-100 (MJ Research, Inc., Waltham, MA, USA). A 60°C to 45°C touchdown protocol was used. The amplification ended with an additional 10 min extension at 72°C, and storage at 4°C. PCR products were visualized on 1.5% agarose with uv light. A band was considered to represent a putative cyclic peptide synthetase gene product if the band was unique to a combination of two primers and did not appear in either of the individual-primer control reactions. Additionally

the band size could be compared with the predicted size, based on known CPS gene sequences. Any such band was gel purified and cloned using a TOPO® TA cloning kit (Invitrogen, Carlsbad, CA, USA). As CPS genes contain multiple domains, each of which may be expected to amplify with a given primer pair, clones were subject to a restriction digest with *Hae* III, which has a four base pair recognition site (four-cutter). A clone of each restriction pattern was sequenced. Sequencing was performed by the Michigan State University Genomics Technology Support Facility, using dye terminator capillary electrophoresis on an ABI Prism® 3700 DNA Analyzer (Applied Biosystems, Foster City, CA, USA). Sequences were submitted to a BLAST search (Altschul et al. 1997) through NCBI GenBank for comparison with known CPS gene sequences.

Pyrophosphate exchange assay

Pyrophosphate exchange assays followed Walton (1987).

Results

All *Galerina* cultures including the *G. autumnalis* monokaryon tested positive with HPLC for α-amanitin. The monokaryon had approximately one tenth the amatoxin of the dikaryon. No peaks consistent with γ-amanitin were observed. It should be noted that Gulden and colleagues (2001) have recently synonymized *G. autumnalis* and *G. marginata* (as *G. marginata*), based on

molecular phylogenetic evidence. Cultures that had been transferred to the carbon-starved GFV broth consistently showed a two- to threefold increase in toxin levels than cultures that had been grown in HSV only.

PCR products were readily obtained. However, most products were consistent with single primer reactions (Figure 24). Seven products that did appear unique to a primer combination were gel purified, cloned and sequenced. Without exception, the sequences matched bacterial DNA, but not cyclic peptide synthetases of bacterial origin. Therefore the sequences were considered to originate from probable contaminants. No matches to peptide synthetases were obtained.

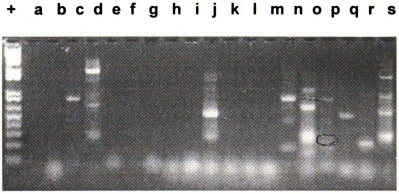


Figure 24. Typical gel showing PCR products from *G. marginata* amplified by CPS primers. + = 1 kb+ ladder, a - s are lanes loaded with PCR products. Lane "d" is *G. marginata* amplified by primers JA1 and LGG; it contains no bands that are not also shown in lane "s", amplified by JA1 alone. Lane "n" was amplified by primer G alone.

Discussion

Attempts to amplify amatoxin synthetase gene fragments using PCR presented many problems. The primers were degenerate, requiring low annealing temperatures that lowered primer specificity. The profusion of bands produced caused difficulty in reading the gels. This difficulty could be overcome by using a higher percentage of agarose in the gel which would interfere with gel purification attempts. Finally, we were using primers based on sequences from four ascomycetes and one heterobasidiomycete in attempts to amplify sequence from a homobasidiomycete. It is possible that homobasidiomycete CPS gene sequences differ sufficiently from those of other groups of fungi to prevent amplification of the desired products. Attempts to BLAST the *Phanerochaete chrysosporium* genome database (<http://www.jgi.doe.gov/programs/whiterot.htm>) with conserved sequence from HC-toxin synthetase yielded no matches. This may mean that *P. chrysosporium*, a white-rotting homobasidiomycete, contains no cyclic peptide synthetases. Alternately, it may indicate divergence.

Summary and future directions

The molecular phylogenetics of *Amanita* suggest one acquisition of amatoxin synthesis within the genus (see Chapter one). Amatoxins are found only in one monophyletic, derived clade. The structurally-similar phallotoxins do

not occur in any *Amanita* species that does not also produce amatoxins (Wieland 1986). Amatoxins are produced by one species of *Conocybe*, *C. filaris*, while phallotoxins are produced by *Conocybe lactea*, which is not closely related to *C. filaris* (Chapter four), suggesting a separate acquisition event for each cyclic peptide in *Conocybe*. In *Galerina* and *Lepiota*, 28S rDNA data places amatoxin-producing species in monophyletic, derived clades (Moncalvo et al. 2002). *Amanita, Conocybe, Galerina* and *Lepiota* are all well separated from each other in molecular phylogenetic analyses (Moncalvo et al. 2002). Together, these data support the hypothesis of multiple independent gains of amatoxin synthesis.

We have begun to search for the amatoxin synthetase enzyme in *Galerina marginata* from culture and dried fruiting bodies using pyrophosphate exchange assay (Walton 1987), but this work is still in the initial stages. We plan to continue efforts to isolate and, ultimately, sequence amatoxin synthetase. Amatoxin synthetase gene sequence would enable us to determine whether amatoxins share a biosynthetic pathway with the heptapeptide phallotoxins.

We also plan to sequence the portion of the RNA polymerase II large subunit (RPB1) responsible for amatoxin binding from toxin producers and nonproducers. The complete RPB1 sequence from *Amanita phalloides* has recently become available (Liu, pers. comm., 11 July 2002), enabling us to design specific primers. The amatoxin binding region of pol II is encoded by regions D - F of RPB1, and amatoxin-resistant mice are known to have mutations in this region (Bartolomei & Corden 1995). Toxin producing fungi are known to possess a modified pol II (Wieland 1986). With the data already available on the

evolutionary history of amatoxin-producing mushrooms, RPB1 and amatoxin synthetase sequence data should be a powerful tool in examining the origins and evolutionary history of amatoxin synthetase.

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APPENDICES

Appendix 1. Aligned *Amanita* ITS sequence for Chapter 1.

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Amanita pleropus          --TTTTT--GCCATTGCTTTCTTCATT-----TTTCCA-CCTGTGCACC-TTTTGTAGACCAAGGGTTAGAGGA----GGTT
A reidii                  --TGTGCACGTCTCTGGT--CATTAAC-AAT---TCCA-CCTGTGCACAT--TTGTAGACACTTGGGAATGAGA-----GAC
A phalloides umbrina      --TGTGCACGTCTCTGGT--CATTAAC-AAT---TCCA-CCTGTGCACAT--TTGTAGACACTTGGGAATGAGA-----GAC
A marmorata myrtacearum   --TGTGCACGTCTCTGGT--CATTAAC-AAT---TCCA-CCTGTGCACAT--TTGTAGACACTTGGGAATGAGA-----GAC
A bisporigera             --TGTGCACGTCTCTGGT--CATTACC-AAT---TCCA-CCTGTGCACAC--TTGTAGACACTTGGGAATGAGA-----GAC
A bisporigera tetraspora  --TGTGNNNGTNTTTGGT--CATTACC-AATC--CCNC-NTTGNGCACAC--TTG-AGACANTTGGNAATGANA-----GCT
A virosa France           --TGTGCACGTCTCTGGT--CATTACC-AAT---TCCA-CCTGTGCACACACTTGTAGACACTTGGGAATGAGA-----GAC
A virosa ss auct amer.    --TGTGCACGTCTCTGGT--CATTACC-AAT---TCCA-CCTGTGCACAC--TTGTAGACACTTGGGAATGAGA-----GAC
A ocreata                 --TGTGCANGGTCTCNGG--CATTCCC-AAT---TCCN-CGTGTGCACNT--TTGTAGCCCANTGGNAATGAGA-----GCN
A arochae                 --AGNGCAGGTNTNTGGT--AATTNCC-AAT---NCCN-CTTGNGCACTT--T-GTANACATTNGGAAAAAAG--------C
A phalloides Australia    --TGTGCACGTCTCTGGT--CATTACC-AAT---TCCA-CCTGTGCACAC--TTGTAGACACTTGGGAATGAGA-----GAC
A phalloides Norway       --TGTGCACGTCTCTGGT--CATTACC-AAT---TCCA-CCTGTGCACAC--TTGTAGACACTTGGGAATGAGA-----GAC
A phalloides alba France  --TGTGNACGTCTCTGGT--CATTTCCCAAT---TCCA-CCTGTGCACAC--TTGTANNCACTTGGAAATGAGA-----GAC
A phalloides California   --TGTGCANGTNTCTGGT--CATTACC-AAT---TCCA-CNTGTGCACAC--TTGTAGACACTTGGGAATGAGA-----GAC
A sp T27                  --TGTGCACGTCTCTGGT--CATTACC-AAT---TCCA-CCTGTGCACAC--TTGTAGACACTTGGGAATGAGA-----GAC
A gilbertii               --TGTGCACCCTGTCGTTGTGGCGCAT-AATATTTTT-CCCCGTGCATA--TGTGTAGACCTTCC----TGGGA--------
A cylindrispora           --TGCGCACCCCTGTGCTGTGGCGCAT-AATATTTAAACCCCGTGCATA--TGTGTAGACCTTCC----TGGGA--------
A virosa ss auct mexic.   --TGC--ACGTCTCTTGCTTATTTGATGAAAATTTCCA-CCCGTGCATGA-TGTGTAGATTGCCTAGGATTTGAGA---AGC
A sp SA22                 --TTTTTT-GCCATTGCTTTNTTCATT-----TTTCCA-CCTGGGCACN-TTTNGTAGNCCAAGGGTTANAGNA----GGTT
A thiersii                CATTTCTCT-ATATCCACCTGTGCA-------CTATAT-GTTGTAGACT-CTTGGGTTATAGGAAGGGAGGCGA----TATA
A muscaria guessowii      CGCGTCTCTCTTGCTTGTTTCTTCATT----CTCTCCA-CTTGTGCACT-GCTTGTAGGCAGCC----------------CTG
A fulva                   CGGCTCTGG-TCACTTGTTTCTTC-------TGTTCCA-CCTGTGCACT-GCCTGTAGACA--------------------
A cf subphalloides        CATTTGCTGCTTATTTCATTCATT--------TTTCCT-CCTGTGAACC-TTTTGTAGACACTTGGGAATGAGAAGTTGGTT
A citrina lavendula       CTGTTGCTACTTATTTCATTCATT--------TTTCCT-CCTGTGCACCGTTTTGTAGACACTTGGGAATGAGAGGTTGGTT
A flavoconia              CTTTTGCTGCTTGCTTCATTCTCT--------TCTCCA-CCTGTGCACT-CTTTGTAGACACTCGGGA-TGTGAGAGAGGTT

Amanita pleropus          -CCCATGCCTCCTTGTTCAAAAGTCC--AA-GGTCT---ATG----ATAATAATTCT-ACATACACTCTATTTGAATGT-TT
A reidii                  ATT-AACC-AGTCTCTTGAGA-AGTCAAA--GTCT---GGGTGTCTATG-CATTTTAAATAAACACA--AGTTGCATGTCTT
A phalloides umbrina      A---AACC-AGTCTCTTGAGA-AGTCAAA--GTCTGATTGGTGTCTATG-CATTTTAAATAAACACA--AGTTGCATGTCTT
A marmorata myrtacearum   ATT-AACC-AGTCTCTTGAGA-AGTCAAA--GTCTG---GGTGTCTATG-CATTTTAAATAAACACA--AGTTGCATGTCTT
A bisporigera             TTT-GACC-GGTCTCTTGAGGGAATTGAA--CTCTG---GGTGTCTATGCCATTTTA-TCAAACACT--AGTTGCATGT-TT
A bisporigera tetraspora  TTT-GACCCAGTNTCTTGAGAGAATTAAA---TCTG---GGTGTCTACGCCATTTTA-TNACACACT--AGTTGCATGT-CT
A virosa France           TTT-GACC-AGTCTCTTGAGAGATTTCAT--ATCTG---GGTGTCTATGCCTTTTTA-TTACACACT--AGTTGCATGT-TT
A virosa ss auct amer.    TTT-GACC-AGTCTCTTGAGAGAATTGAA--GTCTG---GGTGTCTATGCCATTTTA-TTAAACACT--AGTTGTATGT-TT
A ocreata                 TTT-GACC-AGTCTCTTGAGAGAATTGAA--ATCTG---GGTGTCTATGCCATTTTA-TTAACCCCT--AGTGGCATGT-TT
A arochae                 TTT-GCCC-AGTCTCTTGAAAGAATT-AA--ATCTG---GGGGTCAAAGCCATTTTA-TTACACACT--AGTGGCATGT-CT
A phalloides Australia    CTT-GACC-AGTCTCTTGAGA-AGTTGAAA-ATCTG---GGTGTCTATGCCATTTTA-TTAAACACT--AGTTGCATGT-TT
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Appendix 1. Chapter 1 *Amanita* ITS, cont.

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A phalloides Norway                CTT-GACC-AGTCTCTTGAGA-AGTTGAAA-ATCTG---GGTGTCTATGCCATTTTA-TTAAACACT--AGTTGCATGT-TT
A phalloides alba France           CTTTGACC-AGTCTCTTAAGA-AGTTGAAA-ATCTG---GGTGTCTATGCCATTTTA-TTAAACACT--AGTTGCATGT-TT
A phalloides California            NTT-GACC-AGTCTCTTGAGA-AGTTGAAA-ATCTG---GGTGTCTATGCCATTTTA-TTAAACACT--AGTTGCATGT-TT
A sp T27                           TTT-GACCCGGTCTCTTGAGA-AGTTGAAA--TCT---AGGTGTCTATGCCATTTTA-TTAAACACT--AGTTGCATGT-TT
A gilbertii                        -TT-AACCCC---------GGGGATT-----ATCT---ACGT---TA----TTTTTT-TGAAACACAAATTTTGCATGT-TT
A cylindrispora                    -TT-AACCCC---------GGGGATT-----ATCT---ACGT---TA----TTTTTT-TGAAACACAAATTTTGCATGT-TT
A virosa ss auct mexic.            AAGAGTTTTTTGGCTTTTGTTTATAC--AG-ATCTTC-AGGTGTCTATGTTTTTTTC-TATTACACTTAATGAGAATGT-TT
A sp SA22                          -CCCATGCCTCCTTGTTCAAAAG-CC-AAG-GTCT---ATG-----ATAATAATTCT-ACATACACTCTATTTGAATGT-TT
A thiersii                         -GTC-TGCCTTTCTGTGTCA-TGTCCAAA--GTCT---ATG-----ATGACTATACT-ACATACA---TGGTT-ATTGTAAT
A muscaria guessowii               -GCATTG---------------TTCAGGTT-GTCT---ATG-----AT----TTTCT-TTACATACA-----TGAACAC-TT
A fulva                            --CGCTGT-----------------------GTCT---ATG-----AT---ATTTGT-TAACACACA-----ACAATGT-TA
A cf subphalloides                 -GTAATG------ATTGACCTCTTGATATTGATCT---GGGTGTCTATGCCATTTTT-ATAAAGACA-TGGTTGCATGT-GT
A citrina lavendula                -GTAATGTAATGAATTGACCTCTTGAGGTCAGTCT---GGGTGTCTATGACATTTTT-ATAA--ACA-CGGCTGCATGT-GT
A flavoconia                       AG----------CATTGA-TTGTTGACCTCTGTCT---GGGTGTTTATG-TATTTTT-TGACATACA-CGTTTGAATGT-CT

Amanita pleropus                   ATAGAATGTCTTCTCTAGGCTT---GCTCATAGCCTT-TAAACTTTAAATATACAACT-TTCAACAACGGATCTCTTGGCTC
A reidii                           ATAGAATG-------ATGATTG----------------TA---A-TAAA-GTACAACT-TTCAACAACGGATCTCTTGGCTC
A phalloides umbrina               ATAGAATG-------ATAATTG----------------TA---A-TAAA-GTACAACT-TTCAACAACGGATCTCTTGGCTC
A marmorata myrtacearum            ATAGAATG-------ATGATTG----------------TA---A-TAAA-GTACAACT-TTCAACAACGGATCTCTTGGCTC
A bisporigera                      ATAGAATG-------ACGATTT----GAT---------TGAATA-TAAA-ATACAACT-TTCAACAACGGATCTCTTGGCTC
A bisporigera tetraspora           ATAGAATG-------ATAATTT----GAT---------TAAATA-TAAA-ANANCCCT-TTCACCANCGGATCTCTTGGCTC
A virosa France                    ATAGAATG-------ATGATTT----GAT---------TAAATA-TAAA-ATACAACT-TTCAACAACGGATCTCTTGGCTC
A virosa ss auct amer.             ATAGAATG-------TTGAGTT----GAT---------AAAATA-TAAA-ATACAACT-TTCAACAACGGATCTCTTGGCTC
A ocreata                          ATAGAATG-------ATGATTT----GAT---------TAAATA-TAAA-ATACAACT-TTCAACACGGAATCTCTTGGCTC
A arochae                          ATAGAATG-------ATAATTT----GAT---------TAACTA-TAAA-ATCCACCT-TTCANCACGGGATCTCTTGGCTC
A phalloides Australia             ATAGAATG-------ATGATTT----GAT---------TAAATA-TAAA-GTACAACT-TTCAACAACGGATCTCTTGGCTC
A phalloides Norway                ATAGAATG-------ATGATTT----GAT---------TAAATA-TAAA-GTACAACT-TTCAACAACGGATCTCTTGGCTC
A phalloides alba France           ATAGAATG-------ATGATTT----GAT---------TAAATA-TAAA-GTACAACT-TTCAACAACGGATCTCTTGGCTC
A phalloides California            ATAGAATG-------ATGATTT----GAT---------TAAATA-TAAA-GTACAACTCTTCACCAACGGATCTCTTGGCTC
A sp T27                           ATAGAATG-------ATGATTT----GAT---------TATATA-TAAA-GTACAACT-TTCAACAACGGATCTCTTGGCTC
A gilbertii                        ATAGAGTG--------TGGGT-----GATAATGCCTTATAAATATTAAA--TACAACT-TTCAACAACGGATCTCTTGGCTC
A cylindrispora                    ATAGAGTG--------TGGGT-----GATAATGCCTTATAAATATTAAA--TACAACT-TTCAACAACGGATCTCTTGGCTC
A virosa ss auct mexic.            ---GAATG--------TGGTTT-----A-------TTATGA----TAAA--TACAACT-TTCAACAATGGATCTCTTGGCTC
A sp SA22                          ATAGAATGTCTTCTCTAGGCTT---GCTCATAGCCTT-TAAACTTTAAATATNCAACT-TTCAACAACGGATCTCTTGGCTC
A thiersii                         GTTGAATATGTTTTTT-GGCT-----GTTAAAGCCTT-TAAA---TAACTATACAACT-TTCAACAACGGATCTCTTGGCTC
A muscaria guessowii               GTTG--TCAGAATGT-----------GATAAA-------AAATAGTAA---TACAACT-TTCAACAACGGATCTCTTGGCTC
A fulva                            GTTG--GCATGTTAT----------ATATAA-------TAA-TAAAAT--ATACAACT-TTCAACAACGGATCTCTTGGCTC
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Appendix 1. Chapter 1 *Amanita* ITS, cont.

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A cf subphalloides          ATAGAATGAGATTGTATGGTTTTTTTAATAAAGCCTT-TAAATGATAA---TACAACT-TTCAACAACGGATCTCTTGGCTC
A citrina lavendula         ATGGAATGAGACTGTA-GGTTTTTAATGAAAAGCCTT-GAAATGATAAA-GTACAACT-TTCAACAACGGATCTCTTGGCTC
A flavoconia                ATAGAATGAAAATGTA-GGCTTT----TGTCAGCCTT-TAAATGATAAA-ATACAACT-TTCAACAACGGATCTCTTGGCTC

Amanita pleropus            TCGCATCGATGAAGAAC-GCAGC-GAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGC
A reidii                    TCGCATCGATGAAGAAC-GCAGC-GAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGC
A phalloides umbrina        TNGCATCGATGAAGAAC-GCAGC-GAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGC
A marmorata myrtacearum     TCGCATCGATGAAGAACCGCAGC-GAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGC
A bisporigera               TCGCATCGATGAAGAAC-GCAGCCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGC
A bisporigera tetraspora    TCGCATCGATGAAGANC-GCAGC-GAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGGACGC
A virosa France             TCGCATCGATGAAGAAC-GCAGC-GAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGC
A virosa ss auct amer.      TCGCATCGATGAAGAAC-GCAGC-GAAATGCGATAAGTAATGTGAAATGCAGAATTCAGTGAATCATCGAATCTTTGAACGC
A ocreata                   TCGCATCGATGANGAAC-GCAGC-GAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGC
A arochae                   TCGCATCGATGAAGAAC-GCAGC-GAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGC
A phalloides Australia      TCGCATCGATGAAGAAC-GCAGC-GAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGC
A phalloides Norway         TCGCATCGATGAAGAAC-GCAGC-GAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGC
A phalloides alba France    TCGCATCGATGAAGAAC-GCAGC-GAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGC
A phalloides California     TCGCATCGATGAAGAAC-GCAGC-GAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGC
A sp T27                    TCGCATCGATGAAGAAC-GCAGC-GAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGC
A gilbertii                 TCGCATCGATGAAGAAC-GCAGC-GAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATGTTTGAACGC
A cylindrispora             TCGCATCGATGAAGAAC-GCAGC-GAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATGTTTGAACGC
A virosa ss auct mexic.     TCGCATCGATGAAGAAC-GCAGC-GAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATGTTTGAACGC
A sp SA22                   TCGCATCGATGAAGAAC-GCAGC-GAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGC
A thiersii                  TCGCATCGATGAAGAAC-GCAGC-GAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAGTTTTTGAACGC
A muscaria guessowii        TCGCATCGATGAAGAAC-GCAGC-GAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGC
A fulva                     TCGCATCGATGAAGAAC-ACAGC-GAAATGTGATAAGTAATGTGAATTGCAGAATCCAGTGAATCATCGAATCTTTGAACGC
A cf subphalloides          TCGCATCGATGAAGAAC-GCAGC-GAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGC
A citrina lavendula         TCGCATCGATGAAGAAC-GCAGC-GAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGC
A flavoconia                TCGCATCGATGAAGAAC-GCAGC-GAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGC

Amanita pleropus            ACCTTGCGCTCCTTGGTATTCCGAGGAGCATGCCTGTTTGAGTGTCAGTAAATT--CTCAAAACTCAACAT---TTTTATTT
A reidii                    ACCTTGCGCTCCTTGGCATTCCAAGGAGCATGCCTGTTTGAGTGTCATTAAAGT--CTCAAGACC-TGTCTGC------ATT
A phalloides umbrina        ACCTTGCGCTCCTTGGCATTCCAAGGAGCATGCCTGTTTGAGTGTCATTAAAGN--CTCAAGACC-TGTCTGC------ATT
A marmorata myrtacearum     ACCTTGCGCTCCTTGGCATTCCAAGGAGCATGCCTGTTTGAGTGTCATTAAAGT--CTCAAGACC-TGTCTGC------ATT
A bisporigera               ACCTTGCGCTCCTTGGCATTCCGAGGAGCATGCCTGTTTGAGTGTCATTAACAT--CTCAAGACC-TGTCTGC------TTT
A bisporigera tetraspora    ACCTTGCGCTCCTTGGTATTCTGAGGAGCATGCCTGTTTGAGTGTCATTAACAT--CTCAAGACC-TGTCTGC------TTT
A virosa France           ACCTTGCACTCCTTGGCATTCCGAGGAGCATGCCTGTTTGAGTGTCATTAACAT--CTCAAGACC-TGTCTGT------TTT
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Appendix 1. Chapter 1 *Amanita* ITS, cont.

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A virosa ss auct amer.    ACCTTGCGCTCCTTGGCATTCTGAGGAGCATGCCTGTTTGAGTGTCATTAACAT--CTCAAGACCCTGTCTGC------TTT
A ocreata                 ACCTTGCGCTCCTTGGCATTCCGAGGAGCATGCCTGTTTGAGTGTCATTAACAT--TTCAAGACC-TGTCTGC------ATT
A arochae                 ACCTTGCGCTCTTTGGTATTCTGAGGAGCATGCCTGTTTGAGTGTCATTAACAT--CTCAAGACC-TGTCTGC------TTT
A phalloides Australia    ACCTTGCGCTCCTTGGCATTCCGAGGAGCATGCCTGTTTGAGTGTCATTAAATT--CTCAAGACC-TGTCTGC----TTTTT
A phalloides Norway       ACCTTGCGCTCCTTGGCATTCCGAGGAGCATGCCTGTTTGAGTGTCATTAAATT--CTCAAGACC-TGTCTGC----TTTTT
A phalloides alba France  ACCTTGCGCTCCTTGGCATTCCGAGGAGCATGCCTGTTTGAGTGTCATTAAATT--CTCAAGACC-TGTCTGC----TTTTT
A phalloides California   ACCTTGCGCTCCTTGGCATTCCGAGGAGCATGCCTGTTTGAGTGTCATTAAATT--CTCAAGACC-TGTCTGC----TTTTT
A sp T27                  ACCTTGCGCTCCTTGGCATTCCAAGGAGCATGCCTGTTTGAGTGTCATTAAAGT--CTCAAAACC-TGTCTGA------TTT
A gilbertii               ACCTTGCACTCCTTGGTATTCTGAGGAGTATGCCTGTTTGAGTGTCATTAAAAC--ATCAAAAACACCAGTGAATTTATTTT
A cylindrispora           ACCTTGCACTCCTTGGTATTCTGAGGAGTATGCCTGTTTGAGTGTCATTAAAAC--ATCAAAAACACCAGTGAATTTATTTT
A virosa ss auct mexic.   ATCTTGCGCTCCTTGGCATTCCTTGGAGCATGCCTGTTTGAGTGTCATTTAA-TATTGCAAGACTAATTCTGAGTATGATTT
A sp SA22                 ACCTTGCGCTCCTTGGTATTCCGAGGAGCATGCCTGTTTGAGTGTCAGTAAATT--CTCAAAACTCAACAT---TTTTATTT
A thiersii                ACCTTGCGCTCCTTGGTATTCCGAGGAGCATGCCTGTTTGAGTGTCAGTATATT--CTCAAAA----GCAT---TTGGTTG-
A muscaria guessowii      ATCTTGCGCTCCTTGGTATTCCGAGGAGCATGCCTGTTTGAGTGTCATTAAATTCTCTCAAAGCAT-ACAC---TTGAGTG-
A fulva                   ATCTTGCGCTCCTTGGCATTCCGAGGAGCATGCCTGTTTGAGTGTCATTAAA--CTATCAAAACAC-TTTT---TTTGTTG-
A cf subphalloides        ATCTTGCGCTCCTTGGTATTCCGAGGAGCATGCCTGTTTGAGTGTCATTAAG-TATCTCAAAAGCTCTTACTTATGTGCTAT
A citrina lavendula       ATCTTGCGCTCCTTGGTATTCCGAGGAGCATGCCTGTTTGAGTGTCATTCAAGTATCTCAAAAGCTGTTA----TGCAGTAT
A flavoconia              ATCTTGCGCTCCTTGGTATTCCGAGGAGCATGCCTGTTTGAGTGTCATTAAA-TATCTCAAAAAGC-TTGTGC-TTTTTTGG

Amanita pleropus          TGTTGAGCATTTTGGAAT-T-GGGGGT----TGCAGGCTTACTTA---GATGG-------CCTGNTCTCCTT--GAATGTAT
A reidii                  TG--ATAGGTATTGGATGTT-GGGAGT----TGCAGGCTTACTTGTG-GATGG-------CCTGCTCTCCTT--GAATGTAT
A phalloides umbrina      TG--ATAGGTATTGGATGTT-GGGAGT----TGCAGGCTTACTTA---GATGG-------CCTGCTCTCCTT--GAATGTAT
A marmorata myrtacearum   TG--ATAGGTATTGGATGTT-GGGAGT----TGCAGGCTGTTTCA---GATAG-------CTTGCTCTCCTT--GAATGTAT
A bisporigera             TG--ATAGGTATTGGATTTT-GGGAGT----TGCAGGCTTTGTCA---GATAG-------CCTGCTCTCCTT--GAATGTAT
A bisporigera tetraspora  TG--ATAGGTATTGGATTTTTGGGGGT----TGCAGGCTGTTTCA---GATAG-------CTTGCTCTCCTT--GAATGTAT
A virosa France           TG--ATAGGTATTGGATTTTTGGGGGTT---TGCAGGCTTTTTCA---GATAG-------CCTGCTCTCCTT--GAATGTAT
A virosa ss auct amer.    TG--ATAGGTATTGGATTTT-GGGGGT----TGCAGGCTGGTTCA---GATAG-------CTTGCTCTCCTT--GAATGTAT
A ocreata                 TG--ATAGGTATTGGATTTTTGGGGGT----TGCAGGCTGTTTCA---GATTG-------CTTGCTCTCCTT--GAATGTAT
A arochae                 TG--ATAGGTATTGGATTTTTGGGGGN----TGCAGGCTGTTTCA-A-TATAG-------CTTGCTCTCCTT--GAATTTAT
A phalloides Australia    TG--ATAGGTATTGGATTTTTGGGGGT----TGCAGGCTGTTTCA---AATAAAAATAGCCTTGCTCTCTTT--GAATGTAT
A phalloides Norway       T---ATAGGTATTGGATTTTTGGGGGT----TGCAGGCTGTTTCA---AATATAA-TAGCCTTGCTCTCTTT--GAATGTAT
A phalloides alba France  TG--ATAGGTATTGGATTTTTGGGGGT----TGCAGGCTGTTTCA---AATAAAAATAGCCTTGCTCTCTTT--GAATGTAT
A phalloides California   TG--ATAGGTATTGGATTTTTGGGGGT----TGCAGGCTGTTTCA---AATAAAAATAGCCTTGCTCTCTTT--GAATGTAT
A sp T27                  TG--ATAGGTATTGGATTTT-GGGGGT----TGCAGGCTGTTTCA---AATATA-----NCTTGCTCTNCTT--GAATGTAT
A gilbertii               TG-CGGTGGATTTGGATTAT-GGGAGT----TGCAGGCCTCTCATGA-CATTTTGGGG--CCAGCTCTCCTG--AAATGCAA
A cylindrispora           TG-CGGTGGATTTGGATTAT-GGGAGT----TGCAGGCCTCTCATGA-CATTTTGGGG--CCAGCTCTCCTG--AAATGCAA
A virosa ss auct mexic.   CGGAAGATGTTTTGGAATTT-GGGAGT----TGCAGA--TTTCATTA-TATTTGAA----CTAGCTCTCCTG--AAATGGAT
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Appendix 1. Chapter 1 *Amanita* ITS, cont.

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A sp SA22                    TGTTGAGCATTTTGGAAT-T-GGGGGT----TGCAGGCTCACT-----AATGTTGGG---TCAGCTCTCCTC--AAATATAT
A thiersii                   CTTTTGG-AGTTGGGGGT-T-GCAGGC----TGTACT-TTTAC-----AATGGTAG----TCAGC--TCCTCTTAAAAG-AA
A muscaria guessowii         -----TGTGTTTTGGATTGT-GGGAGT-GTCTGCTGG-CTTTAT--------GAG-----CCAGCTCTCCTG--AAAGACAT
A fulva                      ----AAGTGTTTTGGACTTT-GGGAGTACTGTGCTGG-TCTCTTTTGTTTTGGAC-----CCAGCTCTCCTC--AAAAGCAT
A cf subphalloides           ACATGAGAGTTTTGGACATT-GGGAGT----TGCTGG-TCACT------AA-GTGA----TGGGCTCTTCTG--AAAAGCAT
A citrina lavendula          GCATGGAACTTTTGGACATT-GGGAGT----TGCTGG-TCACTG---ATAAAGTGG----TAGGCTCTTCTG--AAAAATAT
A flavoconia                 -CACAGGAGTTTTGGACATT-GGGAGT----TGCCGG-CTGCTGGAT-AACAGTGG----TGGGCTCTTCTG--AAAAGCAT

Amanita pleropus             TA-GTGGAGTTTT----AACTCAAATGTGAACTACCTCTTATTGGTGTGATA-A--TTATCTACNCCAGGAGCAAG-CT-
A reidii                     TAAGTGGATGAAA------------GACCATTTGAACTCCACTGGTGTGATAAAGCCTATCTATGCCAGG-AGTAAT-AT
A phalloides umbrina         TA-GTGGATGAAA------------GACCATTTGAACTCCACTGGTGTGATAAAGCCTATNTATGCCAGG-AGTNAT-AT
A marmorata myrtacearum      TA-GTGGATGAAA------------GACCATTTGAACTCCACTGGTGTGATAAAGCCTATCTATGCCAGG-AGTAAT-AT
A bisporigera                TA-GTGGGAGAAA------------AGCTGGT-GAACTCCATTGGTGTGATNAAATCTATCAATGCCNGG-AGCCAC-CN
A bisporigera tetraspora     TA-GTGGAGAAAG------------AGCCATT-GAACTCCATTGGTGTGATAAAATTTATCAATGCTAGG-AGCCAT-GT
A virosa France              TA-GTGGAGAAAA------------GACCATT-GNACTCCATTGGTGTGATAAAATCTATCAATGNCAGG-AGCCAT-GT
A virosa ss auct amer.       TAAGTGGAGAAAA--------------TCATT-GAACTCCTTTGGTGTGATAACATCTATCAATGCCAGG-AGCAAT-GT
A ocreata                    TA-GTGGAGAAAA------------AGCCATT-GAACTCCATTGGTGTGATAAAACCTATCAATGCCAGG-AGCAAT-GT
A arochae                    TA-GTGGAGAAAG------------AGCCATT-GAACTCCATTGGTGTGATAAAATTTATCAATGCTAGG-AGCCAT-GT
A phalloides Australia       TA-GTGGAGAAA-------------AGCCATT-GAACTCCATTGGTGTGATAAAACCTATCAATGCCAGG-AGCAAT-AT
A phalloides Norway          CA-GTGGAGAAGG------------AGCCGTT-GGACTCCA----GGTGATAA---CCTACAATGCAAGC-AATGA--GT
A phalloides alba France     TA-GTGGAGAAA-------------AGCCATT-GAACTCCATTGGTGTGATAAAACCTATCAATGCCAGG-AGCAAT-AT
A phalloides California      TA-GTGGAGAAA-------------AGCCATT-GAACTCCATTGGTGTGATAAAACCTATCAATGCCAGG-AGCAAT-AT
A sp T27                     TA-AGTGAGAAAG------------AGCCATTTGAACTCCATTGGTGTGATAAAACCTATCAATGCCAGG-AGCAAT-GT
A gilbertii                  TA-GCAGTCTCTGA-------------CGAAGCTTTGTTATTTGGTGTGATAAAGTA-ATTCACCGCCAGGAA-AAGCGT
A cylindrispora              TA-GCAGTCTCTGA-------------CGAAGCTTTGTTATTTGGTGTGATAAAGTA-ATTCACCGCCAGGAA-AAGCGT
A virosa ss auct mexic.      TA-GCAAAAAGGGC-----------ATTATGGGCCATTTTATTGGTGTGATAATGTTTATTGATCCCCCGGA--AAGG--
A sp SA22                    TA-GTGGAGTTTT----AACTCAAATGTGAACTACCTCTTATTGGTGTGATA-A--TTATCTACGCCAGGAGCAAG-CT-
A thiersii                   T----------------AGCTCGAGTGAG-----CCTTCATTTGGTGTGATC-A--CTATCTATGCTAAATGGATG----
A muscaria guessowii         TAGCTTTGGGGGGGAGGTGCCAAGTCACTTCTGCCTTTCCATTGGTGTGATAGA--TGAATT-AACTTATCT-ACG----
A fulva                      TAACTTTGGAGAA-------------------------CCATCAGTGTGATATA--TCATTT-TGTTTAT---ACG----
A cf subphalloides           TAGTTGAGGAGCT----------------TTT-GCACTCTATTGGTGTGATAAA--CTATCTATGCCAGGAG-AAG----
A citrina lavendula          TAGTTGAGGAGCT----------------TTTTGCACTCTATTGGTGTGATAGA--CCATCTATGCCAGGAG-AAG----
A flavoconia                 TAGTTGAGGAGCT-----------------TTGCACTCTATTGGTGTGATAGA--CTATCTATGCCAGGAG-ACG----
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Appendix 2. Aligned *Amanita* 28S sequence for Chapter 1.

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Amanita pleropus                AGCGGGAAAAGCTCAAATTTAAAATCTGGCAGTCTTTTG----------GCTGTCCGAGTTGTAATCTAGAGAAGTG-CTA
A cf subphalloides              ?????????AGCTCAAATTTAAA-TCTGGCAGTG-TTTG----------CTGTCCGAGTTGTA-CCTAGAGAAGTG-TAA
A citrina lavendula             AGAGG-AAAAGCTCAAATTTAAAATCTGACAGTGTTTTG----------CTGTCCGAGTTGCAACCTAGAGAAGTG-TAA
A flavoconia                    AGAGGGAAAAGCTCAAATTTAAAATCTGGCAGTG-TTTC----------ACTGTCCGAGTTGTAACCTAGAGAAGTG-TAA
A thiersii                      AGCGGGAAAAGCTCAAATTTAAAATCTGGCAGTC-TCTG----------GCTGTCCGAGTTGTAATCTAGAGAAGTG-TTA
A virosa ss auct mexic.         AGTGGGAAAAGCTCAAATTTAAAATCTGGCAGTGATTTG----------CTGTCCGAGTTGTAACCTAGAGAAGTG-TAA
A cylindrispora                 AGCGGGATAAGCTCAAATTTAAAATCTGGCGGTCTTAGT----------GCNGTCCGAATTGTAACCTAGAGAAGTG-TTG
A fulva                         AAGCGGAAAAGCTCAAATTTGAAATCTGGCAGTCTATTTTTTCTTCTTTGGCTGTCCGAGTTGTAATCTAGAGAAGTGCTTA
A muscaria alba                 AGAGGGAAGAGCTCAAATTTAAAATCTGGCAGTGTCTTG----------CTGTCCGAGTTGTAATCTAGAGAAGTG-CTG
A muscaria guessowii            AGAGGGAAGAGCTCAAATTTAAAATCTGGCAGTGTTTTG----------CTGTCCGAGTTGTAATCTAGAGAAGTG-CTG
A gilbertii                     AGCGGNATAAGCTCAAATTTAAAATCTGGCGGTC-TTTA----------NCCGTNCGAATTTTAACCTAGAGAAGCG-GTG
A sp T27                        AGCGGGAAAAGCTCAAATTTAAAATCTGGCAGATGTTTG----------TCTGTCCGAGTTGTAACCTAGAGAAGTG-TAA
A magnivelaris                  AGCGGGAAAAGCTCAAATTTAAAATCTGGCAGATGTTTG----------TCTGTCCGAGTTGTAACCTAGAGAAGTG-TAA
A virosa ss auct amer.          AGCGGGAAAAGCTCAAATTTAAAATCTGGCAGGTGTTTG----------TCTGTCCGAGTTGTAACCTAAAGAAGTG-TAA
A bisporigera                   AGCGGGAAAAGCTCAAATTTAAAATCTGGCAGATTTTTG----------TCTGTCCGAGTTGTAACCTAGAGAAGTG-TAA
A virosa China                  AGCGGGAAAAGCTCAAATTTAAAATCTGGCAGATGCTTG----------TCTGTCCGAGTTGTAACCTAGAGAAGTG-TAG
A virosa France                 AGCGGGAAAAGCTCAAATTTAAAATCTGGCAGATGCTTG----------TCTGTCCGAGTTGTAACCTAGAGAAGTG-TAG
A phalloides Germany            AGCGGGAAAAGCTCAAATTTAAAATCTGGCAGATGTTTG----------TCTGTCCGAGTTGTAACCTAGAGAAGTG-TAA
A phalloides alba France        AGCGGGAAAAGCTCAAATTTAAAATCTGGCAGATGTTTG----------TCTGTCCGAGTTGTAACCTAGAGAAGTG-TAA
A phalloides California         AGCGGGAAAAGCTCAAATTTAAAATCTGGCAGATGTTTG----------TCTGTCCGAGTTGTAACCTAGAGAAGTG-TAA
A arochae                       AGCGGGAAAAGCTCAAATTTAAAATCTGGCAGATGCTTG----------TCTGTCCGAGTTGTAACCTAGAAAAGTG-TAA
A virosa Virginia               AGCGGGAAAAGCTCAAATTTAAAATCTGGCAGATGCTTG----------TCTGTCCGAGTTGTAACCTAGAGAAGTG-TAA
A ocreata                       AGCGGGAAAAGCTCAAATTTAAAATCTGGCAGATGTTTG----------TCTGTCCGAGTTGTAACCTAGAGAAGTG-TAA
A marmorata myrtacearum         AGCGGGAAAAGCTCAAATTTAAAATCTGGCAGAC-TTTG----------TCTGTCCGAGTTGTAACCTAGAGAAGTG-TAA
A phalloides umbrina            AGCGGGAAAAGCTCAAATTTAAAATCTGGCAGAC-TTTG----------TCTGTCCGAGTTGTAACCTAGAGAAGTG-TAA
A reidii                        AGCGGGAAAAGCTCAAATTTAAAATCTGGCAGAC-TTTG----------TCTGTCCGAGTTGTAACCTAGAGAAGCG-TAA

Amanita pleropus                TCCGTGCTGGACCATGTATAAGTCTCCTGGAATGGAGTATCACAGAGGGTGAGAATCCCGTCTTTGACATGGACTCCCAGTA
A cf subphalloides              CCTGTGCTGGACCATGTACAAGTCTCCTGGAATGGAGCATCACAGAGGGTGAGAATCCCGTCTTTGACATGGACTACCAGTG
A citrina lavendula             CCTGTGCTGGACCATGTACAAGTCTCCTGGAATGGAGCATCGCAGAGGGTGAGAATCCCGTGTTTGACATGGACTACCAGTG
A flavoconia                    CCTGCGCTGGACCGTGTATAAGTCTCCTGGAATGGAGCGTCACAGAGGGTGAGAATCCCGTCTTTGACACGGACTACCAGTG
A thiersii                      TCCGTGCTGAACCGTGTATAAGTCTCCTGGAATGGAGCGTCATAGAGGGTGAGAATCCCGTCTTGGACACGGACAACCAGTA
A virosa ss auct mexic.         CCTTCACTGGACCGTGTATAAGTCTCCTGGGATGGAGCNTCATAGAGGGTGAGAATCCCGTGTATGACATGGACTACCAGTG
A cylindrispora                 CCCGNGCTGAACCATGTATAAGTCTCNTGGGATGGAGTATCAGAGAGGGTGAGAATCCCGTCTTTGACATGGACTTCCAGTG
A fulva                         CCTGCACTGGACCATGTATAAGTCTCCTGGAATGGAGCGTCACAGAGGGTGAGAATCCCGTCTTTGACATGGACTGCCAGTG
A muscaria alba                 CCCGTGCTGGACCATGTACAAGTCTCCTGGAATGGAGCGTCACAGAGGGTGAGAATCCCGTCTTTGACATGGACTACCAGTG
A muscaria guessowii            CCCGTGCTGGACCATGTACAAGTCTCCTGGAATGGAGCGTCACAGAGGGTGAGAATCCCGTCTTTGACATGGACTACCAGTG
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Appendix 2. Chapter 1 *Amanita* 28S, cont.

A gilbertii	CCCGNNCTGGACCATGTATAAGNCTNCTGGGANGGAGNGACGGAGAGGGTGACAATCCCGACTTTGACATGGACTACCTGTG
A sp T27	CCTGTGCTGGACCGTGTACAAGCCTCCTGGAATGGAGCATCATAGAGGGTGAGAATCCCGTCTTTGACATGGACTACCAGTG
A magnivelaris	CCTGTGCTGGACCGTGTACAAGTCTCCTGGAATGGAGCATCATAGAGGGTGAGAATCCCGTCTTTGACATGGACTACCAGTG
A virosa ss auct amer.	CCTGTGCTGGACCGTGTACAAGTCTCCTGGAATGGAGCATCATAGAGGGTGAGAATCCCGTCTTTGACATGGACTACCAGTG
A bisporigera	TCTGTGCTGGACCGTGTACAAGTCTCCTGGAATGGAGCATCATAGAGGGTGAGAATCCCGTCTTTGACATGGACTACCAGTG
A virosa China	CCTGTGCTGGACCGTGTACAAGTCTCCTGGAATGGAGCATCATAGAGGGTGAGAATCCCGTCTTTGACATGGACTACCAGTG
A virosa France	CCTGTGCTGGACCGTGTACAAGTCTCCTGGAATGGAGCATCATAGAGGGTGAGAATCCCGTCTTTGACATGGACTACCAGTG
A phalloides Germany	CCTGTGCTGGACCGTGTACAAGTCTCCTGGAATGGAGCATCATAGAGGGTGAGAATCCCGTCTTTGACATGGACTACCAGTG
A phalloides alba France	CCTGTGCTGGACCGTGTACAAGTCTCCTGGAATGGAGCATCATAGAGGGTGAGAATCCCGTCTTTGACATGGACTACCAGTG
A phalloides California	CCTGTGCTGG-CCGTGTACAAGTCTCCTGGAATGGAGCATCATAGAGGGTGAGAATCCCGTCTTTGACATGGACTACCAGTG
A arochae	CCTGTGCTGGACCGTGTACAAGTCTCCTGGAATGGAGCATCATAGAGGGTGAGAATCCCGTCTTTGACATGGACTACCAGTG
A virosa Virginia	CCTGTGCTGGACCATGTACAAGTCTCCTGGAATGGAGCATCATAGAGGGTGAGAATCCCGTCTTTGACATGGACTACCAGTG
A ocreata	CCTGTGCTGGACCGTGTACAAGTCTCCTGGAATGGAGCATCATAGAGGGTGAGAATCCCGTCTTTGACATGGACTACCAGTG
A marmorata myrtacearum	CCTGTGCTGGACCATGTACAAGTCTCCTGGAATGGAGCATCACAGAGGGTGAGAATCCCGTCTTTGACATGGACTACCAGTA
A phalloides umbrina	CCTGTGCTGGACCATGTACAAGTCTCCTGGAATGGAGCATCACAGAGGGTGAGAATCCCGTCTTTGACATGGACTACCAGTA
A reidii	CCTGTGCTGGACCATGTACAAGTCTCCTGGAATGGAGCATCACAGAGGGTGAGGATCCCGTCTTTGACATGGACTACCAGTA
Amanita pleropus	CATT-GTGAT-GTGCTCTCAAAGAGTCGAGTTGTTTGGGAATGCAGCTCTAAATGGGTGGTAAATTCCATCTAAAGCTAAAT
A cf *subphalloides*	CATT-GTGGTTGTGCTCTCAACGAGTCGAGTTGTTTGGGAATGCAGCTCTAAATGGGTGGTAAATTCCATCTAAAGCTAAAT
A citrina lavendula	CATT-GTGGTTGTGCTCTCAACGAGTCGAGTTGTTTGGGAATGCAGCTCTAAATGGGTGGTAAATTCCATCTAAAGCTAAAT
A flavoconia	CATT-GTGGTTGTGCTCTCAACGAGTCGAGTTGTTTGGGAATGCAGCTCTAAATGGGTGGTAAATTCCATCTAAAGCTAAAT
A thiersii	CATT-GTGAT-GCGCTCTCAAAGAGTCGAGTTGTTTGGGAATGCAGCTCTAAATGGGTGGTAAATTCCATCTAAAGCTAAAT
A virosa ss auct mexic.	GATT-GTGGT-GTGCTCTCAAAGAGTTGAGTTGTTTGGGAATGCAGCTCTAAAAGGGTGGTAAATTCCATCTAAAGCTAAAT
A cylindrispora	CATT-GTGGT-ATGCTCTCAAAGAGTTGAGTTGTTTGGGAATGCAGCTCTAAATGGGTGGTAAATTCCATCTAAAGCTAAAT
A fulva	TGTT-GTGGT-GTGCTCTCAAAGAGTCGAGTTGTTTGGGAATGCAGCTCTAAATGGGTGGTAAACTCCATCTAAAGCTAAAT
A muscaria alba	CATTTGTGGT-GTGCTCTCAAAGAGTCGAGTTGTTTGGGAATGCAGCTCTAAATGGGTGGTAAACTCCATCTAAAGCTAAAT
A muscaria guessowii	CATTTGTGGT-GTGCTCTCAAAGAGTCGAGTTGTTTGGGAATGCAGCTCTAAATGGGTGGTAAACTCCATCTAAAGCTAAAT
A gilbertii	CATT---GGNTATGCTCTNAAAGAGGTGGGTTGGTTGGGAATGCNTCTCTNAATGGGNGGTAAANTCCATCTNAAGCTAAAN
A sp T27	CACT-GTGGTTGCACACTCAACGAGTCGAGTTGTTTGGGAATGCAGCTCTAAATGGGTGGTAAATTCCATCTAAAGCTAAAT
A magnivelaris	CACT-GTGGTTGCACACTCAACGAGTCGAGTTGTTTGGGAATGCAGCTCTAAATGGGTGGTAAATTCCATCTAAAGCTAAAT
A virosa ss auct amer.	CACT-GTGGTTGCACACTCAACGAGTCGAGTTGTTTGGGAATGCA-CTCTAAATGGGTGGTAAATTCCATCTAAAGCTAAAT
A bisporigera	CACT-GTGGTTGCACACTCAACGAGTCGAGTTGTTTGGGAATGCAGCTCTAAATGGGTGGTAAATTCCATCTAAAGCTAAAT
A virosa China	CACT-GTGGTTGCACACTCAACGAGTCGAGTTGTTTGGGAATGCAGCTCTAAATGGGTGGTAAATTCCATCTAAAGCTAAAT
A virosa France	CACT-GTGGTTGCACACTCAACGAGTCGAGTTGTTTGGGAATGCAGCTCTAAATGGGTGGTAAATTCCATCTAAAGCTAAAT
A phalloides Germany	CACT-GTGGTTGCGCACTCAACGAGTCGAGTTGTTTGGGAATGCAGCTCTAAATGGGTGGTAAATTCCATCTAAAGCTAAAT
A phalloides alba France	CACT-GTGGTTGCGCACTCAACGAGTCGAGTTGTTTGGGAATGCAGCTCTAAATGGGTGGTAAATTCCATCTAAAGCTAAAT
A phalloides California	CACT-GTGGTTGCGCACTCAACGAGTCGAGTTGTTTGGGAATGCAGCTCTAAATGGGTGGTAAATTCCATCTAAAGCTAAAT

Appendix 2. Chapter 1 *Amanita* 28S, cont.

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A arochae                    CATT-GTGGTTGTGCACTCAACGAGTCGAGTTGTTTGGGAATGCAGCTCTAAATGGGTGGTAAATTCCATCTAAAGCTAAAT
A virosa Virginia            CACT-GTGGTTGTGCACTCGACGAGTCGAGTTGTTTGGGAATGCAGCTCTAAATGGGTGGTAAATTCCATCTAAAGCTAAAT
A ocreata                    CACT-GTGGTTGTGCACTCAACGAGTCGAGTTGTTTGGGAATGCAGCTCTAAATGGGTGGTAAATTCCATCTAAAGCTAAAT
A marmorata myrtacearum      CACT-GTGGTTGCACACTCAACGAGTCGAGTTGTTTGGGAATGCAGCTCTAAATGGGTGGTAAATTCCATCTAAAGCTAAAT
A phalloides umbrina         CACT-GTGGTTGCACACTCAACGAGTCGAGTTGTTTGGGAATGCAGCTCTAAATGGGTGGTAAATTCCATCTAAAGCTAAAT
A reidii                     CACT-GTGGTTGCACACTCAACGAGTCGAGTTGTTTGGGAATGCAGCTCTAAATGGGTGGTAAATTCCATCTAAAGCTAAAT

Amanita pleropus             ATTGGCGAGAGACCGATAGTGAACAAGTACCGTGAGGGAAAGATGAAAAGAACTTTGGAAAGAGAGTTAAACAAGTNCGTGA
A cf subphalloides           ATTGGCGAGAGACCGATAGCGAACAAGTACCGTGAGGGAAAGATGAAAAGAACTTTGGAAAGAGAGTTAAACA-GTATGTGA
A citrina lavendula          ATTGGCGAGAGACCGATAGCGAACAAGTACCGTGAGGGAAAGATGAAAAGAACTTTGGAAAGAGAGTTAAACA-GTATGTGA
A flavoconia                 ATTGGCGAGAGACCGATAGCGAACAAGTACCGTGAGGGAAAGATGAAAAGAACTTTGGAAAGAGAGTTAAACA-GTATGTGA
A thiersii                   ATTGGCGAGAGACCGATAGCGAACAAGTACCGTGAGGGAAAGATGAAAAGAACTTTGGAAAGAGAGTTAAACA-GTACGTGA
A virosa ss auct mexic.      ATTGGCGAGAGACCGATAGCGAACAAGTACCGTGAGGGAAAGATGAAAAGAACTTTGGAAAGAGAGTTAAACA-GTATGTGA
A cylindrispora              ATTGGCGAGAGACCGATAGCGAACAAGTACCGTGAGGGAAAGATGAAAAGAACTTTGGAAAGAGAGTTAAACA-GTATGTGA
A fulva                      ATTGGCGAGAGACCGATAGCGAACAAGTACCGTGAGGGAAAGATGAAAAGGACTTTGGAAAGAGAGTTAAACA-GTATGTGA
A muscaria alba              ATTGGCGAGAGACCGATAGCGAACAAGTACCGTGAGGGAAAGATGAAAAGAACTTTGGAAAGAGAGTTAAACA-GTATGTGA
A muscaria guessowii         ATTGGCGAGAGACCGATAGCGAACAAGTACCGTGAGGGAAAGATGAAAAGAACTTTGGAAAGAGAGTTAAACA-GTATGTGA
A gilbertii                  ATTGGCGACNGACCGATAGCGAACAACNACCGGGAGGGAAAGANGNAACNAACTTTGGAAAGANAGNTAAACA-GTNNGAGA
A sp T27                     ATTGGCGAGAGACCGATAGCGAACAAGTACCGTGAGGGAAAGATGAAAGGAACTTTGGAAAGAGAGTTAAACA-GTATGTGA
A magnivelaris               ATTGGCGAGAGACCGATAGCGAACAAGTACCGTGAGGGAAAGATGAAAAGAACTTTGGAAAGAGAGTTAAACA-GTATGTGA
A virosa ss auct amer.       ATTG-CGAGAGACCGATGGCGAACAAGTACCGTGAGGGAAAGATGAAAAGAACTTTGGAAAGAGAGTTAAACG-GTATGTGA
A bisporigera                ATTGGCGAGAGACCGATAGCGAACAAGTACCGTGAGGGAAAGATGAAAAGAACTTTGGAAAGAGAGTTAAACA-GTATGTGA
A virosa China               ATTGGCGAGAGACCGATAGCGAACAAGTACCGTGAGGGAAAGATGAAAAGAACTTTGGAAAGAGAGTTAAACA-GTATGTGA
A virosa France              ATTGGCGAGAGACCGATAGCGAACAAGTACCGTGAGGGAAAGATGAAAAGAACTTTGGAAAGAGAGTTAAACA-GTATGTGA
A phalloides Germany         ATTGGCGAGAGACCGATAGCGAACAAGTACCGTGAGGGAAAGATGAAAAGAACTTTGGAAAGAGAGTTAAACA-GTATGTGA
A phalloides alba France     ATTGGCGAGAGACCGATAGCGAACAAGTACCGTGAGGGAAAGATGAAAAGAACTTTGGAAAGAGAGTTAAACA-GTATGTGA
A phalloides California      ATTGGCGAGAGACCGATAGCGAACAAGTACCGTGAGGGAAAGATGAAAAGAACTTTGGAAAGAGAGTTAAACA-GTATGTGA
A arochae                    ATTGGCGAGAGACCGATAGCGAACAAGTACCGTGAGGGAAAGATGAAAAGAACTTTGGAAAGAGAGTTAAACA-GTATGTGA
A virosa Virginia            ATTGGCGAGAGACCGATAGCGAACAAGTACCGTGAGGGAAAGATGAAAAGAACTTTGGAAAGAGAGTTAAACA-GTATGTGA
A ocreata                    ATTGGCGAGAGACCGATAGCGAACAAGTACCGTGAGGGAAAGATGAAAAGAACTTTGGAAAGAGAGTTAAACA-GTATGTGA
A marmorata myrtacearum      ATTGGCGAGAGACCGATAGTGAACAAGTACCGTGAGGGAAAGATGAAAAGAACTTTGGAAAGAGAGTTAAACA-GTATGTGA
A phalloides umbrina         ATTGGCGAGAGACCGATAGTGAACAAGTACCGTGAGGGAAAGATGAAAAGAACTTTGGAAAGAGAGTTAAACA-GTATGTGA
A reidii                     ATTGGCGAGAGACCGATAGTGAACAAGTACCGTGAGGGAAAGATGAAAAGAACTTTGGAAAGAGAGTTAAACA-GTATGTGA

Amanita pleropus             AATTGTTGAAAGGGAAACGCTTAAAGTCA-GTCACATTGGCTGGGGATCAACCCTGCTCT-----TTTGCT-GGGTGTACTT
A cf subphalloides           AATTGTTGAAAGGGAAACGTTTGAAGTCA-GTCATGTTGGCTGGGGATCAACCTGACAT------TTTGTT-GGGTGTACTT
A citrina lavendula          AATTGTTGAAAGGGAAACGTTTGAAGTCA-GTCATGTCGGGTGGGGATCAGCCTGGCAG------ATTGTT-GGGTGTACTT
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Appendix 2. Chapter 1 *Amanita* 28S, cont.

A flavoconia	AATTGTTGAAAGGGAAACGTTTGAAGTCA-GTCNCGTTGGTTGGGGATCAGNCTGAC--A-----TTTGTT-GGGTGTNCTT
A thiersii	AATTGTTGAAAGGGAAACGCTTGAAGTCA-GTCACATTGGCCAGGGATCAACCTAGCTCA-----TTTGCT-TGGTGTACTT
A virosa ss auct mexic.	AATTGTTGAAAGGGAAACGTTTGAAGTCA-GAGAGGTTGGTCAGGGATCAGCCTAGC--TGTATTTTTGCT-GGGTGGAATT
A cylindrispora	AATTGTTGAAAGGGAAACGTTTGAAGTCA-GTGATATTGGTNAGGGATCAAGCCAAGCATATTCATTTGCTTGGGTGTACTT
A fulva	AATTGTTGAAAGGGAAACGCTTGAAGTCA-GTTATGTTGGCTAGGGATCAACCTAGCTGTCT---TTTGCT-GGGTGAATTT
A muscaria alba	AATTGTTGAAAGGGAAACGCTTAAAGTCA-GTCGTGTCGGCCAGGGATCAACTCAGCTTT----CTTTGCT-GGGTGTACTT
A muscaria guessowii	AATTGTTGAAAGGGAAACGCTTAAAGTCA-GTCGTGTCGGCCAGGGATCAACTCAGCTTT----CTCTGCT-GGGTGTACTT
A gilbertii	AATTGNTGNAAGGNNAACGTTTNAAGTCACGCTATATTGGGNAGGGNTCAAGTCANNAN------TTTGCT-GGACNTACTT
A sp T27	AATTGTTGAAAGGGAAACGCTTGAAGTCA-GCTACATTAGCTGGGGATCAAGCCAGCTAT-----TTTGCT-GGGTGTACTT
A magnivelaris	AATTGTTGAAAGGGAAACGCTTGAAGTCA-GCTGCATTAGCTGGGGATCAAGCCAGCTTT-----TTTGCT-GGGTGTACTT
A virosa ss auct amer.	AATTGTTGAAAGGGAAACGCTTGAAGTCA-GCTGCATTGGCTGGGGATCAAGCCA-CTTT-----TTTGCT-GGGTGTACTT
A bisporigera	AATTGTTGAAAGGGAAACGCTTGAAGTCA-GCTGCATTGGCTGGGGATCAAGCCAGCTTA-----TTTGCT-GGGTGTACTT
A virosa China	AATTGTTGAAAGGGAAACACTTGAAGTCA-GCTGCATTGGCTGGGGATCAAGCCAGCTTA-----TTTGCT-GGGTGTACTT
A virosa France	AATTGTTGAAAGGGAAACACTTGAAGTCA-GCTGCATTGGCTGGGGATCAAGCCAGCTTA-----TTTGCT-GGGTGTACTT
A phalloides Germany	AATTGTTGAAAGGGAAACACTTGAAGTCA-GCTGCATTGGCTGGGGATCAAGCCAGCTTA-----TTTGCT-GGGTGTACTT
A phalloides alba France	AATTGTTGAAAGGGAAACACTTGAAGTCA-GCTGCATTGGCTGGGGATCAAGCCAGCTTA-----TTTGCT-GGGTGTACTT
A phalloides California	AATTGTTGAAAGGGAAACACTTGAAGTCA-GCTGCATTGGCTGGGGATCAAGCCAGCTTA-----TTTGCT-GGGTGTACTT
A arochae	AATTGTTGAAAGGGAAACGCTTGAAGTCA-GCTGCATTGGCTGGGGATCAAGCCAGCTTA-----TTTGCT-GGGTGTACTT
A virosa Virginia	AATTGTTGAAAGGGAAACGCTTGAAGTCA-GCTGCATTGGCTGGGGATCAAGCCAGCTTA-----TTTGCT-GGCTGTACTT
A ocreata	AATTGTTGAAAGGGAAACGCTTGAAGTCA-GCTGCATTGGCTGGGGATCAAGCCAGCTTA-----TTTGCT-GGTTGTACTT
A marmorata myrtacearum	AATTGTTGAAAGGGAAACGCTTGAAGTCA-GCTGCGTTGGCTGGGGATCAAACCAGCTTA-----TTTGCT-GGGTGT-CTT
A phalloides umbrina	AATTGTTGAAAGGGAAACGCTTGAAGTCA-GCTGCGTTGGCTGGGGATCAAGCCAGCTTA-----TTTGCT-GGGTGTACTT
A reidii	AATTGTTGAAAGGGAAACGCTTGAAGTCA-GCTGCGTTGGCTGGGGATCAAGCCAGCTTA-----TTTGCT-GGGTGTACTT
Amanita pleropus	CC-AGNT-GATGGGTCAGCATCAATTTTGATCATTGGAAAAAGGCAGAGGGAATGTGGCATCTCA----GGATGTGTT-ATA
A cf *subphalloides*	CCTGGTG-AACGG-CCAACATCAGTTTTGACTGCCAGAGAAAGTCCAAGGGAATGTGGCACCTTT----GGGTGTGTT-ATA
A citrina lavendula	CCTGGTT-GACGGGCCAACATCAGTTTTGACTGCCAGAGAAAGGTGAAGGGAATGTGGCACCTTT----GGGTGTGTT-ATA
A flavoconia	CCTGGTT-GACGGGCCAACATCATTTTTGACTGCCAGAAAAAGGCCAGAGGAATGTGGCACCTTT----GGGTGTGTT-ATA
A thiersii	CCTGGTT-AATGGGTCAGCATCAATTTTGATCATTGGAAAAAGGTATAGGGAATGTGGCATCTCC----GGATGTGTT-ATA
A virosa ss auct mexic.	TCTGGAC-AANGGGCCAACATGAGTTTTGACTGCTAGAGAAAGGCATTGGGAATGTGGCACCTCTTGGGGGGTGNGTT-ATA
A cylindrispora	CCTGGTT-GATAGGTCAACATCAGTTCTGGCTGCTGGAAAAGGGCTAGCGGAAAGTGGCACCCTT--TTGGGTGTGTTTATA
A fulva	CCTAGTCTGACAGGCCAACATCAGTCTTGACTGCTGGAAAAGAGCAGAGGGAATGTGGCACCTTC----GGGTGTGTT-ATA
A muscaria alba	CCTGATCTGATGGGCCAACATCAGTTTTGACTGCTGGAGAAGGATAGAGGGAATGTGGCACCTTT----GGGTGTGTT-ATA
A muscaria guessowii	CCTGATCTGATGGGCCAACATCAGTTTTGACTGCTGGAGAAGGACAGAGGGAATGTGGCACCTTT----GGGTGTGTT-ATA
A gilbertii	CCTGGTT-GATCNGNCAANATTAGG-CNGACNGCTGGAGANTGGCTAACCGAATGCCGTCTTTTT----AGGNGNNTATATN
A sp T27	CCTGGTT-GATGGGCCAACATCAGTTTTGACCATCAGAGAAAGGCTAAGGGAATGTGGCACCTTT--ATAGGTGTGTT-ATA
A magnivelaris	CCTGGTT-GATGGGCCAACATCAGTTTTGACCATCAGAGAAAGGCTAAGGGAATGTGGCACCTTT--ATAGGTGTGTT-ATA

Appendix 2. Chapter 1 *Amanita* 28S, cont.

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A virosa ss auct amer.    CCTGGTT-GATGGGCCAACATCAGTTTTG-CCATCAGAGAAAGGCCAAGGGAATGTGGCACCTGT--ATAGGTGTGNT-ATA
A bisporigera             CCTGGTT-GATGGGCCAACATCAGTTTTGACCATCAGAGAAAGGCTAAGGGAATGTGGCACCTTT--ATAGGTGTGTT-ATA
A virosa China            CCTGGTT-GATGGGCCAACATCAGTTTTGACCATCAGAGAAAGGCAAAGGGAATGTGGCACCTTT--ATAGGTGTGTT-ATA
A virosa France           CCTGGTT-GATGGGCCAACATCAGTTTTGACCATCAGAGAAAGGCAAAGGGAATGTGGCACCTTT--ATAGGTGTGTT-ATA
A phalloides Germany      CCTGGTT-GATGGGCCAACATCAGTTTTGACCATCAGAGAAAGGCTAAGGGAATGTGGCACCTTT--ATAGGTGTGTT-ATA
A pahlloides alba France  CCTGGTT-GATGGGCCAACATCAGTTTTGACCATCAGAGAAAGGCTAAGGGAATGTGGCACCTTT--ATAGGTGTGTT-ATA
A phalloides California   CCTGGTT-GATGGGCCAACATCAGTTTTGACCATCAGAGAAAGGCTAAGGGAATGTGGCACCTTT--ATAGGTGTGTT-ATA
A arochae                 CCTGGTT-GATGGGCCAACATCAGTTTTGACCATCAGAAAAAGGCAAAGGGAATGTGGCATCTTT--ATAGGTGTGTT-ATA
A virosa Virginia         CCTGGTT-GATGGGCCAACATCAGTTTTGACCATCAGAGAAAGGCAAAGGGAATGTGGCACCTTT--ATAGGTGTGTT-ATA
A ocreata                 CCTGGTT-GATGGGCCAACATCAGTTTTGACCATCAGAGAAAGGCCAAGGGAATGTGGCACCTTT--ATAGGTGTGTT-ATA
A marmorata myrtacearum   CCTGGTT-GACGGGTCAACATCAGTTTTGACCATCAGAGAAAGGCAAAGGGAATGTGGC-CCTTT---TAAGTGTGTT-ATA
A phalloides umbrina      CCTGGTT-GACGGGTCAACATCAGTTTTGACCATCAGAGAAAGGCAAAGGGAATGTGGCACCTTT---TAGGTGTGTT-ATA
A reidii                  CCTGGTT-GACGGGTCAACATCAGTTTTGACCATCAGAGAAAGGCAAAGGGAATGTGGCACCTTT---TAGGTGTGTT-ATA

Amanita pleropus          GCCTTCTGTTGTATACAGTGGTTGGGATTGAGGA
A cf subphalloides        GCTCTTGGTCGTATGTGGTGGTTGGGACTGAGGA
A citrina lavendula       GCTCTTTGTCGTATGTGGTGGTTGGGACTGAGGA
A flavoconia              GCCCTTGGTCGTATATGGTGGTTGGGACTGAGGA
A thiersii                GCCCTATATTGTATACAGTGATTGGGATTGAGGA
A virosa ss auct mexic.   GCCCAAAGTTNTNTAANGCANTTGGAACTGGGGA
A cylindrispora           GCTGCTAGTCATAT-CAGTAGTTGGGACTGAGGA
A fulva                   GCCCTTTGTCATATGTGGCGGTTGGGACTGAGGG
A muscaria alba           GCTCTCTGTCAGATGCANTGGTTGGGACTGAGGA
A muscaria guessowii      GCTCTCTGTCANATGCAGTGGTTGGGACTGAGGA
A gilbertii               GCNNTTAGTCNTAAACCNNGGACTGGACTNAGGA
A sp T27                  -CCCTTGGTTGTATGTGATGGTTGGGACTGAGGA
A magnivelaris            GCCCTTGGTTGTATGTGATGGTTGGGACTGAGGA
A virosa ss auct amer.    GCCCTTGGTTGTATGTGATGGTTGGGACTGANGA
A bisporigera             GCCCTTGGTTGTATGTGATGGTTGGGACTGAGGA
A virosa China            GCCCTTGGGTGNATGTGGTGGNTGGGACTGAAGA
A virosa France           GCCCTTGGTTGTATGTGGTGGTTGGGACTGAAGA
A phalloides Germany      GCCCTTGGTTGTATGTGGTGGATGGGACTGAGGA
A phalloides alba France  ACCCTTGGTTGTATGTGGTGGATGGGACTGAGGA
A phalloides California   GCCCTTGGTTGTATGTGGTGGATGGGACTGAGGA
A arochae                 GCCCTTGGTTGTATGTGGTGGTTGGGACTGAGGA
A virosa Virginia         GCCCTTGGTTGTATGTGGTGGTTGGGAATGAGGA
A ocreata                 GCCCTTGGTTGTATGTGGTGGTTGGGACTGAGGA
```

Appendix 2. Chapter 1 *Amanita* 28S, cont.

A marmorata myrtacearum	GCCCTTTGTTG-ATGTGGTGGGTGGGACTGAAGA
A phalloides umbrina	GCCCTTTGTTGTATGTGGTGGTTGGGACTGAGGA
A reidii	GCCCTTTGTTGTATGTGGTGGTTGGGACTGAGGA

Alignment for the expanded 28S dataset available upon request.

Appendix 3. Aligned *Amanita* mitochondrial large rDNA sequence from Chapter 1.

```
Tricholoma flavovirens AD001652 TTAATAGTCCGGATTATTAAATTAATTTTATAAGGTGGTGGCACAAAATCGGAGGCCCCGACTGTTTACTAAAAA
Amanita jacksonii               TATTAATGAAGGAGATATAATAATTGATCTTAAAATGGTGTCACATAATCGGAGGCCCCGACTGTTTACTAAAAA
A cf virosa ss auct mexic.      TCTAATAAAAAAATTAATAATAATTGATAGATAGGTGGTGGCACAGAATCGGAGGCCCCGACTGTTTACTAAAAA
A calyptrata AD001545           TATTAATGAAGGAGATATCATAATTGATCTTAAAATGGTGGCACAGAATCGGAGGCCCCGACTGTTTACTAAAAA
A francheti AF156915            ????????????????????CATCTTCTAAANAAAGGTGGTGGCACAAAATCGGAGGCCCCGACTGTNTACTAAAAA
A silvicola AD001553            CGAAGATTAAAGATTAATAATAATTGATAGATAGATGGTGGCACAGAATCGGAGGCCCCGACTGTTTACTAAAAA
A phalloides AD001552           AATAAATTAAAAATTCAAATTAATAGATAGATAGATGGTGGCACAGAATCGGAGGCCCCGACTGTTTACTAAAAA
A magnivelaris WH1              AATAAATTCTAAGTTAAAAATAATAGTTAGATAGATGGTGACACAGAATCGGAGGCCCCGACTGTTTACTAAAAA
A pachycolea AD001550           TACATTTATATTTTTTTATTTTATAGATAGATAAATGGTGGCACAGAATCGGAGGCCACGACTGTTTACTAAAAA
A magniverrucata AD001548       TTACTAAGACTTTGTTTTAATAATTGATAGATAAATGGTGGCACAGAATCGGAGGCCCCGACTGTTTACTAAAAA
A gemmata AD001547              TTACTAAGACTTTGTTTTAATAATTGATAGATAAATGGTGGCACAGAATCGGAGGCCCCGACTGTTTACTAAAAA
A muscaria AD001549             TTACTGAAACTTTGTTTTAATAATTGATAGATAAATGGTGGCACAGAATCGGAGGCCCCGACTGTTTACTAAAAA
A muscaria guessowii            TTACTGAAACTTTGTTTTAATAATTGATAGATAAATGGTGGCACAGAATCGGAGGCCCCGACTGTTTACTAAAAA
A muscaria orange               TTACTGAAACTTTGTTTTAATAATTGATAGATAAATGGTGGCACAGAATCGGAGGCCCCGACTGTTTACTAAAAA
A muscaria alba                 TTACTGAAACTTTGTTTTAATAATTGATAGATAAATGGTGGCACAGAATCGGAGGCCCCGACTGTTTACTAAAAA
A pantherina AD001551           TTACTAAGACTTTGTTTTAATAATTGATAGATAAATGGTGGCACAGAATCGGAGGCCCCGACTGTTTACTAAAAA
A citrina                       TAAGAGGTACCATCTATTATTTTTTTAAATATAGATGGTGGCACAGAATCGGAGGCCCCGACTGTTTACTAAAAA
A citrina lavendula             TAAGAGGTACCATCTATTATTTTTTTAAATATAGATGGTGGCACAGAATCGGAGGCCCCGACTGTTTACTAAAAA
A cf subphalloides              ????????????????????????????????????????????????????AGGCCCCGACTGTTTACTAAAAA
A flavoconia                    ????????????????????????????????????????????????????????GACTGTTTACTAAAAA
A brunnescens                   TCTATTATTTTCTTTTCATAAAATT-ANAGATAGGTGGTGGCACAGAATCGGAGGCCCCGACTGTTTACTAAAAA
A excelsa                       CTATTACTGAAACTTTGTTTATATTGATAGATAA-TGGTGGC-CAGAATCGGAGGCCCCGACTGTTTACTAAAAA
A fulva                         CCCATTTATATTTTCTATTTTAATGGATAGATAAATGGTGGCACAGAATCGGAGGCCACGACTGTTTACTAAAAA

Tricholoma flavovirens AD001652 CACAACACAGTGCAATCATTAATATGATAGTATACTGTNNGAAATTTGCCCGATGCCATTAATA-TAAGAGCGG
Amanita jacksonii               CACAACACAGTGCAATCATTAATATGGTAGTATACTGTGTGAAATTTGCCCAATGCCATTAATA-TAAAAGCGG
A cf virosa ss auct mexic.      CACAACACAGTGCAATCATTAATGTGGTAGTATACTGTGTGAAATTTGCCCAATGCCATTAATA-TAAAAGCGG
A calyptrata AD001545           CACAACACAGTGCAATCATTAATATGGTAGTATACTGTGTGAAATTTGCCCAATGCCATTAATA-TAAAAGCGG
A francheti AF156915            CACAACACAGTGCAATCATAAATGTGATAGTATACTGTGTGAAATTTGCCCAATGCCATTNATA-TAAATGCGG
A silvicola AD001553            CACAACACAGTGCAATCATTAATATGGTAGTATACTGTGTGAAATTTGCCCAATGCCATTAATA-TAAAAGCGG
A phalloides AD001552           CACAACACAGTGCAATCATTAATGTGGTAGTATACTGTGTGAAATTTGCCCAATGCCATTAATA-TAANNNCGG
A magnivelaris WH1              CACAACACAGTGCAATCATTAATGTGGTAGTATACTGTGTGAAATTTGCCCAATGCCATTAATA-TAAAAGCGG
A pachycolea AD001550           CACAACACAGTGCAATCATTAATATGGTAGTATACTGTGTGAAATTTGCCCAATGCCATTAATA-TAAAAGCGG
A magniverrucata AD001548       CACAACACAGTGCAATCATTAATATGGTAGTATACTGTGTGAAATTTGCCCAATGCCATTAATA-TAAAAGCGG
A gemmata AD001547              CACAACACAGTGCAATCATTAATATGGTAGTATACTGTGTGAAATTTGCCCAATGCCATTAATA-TAAAAGCGG
A muscaria AD001549             CACAACACAGTGCAATCATTAATATGGTAGTATACTGTGTGAAATTTGCCCAATGCCATTAATA-TAAAAGCGG
A muscaria guessowii            CACAACACAGTGCAATCATTAATATGGTAGTATACTGTGTGAAATTTGCCCAATGCCATTAATA-TAAAAGCGG
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Appendix 3. Chapter 1 *Amanita* mitochondrial large, cont.

```
A muscaria orange                    CACAACACAGTGCAATCATTAATATGGTAGTATACTGTGTGAAATTTGCCCAATGCCATTAATA-TAAAAGCGG
A muscaria alba                      CACAACACAGTGCAATCATTAATATGGTAGTATACTGTGTGAAATTTGCCCAATGCCATTAATA-TAAAAGCGG
A pantherina AD001551                CACAACACAGTGCAATCATTAATATGGTAGTATACTGTGTGAAATTTGCCCAATGCCATTAATA-TAAAAGCGG
A citrina                            CACAACACAGTGCAATCATTAATGTGATAGTATACTGTGTGAAATTTGCCCAATGCCATTAATA-TAAGAGCGG
A citrina lavendula                  CACAACACAGTGCAATCATTAATGTGATAGTATACTGTGTGAAATTTGCCCAATGCCATTAATA-TAAGAGCGG
A cf subphalloides                   CACAACACAGTGCAATCATTAATGTGATAGTATACTGTGTGAAATTTGCCCAATGCCATTAATA-TAAGAGCGG
A flavoconia                         CACAACACAGTGCAATCATAAATGTGATMGTATACTGTGTGAAATTTGCCCAATGCCATTAATA-TAAAAGCGG
A brunnescens                        CACAACACAGTGCAATCATTAATGTGATAGTATACTGTGTGAAATTTGCCCAATGCCATTAATAATAAAAGCGG
A excelsa                            CACAACACAGTGCAATCATTAATATGGTAGTATACTGTGTGAAATTTGCCCAATGCCATTAATA-TAAAAGCGG
A fulva                              CACAACACAGTGCAATCATTAATATGGTAGTATACTGTGTGAAATTTGCCCAATGCCATTAATA-TAAAAGCGG

Tricholoma flavovirens AD001652      TTATGGGTA-ACTGTGACTAATCGAAAATCTGGTTAATAGCGGTCTTAACTATGAGGATCCTAAGGTAGCAAAAT
Amanita jacksonii                    TCATAGGAA-ACTGTGACTAATTGAAAATCTGGTTAATAGCGGTCTTAACCATGAGGATCCTAAGGTAGCAAAAT
A cf virosa ss auct mexic.           TCATAGGAA-ACTGTGACTAATTGAAAATCTGGTTAATAGCGGTCTTAACCATGAGGATCCTAAGGTAGCAAAAT
A calyptrata AD001545                TCATAGGAA-ACTGTGACTAATTGAAAATCTGGTTAATAGCGGTCTTAACCATGAGGATCCTAAGGTAGCAAAAT
A francheti AF156915                 TCATATTAC-ACTGTGACTAATTGAAAATCTGGTTAATAGCGGTCTTAACCATGAGGATCCTAAGGTAGCAAAAT
A silvicola AD001553                 TCATAGGAA-ACTGTGACTAATTGAAAATCTGGTTAATAGCGGTCTTAACCATGAGGATCCTAAGGTAGCAAAAT
A phalloides AD001552                TCATGGGAA-ACTGTGACTAATTGAAAATCTGGTTAATAGCGGTCTTAATCATGAGGATCCTAAGGTAGCAAAAT
A magnivelaris WH1                   TCATGGGAA-ACTGTGACTAATTGAAAATCTGGTTAATAGCGGTCTTAATCATGAGGATCCTAAGGTAGCAAAAT
A pachycolea AD001550                TCATGGGAA-ACTGTGACTAATTGAAAATCTGGTTAATAGCGGTCTTAACCATGAGGATCCTAAGGTAGCAAAAT
A magniverrucata AD001548            TCATAGGAA-ACTGTGACTAATTGAAAATCTGGTTAATAGCGGTCTTAACCATGAGGATCCTAAGGTAGCAAAAT
A gemmata AD001547                   TCATAGGAA-ACTGTGACTAATTGAAAATCTGGTTAATAGCGGTCTTAACCATGAGGATCCTAAGGTAGCAAAAT
A muscaria AD001549                  TCATAGGAA-ACTGTGACTAATTGAAAATCTGGTTAATAGCGGTCTTAACCATGAGGATCCTAAGGTAGCAAAAT
A muscaria guessowii                 TCATAGGAA-ACTGTGACTAATTGAAAATCTGGTTAATAGCGGTCTTAACCATGAGGATCCTAAGGTAGCAAAAT
A muscaria orange                    TCATAGGAA-ACTGTGACTAATTGAAAATCTGGTTAATAGCGGTCTTAACCATGAGGATCYTAAGGTAGCAAAAT
A muscaria alba                      TCATAGGAA-ACTGTGACTAATTGAAAATCTGGTTAATAGCGGTCTTAACCATGAGGATCCTAAGGTAGCAAAAT
A pantherina AD001551                TCATAGGAA-ACTGTGACTAATTGAAAATCTGGTTAATAGCGGTCTTAACCATGAGGATCCTAAGGTAGCAAAAT
A citrina                            TCATATATA-AATGTGACTAATTGAAAATCTGGTTAATAGCGGTCTTAACCATGAGGATCATAAGGTAGCAAAAT
A citrina lavendula                  TCATATATA-AATGTGACTAATTGAAAATCTGGTTAATAGCGGTCTTAACCATGAGGATCCTAAGGTAGCAAAAT
A cf subphalloides                   TCATATGTAGAATGTGACTAATTGAAAATCTGGTTAATAGCGGTCTTAACCATGAGGATCCTAAGGTAGCAAAAT
A flavoconia                         TCATATTAA-ACTGTGACTAATTGAAAATCTGGTTAATAGCGGTCTTAACCATGAGGATCCTAAGGTAGCAAAAT
A brunnescens                        TAATAATGG-AA??????????????????????????????????????????????????CCTAAGGTAGCAAAAT
A excelsa                            TCATAGGAA-ACTGTGACTAATTGAAAATCTGGTTAATAGCGGTCTTAACCATGAGGATCCTAAGGTAGCAAAAT
A fulva                              TCATGGGAA-ACTGTGACTAATTGAAAATCTGGTTAATAGCGGTCTTAACCATGAGGATCCTAAGGTAGCAAAAT

Tricholoma flavovirens AD001652      AAGTTGGCCATTAAATGTGGTCCGGTATCAATAATGTAA-CGATGGCCTCACTG-TC-TCTACNACTTGCTCAGT
Amanita jacksonii                    AAATTGGCCATTAAATGTGGTCCGGTATCAATAATGTAA-CGATGGCCTCACTG-TC-TCTACAACTTGCTCAGT
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Appendix 3. Chapter 1 *Amanita* mitochondrial large, cont.

A cf *virosa* ss auct mexic.	AAATTGGCCATTAAATGTGGTCCGGTATCAATAATGTAA-CGATGGCCTCACTG-TC-TCTACAACTTGCTCAGT
A *calyptrata* AD001545	AAATTGGCCATTAAATGTGGTCCGGTATCAATAATGTAA-CGATGGCCTCACTG-TC-TCTACAACTTGCTCAGT
A *francheti* AF156915	AGATTGGCCATTAAATGTGGTCCGGTATCAATAATGTAA-CGATGGCCTCACTG-TC-TCTACAACTTGCTCAGT
A *silvicola* AD001553	AAATTGGCCATTAAATGTGGTCCGGTATCAATAATGTAA-CGATGGCCTCACTG-TC-TCTACAACTTGCTCAGT
A *phalloides* AD001552	AAATTGGCCATTAAATGTGGTCCGGTATCAATAATGTAA-CGATGGCCTCACTG-TCATCTACAACTTGCTCAGT
A *magnivelaris* WH1	AAATTGGCCATTAAATGTGGTCCGGTATCAATAATGTAA-CGATGGCCTCACTG-TC-TCTACAACTTGCTCAGT
A *pachycolea* AD001550	AAATTGGCCATTAAATGTGGTCCGGTATCAATAATGTAA-CGATGGCCTCACTG-TC-TCTACAACTTGCTCAGT
A *magniverrucata* AD001548	AAATTGGCCATTAAATGTGGTCCGGTATCAATAATGTAA-CGATGGCCTCACTG-TC-TCTACAACTTGCTCAGT
A *gemmata* AD001547	AAATTGGCCATTAAATGTGGTCCGGTATCAATAATGTAA-CGATGGCCTCACTG-TC-TCTACAACTTGCTCAGT
A *muscaria* AD001549	AAATTGGCCATTAAATGTGGTCCGGTATCAATAATGTAA-CGATGGCCTCACTG-TC-TCTACAACTTGCTCAGT
A *muscaria guessowii*	AAATTGGCCATTAAATGTGGTCCGGTATCAATAATGTAA-CGATGGCCTCACTG-TC-TCTACAACTTGCTCAGT
A *muscaria* orange	AAATTGGCCATTAAATGTGGTCCGGTATCAATAATGTAA-CGATGGCCTCACTG-TC-TCTACAACTTGCTCAGT
A *muscaria alba*	AAATTGGCCATTAAATGTGGTCCGGTATCAATAATGTAA-CGATGGCCTCACTG-TC-TCTACAACTTGCTCAGT
A *pantherina* AD001551	AAATTGGCCATTAAATGTGGTCCGGTATCAATAATGTAA-CGATGGCCTCACTG-TC-TCTACAACTTGCTCAGT
A *citrina*	AAATTGGCCATTAAATGTGGTCCGGTATCAATAATGTTA-CGATGGCCTCACTG-TC-TCTACAACTTGCTCAGT
A *citrina* lavendula	AAATTGGCCATTAAATGTGGTCCGGTATCAATAATGTAA-CGATGGCCTCACTG-TC-TCTACAACTTGCTCAGT
A cf *subphalloides*	AAATTGGCCATTAAATGTGGTCCGGTATCAATAATGTAA-CAATGGCCTCACTGGTCTTCTACAACTTG-TCA??
A *flavoconia*	CGATTGGCCATTAAATGTGGTCCGGTATCAATAATGTAA-CGATGGCCTCACTG-TC-TCTACAACTTGCTCAGT
A *brunnescens*	AAATTGGCCATTAAATGTGGTCCGGTATTAATAATGTAAACGAA????????????????????????????????
A *excelsa*	AAATTGGCCATTAAATGTGGTCCGGTATCAATAATGTAA-CGATGGCCTCACTG-TC-TCTACAACTTGCTCAGT
A *fulva*	AAATTGGCCATTAAATGTGGTCCGGTATCAATAATGTAA-CGATGGCCTCACTG-TC-TCTACAACTTGCTCAGT

Tricholoma flavovirens AD001652	GAAATTGAATT
Amanita jacksonii	GAAATTGAATT
A cf *virosa* ss auct mexic.	GAAATTGAATT
A *calyptrata* AD001545	GAAATTGAATT
A *francheti* AF156915	GAAATTGAATT
A *silvicola* AD001553	GAAATTGAATT
A *phalloides* AD001552	GAAATTGAATT
A *magnivelaris* WH1	GAAATTGAATT
A *pachycolea* AD001550	GAAATTGAATT
A *magniverrucata* AD001548	GAAATTGAATT
A *gemmata* AD001547	GAAATTGAATT
A *muscaria* AD001549	GAAATTGAATT
A *muscaria guessowii*	GAAATTGAATT
A *muscaria* orange	GAAATTGAATT
A *muscaria alba*	GAAATTGAATT

Appendix 3. Chapter 1 *Amanita* mitochondrial large, cont.

A pantherina AD001551	GAAATTGAATT
A citrina	GAAATTGAATT
A citrina lavendula	GAAATTGAATT
A cf *subphalloides*	??????????
A flavoconia	GAAATTGAATT
A brunnescens	??????????
A excelsa	GAAATTGAATT
A fulva	GAAATTGAATT

Appendix 4. Aligned ITS from control and parasitized *Amanita* specimens (Chapter 2)

```
Amanita bisporigera          CTTGGTCATTTAAGAGGAAGTAAAAGTCGTNACAAGGTTTCCGTAGGTGAACCTGCGGAAGGGATCATTACTGAAATGGA
A brunnescens                CTTGGTCATTT-AGAGGAAGTAAAAGTCGTAACAAGGTTTCCGTAGGTGAACCTGCGGAAGG-ATCATTATTGAACGAAA
A citrina citrina AB015679   ??????????????GGAAGTAAAAGTCGTAACAAGGTTTCCGTAGGTGAACCTGCGGAAGG-ATCATTATTGAAGGACA
A citrina grisea AB015680    ??????????????GGAAGTAAAAGTCGTAACAAGGTTTCCGTAGGTGAACCTGCGGAAGG-ATCATTATTGAAGGACA
A citrina lavendula          ????????????????????????????????????????????TAGGTGAANCTGCGGAAGG-ATCATTATTGAAGGAGA
A flavipes AB015696          ??????????????GGAAGTAAAAGTCGTAACAAGGTTTCCGTAGGTGAACCTGCGGAAGG-ATCATTACTGAACGAAT
A flavoconia                 ????????????????????????ACCGTNACAAGGTTTCCGTAGGTGAACCTGCGGAAGG-ATCATTACTGAACGAAA
A flavoconia white-stiped    CTTGGTCATTT-AGAGGAAGTAAAAGTCGTAACAAGGTTTCCGTAGGTGAACCTGCGGAAGG-ATCATTACTGAGCGAAA
A novinupta ISOTYPE          CTTGGTCATTT-AGAGGAAGTAAAAGTCGTAACAAGGTTTCCGTAGGTGAACCTGCGGAAGG-ATCATTACCGAACGAAA
A porphyria AB015677         ??????????????GGAAGTAAAAGTCGTAACAAGGTTTCCGTAGGTGAACCTGCGGAAGG-ATCATTATTGAAGGACA
A rubescens AB015682         ??????????????GGAAGTAAAAGTCGTAACAAGGTTTCCGTAGGTGAACCTGCGGAAGG-ATCATTACTGAACGAAA
A rubescens alba             CTTGGTCATTT-AGAGGAAGTAAAAGTCGTAACAAGGTTTCCGTAGGTGAACCTGCGGAAGG-ATCATTATTGAACGAAA
A rubescens congolensis      CTTGGTCATTT-AGAGGAAGTAAAAGTCGTAACAAGGTTTCCGTAGGTGAACCTGCGGAAGG-ATCATTATTGAACGAAA
A rubescens congolensis      CTTGGTCATTT-AGAGGAAGTAAAAGTCGTAACAAGGTTTCCGTAGGTGAACCTGCGGAAGG-ATCATTATTGGACGAAA
A cf subphalloides           ????????????????????????????????????AGGTTTCCGTAGGTGAACCTGCGGAAGG-ATCATTATTGAACAGCA
PA 01                        CTTGGTCATTT-AGAGGAAGTAAAAGTCGTAACAAGGTTTCCGTAGGTGAACCTGCGGAAGG-ATCATTACTGAACGAAA
PA 08                        CTTGGTCATTT-AGAGGAAGTAAAAGTCGTAACAAGGTTTCCGTAGGTGAACCTGCGGAAGG-ATCATTACTGAACGAAA
PA 13                        CTTGGTCATTT-AGAGGAAGTAAAAGTCGTAACAAGGTTTCCGTAGGTGAACCTGCGGAAGG-ATCATTACTGAACGAAA
PA 19                        CTTGGTCATTT-AGAGGAAGTAAAAGTCGTAACAAGGTTTCCGTAGGTGAACCTGCGGAAGG-ATCATTACTGAACGAAA
PA 22                        CTTGGTCATTT-AGAGGAAGTAAAAGTCGTAACAAGGTTTCCGTAGGTGAACCTGCGGAAGG-ATCATTACTGAACGAAA
PA 33                        CTTGGTCATTT-AGAGGAAGTAAAAGTCGTAACAAGGTTTCCGTAGGTGAACCTGCGGAAGAGATCATTACTGAACGAAA
PA 43                        CTTGGTCATTT-AGAGGAAGTAAAAGTCGTAACAAGGTTTCCGTAGGTGAACCTGCGGAAGG-ATCATTATTGAACGAAA

Amanita bisporigera          C-CTCG------AGGCTGTCGCTGGCCCATCTGGGCA--TGTGCAC-GTCTCTGG---------TCATTACC-AATTCCA
A brunnescens                A-CGGGTGGCAAAGGCTGTCGCTGGCTTGAATGAGCA--TGTGCAC-GTCTTTTGCTGCTTATTTCATTCAT-CTTTCCT
A citrina citrina AB015679   AATGGGTGGCAAAGGCTGTCGCTGGCTTGAATGAGCA--TGTGCAC-GTCTTTTGTTGCTTATTTCATTCATCTTTTCCT
A citrina grisea AB015680    A-TGGGTGGCAAAGGCTGTTGCTGGCTTGAATGAGCA--TGTGCAC-GTCTTTCGCTGCTTATTTCATTCAT-TTTTCCT
A citrina lavendula          A-TGGGTGGCAAAGGCTGTTGCTGGCTTGAATGAGCA--TGTGCACCGTCTGTTGCTACTTATTTCATTCAT-TTTTCCT
A flavipes AB015696          T--GGGTGGC-AAGGCTGTCGCTGGCTCAAATGAGCA--TGTGCAC-GTCTTTTGCTGCTTGCTTCATTCTC-TTCTCCA
A flavoconia                 T--GGGTGGC-AAGGCTGTCGCTGGCTCGAATGAGCA--TGTGCAC-GTCTTTTGCTGCTTGCTTCATTCTC-TTCTCCA
A flavoconia white-stiped    T--GGGTGGC-AAGGCTGTCGCTGGCTCAAACGAGCA--TGTGCAC-GTCTTTTGCTGCTTGCTTCATTCTC-TTTTCCA
A novinupta ISOTYPE          T--GGGTGGC-AAGGCTGTCGCTGGCTCAAACGAACA--TGTGCAC-GTCTTTTGCTGCTTGCTTCATTCTA-TTTTCCA
A porphyria AB015677         A-TGGGTGGCAAAGGCTGTCGCTGGCTTGAATGAGCA--TGTGCAC-GTCTTTTGCTGCTTATTTCATTCAT-TTTTCCT
A rubescens AB015682         T--GGGTGGC-AAGGCTGTCGCTGGCTCTAATGAGCA--TGTGCAC-GTCTTTTGCCGCTTGCTTCATTCTA-TTTTCCA
A rubescens alba             A--GGGTGGC-AAGGCTGTTGCTGGCTTGAACAAGCATATGTGCAC-GTCTTTTGCTGCTTATTTCATTCAC-TTTTCCA
A rubescens congolensis      A--GGGTGGC-AAGGCTGTTGCTGGCTTGAACAAGCATATGTGCAC-GTCTTTTGCTGCTTATTTCATTCAC-TTTTCCA
A rubescens congolensis      A--GGGTGGC-AAGGCTGTTGCTGGCTTGAACAAGCATATGTGCAC-GTCTTTTGCTGCTTATTTCATTCAC-TTTTCCA
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Appendix 4. Chapter 2 parasitized *Amanita* ITS, cont.

```
A cf subphalloides          A-TGGGTGGCAAAGACTGTTGCTGGCTTGAATGAGCA--TGTGCAC-GTCATTTGCTGCTTATTTCATTCAT-TTTTCCT
PA 01                       T--GGGTGGC-AAGGCTGTCGCTGGCTCGAATGAGCA--TGTGCAC-GTCTTTTGCTGCTTGCTTCATTCTC-TTCTCCA
PA 08                       T--GGGTGGC-AAGGCTGTCGCTGGCTCTAATGAGCA--TGTGCAC-GCCTTTTGTCGCTTGCTTCATTCTC-TTTTCCA
PA 13                       T--GGGTGGC-AAGGTTGTCGCTGGCTCGAATGAGCA--TGTGCAC-GCCTTTTGCTGCTTGCTTCATTCTC-TTTTCCA
PA 19                       T--GGGTGGC-AAGGCTGTCGCTGGCTCTAATGAGCA--TGTGCAC-GCCTTTTGTCGCTTGCTTCATTCTC-TTTTCCA
PA 22                       T--GGGTGGC-AAGGCTGTCGCTGGCTCAAATGAGCA--TGTGCAC-GCCTTTTGCTGCTTGCTTCATTCTC-TTTTCCA
PA 33                       T--GGGTGGC-AAGGTTGTCGCTGGCTCGAATGAGCA--TGTGCAC-GCCTTTTGCTGCTTGCTTCA-TCTC-TTTTCCA
PA 43                       A-CGGGTGGCAAAGGCTGTCGCTGGCTTGAATGAGCA--TGTGCAC-GTCTTTTGCTGCTTATTTCATTCAT-CTTTCCT

Amanita bisporigera         CCTGTGCACA--CTTGTAG-ACACTTGGGAA-TGAGAGA---CTT-------------------------TG-ACCGGTC
A brunnescens               CCTGTGCACT-ATTTGTAG-ACACTTGGGAATG-AGAG---GTT-TGTTGT----ATTG---------ATTG-ACC----
A citrina citrina AB015679  CCTGTGCACG-TTTTGTAG-ACACTTGGGAAT---GAGAG-GGTTGGTTGT--AATGTAATGTAATG-ATTG-ACC----
A citrina grisea AB015680   CCTGTGCATC-TTTTGTAG-ACACTTGGGAAT---GAGA--GGTTGGTTAT--AATGAATTG-------TTG-ACC----
A citrina lavendula         CCTGTGCACCGTTTTGTAG-ACACTTGGGAAT---GAGA--GGTTGGTTGT--AATGTAATGA-----ATTG-ACC----
A flavipes AB015696         CCTGTGCACT-CTTTGTAG-ACACTCGGGATGTGAGAGA--GGTTAGCA-TTGATTGT----------TG-ACC--TC
A flavoconia                CCTGTGCACT-CTTTGTAG-ACACTCGGGATGTGAGAGA--GGTTAGCA-TTGATTGT----------TG-ACC----
A flavoconia white-stiped   CCTGTGCACT-CTTTGTAG-ACACTCGGGATGGGAGAGA--GGTTGGCA-TTGATGGT----------TA-ACC--TC
A novinupta ISOTYPE         CCTGTGAACT-CTTTGTAG-ACACTCAAGATGAGAGAGAGAGGTTGGCGGTCGAATGT----------TG-ACC--TC
A porphyria AB015677        CCTGTGCACA-TTTTGTAG-ACACTTGGGAAT---GAGA--GGTTGGTTGT--AATGTAATGATATATATTG-ACC----
A rubescens AB015682        CCTGTGCACT-CTTTGTAG-ACACTTGGGAATGG-GAGAG-AGTTGGCA-TCGAATGT----------TGAACCC-TC
A rubescens alba            CCTGTGCACT-TTCTGTAG-ACACTTGGGAATG--GAGA--GGTCAG-ATT--AATTT----------TTTG-ACC----
A rubescens congolensis     CCTGTGCACT-TTCTGTAG-ACACTTGGGAATG--GAGA--GGTCAG-ATT--AATTT----------TTTG-ACC----
A rubescens congolensis     CCTGTGCACT-TTCTGTAG-ACACTTGGGAATG--GAGA--GGTTAG-ATT--AATTT----------TTTG-ACC----
A cf subphalloides          CCTGTGAACC-TTTTGTAG-ACACTTGGGAAT---GAGA--AGTTGGTTGT--AATG----------ATTG-ACC----
PA 01                       CCTGTGCACT-CTTTGTAG-ACACTCGGGATGTGAGAGA--GGTTAGCATT-GATTGT----------TG-ACC----
PA 08                       CCTGTGCACT-CTTTGTAG-ACAACTTTTGATGG-GAGAGAGGTTGGCA-TCGAATGT----------TGGACC--TC
PA 13                       CCTGTGAACT-CTTTGTAG-ACACTTGGGATAGTAGAGA--GGTTGGCGGTCGAATGT----------TG-ACC--TC
PA 19                       CCTGTGCACT-CTTTGTAG-ACAACTCGTGAATGGGAGAGAGGTTGGCA-TCGAATGTT----------GG-ACC--TC
PA 22                       CCTGTGAACT-CTTTGTAG-ACACTTGGGATAGGAGAGA--GGTTGGCGGTCGAATGT----------TG-ACC--TC
PA 33                       CCTGTGAACT-CTT-GTAGNACACTTGGGATAGTAGAGA--GGTTGGCGGTCGAATGT----------TG-ACA--TC
PA 43                       CCTGTGCACT-ATTTGTAG-ACACTTGGGAATG-AGAG---GTT-TGTTGT----ATTG---------ATTG-ACC----

Amanita bisporigera         TCTTGAGGGAATTGAAC----------TCTGGGTGTCTATGC-CATTTTATCAAACACTA-GTTGCATGTTTATAGAA
A brunnescens               TCTTGGCGTTGAAAAAATCCAATGCAATGTCCAAGTGTCTATGA-CATTTTTATATACACACGGTTGTATGTCTATAGAA
A citrina citrina AB015679  TCTTG----AGATGA----------GTCTGGGTGTCTATGG-CATTTTTATAAACACG--GCTGGATGTGTATAGAA
A citrina grisea AB015680   TCTTG----ACATCA----------ATCTGGGTGTCTATGG-CATTTTTATAAACACG--GTTGTATGTCTATAGAA
A citrina lavendula         TCTTG----AGGTCA----------GTCTGGGTGTCTATGA-CATTTTTATAAACACG--GCTGCATGTGTATGGAA
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Appendix 4. Chapter 2 parasitized *Amanita* ITS, cont.

```
A flavipes AB015696          TCTTGATATTGAAAA------------GTCTGGGTGTTTATGTATTTTTTGACATACACG--GTTGAATGTCTATAGAA
A flavoconia                 TCT-------------------------GTCTGGGTGTTTATGTATTTTTTGACATACACG--TTTGAATGTCTATAGAA
A flavoconia white-stiped    TCTTGATATTGAAAA------------GTCTGGGTGTTTATGTATTTTTTGACATACACG--GTTGAATGTCTATAGAA
A novinupta ISOTYPE          TCTTGATATTGAAAA------------GTCTGGGTGTCTATGTGTTTTTTCACATACACA--GTTGAATGTCTATAGAA
A porphyria AB015677         TCTTG----AGATCA------------GTCTGGGTGTCTATGT-CATTTTTATAAACACG--GCTGCATGTCTATAGAA
A rubescens AB015682         TCTTGATATTAAAAA------------GTCTGGGTGTCTATGTGTTTTTTCACATACACAA-GTTGAATGTCTATAGAA
A rubescens alba             TCTTG----TGAAAA------------GTCTGGGTGTCTATG--CATTTTTATATACACA--GTTGCATGTCTATAGAA
A rubescens congolensis      TCTTG----TGAAAA------------GTCTGGGTGTCTATG--CATTTTTATATACACA--GTTGCATGTCTATAGAA
A rubescens congolensis      CCTTG----TGAAAA------------GTCTGGGTGTCTATG--CATTTTTATATACACA--GTTGCATGTCTATAGAA
A cf subphalloides           TCTTG----ATATTG------------ATCTGGGTGTCTATGC-CATTTTTATAAAGACATGGTTGCATGTGTATAGAA
PA 01                        TCT-------------------------GTCTGGGTGTTTATGTATTTTTTGACATACACG--TTTGAATGTCTATAGAA
PA 08                        TCTTGATATTAAAAA------------GTCTGGGTGTCTATGTGTTTTTTCACATACCMRA-GTTGAATGTCTATAGAA
PA 13                        TCTTGATATTGAAAA------------GTCTGGGTGTCTATGTGTTTTTTCACATACACG--GTTGAATGTCTATAGAA
PA 19                        TCTTGATATTAAAAA------------GTCTGGGTGTCTATGTGTTTTTTCACATACCCAA-GTTGAATGTCTATAGAA
PA 22                        TCTTGATATTGAAAA------------GTCTGGGTGTCTATGTGTTTTT-CATATACACA--GTTGAATGTCTATAGAA
PA 33                        TCTTGATATTGAAAA------------GTCTGGGTGTCTATGTGTTTTTTCACATACACG--GTTGAATGTCTATAGAA
PA 43                        TCTTGGCGTTGAAAAAATTCAATGCAATGTCCAAGTGTCTATGA-CATTTTTATATACACATGGTTGTATGTCTATAGAA

Amanita bisporigera          TGACGATTGA------------------------------TTGAA-T-ATAAAATACAACTTTCAACAACGGATCTCTTG
A brunnescens                TGAGAT-GTAGG----------CTTATTT--AAA---GCCATTAAATGATAAAGTACAACTTTCAACAACGGATCTCTTG
A citrina citrina AB015679   TGAGATTGTAGG--------TTTTTAATAAAAA----GCTATTAAATGATAAAGTACAACTTTCAACAACGGATCTCTTG
A citrina grisea AB015680    TGAGATTATAGGGTGATTTTTTTTTAAAAAAAAAAAGGCCTTTAAATGATAAAGTACAACTTTCAACAACGGATCTCTTG
A citrina lavendula          TGAGACTGTAGG--------TTTTTAAT--GAAAA--GCCTTGAAATGATAAAGTACAACTTTCAACAACGGATCTCTTG
A flavipes AB015696          TGAAAATGTAGG----------C-TTTT--GTCAGC--CTTT-AAATGATAAAATACAACTTTCAACAACGGATCTCTTG
A flavoconia                 TGAAAATGTAGG----------C-TTTT--GTCAGC--CTTT-AAATGATAAAATACAACTTTCAACAACGGATCTCTTG
A flavoconia white-stiped    TGAAA-TGTAGG----------C-TTTT--GTCAGC--CTTT-AAATGATAAAATACAACTTTCAACAACGGATCTCTTG
A novinupta ISOTYPE          TGAAATTGTAGG----------C-TTNT--GTCAGC--CTTTTAAATGATAAAATACAACTTTCAACAACGGATCTCTTG
A porphyria AB015677         TGAGATTGTAGG--------TTTTTAATAAAAA----GCCTTTAAATGATAAAGTACAACTTTCAACAACGGATCTCTTG
A rubescens AB015682         TGAAATTGTAGG----------C-TTTT--GTCAGC--CTTTTAAATGATAAAATACAACTTTCAACAACGGATCTCTTG
A rubescens alba             TGAGATGGTAGG----------CTTTTTT-GTGA---GCCTGTAAATGATAAAGTACAACTTTCAACAACGGATCTCTTG
A rubescens congolensis      TGAGATGGTAGG----------CTTTTTT-GTGA---GCCTGTAAATGATAAAGTACAACTTTCAACAACGGATCTCTTG
A rubescens congolensis      TGAGATTGTAGG----------CTTTTTTTGTGA---GCCTGTAAATGATAAAGTACAACTTTCAACAACGGATCTCTTG
A cf subphalloides           TGAGATTGTATG--------GTTTTTTTAATAAA---GCCTTTAAATGATAA--TACAACTTTCAACAACGGATCTCTTG
PA 01                        TGAAAATGTAGG----------C-TTTT--GTGAGC--CTTT-AAATGATAAAATACAACTTTCAACAACGGATCTCTTG
PA 08                        TGAAATTGTAGG----------C-TTTT--GTCAGC--CTTTTAAATGATAAAATACAACTTTCAACAACGGATCTCTTG
PA 13                        TGAAATTGTAGG----------C-TTTT--GTCAGC--CTTTTAAATGATAAAATACAACTTTCAACAACGGATCTCTTG
PA 19                        TGAAACTGTAGG----------C-TTTT--GTCAGC--CTTTTAAATGATAAAATACAACTTTCAACAACGGATCTCTTG
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Appendix 4. Chapter 2 parasitized *Amanita* ITS, cont.

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PA 22                          TGAAATTGTAGG----------C-TTTT--GTCAGC--CTTTTAAATGATAAAATACAACTTTCAACAACGGATCTCTTG
PA 33                          TGAAATTGTAGG----------C-TTTT--GTCAGC--CTTTTAAATGATAAAATACAACTTTCAACAACGGATCTCTTG
PA 43                          TGAGAT-GTAGG----------CTTATTT--AAA---GCCATTAAATGATAAAGTACAACTTTCAACAACGGATCTCTTG

Amanita bisporigera            GCTCTCGCATCGATGAAGAACGCAGCCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTG
A brunnescens                  GCTCTCGCATCGATGAAGAACGCAGC-GAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTG
A citrina citrina AB015679     GCTCTCGCATCGATGAAGAACGCAGC-GAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTG
A citrina grisea AB015680      GCTCTCGCATCGATGAAGAACGCAGC-GAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTG
A citrina lavendula            GCTCTCGCATCGATGAAGAACGCAGC-GAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTG
A flavipes AB015696            GCTCTCGCATCGATGAAGAACGCAGC-GAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTG
A flavoconia                   GCTCTCGCATCGATGAAGAACGCAGC-GAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTG
A flavoconia white-stiped      GCTCTCGCATCGATGAAGAACGCAGC-GAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTG
A novinupta ISOTYPE            GTTCTCGCATCGATGAAGAACGCAGC-GAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTG
A porphyria AB015677           GCTCTCGCATCGATGAAGAACGCAGC-GAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTG
A rubescens AB015682           GCTCTCGCATCGATGAAGAACGCAGC-GAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTG
A rubescens alba               GCTCTCGCATCGATGAAGAACGCAGC-GAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTG
A rubescens congolensis        GCTCTCGCATCGATGAAGAACGCAGC-GAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTG
A rubescens congolensis        GCTCTCGCATCGATGAAGAACGCAGC-GAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTG
A cf subphalloides             GCTCTCGCATCGATGAAGAACGCAGC-GAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTG
PA 01                          GCTATCGCATCGATGAAGAACGCAGC-GAAATGCGATAAGTAATGTGAATTGTAGAATTCAGTGAATCATTGAATCTTTG
PA 08                          GCTCTCGCATCGATGAAGAACGCAGC-GAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTG
PA 13                          GCTCTCGCATCGATGAAGAACGCAGC-GAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTG
PA 19                          GCTCTCGCATCGATGAAGAACGCAGC-GAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTG
PA 22                          GCTCTCGCATCGATGAAGAACGCAGC-GAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTG
PA 33                          GCTCTCGCATCGATGAAGAACGCAGC-GAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTG
PA 43                          GCTCTCGCATCGATGAAGAACGCAGC-GAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTG

Amanita bisporigera            AACGCACCTTGCGCTCCTTGGCATTCCGAGGAGCATGCCTGTTTGAGTGTCATTAA-CATCTCAAGACCTGTCTGCT---
A brunnescens                  AACGCATCTTGCGCTCCTTGGTATTCCGAGGAGCATGCCTGTTTGAGTGTCATTAA??????????????????????
A citrina citrina AB015679     AACGCATCTTGCGCTCCTTGGTATTCCGAGGAGCATGCCTGTTTGAGTGTCATTCAAGTATCTCAAAAGAGCCTTA----
A citrina grisea AB015680      AACGCATCTTGCGCTCCTTGGTATTCCGAGGAGCATGCCTGTTTGAGTGTCATTGAAGTATCTCAAAAG--CCCTA----
A citrina lavendula            AACGCATCTTGCGCTCCTTGGTATTCCGAGGAGCATGCCTGTTTGAGTGTCATTCAAGTATCTCAAAAGC-TGTTA----
A flavipes AB015696            AACGCATCTTGCGCTCCTTGGTATTCCGAGGAGCATGCCTGTTTGAGTGTCATTAAATATCTCAAAAAGCTTGTGCTT--
A flavoconia                   AACGCATCTTGCGCTCCTTGGTATTCCGAGGAGCATGCCTGTTTGAGTGTCATTAAATATCTCAAAAAGCTTGTGCT---
A flavoconia white-stiped      AACGCATCTTGCGCTCCTTGGTATTCCGAGGAGCATGCCTGTTTGAGTGTCATTAAATATCTCAAAAAGTTTGTGCT---
A novinupta ISOTYPE            AACGCATCTTGCGCTCCTTGGTATTCCGAGGAGCATGCCTGTTTGAGTGTCATTAAATATCTCAAAAAGCTTGTGCT---
A porphyria AB015677           AACGCATCTTGCGCTCCTTGGTATTCCGAGGAGCATGCCTGTTTGAGTGTCATTCAAGTATCTCAAAAGT-TCTTA----
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Appendix 4. Chapter 2 parasitized *Amanita* ITS, cont.

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A rubescens AB015682          AACGCATCTTGCGCTCCTTGGTATTCCGAGGAGCATGCCTGTTTGAGTGTCATTAAATATCTCAAAAAGCTTGTGCT---
A rubescens alba              AACGCATCTTGCGCTCCTTGGTATTCCGAGGAGCATGCCTGTTTGAGTGTCAT-CAATTATCTCAAAAGCTTACACCT--
A rubescens congolensis       AACGCATCTTGCGCTCCTTGGTATTCCGAGGAGCATGCCTGTTTGAGTGTCAT-CAATTATCTCAAAAGCTTACACCT--
A rubescens congolensis       AACGCATCTTGCGCTCCTTGGTATTCCGAGGAGCATGCCTGTTTGAGTGTCAT-CAATTATCTCAAAAGCTTACACCT--
A cf subphalloides            AACGCATCTTGCGCTCCTTGGTATTCCGAGGAGCATGCCTGTTTGAGTGTCATT-AAGTATCTCAAAAGC-TCTTACTTA
PA 01                         AACGCATCTTGCGCTCCTTGGTATTYCGAGGAGCATGCCTGTTTGAGTGTCATTAAATATCTGAAAAAGCTTGTGCT---
PA 08                         AACGCATCTTGCGCTCCTTGGTATTCCGAGGAGCATGCCTGTTTGAGTGTCATTAAATATCTCAAAAAGCTTGTGCT---
PA 13                         AACGCATCTTGCGCTCCTTGGTATTCCGAGGAGCATGCCTGTTTGAGTGTCATTAAATATCTCAAAAAGCTTGTGCT---
PA 19                         AACGCATCTTGCGCTCCTTGGTATTCCGAGGAGCATGCCTGTTTGAGTGTCATTAAATATCTCAAAAAGCTTGTGCT---
PA 22                         AACGCATCTTGCGCTCCTTGGTATTCCGAGGAGCATGCCTGTTTGAGTGTCATTAAATATCTCAAAAAGCTTGTGCT---
PA 33                         AACGCATCTTGCGCTCCTTGGTATTCCGAGGAGCATGCCTGTTTGAGTGTCATTAAATATCTCAAAAAGCTTGTGCT---
PA 43                         AACGCATCTTGCGCTCCTTGGTATTCCGAGGAGCATGCCTGTTTGAGTGTCATTAAAGTATCTCAAAAGCTCTTATGT--

Amanita bisporigera           ----TTTGAT----AGGTATT---GGATT-TTGGGAGTTGCAGGCTGT--TTCAAA-TATANCTTGCTCTNCTTGAATGT
A brunnescens                 ??????????????????????????????????????????GCCGGTCACTG-ATACA-AGTGGTGGGCTCTTCTGAAAAGC
A citrina citrina AB015679    TGCAGTTTGC--AGGGAACTTTT-GGACA-TTGGGAGTTGCTGGTCACTG-ATAAAAAGTGGTTGGCTCTTCTGAAAAGG
A citrina grisea AB015680     TGCACTATGC--AGGGAACTTTT-GGACA-TTGGGAGTTGCTGGTCACTG-ATAA--AGTGGTTAGCTCTTCTGAAAAGC
A citrina lavendula           TGCAGTATGC--ATGGAACTTTT-GGACA-TTGGGAGTTGCTGGTCACTG-ATAA--AGTGGTAGGCTCTTCTGAAAAAT
A flavipes AB015696           --TTTTTGGC--ACAGGAGTTTT-GGACA-TTGGGAGTTGCCGGCTGCTGGATAAC-AGTGGTGGGCTCTTCTGAAAAGC
A flavoconia                  --TTTTTGGC--ACAGGAGTTTT-GGACA-TTGGGAGTTGCCGGCTGCTGGATAAC-AGTGGTGGGCTCTTCTGAAAAGC
A flavoconia white-stiped     --TTTTTGGC--ATAAGATTTTTTGGACA-TTGGGAGTTGCCGGCTGCTG-ATAAA-AGTGGTGGGCTCTTCTGAAAAGC
A novinupta ISOTYPE           --TTTTTGGC--ATGGGATTTTT-GGACA-TTGGGAGTTGCCGGCCACTG-ATAA--AGTGGTGGGCTCTTCTGAAAAGC
A porphyria AB015677          TGCACTATGC--AAGGAACTTTT-GGATA-TTGGGAGTTGCTGGTCACTG-ATAA--AGTGGTTGGCTCTTCTGAAAAGC
A rubescens AB015682          --TTTTTGGC--ATGGGAATTTT-GGACA-TTGGGAGTTGCCGGCTACTG-ATAA--AGTGGTGGGCTCTTCTGAAAAGC
A rubescens alba              --ATGTAGTGGTGTAGGATTTTT-GGACAA-TGGAGGCTGCCGGTCACTG-ATGAAAAGTGGTGGGCTCTTCTGAAAAGC
A rubescens congolensis       --ATGTAGCGGTGTAGGATTTTT-GGACAA-TGGAGGCTGCCGGTCACTG-ATGAAAAGTGGTGGGCTCTTCTGAAAAGC
A rubescens congolensis       --ATGTAGTGGTGTAGGATTTTT-GGACAA-TGGAAGCTGCCGGTCACTG-ATGAAAAGTGGTGGGCTCTTCTGAAAAGC
A cf subphalloides            TGTGCTATAC--ATGAGAGTTTT-GGACA-TTGGGAGTTGCTGGTCAC----TA---AGTGATGGGCTCTTCTGAAAAGC
PA 01                         --TTTTTGGC--ACAGGAGTTTT-GGACA-TTGGGAGTTGCCGGCTGCTGGATAAC-AGTGGTGGGCTCTTCTGAAAAGC
PA 08                         --TTTTTGGC--ATGGGATTTTT-GGACA-TTGGGAGTTGCCAGCTGCTG-ATAA--AGTGGTGGGCTCTTCTGAAAAGC
PA 13                         --GTTTTGGC--ATGGGATTTTT-GGACA-TTGGGAGTTGCCGGCCACTG-ATAA--AGTGGTGGGCTCTTCTGAAAAGC
PA 19                         --TTTTTGGC--ATGGGATTTTT-GGACA-TTGGGAGTTGCCAGCTGCTG-ATAA--AGTGGTGGGCTCTTCTGAAAAGC
PA 22                         --TTTTTGGC--ATGGGATTTTT-GGACA-TTGGGAGTTGCCGGCCACTG-ATAA--AGTGGTGGGCTCTTCTGAAAAGC
PA 33                         --GTTTTGGC--ATGGGATTTTT-GGACA-TTGGGAGTTGCCGGCCACTG-ATAA--AGTGGTGGGCTCTTCTGAAAAGC
PA 43                         --GTTATGCAT-ATGGAACTTTT-GGACAATTGGGAGTTGCCGGTCACTG-ATACA-AGTGGTGGGCTCTTCTGAAAAGC

Amanita bisporigera           ATTAAGT--GGAGAAAAGCTGGTGAACTCCATTGGTGTGATNAAATCTATCAATGCCNGGAGCCACCNCNAGNGG--CTC
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Appendix 4. Chapter 2 parasitized *Amanita* ITS, cont.

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A brunnescens                ATTAGTTGAGAAGCTTT------GCACTCTATTGGTGTGATAGA--CTATCTATGCCAGGAGATGCATTATTG---CCTC
A citrina citrina AB015679   ATTAGTTGAGGAGCTTTTT----GTGCTCTATTGGTGTGATAGA--GTATCTATGCCAGGAGAAGCATTAATGAAGCCTC
A citrina grisea AB015680    ATTAGTTGAGGAACTTTTT----GTACTCTACTGGTGTGATAGA--CTATCTATGCCAGGAGAAGCATT-ATGAAGCCTC
A citrina lavendula          ATTAGTTGAGGAGCTTTTT----GCACTCTATTGGTGTGATAGA--CCATCTATGCCAGGAGAAGTGTT-ATGAAGTGTC
A flavipes AB015696          ATTAGTTGAGGAGCTTT------GCACTCTATTGGTGTGATAGA--CTATCTATGCCAGGAGACG-CTTCATGAT-CCTC
A flavoconia                 ATTAGTTGAGGAGCTTT------GCACTCTATTGGTGTGATAGA--CTATCTATGCCAGGAGACG-CTTCATGAT-CCTC
A flavoconia white-stiped    ATTAGTTGAGGAGCTTT------GCACTCTATTGGTGTGATAGA--CTATCTATGCCAGGAGAAG-CTTCATGAA-CCTC
A novinupta ISOTYPE          ATTAGTTGAGGAGCTTT------GCACTCTATTGGTGTGATAGA--CTATCTATGCCAGGAGATG-CTTCATGAA-CCTC
A porphyria AB015677         ATTAGTTGAGGAGCTCCTT----GCACTCTATTGGTGTGATAGA--CTATCTATGCCAGGAGAAGCATT-ATGAAGCCTC
A rubescens AB015682         ATTAGTCGAGGAGCTTT------GCACTCTATTGGTGTGATAGA--CTATCTATGCCAGGAGATG-CTTCATGAT-CCTC
A rubescens alba             ATTAGTTGAGGAGCTTT------GCACTCTATTGGCGTGATAGT--CTATCTATGCCAGTTGTTGCTTTAAT----CCTT
A rubescens congolensis      ATTAGTTGAGGAGCTTT------GCACTCTATTGGCGTGATAGT--CTATCTATGCCAGTTGTTGCTTTAAT----CCTT
A rubescens congolensis      ATTAGTTGAGGAGCTTT------GCACTCTATTGGTGTGATAGT--CTATCTATGCCAGTTGTTGCTTTGAT----CCTT
A cf subphalloides           ATTAGTTGAGGAGCTTTT-----GCACTCTATTGGTGTGATAAA--CTATCTATGCCAGGAGAAGCATT-ATGAACCCTC
PA 01                        ATTAGTTGAGGAGCTTT------GCACTCTATTGGTGTGATAGA--CTATCTATGCCAGGAGACG-CTTCATGAT-CCTC
PA 08                        ATTAGTTGAGGAGCTTT------GCACTCTATTGGTGTGATAGA--CTATCTATGCCAGGAGATG-CTTCATGAT-CCTC
PA 13                        ATTAGTTGAGGAGCTTT------GCACTCTATTGGTGTGATAGA--CTATCTATGCCAGGAGATG-CTTCATGAA-CCTC
PA 19                        ATTAGTTGAGGAGCTTT------GCACTCTATTGGTGTGATAGA--CTATCTATGCCAGGAGATG-CTTCATGAT-CCTC
PA 22                        ATTAGTTGAGGAGCTTT------GCACTCTATTGGTGTGATAGA--CTATCTATGCCAGGAGATG-CTTCATGAA-CCTC
PA 33                        ATTAGTTGAGGAGCTTT------GCACTCTATTGGTGTGATAGA--CTATCTATGCCAGGAGATG-CTTCATGAA-CCTC
PA 43                        ATTAGTTGAGGAGCTT-------GCACTCTATTGGTGTGATAGA--CTATCTATGCCAGGAGATGCATTATTG---CCTC

Amanita bisporigera          TGN-TGNT--AACCTACT---------------GCTGCCCCTTGNCC-NAAATAAGGN-GGA-TACCCGNTGA-CTTAAC
A brunnescens                TGC-TCTCT-AACAGTCC-TTATTGGACAA-GATGACGAACTTGACCTCAAATCAGGTAGGACTACCCGCTGAACTTAAG
A citrina citrina AB015679   TGC-TGTCT-AACAGTTG-TAATTGGACAA-TGTGATAAAGTTGACCTCAAATCAGGTAGGACTACCCGCTGAACTTAAG
A citrina grisea AB015680    TGC-TGTCT-AACAGTCC-ATAATGGACAA-GATGATAAAGTTGACCTCAAATCAGGTAGGACTACCCGCTGAACTTAAG
A citrina lavendula          TGC-TGNCT-AATGATCC-TCATTGGACAA-GATGATAAACTTGGCCTCAAATCANGNAGGACTACCCGCTGA-CTTAAG
A flavipes AB015696          TGCCATCTT-AACCGTCT-TTATAAGACAA-TATGATAAACTTGACCTCAAATCAGGTAGGACTACCCGCTGAACTTAAG
A flavoconia                 TGC-ATCT--AACCG-CT-TTATAAGACAA-TATGATAAACTTGACCTCAAATCANGTAGGACTACCCGCTGAACTTAAG
A flavoconia white-stiped    TGC-CATCT-AACTGTCT-TTATTAGACAA-CATGATAAACTTGACCTCAAATCAGGTAGGACTACCCGCTGAACTTAAG
A porphyria AB015677         TGC-TGTCT-AACAGTCC-TAATTGGACAA-TATGATAAAATTGACCTCAAATCAGGTAGGACTACCCGCTGAACTTAAG
A novinupta ISOTYPE          TGC-TGTCT-AACTGTCT-TTATCAGACAA-TATGATAAACTTGACCTCAAATCAGGTAGGACTACCCGCTGAACTTAAG
A rubescens AB015682         CGC-TGTCTTAACTGTCT-TTATCAGACAA-TATGATAAATTTGACCTCAAATCAGGTAGGACTACCCGCTGAACTTAAG
A rubescens alba             TGC-TGCCT-AACTGTCT-TTATCAGACAAACATGATGAACTTGACCTCAAATCAGGTAGGACTACCCGCTGAACTTAAG
A rubescens congolensis      TGC-TGCCT-AACTGTCT-TTATCAGACAAACATGATGAACTTGACCTCAAATCAGGTAGGACTACCCGCTGAACTTAAG
A rubescens congolensis      TGC-TGCCT-AACTGTCT-TTATCAGACAA-CATGATGAACTTGACCTCAAATCAGGTAGGACTACCCGCTGAACTTAAG
A cf subphalloides           TGC-TGCCT-AACAGTCCATTATTGGACAA-GATGATCAACTTG-CCTCAAATCANGTAGGACTACCCGCTGA-CTTAAG
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Appendix 4. Chapter 2 parasitized *Amanita* ITS, cont.

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PA 01                          TGC-CATCT-AACCGTCT-TTATAAGACAA-TATGATAAACTTGACCTCAAATCAGGTAGGACTACCCGCTGAACTTAAG
PA 08                          TGC-TGTCT-AACTGTCT-TTATCAGACAA-TACGATAAACTTGACCTCAAATCAGGTAGGACTACCCGCTGAACATAAG
PA 13                          TGC-TGTCT-AACTGTCT-TTATCAGACAA-TATGATAAACTTGACCTCAAATCAGGTAGGACTACCCGCTGAACTTAAG
PA 19                          TGC-TGTCT-AACTGTCT-TTATCAGACAA-TACGATAAACTTGACCTCAAATCAGGTAGGACTACCCGCTGAACTTAAG
PA 22                          TGC-TGTCT-AACTGTCT-TTATCAGACAA-TATGATAAGCTTGACCTCAAATCAGGTAGGACTACCCGCTGAACTTAAG
PA 33                          TGC-TGTCT-AACTGTCT-TTATCAGACAA-TATGATAAACTTGACCTCAAATCAGGTAGGACTACCCGCTGAACTTAAG
PA 43                          TGC-TCTCT-AACAGTCC-TTATTGGACAA-GATGACGAACTTGACCTCAAATCAGGTAGGACTACCCGCTGAACTTAAG

Amanita bisporigera            CCTNTNATNNC-NGGGGGAAAAAA--CTA-CAAGG-TTNCCT-AGNA-CT??????????????????????????????
A brunnescens                  CATATCAATAAGCGGAGGAAAAGAAACTAACAAGGATTCCCTTAGTAACTGCGAGTGAAGAGGGAAAAGCTCAAATTTAA
A citrina citrina AB015679     CATATCAATAAGCGGAGGA??????????????????????????????????????????????????????????
A citrina grisea AB015680      CATATCAATAAGCGGAGGA??????????????????????????????????????????????????????????
A citrina lavendula            CATATCAATAAGCGGAGGAAAAGAA--CTACCANGGTTNCCTTANTACCTGCGAGTGAAGANGGGAAAGC-CAAATTNAA
A flavipes AB015696            CATATCAATAAGCGGAGGA??????????????????????????????????????????????????????????
A flavoconia                   CATATCAATAAGCNGAGGAAAAGAAACTAACAAGGATTNCCTTATTA-CTGC-AATGAAAAAGGAAAA-CTCAAATTNAA
A flavoconia white-stiped      CATATCAATAAGCGGAGGAAAAGAAACTAACAAGGATTCCCTTAGTAACTGCGAGTGAAGAGGGAAAAGCTCAAATTTAA
A novinupta ISOTYPE            CATATCAATAAGCGGAGGAAAAGAAACTAACAAGGATTCCCTTAGTAACTGCGAGTGAAGAGGGAAAAGCTCAAATTTAA
A porphyria AB015677           CATATCAATAAGCGGAGGA??????????????????????????????????????????????????????????
A rubescens AB015682           CATATCAATAAGCGGAGGA??????????????????????????????????????????????????????????
A rubescens alba               CATATCAATAAGCGGAGGAAAGGAAACCAACAAGGATTCCCTTAGTAACTGCGAGTGAAGAGGGAAAAGCTCAAATTTAA
A rubescens congolensis        CATATCAATAAGCGGAGGAAAAGAAACTAACAAGGATTCCCTTAGTAACTGCGAGTGAAGAGGGAAAAGCTCAAATTTAA
A rubescens congolensis        CATATCAATAAGCGGAGGAAAAGAAACTAACAAGGATTCCCTTAGTAACTGCGAGTGAAGAGGGAAAAGCTCAAATTTAA
A cf subphalloides             CCTATNAATAAGCGGAGGAAAAGAA--CTAC-AAGGATNCCTTAATACCTGCGAGTGAAGAAGGATAGCC-CAAANTTNA
PA 01                          CATATCAATAAGCGGAGGAAAAGAAACTAACAAGGATTCCCTTAGTAACTGCGAGTGAAGAGGGAAAAGCTCAAATTTAA
PA 08                          CATATCAATAAGCGGAGGAAAAGAAACTAACAAGGATTCCCTTAGTAACTGCGAGTGAAGAGGGAAAAGCTCAAATTTAA
PA 13                          CATATCAATAAGCGGAGGAAAAGAAACTAACAAGGATTCCCTTAGTAACTGCGAGTGAAGAGGGAAAAGCTCAAATTTAA
PA 19                          CATATCAATAAGCGGAGGAAAAGAAACTAACAAGGATTCCCTTAGTAACTGCGAGTGAAGAGGGAAAAGCTCAAATTTAA
PA 22                          CATATCAATAAGCGGAGGAAAAGAAACTAACAAGGATTCCCTTAGTAACTGCGAGTGAAGAGGGAAAAGCTCAAATTTAA
PA 33                          CATATCAATAAGCGGAGGAAAAGAAACTAACAAGGATTCCCTTAGTAACTGCGAGTGAAGAGGGAAAAGCTCAAATTTAA
PA 43                          CATATCAATAAGCGGAGGAAAAGAAACTAACAAGGATTCCCTTAGTAACTGCGAGTGAAGAGGGAAAAGCTCAAATTTAA

Amanita bisporigera            ??????????????????????????????????????????????????????????????????????????
A brunnescens                  AATCTGGCAGTGTTTCATTGTCCGAGTTGTAACCTAGAGAAGTGTAACCTGTGCTGGACCGTGTACAAGTCT
A citrina citrina AB015679     ??????????????????????????????????????????????????????????????????????????
A citrina grisea AB015680      ??????????????????????????????????????????????????????????????????????????
A citrina lavendula            A-TCTGACAG-G????????????????????????????????????????????????????????????????
A flavipes AB015696            ??????????????????????????????????????????????????????????????????????????
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Appendix 4. Chapter 2 parasitized *Amanita* ITS, cont.

A flavoconia	A-TCTGGCAGGGTT-CACTGGCT??
A flavoconia white-stiped	AATCTGGCAGTATTTCACTGTCCGAGTTGTAACCTAGAGAAGTGTAACCTGTGCTGGACCGTGTACAAGTCT
A novinupta ISOTYPE	AATCTGGCAGTGTTTCACTGTCCGAGTTGTAACCTAGAGAAGTGTAACCTGCGCTGGACCGTGTACAAGTCT
A porphyria AB015677	??
A rubescens AB015682	??
A rubescens alba	AATCTGGTAGTGTTTCACTGTCCGAGTTGTAACCTAGAGAAGTGTAACCTGTGCTGGACCGTGTACAAGTCT
A rubescens congolensis	AATCTGGTAGTGTTTCACTGTCCGAGTTGTAACCTAGAGAAGTGTAACCTGTGCTGGACCGTGTACAAGTCT
A rubescens congolensis	AATCTGGCAGTGTTTCACTGTCCGAGTTGTAACCTAGAGAAGTGTAACCTGTGCTGGACCGTGTACAAGTCT
A cf subphalloides	AATNTGGCGNGGTT??
PA 01	AATCTGGCAGTGTTTCACTGTCCGAGTTGTAACCTAGAGAAGTGTAACCTGCGCTGGACCGTGTACAAGTCT
PA 08	AATCTGGCAGTGTTTCACTGTCCGAGTTGTAACCTAGAGAAGTGTAACCTGCGCTGGACCGTAGACAAGTCT
PA 13	AATCTGGCAGTGTTTCACTGTCCGAGTTGTAACCTAGAGAAGTGTAACCTGCGCTGGACCGTGTACAAGTCT
PA 19	AATCTGGCAGTGTTTCACTGTCCGAGTTGTAACCTAGAGAAGTGTAACCTGCGCTGGACCGTGTACAAGTCT
PA 22	AATCTGGCAGTGTTTCACTGTCCGAGTTG-AACCTAGAGAAGTGTAACCTGCGCTGGACCGTGTACAAGTCT
PA 33	AATCTGGCAGTGTTTCACTGTCCGAGTTGTAACCTAGAGAAGTGTAACCTGCGCTGGACCGTGTACAAGTCT
PA 43	AATCTGGCAGTGTTTCATTGTCCGAGTTGTAACCTAGAGAAGTGTAACCTGTGCTGGACCGTGTACAAGTCT

Appendix 5. Alignment of ITS 1 - 5.8S - ITS 2 regions of the nuclear ribosomal RNA operon in *Conocybe* and related genera (Chapter 4).
Total aligned length = 930 positions. Shaded areas show ambiguous alignment and were excluded from analysis. Gap coding is shown at the end of the alignment in `lower case`. Gaps were coded as follows: "g" = "gap present"; "a" = "gap absent"; "-" = "indeterminate" (the gap being coded is a small gap which entirely falls within the area covered by a larger gap); "?" = "missing data".

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'Weraroa cucullata'              AGGCA-TGTGCATGCCCGTATCATCTTTATATCTCCACC-TGTGCACCTTTTG--TAGA-CTTGAGAGT-ATT
'Cyttarophyllum besseyi'         GA-CA-TGTGCTNTTCCG--TCATCTTTATATCTCCACC-TGTGCACCCTTTG--TAGA-CCTGGACG-----
'Agrocybe semiorbicularis'       GTGAA-CCTGCGGAAGGA--TCATTATTGAATAAACCTGGTGTGCACCCTTTG--TAGA-CCTGGACG-----
'Agrocybe praecox'               GGGCA-TGTGCTCCCCCG--TCAACTTTATATCTTCACCTGGTGCACCCTTTG--TAGA-CCTGGACG-----
'Galeropsis desertorum'          GAGCA-TGTGCACGCTCA--TCGTCTTTATCCATCCACC-TGTGCACCTTTTG--TAGT-CTTGGGAAATGAA
'Bolbitius variicolor'           GAGCA-TGTGCACGCCGG--TCACTTTTATCTTACCACC-TGTGAACACTTTG--TAG--------------A
'Bolbitius vitellinus'           GAGCA-TGTGCACGCCGG--TCACTTTTATCTTACCACC-TGTGAACACTTTG--TAGA-TCTGGAAG-----
'Bolbitius lacteus'              GAGCA-TGTGCACGCCGG--TCACTTTTATCTTACCACC-TGTGAACACTTTG--TAGA-TCTGGAGG-----
'Gastrocybe deceptiva TYPE'      GAGCA-TGTGCACGCCGG--TCACTTTTATCTTACCACC-TGTGAACACTTTG--TAGA-TCTGGA-GGCA--
'yellow Gastrocybe'              GAGCA-TGTGCACGCCGG--TCACTTTTATCTTACCACC-TGTGAACACTTTG--TAGA-TNTGGA-GGCA--
'Conocybe subnuda'               NAGCA-AGGGCACGTCTA--TCATTTTTGTCTTNCCACC-TGTGCACANTTTG---AGG-TTTGAAAG-----
'Conocybe coprophila'            GGGCACTGTGCACGTCTG--TCATTTTTACTTTTCCACC-TGTGCACCTTTTG--TAGA-TTCCG--------
'Conocybe rickenii'              GAGCA-TGTGCACACCTG--TCATCTTTATCTTTCCACCATGTGCACCTTTTG--TAGG-ACTGGAA-TAGAC
'Conocybe filaris'               GAGCA-TGTGCACACCTG--TCATTTTTATATTTCCACC-TGTGCACCCTTTG--TAGT-TCTGGA--CCAA-
'Conocybe lactea 2'              GAGTA-TGTGCACGCCTG--TCATTTTAATCTTTCCACCATGTGCAC--TTTGCGTAGGGTCTGAAAATCA--
'Conocybe lactea 1'              GAGTA-TGTGCACGCCTGG-TCATTTTTATCTTTCCACCATGTGCAC--TTTGCGTAGGGTCTGAAA-TCA--
'Conocybe lactea 4'              GAGTA-TGTGCACGCCTG--TCATTTTTATCTTTCCACCATGTGCAC--TTTGCGTAGGGTCTGAAAATCA--
'Conocybe crispa 5'              ??????????????????????????????????????????????????????????????????????
'Conocybe lactea 5'              ??????????????????????????????????????????????????CGTAGGGTCTGAAAATCA--
'Conocybe crispa 2'              GAGTA-TGTGCACGCCTG--TCATTTTTATCTTTCCACCATGTGCAC--TTTGCGTAGGGTCTGAAAATCA--
'Conocybe crispa 4'              GAGTA-TGTGCACGCCTG--TCATTTTTATCTTTCCACCATGTGCAC--TTTGCGTAGGGTCTGAAAATCA--
'Conocybe crispa 3'              GAGTA-TGTGCACGCCTG--TCATTTTTATCTTTCCACCATGTGCAC--TTTGCGTAGGGTCTGAAAATCA--
'Lactea-crispa intermediate'     GAGTA-TGTGCACGCCTG--TCATTTTTATCTTTCCACCATGTGCAC--TTTGCGTAGGGTCTGAAAATCA--
'Conocybe crispa 1'              GAGTA-TGTGCACGCCTG--TCATTTTTATCTTTCCACCATGTGCAC--TTTGCGTAGGGTCTGAAAATCA--
'Conocybe lactea UK clone 2'     GAGTA-TGTGCACRCCTG--TCATTTTTATCTTTCCACCATGTGCAC--TTTGCATAGG-TCTGAAAATCA--
'Conocybe lactea 3'              GAGTC-TGTGCACGCCTG--TCATTTTTCTCTTTCCACCATGTGCAC--TTTGCGTAGGGTCTGAAAATCA--
'Conocybe lactea Corvallis'      GAGTC-TGTGCACGCCTG--TCATTTTTATCTTTCCACCATGTGCAC--TTTGCGTAGGGTCTGAAAATCA--
'Conocybe lactea Eugene'         ??????????????????????????????????????????????????????????????????????
'Intermediate 3'                 GAGTA-TGTGCACACCTG--TCATTTTTATCTTTCCACCATGTGCAC--TTTGCATAGG-TCTGAAAATCAC-
'Conocybe lactea UK clone 5'     GAGTA-TGTGCACRCCTG--TCATTTTTATCTTTCCACCATGTGCAC--TTTGCATAGG-TCTGAAAATCAC-
'Conocybe huijsmannii conica'    GAGTA-TGTGCACGCCTG--TCATTTTANTCTTTCCACCATGTGCAC--TTTGCGTAGGGTCTGAAAATCAA-
'Conocybe crispa UK'             ??????????????????????????????????????????????????????????????????????
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Appendix 5. Chapter 4 *Conocybe* ITS, cont.

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'Intermediate 2'                GAGTA-TGTGCACACCTG--TCATTTTTATCTTTCCACCATGTGCAC--TTTGCATAGG-TCTGAAAATCAC-
'Conocybe subcrispa'            GAGTA-TGTGCACACCTG--TCATTTTTATCTTTTCACCATGTGCAC--TTTGCATAGG-TCTGAAAATCAC-
'Gastrocybe lateritia 4'        GAGCA-TGTGCACGCTTG--TCATTTTTATCTTTCCACCATGTGCAC--TTTGCATAGG-TCTGAAAATCAA-
'Gastrocybe lateritia 3'        GAGCA-TGTGCACGCTTG--TCATTTTTATCTTTCCACCATGTGCAC--TTTGCATAGG-TCGGAAAATCAA-
'Gastrocybe lateritia 5'        GAGCA-TGTGCACGCTTG--TCATTTTTATCTTTCCACCATGTGCAC--TTTGCATAGG-TCTGAAAATCAA-
'Intermediate 1'                GAGCA-TGTGCACGCTTG--TCATTTTTATCTTTCCACCATGTGCAC--TTTGCATAGG-TCTGAAAATCAA-
'Gastrocybe lateritia 1'        GAGCA-TGTGCACGCTNG--TCATTTTTATCTTTCCACCATGTGCAC--TTTGCATAGG-TCTGAAAATCAA-
'Gastrocybe lateritia 2'        GAGCA-TGTGCACGCTTG--TCATTTTTATCTTTCCACCATGTGCAC--TTTGCATAGG-TCTGAAAATCAA-


'Weraroa cucullata'             GGCGTAAGTC------------------------------------------------------------------
'Cyttarophyllum besseyi'        ---ATAACTT--TCCGA--GGCAA-CTCGGTC-----GGGAGG--ACTG----CTGGCgTT--CACGAGTCGG
'Agrocybe semiorbicularis'      ---ATAACTT--TCCGA--GGCAATC--GGTC-----GGGAGG--ACTG----CTGGCTTT--CACGAGTCGG
'Agrocybe praecox'              ---ATAACTTTTTCCGA-GKGCAAACTCGGGTC----GGGAGG--ACTK---GCTGGCTTTTCCACGAGTCGG
'Galeropsis desertorum'         TGCAATGGAACCTC-GATAGGTT-TTTCAGCC--TTTCGGATG-TGAGGAATGCTTTGT--------GAAAGC
'Bolbitius variicolor'          TCTGGAGGCATCTT-CACAGACTCTT-TTGTC--TGTGGTTTT-TGGAA---G-TG-----------------
'Bolbitius vitellinus'          ----CATCTTCAC-----AGACTCTT-TTGTC--GGTGAGCTT-TGGGAA--GCTGT---------CGTGTTC
'Bolbitius lacteus'             ----CATCTTCAC-----AGACTCTT-TTGTC--GGTGAGCTT-TGGGAA--GCTGT---------CGTGTTC
'Gastrocybe deceptiva TYPE'     ------TCTTCAC-----AGACTCTT-TTGTC--TGTGGTTTT-TGGAA---GCTGT---------------C
'yellow Gastrocybe'             ------TCTTCAC-----AGACTCTT-TTGTC--TGTGGTTTT-TGGAA-GCTTTGT---------CGTG---
'Conocybe subnuda'              --TTAATCTTNCCN--A-AGATTCT----------TTGGGTTTGAGGATTGTGCGCT------CACCGCCGAC
'Conocybe coprophila'           --TTAA-C----------AGGTTTCTGAATTTC-CGTGGGGGCGGTCT---TGCTT----------CTGCGGT
'Conocybe rickenii'             ----AAT-AA-GCC---TAGTCTCTTG-TAGACTATTGGGAGGTTGCTG-ATT-TT----------ATCAGC
'Conocybe filaris'              ------TCTAT------GCTTTCACTGCATGAC-AGAGGGA--CTGCTG--TGCTGT------GCAAGCCAGC
'Conocybe lactea 2'             --AGTAAATT-------------CTTGATGTC---TTGGGA--CTGCTG--TGCTT-------CACAGCCGGC
'Conocybe lactea 1'             --AGTAAATT-------------CTTGATGTC---TTGGGA--CTGCTG--TGCTT-------CACAGCCGGC
'Conocybe lactea 4'             --AGTAAATT-------------CTTGATGTC---TTGGGAA-CTGCTG--TGCTT-------CACAGCCGGC
'Conocybe crispa 5'             ??????????????????????????????????????????????????????????????CCGGC
'Conocybe lactea 5'             --AGTGAATT-------------CTTGATGTC---TTGGGAA-CTGCTG--TGCTT-------CACAGCCGGC
'Conocybe crispa 2'             --AGTAAATT-------------CTTGATGTC---TTGGGAA-CTGCTG--TGCTT-------CACAGCCGGC
'Conocybe crispa 4'             --AGTAAATT-------------CTTGATGTC---TTGCTAA-CTGCTG--TGCTT-------CACAGCCGGC
'Conocybe crispa 3'             --AGTAAATT-------------CTTGATGTC---TTGCTAA-CTGCTG--TGCTT-------CACAGCCGGC
'Lactea-crispa intermediate'    --AGTGAATT-------------CTTGATGTC---TTGGGAA-CTGCTG--TGCTT-------CACAGCCGGC
'Conocybe crispa 1'             --AGTGAATT-------------CTTGATGTC---TTGGGA--CTGCTG--TGCTT-------CACAGCCGGC
'Conocybe lactea UK clone 2'    --AGTGTTTTT-------TTTCACTTGATGTC---TTGGGAA-CTGCTG--TGCTT-------CACAGCCAGC
'Conocybe lactea 3'             --AGTGAATT-------------CTTGATGTC---TTGGGAC-CTGCTG--TGCTT-------CACAGCCGGC
'Conocybe lactea Corvallis'     --AGTGAATT-------------CTTGATGTC---TTGGGAA-CTGCTG--TGCTT-------CACAGCCAGC
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Appendix 5. Chapter 4 *Conocybe* ITS, cont.

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'Conocybe lactea Eugene'          ??????????????????????????????????TTGGGAA-NTGTTG--TGNTT-------CACAGCCGGC
'Lactea-Gastrocybe intermediate'  -TAGTCCTTGT-------GGACTTTTGATGTC---TTGGGAA-CTGCTA--TTCTT-------CACAGTCAGC
'Conocybe lactea UK clone 5'      -TAGTCCTTGT-------GGACTTTTGATGTC---TTGGGAA-CTGCTA--TTCTT-------CACAGTCAGC
'Conocybe huijsmannii conica'     --AGTGAATT-------------CTTGATGTC---TTGGGAA-CTGCTG--TGCTT-------CACAGCCGGC
'Conocybe crispa UK'              ??????????????????????????????????????????????????????????????????????
'Intermediate 2'                  -TAGTCCTTGT-------GGACTTTTGATGTC---TTGGGAA-CTGCTG--TTCTT-------CACAGTCAGC
'Conocybe subcrispa'              -TAGTCCTTTTT------GGACTTTTGATGTC---TTGGGAA-CTGCTG--TTCTT-------CACAGTCAGC
'Gastrocybe lateritia 4'          --GGTCTCTAC-----------CTTTGATGTC---TTGGGAA-TTGCAGGTTGCTTT------TGCAATCAGC
'Gastrocybe lateritia 3'          --GGTCTCTAC-----------CTTTGATGTC---TTGGGAA-TTGCAGGTTGCTTT------TGCAATCAGC
'Gastrocybe lateritia 5'          --GGTCTCTAC-----------CTTTGATGTC---TTGGGAA-TTGCAGGTTGCTTW------TGCAATCAGC
'Intermediate 1'                  --GGTCTCTAC-----------CTTTGATGTC---TTGGGAA-TTGCAGGTTGCTTT------TGCAATCAGC
'Gastrocybe lateritia 1'          --GGTCTCTAC-----------CTTGGATGTC---TTGGGAA-TTGCAGGTTGCTTT------TGCAATCAGC
'Gastrocybe lateritia 2'          --GGTCTCTAC-----------CTTTGATGTC---TTGGGAA-TTGCAGGTTGCTTT------TGCAATCAGC


'Weraroa cucullata'               --------------AGT------AATC--TGAA------GCC-TATGTTTTTCATATACACCA--TAA-GAA
'Cyttarophyllum besseyi'          CTTTCCT--------------------TGTATTATCCAGGCC-TATGTCTTACACAT--ACCCC-AAA-GAA
'Agrocybe semiorbicularis'        CTTTCCT--------------------TGTATTATCCAGGCC-TATGTCTTACACAT--ACCCC-AAA-GAA
'Agrocybe praecox'                CTTTCCT--------------------TTGATTATCCAGGCCTTATGTTATTCACAT---CCCC-AAA-GAA
'Galeropsis desertorum'           ATGGCTCTTCTCAATAGCCTTGCAATCG---TTACTCAGAC--TATGTTTTTCA----TACACC-AT--GTA
'Bolbitius variicolor'            --TTCCGACTCTTC--CTGGCC------------TTCAGG-TTTATGTCTTT-ACATCTACACC-ATAAGCA
'Bolbitius vitellinus'            GCTTCATCGCGTCCGACTCTTCCTGG------CCTTCAGG-TTTATGTCTTTA-CATCTACMCC-ATAAGCA
'Bolbitius lacteus'               GCTTCATCGCGTCCGACTCTTCCTGG------CCTTCAGG-TTTATGTCTTTA-CATCTACACC-ATAAGCA
'Gastrocybe deceptiva TYPE'       G-TTCGACTCTTC----------------TGGCCTTCAGG-TTTATGTCTTTA-CATCTACACC-ATAAGCA
'yellow Gastrocybe'               --TTCCCTTCATTGAGTCCGACT-CTTCCTGGCCTTCAGG-TNTATGTCTTTA-CATCTACACC-NTAAGCA
'Conocybe subnuda'                T-TTCC----------TTGTGCT-----------TTCCAGATCTACGTTTTCTCTATCTACTCC-AC-AGAA
'Conocybe coprophila'             TTTTTCAGAGC----------------------TTGGGAGTCTACGTTTTTTTATACAAACCC-AAATGTA
'Conocybe rickenii'               TCTTTCTGGTTCTGG-CTGTC---------T-ATTTCATTCTCTATGATATTCATATACACACC-AT--GTA
'Conocybe filaris'                T-TTCC----------TTTAC---------TCTTTTCCAGATCTATGTTTTTCAATAACACACCCAT--GTA
'Conocybe lactea 2'               T-TTTC----------CTGTC-------------TTCAGGCTCTATGATTTTTATATATACACC-AT--GTA
'Conocybe lactea 1'               T-TTTC----------CTGTC-------------TTCAGGCTCTATGATTTTTATATATACACC-AT--GTA
'Conocybe lactea 4'               T-TTCC----------CTGTC-------------TTCAGGCTCTATGATTTTTATATATACACC-AT--GTA
'Conocybe crispa 5'               T-TTTC----------CTGTC-------------TTCAGGCTCTATGATTTTTATATATACACC-AT--GTA
'Conocybe lactea 5'               T-TTTC----------CTGTC-------------TTCAGGCTCTATGATTTTTATATATACACC-AT--GTA
'Conocybe crispa 2'               T-TTTC----------CTGTC-------------TTCAGGCTCTATGATTTTTATATATACACC-AT--GTA
'Conocybe crispa 4'               T-TTTC----------CTGTC-------------TTCAGGCTCTATGATTTTTATATATACACC-AT--GTA
'Conocybe crispa 3'               T-TTTC----------CTGTC-------------TTCAGGCTCTATGATTTTTATATATACACC-AT--GTA
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Appendix 5. Chapter 4 *Conocybe* ITS, cont.

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'Lactea-crispa intermediate'      T-TTTC----------CTGTC-------------TTCAGGCTCTATGATTTTTATATATACACC-AT--GTA
'Conocybe crispa 1'               T-TTTC----------CTGTC-------------TTCAGGCTCTATGATTTTTATATATACACC-AT--GTA
'Conocybe lactea UK clone 2'      T-TTTC----------CTGTC-------------TTCAGGCTCTATGATTTTTATATATACACC-AT--GTA
'Conocybe lactea 3'               T-TTTC----------CTGTC-------------TTCAGGCTCTATGATTTTT-TTTAAACCCC-AT--GTA
'Conocybe lactea Corvallis'       T-TTTC----------CTGTC-------------TTCAGGCTCTATGATTTTTATATATACACC-AT--GTA
'Conocybe lactea Eugene'          T-TTTC----------CTGTG-------------TTCAGGCTNTATGATTTTTATATATACACC-AT--GTA
'Lactea-Gastrocybe intermediate'  T-TTTC----------CTGTC-------------TTCAGGCTCTATGATTTTT--ATATACACC-AT--GTA
'Conocybe lactea UK clone 5'      T-TTTC----------CTGTC-------------TTCAGGCTCTATGATTTTT--ATATACACC-AT--GTA
'Conocybe huijsmannii conica'     T-TTTC----------CTGTC-------------TTCAGGCTCTATGATTTTTATATATACACC-AT--GTA
'Conocybe crispa UK'              ??????????????????????????????????TTCATTCTCTATGAAATTCATATACACACC-AT--GTA
'Intermediate 2'                  T-TTTC----------CTGTA-------------TTCAGGCTCTATGATTTTT--ATATACACC-AT--GTA
'Conocybe subcrispa'              T-TTTC----------CTGTC-------------TTCAGGCTCTATGATTTTT--ATATACACC-AT--GTA
'Gastrocybe lateritia 4'          T-TTTC----------CTGTC-------------TTCAGGCTCTATGATTTTT--ATATACACC-AT--GTA
'Gastrocybe lateritia 3'          T-TTTC----------CTGTC-------------TTCAGGCTCTATGATTTTT--ATATACACC-AT--GTA
'Gastrocybe lateritia 5'          T-TTTC----------CTGTC-------------TTCAGGCTCTATGATTTTT--ATATACACC-AT--GTA
'Intermediate 1'                  T-TTTC----------CTGTC-------------TTCAGGCTCTATGATTTTT--ATATACACC-AT--GTA
'Gastrocybe lateritia 1'          T-TTTC----------CTGTC-------------TTCAGGCTCTATGATTTTT--ATATACACC-AT--GTA
'Gastrocybe lateritia 2'          T-TTTC----------CTGTC-------------TTCAGGCTCTATGATTTTT--ATATACACC-AT--GTA

'Weraroa cucullata'                TGTA-ACAGAATGT---C-AAT------TGGGCTTTATTG---TCCTATAAA-C-TATATACAACTTTCAG
'Cyttarophyllum besseyi'           TGTA-ACAGAATGTATTGTATN------TGGCC--TAGTG----CCTATAAA-C-TATATACAACTTTCAG
'Agrocybe semiorbicularis'         TGTA-ACAGAATGTATTGTATA------TGGCC--TAGTG----CCTATAAA-C-TATATACAACTTTMAG
'Agrocybe praecox'                 TGTA--CAGAATGTA-TC-ATA------TGCCC--TTGTG----CCTATAAA-C-TATATACAACTTTCAG
'Galeropsis desertorum'            GTATGTTTGCAGAATGTA-TCAATG----GGC---CTCTGT--GCCTATAAAAC-TCAATACAACTTTCAG
'Bolbitius variicolor'             TGTA-AACGAATGAA-TC-AATGGCAGCTTCA---TTGCA---GCCTATAAAAC--TTAAACAACTTTCAG
'Bolbitius vitellinus'             TGTA-AACGAATGAA-TC-AATGGCAGCTTCA---TTGCA---GCCTATAAAAC--TTAAACAACTTTCAG
'Bolbitius lacteus'                TGTA-AACGAATGAA-TC-AATGGCAGCTTCA---TTGCA---GCCTATAAAAC--TTAAACAACTTTCAG
'Gastrocybe deceptiva TYPE'        TGTA-AACGAATGAAATC-AATGGCAGCTTCA---TTGCA---GCCTATAAAAC--TTAAACAACTTTCAG
'yellow Gastrocybe'                TGTN-AACGAATGAA-TC-AATGGCAGCTTCA---TTGCA---GGCTATAAAAC--TTAAACAACTTTCAG
'Conocybe subnuda'                 TG-A-ACAGAATGAA-TC-AATGGCTA-TTCA---T-------GCCAATAAA-C-TTTATACAACTTTCAG
'Conocybe coprophila'              TGTT-TTAGAACGTGTAT-ACAAG-----GCC---TTAAAACAGCCTATAATAA-TCTATACAACTTTCAG
'Conocybe rickenii'                TGTC-AATGAACGCAATC-AATG------GGC---CCACAAA-GCCTATAAA-CTATAATACAWCTTTCAS
'Conocybe filaris'                 TGTT-ACAGAATGATATT-AAAA------GGC---CCTAAAAAGTCTATATAAA-CATATACAACTTTCAG
'Conocybe lactea 2'                TGTT-AAAGAACGCATTT-ACA-------GGC---TTGCAAAAGCCTATAAA-C-TTTATACAACTTTCAG
'Conocybe lactea 1'                TGTT-AAAGAACGCATTT-ACA-------GGC---TTGCAAAAGCCTATAAA-C-TTTATACAACTTTCAG
'Conocybe lactea 4'                TGTT-AAAGAACGCATTT-ACA-------GGC---TTGCAAAAGCCTATAAA-C-TTTATACAACTTTCAG
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Appendix 5. Chapter 4 *Conocybe* ITS, cont.

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'Conocybe crispa 5'                TGTT-AAAGAACGCATTT-ACA-------GGC---TTGCAAAAGCCTATAAA-C-TTTATACAACTTTCAG
'Conocybe lactea 5'                TGTT-AAAGAACGCATTT-ACA-------GGC---TTGCAAAAGCCTATAAA-C-TTTATACAACTTTCAG
'Conocybe crispa 2'                TGTT-AAAGAACGCATTT-ACA-------GGC---TTGCAAAAGCCTATAAA-C-TTTATACAACTTTCAG
'Conocybe crispa 4'                TGTT-AAAGAACGCATTT-ACA-------GGC---TTGCAAAAGCCTATAAA-C-TTTATACAACTTTCAG
'Conocybe crispa 3'                TGTT-AAAGAACGCATTT-ACA-------GGC---TTGCAAAAGCCTATAAA-C-TTTATACAACTTTCAG
'Lactea-crispa intermediate'       TGTT-AAAGAACGCATTT-ACA-------GGC---TTGCAAAAGCCTATAAA-C-TTTATACAACTTTCAG
'Conocybe crispa 1'                TGTT-AAAGAACGCATTT-ACA-------GGC---TTGCAAAAGCCTATAAA-C-TTTATACAACTTTCAG
'Conocybe lactea UK clone 2'       TGTT-AAAGAACGCATTT-ACA-------GGC---TTGCAAAAGCCTATAAA-C-TTTATACAACTTTCAG
'Conocybe lactea 3'                TGTT-AAAGAACGCATTT-ACAA------GGC---TTGCAAAAGCCTTTAAA-C-ATTATACAACTTTCAA
'Conocybe lactea Corvallis'        TGTT-AAAGAACGCATTT-ACA-------GGC---TTGCAAAAGCCTATAAA-C-TTTATACAACTTTCAG
'Conocybe lactea Eugene'           TGTT-AAAGAACGCATTT-ACA-------GGC---TTGCAAAAGCCTATAAA-C-ATTATACAACTTTCAG
'Lactea-Gastrocybe intermediate'   TGTT-AAAGAACGC-TTT-ACA-------GGC---CTTCAAA-GCCTATAAA-C-ATAATACAACTTTCAG
'Conocybe lactea UK clone 5'       TGTT-AAAGAACGC-TTT-ACA-------GGC---CTTCAAA-GCCTATAAA-C-ATAATACAACTTTCAG
'Conocybe huijsmannii conica'      TGTT-AAAGAACGCATTT-ACA-------GGC---TTGCAAAAGCCTATAAA-C-TTTATACAACTTTCAG
'Conocybe crispa UK'               TGTC-AATGAACGCAGTC-AAT------GGGC---CCACAAA-GCCTATAAA-CTATAATACAACTTTCAG
'Intermediate 2'                   TGTT-AAASAACGC-TTT-ACA-------GGC---CTTCAAA-GCCTATAAA-C-ATAATACAACTTTCAG
'Conocybe subcrispa'               TGTT-AAAGAACGC-TTT-ACA-------GGC---TTTCAAAGGCCTATAAA-C-ATAATACAACTTTCAG
'Gastrocybe lateritia 4'           TGTT-AAAGAACGTCTGT-AAA-------GGC---TTTTA---GCCTATAAA-C-ATAATACAACTTTCAG
'Gastrocybe lateritia 3'           TGTT-AAAGAACGTCTTT-AAA-------GGC---TTTTA---GCCTATAAA-C-ATAATACAACTTTCAG
'Gastrocybe lateritia 5'           TGTT-AAAGAACGTCTTT-AAA-------GGC---TTTTA---GCCTATAAA-C-ATAATACAACTTTCAG
'Intermediate 1'                   TGTT-AAAGAACGTCTTT-AAA-------GGC---TTTTA---GCCTATAAA-C-ATAATACAACTTTCAG
'Gastrocybe lateritia 1'           TGTT-AAAGAACGTCTTT-AAA-------GGC---TTTTA---GCCTATAAA-C-ATAATACAACTTTCAG
'Gastrocybe lateritia 2'           TGTT-AAAGAACGTCTTT-AAA-------GGC---TTTTA---GCCTATAAA-C-ATAATACAACTTTCAG


'Weraroa cucullata'                CAACGGATCTCTTGGCTCTCGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAAT
'Cyttarophyllum besseyi'           CAACGGATCTCTTGGCTCTCGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAAT
'Agrocybe semiorbicularis'         CAACGGATCTCTTGGCTCTCGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAAT
'Agrocybe praecox'                 CAACGGATCTCTTGGCTCTCGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAAT
'Galeropsis desertorum'            CAACGGATCTCTTGGCTCTCGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAAT
'Bolbitius variicolor'             CAACGGATCTCTTGGCTCTCGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAAT
'Bolbitius vitellinus'             CAACGGATCTCTTGGCTCTCGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAAT
'Bolbitius lacteus'                CAACGGATCTCTTGGCTCTCGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAAT
'Gastrocybe deceptiva TYPE'        CAACGGATCTCTTGGCTCTCGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAAT
'yellow Gastrocybe'                CAACGGATCTCTTGGCTCTCGCATCGATGAAGAACGCACCGAAATGCGATNAGTAATGTGAATTGCAGAAT
'Conocybe subnuda'                 CAACGGATCTCTTGGCTCTCGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAAT
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Appendix 5. Chapter 4 *Conocybe* ITS, cont.

'Conocybe coprophila'	CAACGGATCTCTTGGCTCTCGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAAT
'Conocybe rickenii'	CAACGGATCTCTTGGCTCTCGCATCGATGAAGAACGCAGCKAAMTGCGATAMGTAMTGTSAATTGCAGAAT
'Conocybe filaris'	CAACGGATCTCTTGGCTCTCGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAAT
'Conocybe lactea 2'	CAACGGATCTCTTGGCTCTCGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAAT
'Conocybe lactea 1'	CAACGGATCTCTTGGCTCTCGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAAT
'Conocybe lactea 4'	CAACGGATCTCTTGGCTCTCGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAAT
'Conocybe crispa 5'	CAACGGATCTCTTGGCTCTCGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAAT
'Conocybe lactea 5'	CAACGGATCTCTTGGCTCTCGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAAT
'Conocybe crispa 2'	CAACGGATCTCTTGGCTCTCGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAAAAT
'Conocybe crispa 4'	CAACGGATCTCTTGGCTCTCGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAAT
'Conocybe crispa 3'	CAACGGATCTCTTGGCTCTCGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAAT
'Lactea-crispa intermediate'	CAACGGATCTCTTGGCTCTCGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAAT
'Conocybe crispa 1'	CAACGGATCTCTTGGCTCTCGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAAT
'Conocybe lactea UK clone 2'	CAACGGATCTCTTGGCTCTCGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAAT
'Conocybe lactea 3'	CAACGGATCTTTTGGCTCTTGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAAT
'Conocybe lactea Corvallis'	CAACGGATCTCTTGGCTCTCGCATCGATGAAGAACGCANCGAAATGCGATAAGTAATGTGAATTGCANAAT
'Conocybe lactea Eugene'	CAACGGATCTCTTGGCTCTCGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAAT
'Lactea-Gastrocybe intermediate'	CAACGGATCTCTTGGCTCTCGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAAT
'Conocybe lactea UK clone 5'	CAACGGATCTCTTGGCTCTCGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAAT
'Conocybe huijsmannii conica'	CAACGGATCTCTTGGCTCTCGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAAT
'Conocybe crispa UK'	CAACGGATCTCTTGGCTCTCGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAAT
'Intermediate 2'	CAACGGATCTCTTGGCTCTCGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAAT
'Conocybe subcrispa'	CAACGGATCTCTTGGCTCTCGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAAT
'Gastrocybe lateritia 4'	CAACGGATCTCTTGGCTCTCGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAAT
'Gastrocybe lateritia 3'	CAACGGATCTCTTGGCTCTCGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAAT
'Gastrocybe lateritia 5'	CAACGGATCTCTTGGCTCTCGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAAT
'Intermediate 1'	CAACGGATCTCTTGGCTCTCGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAAT
'Gastrocybe lateritia 1'	CAACGGATCTCTTGGCTCTCGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAAT
'Gastrocybe lateritia 2'	CAACGGATCTCTTGGCTCTCGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAAT
'Weraroa cucullata'	TCA-GTGAATCATCGAATCTTTGAA-CGCACCTTGCGCTCCTTGGTATTCCGAGGAGCATGCCTGTTTGAG
'Cyttarophyllum besseyi'	TCA-GTGAATCATCGAATCTTTGAA-CGCACCTTGCGCTCCTTGGTATTCCGAGGAGCATGCCTGTTTGAG
'Agrocybe semiorbicularis'	TCA-GTGAATCATCGAATCTTTGAA-CGCACCTTGCGCTCCTTGGTATTCCGAGGAGCATGCCTGTTTGAG
'Agrocybe praecox'	TCA-GTGAATCATCGAATCTTTGAA-CGCACCTTGCGCTCCTTGGTATTCCGAGGAGCATGCCTGTTTGAG
'Galeropsis desertorum'	TCA-GTGAATCATCGAATCTTTGAA-CGCACCTTGCGCTCCTTGGTATTCCGAGGAGCATGCCTGTTTGAG
'Bolbitius variicolor'	TCA-GTGAATCATCGAATCTTTGAA-CGCACCTTGCGCTCCTTGGTATTCCGAGGAGCATGCCTGTTTGAG

Appendix 5. Chapter 4 *Conocybe* ITS, cont.

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'Bolbitius vitellinus'              TCA-GTGAATCATCGAATCTTTGAA-CGCACCTTGCGCTCCTTGGTATTCCGAGGAGCATGCCTGTTTGAG
'Bolbitius lacteus'                 TCA-GTGAATCATCGAATCTTTGAA-CGCACCTTGCGCTCCTTGGTATTCCGAGGAGCATGCCTGTTTGAG
'Gastrocybe deceptiva TYPE'         TCA-GTGAATCATCGAATCTTTGAA-CGCACCTTGCGCTCCTTGGTATTCCGAGGAGCATGCCTGTTTGAG
'yellow Gastrocybe'                 TCA-CTGAATCATCGAATCTTTGAA-CACACCTTGCGCTCCTTGGTGTTCCAAAGAGCATGCCTGTTTGAG
'Conocybe subnuda'                  TCA-GTGAATCATCGAATCTTTGAA-CGCACCTTGCGCTCCTTGGTATTCCGAGGAGCATGCCTGTTTGAG
'Conocybe coprophila'               TCA-GTGAATCATCGAATCTTTGAA-CGCACCTTGCGCTCCTTGGTATTCCGAGGAGCATGCCTGTTTGAG
'Conocybe rickenii'                 TCA-GTGAATCATCGAATCTTTGAA-CGCACCTTGCGCTCCTTGGYMTTCCKAGGAKCATGCCTGYTTSAG
'Conocybe filaris'                  TCA-GTGAATCATCGAATCTTTGAA-CGCACCTTGCGCTCCTTGGTATTCCGAGGAGCATGCCTGTTTGAG
'Conocybe lactea 2'                 TCA-GTGAATCATCGAATCTTTGAA-CGCACCTTGCGCTCCTTGGTATTCCGAGGAGCATGCCTGTTTGAG
'Conocybe lactea 1'                 TCA-GTGAATCATCGAATCTTTGAA-CGCACCTTGCGCTCCTTGGTATTCCGAGGAGCATGCCTGTTTGAG
'Conocybe lactea 4'                 TCA-GTGAATCATCGAATCTTTGAA-CGCACCTTGCGCTCCTTGGTATTCCGAGGAGCATGCCTGTTTGAG
'Conocybe crispa 5'                 TCA-GTGAATCATCGAATCTTTGAA-CGCACCTTGCGCTCCTTGGTATTCCGAGGAGCATGCCTGTTTGAG
'Conocybe lactea 5'                 TCA-GTGAATCATCGAATCTTTGAA-CGCACCTTGCGCTCCTTGGTATTCCGAGGAGCATGCCTGTTTGAG
'Conocybe crispa 2'                 TCA-GTGAATCATCGAATCTTTGAA-CGCACCTTGCGCTCCTTGGTATTCCGAGGAGCATGCCTGTTTGAG
'Conocybe crispa 4'                 TCA-GTGAATCATCGAATCTTTGAA-CGCACCTTGCGCTCCTTGGTATTCCGAGGAGCATGCCTGTTTGAG
'Conocybe crispa 3'                 TCA-GTGAATCATCGAATCTTTGAA-CGCACCTTGCGCTCCTTGGTATTCCGAGGAGCATGCCTGTTTGAG
'Lactea-crispa intermediate'        TCA-GTGAATCATCGAATCTTTGAA-CGCACCTTGCGCTCCTTGGTATTCCGAGGAGCATGCCTGTTTGAG
'Conocybe crispa 1'                 TCA-GTGAATCATCGAATCTTTGAA-CGCACCTTGCGCTCCTTGGTATTCCGAGGAGCATGCCTGTTTGAG
'Conocybe lactea UK clone 2'        TCA-GTGAATCATCGAATCTTTGAA-CGCACCTTGCGCTCCTTGGTATTCCGAGGAGCATGCCTGTTTGAG
'Conocybe lactea 3'                 TCAAGTGAATAATCGAATCTTTGAAACGCACCTTGCGCTTCTTGGTATTCCGAGGAACATGCCTGTTTGAG
'Conocybe lactea Corvallis'         TCA-GTGAATCATCGAATCTTTGAA-CGCACCTTGCGCTCCTTGGTATTCCGAGGAGCATGCCTGTTTGAA
'Conocybe lactea Eugene'            TCA-GTGAATCATCGAATCTTTGAA-CGCACCTTGCGCTCCTTGGTATTCCGAGGAGCATGCCTGTTTGAG
'Lactea-Gastrocybe intermediate'    TCA-GTGAATCATCGAATCTTTGAA-CGCACCTTGCGCTCCTTGGTATTCCGAGGAGCATGCCTGTTTGAG
'Conocybe lactea UK clone 5'        TCA-GTGAATCATCGAATCTTTGAA-CGCACCTTGCGCTCCTTGGTATTCCGAGGAGCATGCCTGTTTGAG
'Conocybe huijsmannii conica'       TCA-GAGAATCATCGAATCTTTGAA-CGCACCTTGCGCTCCTTGGTATTCCGAGGAGCATGCCTGTTTGAG
'Conocybe crispa UK'                TCA-GTGAATCATCGAATCTTTGAA-CGCACCTTGCGCTCCTTGGTATTCCGAGGAGCATGCCTGTTTGAG
'Intermediate 2'                    TCA-GTGAATCATCGAATCTTTGAA-CGCACCTTGCGCTCCTTGGTATTCCGAGGAGCATGCCTGTTTGAG
'Conocybe subcrispa'                TCA-GTGAATCATCGAATCTTTGAA-CGCACCTTGCGCTCCTTGGTATTCCGAGGAGCATGCCTGTTTGAG
'Gastrocybe lateritia 4'            TCA-GTGAATCATCGAATCTTTGAA-CGCACCTTGCGCTCCTTGGTATTCCGAGGAGCATGCCTGTTTGAG
'Gastrocybe lateritia 3'            TCA-GTGAATCATCGAATCTTTGAA-CGCACCTTGCGCTCCTTGGTATTCCGAGGAGCATGCCTGTTTGAG
'Gastrocybe lateritia 5'            TCA-GTGAATCATCGAATCTTTGAA-CGCACCTTGCGCTCCTTGGTATTCCGAGGAGCATGCCTGTCTGAG
'Intermediate 1'                    TCA-GTGAATCATCGAATCTTTGAA-CGCACCTTGCGCTCCTTGGTATTCCGAGGAGCATGCCTGTTTGAG
'Gastrocybe lateritia 1'            TCA-GTGAATCATCGAATCTTTGAA-CGCACCTTGCGCTCCTTGGTATTCCGAGGAGCATGCCTGTTTGAG
'Gastrocybe lateritia 2'            TCA-GTGAATCATCGAATCTTTGAA-CGCACCTCGCGCTCCTTGGTATTCCGAGGAGCATGCCTGTTTGAG


'Weraroa cucullata'             TGTCATTAAATTCTCAACCTTTAT--CATTT---------ATTGATAAATGGCTTGGAC-TTGGGGGT-TTG-
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Appendix 5. Chapter 4 *Conocybe* ITS, cont.

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'Cyttarophyllum besseyi'          TGTCATTAAATTCTCAACCTTATTAGCTTTT---------GCTGATAA-TGGCTTGGAC-TTGGGGGTCTTTT
'Agrocybe semiorbicularis'        TGTCATTAAATTCTCAACCTTATTAGCTTTT---------GCTGATAA-TGGCTTGGAC-TTGGGGGTCTTTT
'Agrocybe praecox'                TGTCATTAAATTCTCAACCTTATTAGCTTTT---------GCTGATAA-TGGCTTGGAC-TTGGGGGTCTTTT
'Galeropsis desertorum'           TGTCATTTAATTCTCAACCACAAAGGCTTTTGC--GAGCTTTTG-----TGGATTGGAC-GTGGGGGTAACTG
'Bolbitius variicolor'            TGTCATTAAATTCTCAACCATCCTGTCTTTCTTTCGAGAGG-CA-GCGATGCGTTGGAT-GTGGGGAC-TTGC
'Bolbitius vitellinus'            TGTCATTAAATTCTCAACCATCCTGTCTTTCTTTCGAGAGG-CG-GCGATGCGTTGGAT-GTGGGGAC-TTGC
'Bolbitius lacteus'               TGTCATTAAATTCTCAACCATCCTGTCTTTCTTTCGAGAGG-CG-GCGATGCGTTGGAT-GTGGGGAC-TTGC
'Gastrocybe deceptiva TYPE'       TG-CATTAAAT-CTCAACCATCCTGGCTTTCTTTCGAGAGGGCG-GTGATGCGTTGGAT-GTGGG-AC-CTGC
'yellow Gastrocybe'               TGTCATTAAATTCTCAACCATCCTGTCTTTCTTTCNAGAAGCAA-TNGATGCGTNGGAT-GTAGGGAC-TNGC
'Conocybe subnuda'                TGTCATTAAATTATCAACCGCTCTGA--TTCATTTCAGA---------AGTGGATTGGAT-TTGGGGG--TTGC
'Conocybe coprophila'             TGTCATTAAATCATCAACTACCCTTTCATTTATTT-----GTAA-AGAGTAGCTTGGAC-TTGGGAGTCTT--
'Conocybe rickenii'               TGTCATTAAATTCTCAATCTTTACAACTTTGT--------GTTG-TC--CAGATTGGAT-TTGGGGG--TTTC
'Conocybe filaris'                TGTCATTAAATCATCAA--------ACCTTAC-----AATTTTG-AAA--GGCTTGGAT-TTGGGAGTGTTG-
'Conocybe lactea 2'               TGTCATTAAATTCTCAATCTCTACAACTTTT---------GTTG-TTAGCAGGTTGGAT-GTGGGGG--TTTA
'Conocybe lactea 1'               TGTCATTAAATTCTCAATCTCTACAACTTTT---------GTTG-TTAGCAGGTTGGAT-GTGGGGG--TTTA
'Conocybe lactea 4'               TGTCATTAAATTCTCAATCTCTACAACTTTT---------GTTG-TTAGCAGGTTGGAT-GTGGGGGG-TTTA
'Conocybe crispa 5'               TGTCATTAAATTCTCAATCTCTACAACTTTT---------GTTG-TTAGCAGGTTGGAT-GTGGGGG--TTTA
'Conocybe lactea 5'               TGTCATTAAATTCTCAATCTCTACAACTTTT---------GTTG-TTAGCAGGTTGGAT-GTGGGGG--TTTA
'Conocybe crispa 2'               TGTCATTAAATTCTCAATCTCTACAACTTTT---------GTTG-TTAGCAGGTTGGAT-GTGGGGG--TTTA
'Conocybe crispa 4'               TGTCATTAAATTCTCAATCTCTACAACTTTT---------GTTG-TTAGCAGGTTGGAT-GTGGGGG--TTTA
'Conocybe crispa 3'               TGTCATTAAATTCTCAATCTCTACAACTTTT---------GTTG-TTAGCAGGTTGGAT-GTGGGGG--TTTA
'Lactea-crispa intermediate'      TGTCATTAAATTCTCAATCTCTACAACTTTT---------GTTG-TTAGCAGGTTGGAT-GTGGGGG--TTTA
'Conocybe crispa 1'               TGTCATTAAATTCTCAATCTCTACAACTTTT---------GTTG-TTAGCAGGTTGGAT-GTGGGGG--TTTA
'Conocybe lactea UK clone 2'      TGTCATTAAATTCTCAATCTCTACAACTTTT---------GTTG-TTAGCAGGTTGGAT-GTGGGGGCTTTTA
'Conocybe lactea 3'               TGTCATTAAATTCTCAATCTCTACAACTNTT---------GTTG-TTAGCAAGTTG-AT-GTGGGGG--TTTA
'Conocybe lactea Corvallis'       TGTCATTAAATTCTCAATCTCTACAACTTTT---------GTTG-TTANCAAGTTGGAT-GTGGGGG--TTTA
'Conocybe lactea Eugene'          TGTCATTAAATTCTCAATCTCTACAACTTTT---------GTTG-TTAGCAGGTTGGAT-GTGGGGG--TTTA
'Lactea-Gastrocybe intermediate'  TGTCATTAAATTCTCAATCTCTACAACTTT----------GTTG-TTAGTAGGTTGGAA-GTGGGGGC-TTTT
'Conocybe lactea UK clone 5'      TGTCATTAAATTCTCAATCTCTACAACTTT----------GTTG-TTAGTAGTTTGGAAAGTGGGGGC-TTNT
'Conocybe huijsmannii conica'     TGTCATTAAATTCTCAATCTCTACAACTTTT---------GTTG-TTAGCAGGTTGGAT-GTGGGGGT-TT--
'Conocybe crispa UK'              TGTCATTAAATTCTCAATCTTTACAACTTTGT--------GTTG-TC--CAGATTGGAT-TTGGGGGT-TTCA
'Intermediate 2'                  TGTCATTAAATTCTCAATCTCTACAACTTT----------GTTG-TTAGTAGGTTGGAA-GTGGGGGC-TTTT
'Conocybe subcrispa'              TGTCATTAAATTCTCAATCTCTACAACTTT----------GTTG-TTAGCAGGTTGGAT-GTGGGGGA-TT--
'Gastrocybe lateritia 4'          TGTCATTAAATTCTCAATCTCTACAACTTT----------GTTG-TTAGTCGATTG-ATGTTGGGGGT-CTTG
'Gastrocybe lateritia 3'          TGTCATTAAATTCTCAATCTCTACAACTTT----------GTTG-TTAGTCGATTGGATGGTGGGGGT-CTTG
'Gastrocybe lateritia 5'          TGTCATTAAATTCTCAATCTCTACAACTTT----------GTTG-TTAGTCGATTGGATGTTGGGGGT-CTTG
'Intermediate 1'                  TGTCATTAAATTCTCAATCTCTACAACTTT----------GTTG-TTAGTCGATTGGAT-GTGGGGGT-CTTG
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Appendix 5. Chapter 4 *Conocybe* ITS, cont.

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'Gastrocybe lateritia 1'          TGTCATTAAATTCTCAATCTCTACAACTTT----------GTTG-TTAGTCGATTGGAT-GTGGGGGT-CTTG
'Gastrocybe lateritia 2'          TGTCATTAAATTCTCAATCTCTACAACTTT----------GTTG-TTAGTCGATTGGATGTTGGGGGT-CTTG


'Weraroa cucullata'               CCGG------CGAAAG-------TCA-GCTCCTCTGAAAAGTATTAGC-TGGTT-GCCTTGTGTAAACTTGT
'Cyttarophyllum besseyi'          TNNNGGGTTTCANTNAA------CCN-GGTTCCCTTAAN-GGATTAACCCGGTCCCCCC?????????????
'Agrocybe semiorbicularis'        TGCTGGCTTTCATTAG-------TCT-GCTCCCCTTAAATGTATTAGC-TGGTGCCCCGCAGTGGAA-CCGT
'Agrocybe praecox'                -GCTGGCTTTCATTAG-------TCT-GCTCCCCTTAAATGTATTAGC-TGGCGCCCCGCAGTGGA--CCGT
'Galeropsis desertorum'           -CAGGCCTT-CCCAGGTCA--------GCTCCCCTAAAATGCATTAGC--GGAA-CCGTTTGCGG----TAA
'Bolbitius variicolor'            -CTGTTTCT-TACGAGAC---TTCGG-CTTCCCTTAAAA-GCATTAGC-TAGA--GCGCTTCTGT----TGA
'Bolbitius vitellinus'            -CTGTTTCT-TACGAGAC---TTCGG-CTTCCCTTAAAA-GCATTAGC-TAGA--GCGCTTCTGT----TGA
'Bolbitius lacteus'               -CTGTTTCT-TACGAGAC---TTCGG-CTTCCCTTAAAA-GCATTAGC-TAGA--GCGCTTCTGT----TGA
'Gastrocybe deceptiva TYPE'       -CTGGTTNT-TACNAGAC---TTCGG-NTTCC-TTAAAA-GCATTANC-TNGA--GCGCTTNTGN----TGN
'yellow Gastrocybe'               -CTGTTTCT-TACTAAAC---TTAAG-CTTGCNKKAAAA-ANAAAAAC-TAGA--GCGCTTCTGT----TGA
'Conocybe subnuda'                -AAGCTTCT-AAAGAGGCT------G-GCTCCCCTTAAATATATTAGC-TGGAATGTGTCTCTGTGAG----
'Conocybe coprophila'             -CTGTGGGC-CTTAGGTCT--------GCTCTCCTTAAATGCCTTAGC-TGGAATGTACCCCTGCAGTCCAA
'Conocybe rickenii'               ATTGTGCGG-AATT-CGA---TTCCTCACTCCCCTTAAATGCATTAGC-TGGAATGCCTCCGCATAT-CTGA
'Conocybe filaris'                -TTGGCCTT-CCTTTTGGTCA------GCTCTCCTTAAATGCATTAGC-TGGAA-GCTTTTGTAG-------
'Conocybe lactea 2'               -TTGTGGG--CGTTACAA-----CCT-ACTCCCCTGAAATGAATTAGC-TGGAATGCCTCGGCAAAT-CCAA
'Conocybe lactea 1'               -TTGTGGG--CGTTACAA-----CCT-ACTCCCCTGAAATGAATTAGC-TGGAATGCCTCGGCAAAT-CCAA
'Conocybe lactea 4'               -TTGTGGGG-CGTTACAA-----CCT-ACTCCCCTGAAATGAATTAGC-TGGAATGCCTCGGCAAAT-CCAA
'Conocybe crispa 5'               -TTGTGGG--CGTTACAA-----CCT-ACTCCCCTGAAATGAATTAGC-TGGAATGCCTCGGCAAAT-CCAA
'Conocybe lactea 5'               --TGTGGG--CGTTACAA----CCTT-ACTCCCCTGAAATGAATTAGC-TGGAATGCCTCGGCAAAT-CCAA
'Conocybe crispa 2'               -TTGTGGG--CGTTACAA-----CCT-ACTCCCCTGAAATGAATTAGC-TGGAATGCCTCGGCAAAT-CCAA
'Conocybe crispa 4'               -TTGTGGG--CGTTACAA-----CCT-ACTCCCCTGAAATGAATTAGC-TGGAATGCCTCGGNCAAT-CCAA
'Conocybe crispa 3'               -TTGTGGG--CGTTACAA-----CCT-ACTCCCCTGAAATGAATTAGC-TGGAATGCCTCGGNCAAT-CCAA
'Lactea-crispa intermediate'      -TTGTGGG--CATTACAA-----CCT-ACTCCCCTGAAATGAATTAGC-TGGAATGCCTCGGCAAAT-CCAA
'Conocybe crispa 1'               -TTGTGGG--CGTTACAA-----CCT-ACTCCCCTGAAATGAATTAGC-TGGAATGCCTCGGCAAAT-CCAA
'Conocybe lactea UK clone 2'      -TTGTGGG--CGTTACAA-----CCC-ACTCCCCTGAAATGAATTAGC-TGGAATGCCTCGGCAAAT-CCAA
'Conocybe lactea 3'               -TTGTAGG--CGTTACAA-----CCT-ACTCCCCTGAAATGAATTAGC-TGGAATGCCTCGGCAAAT-CCAA
'Conocybe lactea Corvallis'       -ATGTGGG--CGTAACAA-----CCT-ACTCCCCTCAAATGAATTAGC-TGGAATGCCTCGGCAAAT-CCAA
'Conocybe lactea Eugene'          -TTGTGGG--CGTTACAA-----CCT-ACTCCCCTGAAATGAATTAGC-TGGAATGCCTCGGCAAAT-CCAA
'Lactea-Gastrocybe intermediate'  --TGTGAGA-TGTTTTAGTACGTCCC-ACTCCCCTGAAATGAATTAGC-TGGAATGCCTCGGCAAAT-CCAA
'Conocybe lactea UK clone 5'      -TTGTGAGA-TGTTTTAGTACGTCCC-ACTCCCCTGAAATGAATTAGC-TGGAATGCCTCGGCAAAT-CCAA
'Conocybe huijsmannii conica'     -ATGTGGG--CGTTACAA-----CCT-ACTCCCCTGAAATGAATTAGC-TGGAATGCCTCGGCAAAT-CCAA
'Conocybe crispa UK'              T-TGTGCGG-AATT-CAA---TTCCTCACTCCCCTTAAATGCATTAGC-TGGAATGCCTCCGCATAT-CTGA
'Intermediate 2'                  --TGTGAGA-TGTTTTARTACGTCCC-ACTCCCCTGAAATGAATTAGC-TGGAATGCCTCGGCAAAT-CCAA
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Appendix 5. Chapter 4 *Conocybe* ITS, cont.

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'Conocybe subcrispa'              ---GTGGGA-TGTTTCAGTACGTCCC-ACTCCCCTGAAATGAATTAGC-TGGAATGCCTCGGCAAAT-CCAA
'Gastrocybe lateritia 4'          -TTGGGTGG-TTTAAGTACATTTCC--ACTCCCCTAAAACGAATTAGC-TGGAATGCCTCGGCAAGT-CCAA
'Gastrocybe lateritia 3'          -GTGGGTGG-TTTAAGTACATTTCC--ACTCCCCTAAAACGAATTAGC-TGGAATGCCTCGGCAAGT-CCAA
'Gastrocybe lateritia 5'          -GTGGGTGG-TTTAAGTACATTTCC--ACTCCCCTAAAACGAATTAGC-TGGAATGCCTCGGCAAGT-CCAA
'Intermediate 1'                  --TGGGTG--TTTAAGTACATTTCC--ACTCCCCTAAAACGAATTAGC-TGGAATGCCTCGGCAAGT-CCAA
'Gastrocybe lateritia 1'          --TGGGTG--TTTAAGTACATTTCC--ACTCCCCTAAAACGAATTAGC-TGGAATGCCTCGGCAAGT-CCAA
'Gastrocybe lateritia 2'          NNTGGGTG--TTTAAGTACATTTCC--ACTCCCCTAAAACGAATTAGC-TGGAATGCCTCGGCAAGT-CCAA


'Weraroa cucullata'               CTATT-----GGTGTGATAATTATC--TACGCCG-TGGGCTGTCTACCGTTTTATAGCA-------CTGCTT
'Cyttarophyllum besseyi'          ??????????GTGTGATAATTATC--YACRCYG-TGGAAGT-CTGCWAY-A-AWGGRW--WTGCKCTGCTT
'Agrocybe semiorbicularis'        CTATT-----GGTGTGATAATTATC--TACGCCG-TGGACGT-CTGCTAT-A-ATGGGT--TTGCGCTGCTT
'Agrocybe praecox'                CTATT-----GGTGTGATAATTATC--TACGCCG-TGGATGT-CTGCTTTCA-ATGGGTCTTTGCGCTGCTT
'Galeropsis desertorum'           CCAGTCGCCAGGTGTGATAATTATC--TACGCC---AATAGACATGAACTGCTCTCTGTTG---TTCTGCTT
'Bolbitius variicolor'            CTAGCTGCT-AGTGTGATAATTATC--TACACTG-TGG----------CCAACAAAGGAAA---CTCTGCTT
'Bolbitius vitellinus'            CTAGCTGCT-AGTGTGATAATTATC--TACACTG-TGG----------CCAACGAAGGAAA---CTCTGCTT
'Bolbitius lacteus'               CTAGCTGCT-AGTGTGATAATTATC--TACACTG-TGG----------CCAACGAAGGAAA---CTCTGCTT
'Gastrocybe deceptiva TYPE'       CTANCTGNT-AGNGTGANAANTATCT-CAC--TG-NGG----------CCAACGAAGGAAA---CTNTNNTT
'yellow Gastrocybe'               CTAGCTGNTNAGTGTGATAATTATCTACAC--TG--GG----------CCAACGAAGRAAA---NTGTGCTT
'Conocybe subnuda'                CTGTCCCATCAGTGTGATAATTATCTACTG--TG-CGGTCGGTTTGCCTC---GAAAATGAGCGCTCTGCTT
'Conocybe coprophila'             CTGTT-----AGTGTGATAATTATCTACGC--TACACAGGGC--AGCCTTTT-GAGTGGGA---CTCTGGCT
'Conocybe rickenii'               CTATC-----AGTGTGATAATTATCTATAC--TG-TAGTTGT--TGCATGA----GGG-----TATCTGCTT
'Conocybe filaris'                CTATCTACT-GGTGTGATAATTATCTATAC--CATTGAATGC--TGCACTTTGTATAA---GCCTTCTGCTT
'Conocybe lactea 2'               CTATC-----AGTGTGATAAATATCT-TAC--TG-TGGTTGT--TGCTTTT--GAGGG-----TATCCGCTT
'Conocybe lactea 1'               CTATC-----AGTGTGATAAATATCT-TAC--TG-TGGTTGT--TGCTTTT--GAGGG-----TATCCGCTT
'Conocybe lactea 4'               CTATC-----AGTGTGATAAATATCT-TAC--TG-TGGTTGT--TGCTTTT--GAGGG-----TATCCGCTT
'Conocybe crispa 5'               CTATC-----AGTGTGATAAATATCT-TAC--TG-TGGTTGT--TGCTTTT--GAGGG-----TATCCGCTT
'Conocybe lactea 5'               CTATC-----AGTGTGATAAATATCC-TAC--TG-TGGTTGT--TGCTTTT--GAGGG-----TATCCGCTT
'Conocybe crispa 2'               CTATC-----AGTGTGATAAATATCT-TAC--TG-TGGTTGT--TGCTTTT--GAGGG-----TATCCGCTT
'Conocybe crispa 4'               CTATC-----AGTGTGATAAATATCT-TAC--TG-TGGTTGT--TGCTTTT--GAGGG-----TATCCGCTT
'Conocybe crispa 3'               CTATC-----AGTGTGATAAATATCT-TAC--TG-TGGTTGT--TGCTTTT--GAGGG-----TATCCGCTT
'Lactea-crispa intermediate'      CTATC-----AGTGTGATAAATATCT-TAC--TG-TGGTTGT--TGCTTTT--GAGGG-----TATCCGCTT
'Conocybe crispa 1'               CTATC-----AGTGTGATAAATATCT-TAC--TG-NGGTTGT--TGCTTTT--GAGGG-----NATCCGCTT
'Conocybe lactea UK clone 2'      CTATC-----AGTGTGATAAATATC--TAC--TG-TGGTTGT--TGCTTTTT-GAGGG-----TATCTGCTT
'Conocybe lactea 3'               CTATC-----AGTGTGATAAATATCT-TAC--TG-TAGTTGT--TGCTTTT--GAGGG-----TATCCGCTT
'Conocybe lactea Corvallis'       CTATC-----AGTGTGATAAATATCT-TAC--TG-TGGTTGT--TGCTTTT--GAGGG-----TATCCGCTT
'Conocybe lactea Eugene'          CTATC-----AGTGTGATAAATATCT-TAC--TG-TAGTTGT--TGCTTTT--GAGGG-----TATCCGCTT
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Appendix 5. Chapter 4 *Conocybe* ITS, cont.

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'Lactea-Gastrocybe intermediate'  CTATC-----AGTGTGATAAATATC--TAC--TG-TGGTTGT--TGCATT---GAGGG-----TATCTGCTT
'Conocybe lactea UK clone 5'      CTATC-----AGTGTGATAA-TATC--TAC--TG-G??????????????????????????????????????
'Conocybe huijsmannii conica'     CTATC-----AGTGTGATAAATATCT-TAC--TG-TGGTTGT--TGCTTTT--GAGGG-----TATCCGCTT
'Conocybe crispa UK'              CTATC-----AGTGTGATAATTATCTATAC--TG-TAGTTGT--TGCAT----GAGGG-----TATCTGCTT
'Intermediate 2'                  CTATC-----AGTGTGATAAATATC--TAC--TG-TGGTTGT--TGCAT----GAGGG-----TATCTGCTT
'Conocybe subcrispa'              CTATC-----AGTGTGATAAATATC--TAC--TG-TGGTTGT--TGCAT----GAGGG-----TATCTGCTT
'Gastrocybe lateritia 4'          CTATC-----AGTGTGATAAATATCAGTAC--TG-TAGTTGT--TGCATT---GAGGG-----TATCTGCTT
'Gastrocybe lateritia 3'          CTATC-----AGTGTGATAAATATCAGTAC--TG-TAGTTGT--TGCATT---GAGGG-----TATCTGCTT
'Gastrocybe lateritia 5'          CTATC-----AGTGTGATAAATATCAGTAC--TG-TAGTTGT--TGCATT---GAGGG-----TATCTGCTT
'Intermediate 1'                  CTATC-----AGTGTGATAAATATCAGTAC--TG-TAGTTGT--TGCATT---GAGGG-----TATCTGCTT
'Gastrocybe lateritia 1'          CTATC-----AGTGTGATAAATATCAGTAC--TG-TAGTTGT--TGCATT---GAGGG-----TATCTGCTT
'Gastrocybe lateritia 2'          CTATC-----AGTGTGATAAATATCAGTAC--TG-TAGTTGT--TGCATT---GAGGG-----TATCTGCTT


'Weraroa cucullata'               CT-AACC-GTCTGTTAACTCGGACAATATAT--GA---CAATTTGACCTCAAATCagaagagaagaaaaaaa
'Cyttarophyllum besseyi'          CT-AAYC-GTC-TGCTYGSA-GACAACWYMAATGA---YAATTTGACCTCAAATCggg-gagaagaaaa-g-
'Agrocybe semiorbicularis'        CT-AACC-GTC-TCTCGG---GACAACACAAATGA---CAATTTGACCTCAAATCagg-aagaagaaaa-g-
'Agrocybe praecox'                C-CAACC-GTCCTCTCG----GACAATACAAATGA---CAATTTGACCTCAAATCagg-aagaagaaaa-g-
'Galeropsis desertorum'           --CAAATTGTC-TTGCT-------AGACAACTTTTGA-ATGTTTGACCTCAAATCagg-gagaagaaaaaaa
'Bolbitius variicolor'            CTAATCGTCTGCTTGC--------AGACAACTTCTGA-TATCTTGACCTCAAATCagg-gagag-a---aaa
'Bolbitius vitellinus'            CTAATCGTCTGCTTGC--------AGACAACTTCTGA-TATCTTGACCTCAAATCagg-gagaagaaaa--g
'Bolbitius lacteus'               CTAATCGTCTGCTTGC--------AGACAACTTCTGA-TATCTTGACCTCAAATCagg-gagaagaaaa--g
'Gastrocybe deceptiva TYPE'       NTAATCGNNTGNTTGN--------AGACAACTNCTGN-NATTT-G-NCNCAA-TNagg-gagaaga-gaaaa
'yellow Gastrocybe'               CNAATNGTN-NGTTT-------GCAGACAACTTCTGA-TATCTTGACCTCAAATCagg-gagaaga-gaaaa
'Conocybe subnuda'                CTAATCGTC-C-TTT---------GGACAACTTTTWA-CAACTTGACCTCAAATCagg-gaggagaaaagaa
'Conocybe coprophila'             CTCTAACCGTTGTCATCT---GACAAC-AACTCCTGATCATTTTGACCTCAAATCaag-gagaagg----aa
'Conocybe rickenii'               CATCAATCGTC--GCA--------AGACAAAT-G-AC-CATCTTGACCTCAAATCagg-aagaagaaagaaa
'Conocybe filaris'                CT-AAC-TGTC-CACCTCTATG---GACAATT-TTGA-CAATTTGACCTCAAATCagg-gagaagag--aaa
'Conocybe lactea 2'               CTCAACGTGTC-TGCAA-------AGACAACTTGAAT-CATTTTGACCTCAAATCagg-agaaaaaaaaaaa
'Conocybe lactea 1'               CTCAAC-TGTC-TGCAA-------AGACAACTTGAAT-CATTTTGACCTCAAATCagagagaaaaaaaaaaa
'Conocybe lactea 4'               CTCAAC-TGTC-TGCAA-------AGACAACTTGAAT-CATTTTGACCTCAAATCagg-agaaaaaaaaaaa
'Conocybe crispa 5'               CTCAAC-TGTC-TGCAA-------AGACAACTTGAAT-CATTTTGACCTCAAATC???????????????
'Conocybe lactea 5'               CTCAAC-TGTC-TGCAA-------AGACAACTTGAAT-CATTTTGACCTCAAATC??????aaaaaaaaaaa
'Conocybe crispa 2'               CTCAAC-TGTC-TGCAA-------AGACAACTTGAAT-CATTTTGACCTCAAATCagg-agaaaaaaaaaaa
'Conocybe crispa 4'               CTCAAC-TGTC-TGNCA-------AGACAACTTGAAT-CATTTTGACCTCAAATCagg-agaaaaaaaaaaa
'Conocybe crispa 3'               CTCAAC-TGTC-TGNCA-------AGACAACTTGAAT-CATTTTGACCTCAAATCagg-agaaaaaaaaaaa
'Lactea-crispa intermediate'      CTCAAC-TGTC-TGCAA-------AGACAACTTGAAT-CATTTTGACCTCAAATCagg-agaaaaaaaaaaa
```

Appendix 5. Chapter 4 *Conocybe* ITS, cont.

```
'Conocybe crispa 1'              CTCAAC-TGNC-TGCAA-------AGACA-CTTGAAT-CATTTTGACCTCAAATCagg-agaaaaaaaaaaa
'Conocybe lactea UK clone 2'     CTCAAC-TGTC-TGCAA-------AGACAACTTGAAT-CATTTTGACCTCAAATCagg-agaaagaaaaaaa
'Conocybe lactea 3'              CTCAAC-TGTC-TGCAA-------AGACAACTTGAAT-CATTTTGACCTCAAATCagg-agaaaaaaaaaaa
'Conocybe lactea Corvallis'      CTCAAC-TGTC-TGCAA-------AGACAACTTGAAT-CATTTTGACCTCAAATCagg-agaaaaaaaaaaa
'Conocybe lactea Eugene'         CTCAAC-TGTC-TGCAA-------AGACAACTTGAAT-CATTTTGACCTCAAATC???????????????
'Lactea-Gastrocybe intermediate' CTCAAC-TGTC-TGCAA-------AGACAACTTGAAT-CATTTTGACCTCAAATCagg-agaaagaaaaaaa
'Conocybe lactea UK clone 5'     ??????????????????????????????????????????????????agg-agaaagaaaaaaa
'Conocybe huijsmannii conica'    CTCAAC-TGTC-TGCAA-------AGACAACTTGAAT-CATTTTGACCTCAAATCagg-agaaaaaaaaaaa
'Conocybe crispa UK'             CATCAATCGTC--GCAA--------GACAAAT-GAC--CATCTTGACCTCAAATC???????????????
'Intermediate 2'                 CTCAAC-TGTC-TGCAA-------AGACAACTTGAAT-CATTTTGACCTCAAATCagg-agaaagaaaaaaa
'Conocybe subcrispa'             CTCAAC-TGTC-TGCAA-------AGACAACTTGAAT-CATTTTGACCTCAAATCagg-agaaagaaaaaaa
'Gastrocybe lateritia 4'         CTCAAC-TGTC-TTT---------GGACAACTTGAAT-CATTTTGACCTCAAATCagg-agaaagaaaaaaa
'Gastrocybe lateritia 3'         CTCAAC-TGTC-TTT---------GGACAACTTGAAT-CATTNTGACCTCAAATCagg-agaaagaaaaaaa
'Gastrocybe lateritia 5'         CTCAAC-TGTC-TTT---------GGACAACTTGAAT-CATTTTGACCTCAAATCagg-agaaagaaaaaaa
'Intermediate 1'                 CTCAAC-TGTC-TTT---------GGACAACTTGAAT-CATTTTGACCTCAAATCagg-agaaagaaaaaaa
'Gastrocybe lateritia 1'         CTCAAC-TGTC-TTT---------GGACAACTTGAAT-CATTTTGACCTCAAATCagg-agaaagaaaaaaa
'Gastrocybe lateritia 2'         CTCAAC-TGTC-TTT---------GGACAACTTGAAT-CATTTTGACCTCAAATCagg-agaaagaaaaaaa

'Weraroa cucullata'              gaaaaaaaaa---g--a-----------------------------------------aaaaaagaaaaagag
'Cyttarophyllum besseyi'         --a--aaaaa-gaaaaaaaaaa-gagaaaa--g--ag-ga---g-aaaaaaaaagaaagaa---aaaaaaaa
'Agrocybe semiorbicularis'       --a--aaaaa-gaaaaaaaaaa-gaaa-g---g--ag-ga---g-aaaaaaaaagaaagaa---aaaaaaaa
'Agrocybe praecox'               --a--aaaaaaaaaaaaaaaagaaaaaaaaaa-gag-ga--ga-aaaaaaaaaaaaagaa---aaaaaaaa
'Galeropsis desertorum'          aaaaaaaaaaaaaaaaaaagaaaagaaaaagaa-aaggaaaaaaaaaaaaaaag-aaaaaaaaaaaaaaaga
'Bolbitius variicolor'           -aaaaaaaaaaaaaaaaaagaaaaaaagaagaa-aaggaa--ga-g---g-----a-aaaag-aaag----a
'Bolbitius vitellinus'           --a--a-aaaaaaaaaaag----aaaagaagaa-aaggaaaaaagaaaaa-gaa-aaaaaaaaaaaaag--a
'Bolbitius lacteus'              --a--a-aaaaaaaaaaag----aaaagaagaa-aaggaaaaaagaaaaa-gaa-aaaaaaaaaaaaag--a
'Gastrocybe deceptiva TYPE'      a-g----aaaaaaaaaaag----aaaagaagaa-aaggaa--ga-aaaaa--ga-agaaag---aaaaa-aa
'yellow Gastrocybe'              a-g----aaaaaaaaaaag----aaaagaagaa-aaggaagaaaaaaaaa-gaa-g-aaaaaaaaaaaaaaa
'Conocybe subnuda'               --a--aaaaaaaaaaaaa-gagaaag-----a-aagaaaaaaaaaaaaagaaa-aggaaa--aaaag---a
'Conocybe coprophila'            --a--aagag--aa--a------aaaaaaaaaagaagaag--aaaa-gaa--aa-aaaaga----------a
'Conocybe rickenii'              aaaaaagagagaaaaaaaa--gaaaaaaagaaaaaagaaagaaaaa--ga--a--aaaaaaagagaaaa-aa
'Conocybe filaris'               aaa--g-aaaaaaag-a-a---aaaaaaaaaaagag-aaa-gaaaaaaaagaaa-aggaaa--agaaaa-aa
'Conocybe lactea 2'              ag---aaaaa--ga----------aaaaaa--a-ag-aaa-gaaaagaaa-aaa-aggaaa--a-g-----a
'Conocybe lactea 1'              ag---aaaaa--ga----------aaaaaa--a-ag-aaa-gaaaagaaa-aaa-aggaaa--a-g-----a
'Conocybe lactea 4'              ag---aaaaa--ga----------aaaaaa--a-aagaaa-gaaaagaaa-aaa-aggaaa--a-g-----a
```

Appendix 5. Chapter 4 *Conocybe* ITS, cont.

```
'Conocybe crispa 5'                ??????????????a??????????????????????????????????a??a??aggaaa--a-g-----a
'Conocybe lactea 5'                ag---aaaaa--ga----------aaaaaa--a-aagaaa-gaaaagaaa-aaa-aggaaa--a-g-----a
'Conocybe crispa 2'                ag---aaaaa--ga----------aaaaaa--a-aagaaa-gaaaagaaa-aaa-aggaaa--a-g-----a
'Conocybe crispa 4'                ag---aaaaa--ga----------aaaaaa--a-aagaaa-gaaaagaaa-aaa-aggaaa--a-g-----a
'Conocybe crispa 3'                ag---aaaaa--ga----------aaaaaa--a-aagaaa-gaaaagaaa-aaa-aggaaa--a-g-----a
'Lactea-crispa intermediate'       ag---aaaaa--ga----------aaaaaa--a-aagaaa-gaaaagaaa-aaa-aggaaa--a-g-----a
'Conocybe crispa 1'                ag---aaaaa--ga----------aaaaaa--a-ag-aaa-gaaaagaaa-aaa-aggaaa--a-g-----a
'Conocybe lactea UK clone 2'       ag---aaaaaaaaa--a-a----aaaaaaa--a-aagaaa-gaaaagaaa-aaa-aggaaa--a-g-----a
'Conocybe lactea 3'                ag---aaaaa--ga----------aaaaaa--a-aagaaa-gaaaagaaa-aaa-aggaaa--a-g-----a
'Conocybe lactea Corvallis'        ag---aaaaa--ga----------aaaaaa--a-aagaaa-gaaaagaaa-aaa-aggaaa--a-g-----a
'Conocybe lactea Eugene'           ??????????????a???????????????????a?aagaaa-gaaaagaaa-aaa-aggaaa--a-g-----a
'Lactea-Gastrocybe intermediate'   aaaga-aaaaaaaa-ga-a----aaaaaaa--a-aagaaa-gaaaagaaa-aaa-aggaaa--a-g-----a
'Conocybe lactea UK clone 5'       aaaga-aaaaaaaa-ga-a----aaaaaaa--a-aagaaa-gaaaagaaa-aaa-aggaaa--a-g-----a
'Conocybe huijsmannii conica'      aaa-g-aaaa--ga----------aaaaaa--a-aagaaa-gaaaagaaa-aaa-aggaaa--a-g-----a
'Conocybe crispa UK'               ????????????????????????????????????????????????????????????????????a
'Intermediate 2'                   aaaga-aaaaaaaa-ga-a----aaaaaaa--a-aagaaa-gaaaagaaa-aaa-aggaaa--a-g-----a
'Conocybe subcrispa'               aaaga-aaaaaaaaaaaga----aaaaaaa--a-aagaaa-gaaaagaaa-aaa-aggaaa--a-g-----a
'Gastrocybe lateritia 4'           aaa-g-aaaaaaaa--g-------aaaaaa--a-aagaaaaaaaaaaaaaagaaa-aggaaa--a-g-----a
'Gastrocybe lateritia 3'           aaa-g-aaaaaaaa--g-------aaaaaa--a-aagaaaaaaaaaaaaaagaaa-aggaaa--a-g-----a
'Gastrocybe lateritia 5'           aaa-g-aaaaaaaa--g-------aaaaaa--a-aagaaaaaaaaaaaaaagaaa-aggaaa--a-g-----a
'Intermediate 1'                   aaa-g-aaaaaaaa--g-------aaaaaa--a-aagaaaaaaaaaaaaaagaaa-aggaaa--a-g-----a
'Gastrocybe lateritia 1'           aaa-g-aaaaaaaa--g-------aaaaaa--a-aagaaaaaaaaaaaaaagaaa-aggaaa--a-g-----a
'Gastrocybe lateritia 2'           aaa-g-aaaaaaaa--g-------aaaaaa--a-aagaaaaaaaaaaaaaagaaa-aggaaa--a-g-----a


'Weraroa cucullata'                aagaaaaaag-aagga-g-ggaaaaaaaga--ggaggaaagaag----aaaaaaaagaagaaagag--ag---
'Cyttarophyllum besseyi'           aagaaaagaagaaggaaaaagaaaaaga-g--ggaggaaaaaag----aaaaagaagaaaaaaaaaaaaag--
'Agrocybe semiorbicularis'         aagaaaagaagaaggaaaaagaaaaaga-g--ggaggaaaaaag----aaaaagaagaaaaaaaaaaaag---
'Agrocybe praecox'                 aaaaaaa-gagaag-gaagggaaaaaga-g--ggaggaaaaaag----aaaaagaagaaaaaagaaaaag---
'Galeropsis desertorum'            ag-aaagaaag-g-aaaaagaaaagg-aaag-agaggaaaaaaaaagaaa-g--aagaaaaaagaaagaaaaa
'Bolbitius variicolor'             gaagaaaaaagaaagaaaggaaaaag-aga--a-gggaaaaaaaaaaaaggaaaaagaagaaagaaagaaaga
'Bolbitius vitellinus'             gaaagaaaaagaaagaaaggaaaaag-aga--a-gggaaaaaaaaaaaaggaaaaagaagaaagaaagaaaga
'Bolbitius lacteus'                gaaagaaaaagaaagaaaggaaaaag-aga--a-gggaaaaaaaaaaaaggaaaaagaagaaagaaagaaaga
'Gastrocybe deceptiva TYPE'        gaaagaaaaagaaagaaaagaaaaag-aga--a-gggggaaaaaaaaaaagaaaaaggagaaagaaagaaaga
'yellow Gastrocybe'                gaaagaaaaagaaagaaaggaaaaag-aga--a-gggaaaaaaaaaaaaagaaaaagaagaaagaaagaaaga
'Conocybe subnuda'                 aaaaaaaaaaggaagaaaggaaaaag-g----ggaggaaaagaaaaaag--aaaaagag-aaagaaagaaaaa
'Conocybe coprophila'              aaaaaaaaaagaaagaaaagaaag-g-aaaaaagaggaaaaaaaaaagaagaaaaagaaag---aaagaaaaa
```

Appendix 5. Chapter 4 *Conocybe* ITS, cont.

```
'Conocybe rickenii'              aaaaaaaaaag-g-gaaaagaag--g-aaaaggaaggaaaaaaag---aagag-aagag-aaaaaaaggaaga
'Conocybe filaris'               aaaaaaaaaaa-g-gaaaagaag--g-aaaaaagaggaagaaaaag-aaagaa-gagaaaaag-aaagaaaaa
'Conocybe lactea 2'              aaaaaaaaaag-g-gaaaag-g---g-aaaaaggaggaaaaaag----aagaaaaagag-aaagaag-aaa--
'Conocybe lactea 1'              aaaaaaaaaag-g-gaaaag-g---g-aaaaaggaggaaaaaag----aagaaaaagag-aaagaag-aaa--
'Conocybe lactea 4'              aaaaaaaaaag-g-gaaaag-g---g-aaaaaggaggaaaaaag----aagaaaaagaagaaagaaagaaa--
'Conocybe crispa 5'              aaaaaaaaaag-g-gaaaag-g---g-aaaaaggaggaaaaaag----aagaaaaagag-aaagaag-aaa--
'Conocybe lactea 5'              aaaaaaaaaag-g-gaaaag-g---g-aaaaaggaggaaaaaag----aagaaaaagag-aaa-gag-aaa-g
'Conocybe crispa 2'              aaaaaaaaaag-g-gaaaag-g---g-aaaaaggaggaaaaaag----aagaaaaagag-aaagaag-aaa--
'Conocybe crispa 4'              aaaaaaaaaag-g-gaaaag-g---g-aaaaaggaggaaaaaag----aagaaaaagag-aaagaag-aaa--
'Conocybe crispa 3'              aaaaaaaaaag-g-gaaaag-g---g-aaaaaggaggaaaaaag----aagaaaaagag-aaagaag-aaa--
'Lactea-crispa intermediate'     aaaaaaaaaag-g-gaaaag-g---g-aaaaaggaggaaaaaag----aagaaaaagag-aaagaag-aaa--
'Conocybe crispa 1'              aaaaaaaaaag-g-gaaaag-g---g-aaaaaggaggaaaaaag----aagaaaaagag-aaagaag-aaa--
'Conocybe lactea UK clone 2'     aaaaaaaaaag-g-gaaaag-g---g-aaaaaggaggaaaaaag----aagaaaaagaaaaaagaag-aaa--
'Conocybe lactea 3'              aaaagaaaaag-g-gaaaagaag--g-aaaaaggaaaaaaaaag----aagaaaaggag-aaagaag-aaa--
'Conocybe lactea Corvallis'      aaaaaaaaaag-g-gaaaag-g---g-aaaaaggaggaaaaaag----aagaaaaagag-aaagaag-aaa--
'Conocybe lactea Eugene'         aaaaaaaaaag-g-gaaaag-g---g-aaaaaggaggaaaaaag----aagaaaaagag-aaagaag-aaa--
'Lactea-Gastrocybe intermediate' aaaa-gaaaag-g-gagaag-g---g-aaaaaggaggaaaaag-----aagaaaaagaagaaa-gaagaaaaa
'Conocybe lactea UK clone 5'     aaaa-gaaaag-g-gagaag-g---g-aaaaaggaggaaaaag-----aagaaaaaaaagaaagaaagaaaaa
'Conocybe huijsmannii conica'    aaaaaaaaaag-g-gaaaag-g---g-aaaaaggaggaaaaaag----aagaaaaagaagg---aag-aaa--
'Conocybe crispa UK'             aaaaaaaaaag-g-gaaaagga---g-aaaaggaaggaaaaaaag---aagag-aagaagaaagaaaggaaga
'Intermediate 2'                 aaaa-gaaaag-g-gagaag-g---g-aaaagggaggaaaaag-----aagaaaaagaagaaa-gaagaaaaa
'Conocybe subcrispa'             aaaa-gaaaag-g-gagaag-g---g-aaaagggaggaaaaag-----aagaaaaagaagag--aaagaaaaa
'Gastrocybe lateritia 4'         aaaa-gaaaag-g-gaaaag-g---g-aga--ggaggaaaaag-----aagaaaaaaaagaaagaaagaaaaa
'Gastrocybe lateritia 3'         aaaa-gaaaag-g-gaaaag-g---g-aga--ggaggaaaaag-----aagaaaaaaaagaaagaaagaaaaa
'Gastrocybe lateritia 5'         aaaa-gaaaag-g-gaaaag-g---g-aga--ggaggaaaaag-----aagaaaaaaaagaaagaaagaaaaa
'Intermediate 1'                 aaaa-gaaaag-g-gaaaag-g---g-aga--ggaggaaaaag-----aagaaaaagaagaaa-gag-aaaaa
'Gastrocybe lateritia 1'         aaaa-gaaaag-g-gaaaag-g---g-aga--ggaggaaaaag-----aagaaaaagaagaaa-gag-aaaaa
'Gastrocybe lateritia 2'         aaaa-gaaaag-g-gaaaag-g---g-aga--ggaggaaaaag-----aagaaaaaaaagaaa-gaaaaaaaa


'Weraroa cucullata'              -aaaagaagaagaaaaag-ag-aagaaaaaaaaaaa-g-aagagaaaaaaaaaaaaag--g-aaa
'Cyttarophyllum besseyi'         -aaaagagaaaa???????ag-aagaagaaagagaagaaaagagagaaaaaaagaaaaa-g-aaa
'Agrocybe semiorbicularis'       -aaaagaagaaaaaagag-ag-aagaagaaagagaagaaaagagagaaaaaga-aaaaa-g-aaa
'Agrocybe praecox'               -aaaagaagaaaaag-ag-ag-aagaagaaaagaaaaaagaagaaaaaaa-g-aaaaa-g-aaa
'Galeropsis desertorum'          a-g---aa-gagga--aaaag-ag--aaaaaaaaaaaagg-aaaagaaaag---aaaaaaagaaa
'Bolbitius variicolor'           aaaaagaggag-ga--aagag-aagag--aaaaaaaaagaaaaaaaaaag----aaaaaaagaaa
'Bolbitius vitellinus'           aaaaagaggag-ga--aagag-aagag--aaaaaaaaagaaaaaaaaaag----aaaaaaagaaa
```

Appendix 5. Chapter 4 *Conocybe* ITS, cont.

```
'Bolbitius lacteus'              aaaaagaggag-ga--aagag-aagag--aaaaaaaaagaaaaaaaaaag----aaaaaaagaaa
'Gastrocybe deceptiva TYPE'      aaaaaggggag-ga--aagaaggagag--aaaaaaaaagaaaaaaaaaag----aaaaaaagggg
'yellow Gastrocybe'              aaaaagaggag-ga--aaaaaaga-gg--aaaaaaaaagaaaaagaagaaa---aaaaaaagaaa
'Conocybe subnuda'               agaaagaagaaaaaaagaaaaagagaaaaag---aaaaaaaaaagga-g-----aaaaaaagaaa
'Conocybe coprophila'            a-g---aagaaaaaaaag-aaagaaaa-gaaaagaaaagaaaaaaaaaaaga-agaaaaaaaaa
'Conocybe rickenii'              aaaaagaagaaaaaagag-aaagagaa-gg-----g-aaaaaaaa-gaag----aaaaggagaaa
'Conocybe filaris'               aaag--aagaag-g---agaaagaaaa-gaaaaaga-aaaaagaagaaaaaaaagaaagaagaaa
'Conocybe lactea 2'              gaaaagaagaaaaaagag-aaggagaa-gaaag--g-aaaaaaaagaaaag---aaaaaaagaaa
'Conocybe lactea 1'              gaaaagaagaaaaaagag-aaggagaa-gaaag--g-aaaaagaagaaaag---aaaaaaagaaa
'Conocybe lactea 4'              gaaaagaagaaaaaagag-aaggagaa-gaaag--g-aaaaagaagaaaag---aaaaaaagaaa
'Conocybe crispa 5'              gaaaagaagaaaaaagag-aaggagaa-gaaag--g-aaaaagaagaaaag---aaaaaaagaaa
'Conocybe lactea 5'              aaaaagaagaaaaaagag-aaggagaa-gaaag--g-aaaaagaagaaaag---aaaaaaagaaa
'Conocybe crispa 2'              gaaaagaagaaaaaagag-aaggagaa-gaaag--g-aaaaagaagaaaag---aaaaaaagaaa
'Conocybe crispa 4'              gaaaagaagaaaaaagag-aaggagaa-gaaag--g-aaaaagaagaaaag---aaaaaaagaaa
'Conocybe crispa 3'              gaaaagaagaaaaaagag-aaggagaa-gaaag--g-aaaaagaagaaaag---aaaaaaagaaa
'Lactea-crispa intermediate'     gaaaagaagaaaaaagag-aaggagaa-gaaag--g-aaaaagaagaaaag---aaaaaaagaaa
'Conocybe crispa 1'              gaaaagaagaaaaaagag-aaggagaa-gaaag--g-aaaaagaagaaaag---aagaaaagaaa
'Conocybe lactea UK clone 2'     gaaaagaagaaaaaagag-ag-gagaa-gaaaag-g-aaaaagaagaaaag---aaaaaaagaaa
'Conocybe lactea 3'              gaaaagaagaaaaaagag-aaggagaa-gaaag--g-aaaaagaagaaaag---aaaaaaagaaa
'Conocybe lactea Corvallis'      gaaaagaagaaaaaagag-aaggagaa-gaaag--g-aaaaagaagaaaag---aaaaaaagaaa
'Conocybe lactea Eugene'         gaaaagaagaaaaaagag-aaggagaa-gaaag--g-aaaaagaagaaaag---aaaaaaagaaa
'Lactea-Gastrocybe intermediate' aaaaagaagaaaaaagag-ag-gagaa-gag----g-aaaaagaagaaaag---aaaaaaagaaa
'Conocybe lactea UK clone 5'     aaaaagaagaaaaaagag-gg-gaga??????????????????????????????????????
'Conocybe huijsmannii conica'    gaaaagaagaaaaaagag-aaggagaa-gaaag--g-aaaaagaagaaaag---aaaaaaagaaa
'Conocybe crispa UK'             aaaaaaaagaaaaaagag-aaagagaa-gg-----g-aaaaagaa-gaaa----aaaaggagaaa
'Intermediate 2'                 aaaaagaagaaaaaagag-ag-gagaa-gg-----g-aaaaagaagaaaag---aaaaaaagaaa
'Conocybe subcrispa'             aaaaagaagaaaaaagag-ag-gagaa-gg-----g-aaaaagaagaaaag---aaaaaaagaaa
'Gastrocybe lateritia 4'         aaaag-aagaaaaaagag-aaagagaa-gag----g-aaaaagaaga-g-----aaaaaaagaaa
'Gastrocybe lateritia 3'         aaaag-aagaaaaaagag-aaagagaa-gag----g-aaaaagaaga-g-----aaaaaaagaaa
'Gastrocybe lateritia 5'         aaaag-aagaaaaaagag-aaagagaa-gag----g-aaaaagaaga-g-----aaaaaaagaaa
'Intermediate 1'                 aaaag-aagaaaaaagag-aaagagaa-gag----g-aaaaagaaga-g-----aaaaaaagaaa
'Gastrocybe lateritia 1'         aaaag-aagaaaaaagag-aaagagaa-gag----g-aaaaagaaga-g-----aaaaaaagaaa
'Gastrocybe lateritia 2'         aaaag-aagaaaaaagag-aaagagaa-gag----g-aaaaagaaga-g-----aaaaaaagaaa
```

Appendix 6. Alignment of the partial 28S region of the nuclear ribosomal RNA operon in *Conocybe* and related genera (Chapter 4).
Total aligned length = 537 positions. Gap coding is shown at the end of the alignment in `lower case`. Gaps were coded as follows: "`g`" = "gap present"; "`a`" = "gap absent"; "?" = "missing data".

```
'Weraroa cucullata'            CCCCTAGTAACT-GCG-AGTG-AAGCGGG-AAAAGCTCAAATTTAAAATCTGGCAGCCTTCGGTTGTCCGAGTTGT
'Galeropsis desertorum'        CCCCTAGTAACT-GCG-AGTG-AAGCGGG-AAAAGCTCAAATTTAAAATCTGGCGGTCCTTGGCCGTCCGAGTTGT
'Agrocybe seimorbicularis'     CCCCTAGTAACT-GCG-AGTG-AAGCGGG-AAAAGCTCAAATTTAAAATCTGGCGGTCTTTGGCCGTCCGAGTTGT
'Bolbitius vitellinus'         CCCCTAGTAACT-GCG-AGTG-AAGCGGG-AAAAGCTCAAATTTAAAATCTGGCAGTCTTTGATTGTCCGAGTTGT
'Yellow Gastrocybe'            ??????????????????G-AGTGGAGGCGGTGAAAAGCTCAAATTTAAAATCTGGCAGTCTTTGATTGTCCGAGTTGT
'Bolbitius lacteus'            ????????????????????TG-AAGCGGG-AAAAGCTCAAATTTAAAATCTGGCAGTCTTTGATTGTCCGAGTTGT
'Bolbitius variicolor'         CCCCTAGTAACT-GCG-TTNG-AAGCCCNTAAAAGCTCCAATTTAAAATCTGGCAGTCTTTGATTGTCCGAGTTGT
'Gastrocybe lateritia TYPE'    CCCCTAGTAACT-GCG-AGTG-AAGCGGG-AAAAGCTCAAATTTAAAATCTGGCAGTCTTTGGCTGTCCGAGTTGT
'Intermediate 3'               CCCCTAGTAACT-GCG-AGTG-AAGCGGG-AAAAGCTCAAATTTAAAATCTGGCAGTCTTTGATTGTCCGAGTTGT
'Intermediate 1'               CCCCTAGTAACT-GCG-AGTG-AAGCGGG-AAAAGCTCAAATTTAAAATCTGGCAGTCTTTGGCTGTCCGAGTTGT
'Intermediate 2'               CCCCTAGTAACT-GCG-AGTG-AAGCGGG-AAAAGCTCAAATTTAAAATCTGGCAGTCTTTGATTGTCCGAGTTGT
'Gastrocybe lateritia 5'       CCCCTAGTAACT-GCG-AGTG-AAGCGGG-AAAAGCTCAAATTTAAAATCTGGCAGTCTTTGGCTGTCCGAGTTGT
'Gastrocybe lateritia 3'       CCCCTAGTAACT-GCG-AGTG-AAGCGGG-AAAAGCTCAAATTTAAAATCTGGCAGTCTTTGGCTGTCCGAGTTGT
'Gastrocybe lateritia 2'       CCCCTAGTAACT-GCG-AGTG-AAGCGGG-AAAAGCTCAAATTTAAAATCTGGCAGTCTTTGGCTGTCCGAGTTGT
'Gastrocybe lateritia 4'       CCCCTAGTAACT-GCG-AGTG-AAGCGGG-AAAAGCTCAAATTTAAAATCTGGCAGTCTTTGGCTGTCCGAGTTGT
'Bolbitius tener'              CCCCTAGTAACT-GCGAAGTG-AAGCGGA-AAAAGCTCAAATTTAAAATCTGGCAGTCTTTGCCTGTCCGAGTTGT
'Conocybe lactea UK'           CCCCTAGTAACT-GCG-AGTG-AAGCGGG-AAAAGCTCAAATTTAAAATCTGGCAGTCTTTGATTGTCCGAGTTGT
'Conocybe rickenii'            CCCCTAGTAACT-GCG-AGTG-AAGCGGG-AAAAGCTCAAATTTAAAATCTGGCGGTCTTTGGCCGTCCGAGTTGT
'Conocybe subnuda'             CCCCTAGTTTCT-GCG-AGTG-AAGCGGG-AAAAGCTCAAATTTAAAATCTGGCGGTCTTTGGCTGTCCGAGTTGT
'Conocybe lactea 5'            CCCCTAGTAACT-GCG-AGTG-AAGCGGG-AAAAGCTCAAATTTAAAATCTGGCAGTCTTTGATTGTCCGAGTTGT
'Conocybe lactea 3'            CCCCTAGTAACT-GCG-AGTG-AAGCGGG-AAAAGCTCAAATTTAAAATCTGGCAGTCTTTGATTGTCCGAGTTGT
'Conocybe lactea 2'            CCCCTAGTAACT-GCG-AGTG-AAGCGGG-AAAAGCTCAAATTTAAAATCTGGCAGTCTTTGATTGTCCGAGTTGT
'Conocybe lactea Cheyenne'     ?GCGTAGTAATAAGCG-AGTG-AAGCGGG-AAAAGCTCAAATTTAAAATCTGGCAGTCTTTGATTGTCCGAGTTGT
'Conocybe lactea Corvallis'    CCCCTAGTAACT-GCG-AGTG-AAGCGGG-AAAAGCTCAAATTTAAAATCTGGCAGTCTTTGATTGTCCGAGTTGT
'Conocybe lactea Eugene'       ????????????????????????????????????????????????????????????????????????
'Conocybe lactea 1'            CCCCTAGTAACT-GCG-AGTG-AAGCGGG-AAAAGCTCAAATTTAAAATCTGGCAGTCTTTGATTGTCCGAGTTGT
'Lactea-crispa intermediate'   CCCCTAGTAACT-GCG-AGTG-AAGCGGG-AAAAGCTCAAATTTAAAATCTGGCAGTCTTTGATTGTCCGAGTTGT
'Conocybe huijsmanii conica'   ????????????????????????????????????????????????????CTTTGATAGTCCGAGTTGT
'Conocybe crispa UK'           CCCCTAGTAACT-GCG-AGTG-AAGCGGG-AAAAGCTCAAATTTAAAATCTGGCGGTCTTTGGCCGTCCGAGTTGT
'Conocybe crispa 1'            CCCCTAGTAACT-GCG-AGTG-AAGCGGG-AAAAGCTCAAATTTAAAATCTGGCAGTCTTTGATTGTCCGAGTTGT
'Conocybe crispa 2'            CCCCTAGTAACT-GCG-AGTG-AAGCGGG-AAAAGCTCAAATTTAAAATCTGGCAGTCTTTGATTGTCCGAGTTGT
'Conocybe crispa 3'            CCCCTAGTAACT-CGG-AGTG-AAGCGGG-AAAAGCTCAAATTTAAAATCTGGCAGTCTTTGATTGTCCGAGTTGT
'Conocybe crispa 4'            CCCCTAGTAACT-GCG-AGTG-AAGCGGG-AAAAGCTCAAATTTAAAATCTGGCAGTCTTTGATTGTCCGAGTTGT
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Appendix 6. Chapter 4 *Conocybe* 28S, cont.

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'Conocybe crispa 5'            CCCCTAGTAACT-GCG-AGTG-AAGCGGG-AAAAGCTCAAATTTAAAATCTGGCAGTCTTTGATTGTCCGAGTTGT
'Conocybe subcrispa'           CCCCTAGTAACT-GCG-AGTG-AAGCGGG-AAAAGCTCAAATTTAAAATCTGGCAGTCTTTGATTGTCCGAGTTGT
'Conocybe filaris'             CCCCTAGTAACT-GCG-AGTG-AAGTGGG-AAAAGCTCAAATTTAAAATCTGACTGCCTTTGGTTGTCCGAGTTGT
'Cyttarophyllum besseyii'      CCCCTAGTAACT-GCG-AGTG-AAGCGGG-AAAAGCTCAAATTTAAAATCTGGCGGTCTTTGGCCGTCCGAGTTGT
'Agrocybe praecox AF041545'    CCCCTAGTAACT-GCG-AGTG-AAGCGGG-AAAAGCTCAAATTTAAAATCTGGCGGTCTCTGGCCGTCCGAGTTGT


'Weraroa cucullata'                AATCTAGAGAAGTGTTATCCGCGCTGGACCGTGTACAAGTCTCCTGGAATGGAGCGTCATAGAGGGTGAGAATCC
'Galeropsis desertorum'            AATCTAGAGAAGTGTTACCCGCGCTGGACCATGTACAAGTCTCCTGGAATGGAGCGTCATAGAGGGTGAGAATCC
'Agrocybe seimorbicularis'         AATCTAGAGAAGTGTTATCCGCGCTGGACCGTGTACAAGTCTCCTGGAATGGAGCGTCATAGAGGGTGAGAATCC
'Bolbitius vitellinus'             AAACTAGAGAAGTGTTATCCGCGCTGGACCGTGTAAAAGTCTCCTGGAATGGAGCATCATAGAGGGTGAGAATCC
'Yellow Gastrocybe'                AAACTAGAGAAGTGTTATCCGCGCTGGACCGTGTAAAAGTCTCCTGGAATGGAGCATCATAGAGGGTGAGAATCC
'Bolbitius lacteus'                AAACTAGAGAAGTGTTATCCGCGCTGGACCGTGTAAAAGTCTCCTGGAATGGAGCATCATAGAGGGTGAGAATCC
'Bolbitius variicolor'             AAACTAGAGAAGTGTTATCCGTGCTGGACCGTGTAAAAGTCTCCTGGAATGGAGCATCATAGAGGGTGAGAATCC
'Gastrocybe lateritia TYPE'        AATCTAGAGAAGTGTTATCCGCGTTGGACCGTGTACAAGTCTCCTGGAATGGAGCATCATAGAGGGTGAGAATCC
'Intermediate 3'                   AATCTAGAGAAGTGTTATCCGCGTTGGACCGTGTACAAGTCTCCTGGAATGGGGCATCATAGAGGGTGAGAATCC
'Intermediate 1'                   AATCTAGAGAAGTGTTATCCGCGTTGGACCGTGTACAAGTCTCCTGGAATGGAGCATCATAGAGGGTGAGAATCC
'Intermediate 2'                   AATCTAGAGAAGTGTTATCCGCGTTGGACCGTGTACAAGTCTCCTGGAATGGGGCATCATAGAGGGTGAGAATCC
'Gastrocybe lateritia 5'           AATCTAGAGAAGTGTTATCCGCGTTGGACCGTGTACAAGTCTCCTGGAATGGAGCATCATAGAGGGTGAGAATCC
'Gastrocybe lateritia 3'           AATCTAGAGAAGTGTTATCCGCGTTGGACCGTGTACAAGTCTCCTGGAATGGAGCATCATAGAGGGTGAGAATCC
'Gastrocybe lateritia 2'           AATCTAGAGAAGTGTTATCCGCGTTGGACCGTGTACAAGTCTCCTGGAATGGAGCATCATAGAGGGTGAGAATCC
'Gastrocybe lateritia 4'           AATCTAGAGAAGTGTTATCCGCGTTGGACCGTGTACAAGTCTCCTGGAATGGAGCATCATAGAGGGTGAGAATCC
'Bolbitius tener'                  AATCTAGA-AAGTGTTATCCGCGTTG-ACCGTGTACAAGTCTCCTGGAATG?AGCATCATAGAGGGTGAGAATCC
'Conocybe lactea UK'               AATCTAGAGAAGTGTTATCCGCGTTGGACCGTGTACAAGTCTCCTGGAATGGAGCATCATAGAGGGTGAGAATCC
'Conocybe rickenii'                AATCTAGAGAAGTGTTATCCGCGTTGGACCGTGTACAAGTCTCCTGGAATGGAGCATCATAGAGGGTGAGAATCC
'Conocybe subnuda'                 AATCTAGAGAAGTGTTATCCGTGTCGGACCGTGTAAAAGTCTCCTGGAATGGAGCATCATAGAGGGTGAGAATCC
'Conocybe lactea 5'                AATCTAGAGAAGTGTTATCCGCGTTGGACCGTGTACAAGTCTCCTGGAATGGAGCATCATAGAGGGTGAGAATCC
'Conocybe lactea 3'                AATCTAGAGAAGTGTTATCCGCGTTGGACCGTGTACAAGTCTCCTGGAATGGAGCATCATAGAGGGTGAGAATCC
'Conocybe lactea 2'                AATCTAGAGAAGTGTTATCCGCGTTGGACCGTGTACAAGTCTCCTGGAATGGAGCATCATAGAGGGTGAGAATCC
'Conocybe lactea Cheyenne'         AATCTAGAGAAGTGTTATCCGCGTTGGACCGTGTACAAGTCTCCTGGAATGGAGCATCATAGAGGGTGAGAATCC
'Conocybe lactea Corvallis'        AATCTAGAGAAGTGTTATCCGCGTTGGACGGTGTACAAGTCTCCTGGAATGGAGCATCATAGAGGGTGAGAATCC
'Conocybe lactea Eugene'           ??????????????????????????????????TGCCAAGTCTCCTGGAATGGAGCRTCATACAGGGTGAGAATCC
'Conocybe lactea 1'                AATCTAGAGAAGTGTTATCCGCGTTGGACCGTGTACAAGTCTCCTGGAATGGAGCATCATAGAGGGTGAGAATCC
'Lactea-crispa intermediate'       AATCTAGAGAAGTGTTATCCGCGTTGGACCGTGTACAAGTCTCCTGGAATGGAGCATCATAGAGGGTGAGAATCC
'Conocybe huijsmanii conica'       AATCTAAAGAAGTGTTATCCGCGTTGGACCGTGT-CAAGTCTCCTGGAATGGAGCATCATAGAGGGTGAGAATCC
'Conocybe crispa UK'               AATCTAGAGAAGTGTTATCCGCGTTGGACCGTGTACAAGTCTCCTGGAATGGAGCATCATAGAGGGTGAGAATCC
'Conocybe crispa 1'                AATCTAGAGAAGTGTTATCCGCGTTGGACCGTGTACAAGTCTCCTGGAATGGAGCATCATAGAGGGTGAGAATCC
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Appendix 6. Chapter 4 *Conocybe* 28S, cont.

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'Conocybe crispa 2'            AATCTAGAGAAGTGTTATCCGCGTTGGACCGTGTACAAGTCTCCTGGAATGGAGCATCATAGAGGGTGAGAATCC
'Conocybe crispa 3'            AATCTAGAGAAGTGTTATCCGCGTTGGACCGTGTACAAGTCTCCTGGAATGGAGCATCATAGAGGGTGAGAATCC
'Conocybe crispa 4'            AATCTAGAGAAGTGTTATCCGCGTTGGACCGTGTACAAGTCTCCTGGAATGGAGCATCATAGAGGGTGAGAATCC
'Conocybe crispa 5'            AATCTAGAGAAGTGTTATCCGCGTTGGACCGTGTACAAGTCTCCTGGAATGGAGCATCATAGAGGGTGAGAATCC
'Conocybe subcrispa'           AATCTAGAGAAGTGTTATCCGCGTTGGACCGTGTACAAGTCTCCTGGAATGGAGCATCATAGAGGGTGAGAATCC
'Conocybe filaris'             AACCTAGAGAAGTGTTATCCG?GTTGGACCATGTTTAAGTCTCCTGGAATGGAGCGTCATAGAGGGTGAGAATCC
'Cyttarophyllum besseyii'      AATCTAGAGAAGTGTTATCCGCGCTGGACCGTGTACAAGTCTCCTGGAATGGAGCGTCATAGAGGGTGAGAATCC
'Agrocybe praecox AF041545'    AATCTAGAGAAGTGTTATCCGCGCTGGACCGTGTACAAGTCTCCTGGAATGGAGCGTCATAGAGGGTGAGAATCC


'Weraroa cucullata'            CGTCTTTGACACGGACTGCCAGGGCTTTGTGATGCGCTCTCAAAGAGTCGAGTTGTTTGGGAATGCAGCTCAAA
'Galeropsis desertorum'        CGTCTCTGACATGGACTACCAGGGCTTTGTGGTATGCTCTCAAAGAGTCGAGTTGTTTGGGAATGCAGCTCAAA
'Agrocybe seimorbicularis'     CGTCTTTGACACGGACTGCCAGGGCTTTGTGATGCGCTCTCAAAGAGTCGAGTTGTTTGGGAATGCAGCTCCAA
'Bolbitius vitellinus'         CGTCTTTGACATGGACTACCAGTGCTTTGTGATGCGCTCTCAAAGAGTCGAGTTGTTTGGGAATGCAGCTCAAA
'Yellow Gastrocybe'            CGTCTTTGACATGGACTACCAGTGCTTTGTGATGCGCTCTCAAAGAGTCGAGTTGTTTGGGAATGCAGCTCAAA
'Bolbitius lacteus'            CGTCTTTGACATGGACTACCAGTGCTTTGTGATGCGCTCTCAAAGAGTCGAGTTGTTTGGGAATGCAGCTCAAA
'Bolbitius variicolor'         CGTCTTTGACATGGACTACCAGTGCTTTGTGATGCGCTCTCAAAGAGTCGAGTTGTTTGGGAATGCAGCTCAAA
'Gastrocybe lateritia TYPE'    CGTCTTTGACATGGACTACCAGTGCTTTGTGATGCGCTCTCAAAGAGTCGAGTTGTTTGGGAATGCAGCTCAAA
'Intermediate 3'               CGTCTTTGACACGGACTACCAGTGCTTTGTGATGCGCTCTCAAAGAGTCGAGTTGTTTGGGAATGCAGCTCAAA
'Intermediate 1'               CGTCTTTGACATGGACTACCAGTGCTTTGTGATGCGCTCTCAAAGAGTCGAGTTGTTTGGGAATGCAGCTCAAA
'Intermediate 2'               CGTCTTTGACACGGACTACCAGTGCTTTGTGATGCGCTCTCAAAGAGTCGAGTTGTTTGGGAATGCAGCTCAAA
'Gastrocybe lateritia 5'       CGTCTTTGACATGGACTACCAGTGCTTTGTGATGCGCTCTCAAAGAGTCGAGTTGTTTGGGAATGCAGCTCAAA
'Gastrocybe lateritia 3'       CGTCTTTGACATGGACTACCAGTGCTTTGTGATGCGCTCTCAAAGAGTCGAGTTGTTTGGGAATGCAGCTCAAA
'Gastrocybe lateritia 2'       CGTCTTTGACATGGACTACCAGTGCTTTGTGATGCGCTCTCAAAGAGTCGAGTTGTTTGGGAATGCAGCTCAAA
'Gastrocybe lateritia 4'       CGTCTTTGACATGGACTACCAGTGCTTTGTGATGCGCTCTCAAAGAGTCGAGTTGTTTGGGAATGCAGCTCAAA
'Bolbitius tener'              CGTCTTTGACATGGACTACCAGTGCTTTGTGATGCGCTCTCAAAGAGTCGAGTTGTTTGGGAATGCAGCTCAAA
'Conocybe lactea UK'           CGTCTTTGACATGGACTACCAGTGCTTTGTGATGCGCTCTCAAAGAGTCGAGTTGTTTGGGAATGCAGCTCAAA
'Conocybe rickenii'            CGTCTTTGACACGGACTACCAGTGCTTTGTGATGCGCTCTCAAAGAGTCGAGTTGTTTGGGAATGCAGCTCTAA
'Conocybe subnuda'             CGTCTTTGACATGGACTACCGGTGCTTTGTGATACACTCTCAAAGAGTCGAGTTGTTTGGGAATGCAGCTCAAA
'Conocybe lactea 5'            CGTCTTTGACACGGACTACCAGTGCTTTGTGATGCGCTCTCAAAGAGTCGAGTTGTTTGGGAATGCAGCTCAAA
'Conocybe lactea 3'            CGTCTTTGACACGGACTACCAGTGCTTTGTGATGCGCTCTCAAAGAGTCGAGTTGTTTGGGAATGCAGCTCAAA
'Conocybe lactea 2'            CGTCTTTGACACGGACTACCAGTGCTTTGTGATGCGCTCTCAAAGAGTCGAGTTGTTTGGGAATGCAGCTCAAA
'Conocybe lactea Cheyenne'     CGTCTTTGACACGGACTACCAGTGCTTTGTGATGCGCTCTCAAAGAGTCGAGTTGTTTGGGAATGCAGCTCAAA
'Conocybe lactea Corvallis'    CGTCTTTGACACGGACAACCAGTGCTTTGTGATGCGCTCTCAAAGAGTCGAGTTGTTTGGGAATGCAGCTCAAA
'Conocybe lactea Eugene'       CRTCTTTGACACGGACTACCACTGCTTTGTGATGCGCTCTCAAAGAGTCGAGTTGTTTGGGAATGCAGCTCAMA
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Appendix 6. Chapter 4 *Conocybe* 28S, cont.

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'Conocybe lactea 1'              CGTCTTTGACACGGACTACCAGTGCTTTGTGATGCGCTCTCAAAGAGTCGAGTTGTTTGGGAATGCAGCTCAAA
'Lactea-crispa intermediate'     CGTCTTTGACACGGACTACCAGTGCTTTGTGATGCGCTCTCAAAGAGTCGAGTTGTTTGGGAATGCAGCTCAAA
'Conocybe huijsmanii conica'     CGTCTTGAACCACGGCCACCCCTGCTTTGTGATGCGCTCTCAAAGAGTCGAGTTGTTTGGGAATGCAGCTCAAA
'Conocybe crispa UK'             CGTCTTTGACACGGACTACCAGTGCTTTGTGATGCGCTCTCAAAGAGTCGAGTTGTTTGGGAATGCAGCTCTAA
'Conocybe crispa 1'              CGTCTTTGACACGGACTACCAGTGCTTTGTGATGCGCTCTCAAAGAGTCGAGTTGTTTGGGAATGCAGCTCAAA
'Conocybe crispa 2'              CGTCTTTGACACGGACTACCAGTGCTTTGTGATGCGCTCTCAAAGAGTCGAGTTGTTTGGGAATGCAGCTCAAA
'Conocybe crispa 3'              CGTCTTTGACACGGACTACCAGTGCTTTGTGATGCGCTCTCAAAGAGTCGAGTTGTTTGGGAATGCAGCTCAAA
'Conocybe crispa 4'              CGTCTTTGACACGGACTACCAGTGCTTTGTGATGCGCTCTCAAAGAGTCGAGTTGTTTGGGAATGCAGCTCAAA
'Conocybe crispa 5'              CGTCTTTGACACGGACTACCAGTGCTTTGTGATGCGCTCTCAAAGAGTCGAGTTGTTTGGGAATGCAGCTCAAA
'Conocybe subcrispa'             CGTCTTTGACACGGACTACCAGTGCTTTGTGATGCGCTCTCAAAGAGTCGAGTTGTTTGGGAATGCAGCTCAAA
'Conocybe filaris'               CGTCTTTAACATGGACTACCAATGCATTGTGATGCGCTCTCAAAGAGTCGAGTTGTTTGGGAATGCAGCTCAAA
'Cyttarophyllum besseyii'        CGTCTTTGACACGGACTGCCAGGGCTTTGTGATGCGCTCTCAAAGAGTCGAGTTGTTTGGGAATGCAGCTCCAA
'Agrocybe praecox AF041545'      CGTCTTTGACACGGACTGCCAGGGCTTTGTGATGCGCTCTCAAAGAGTCGAGTTGTTTGGGAATGCAGCTCCAA


'Weraroa cucullata'              ATGGGTGGTAAATTCCATCTAAAGCTAAATATTGGCGAGAGACCGATAGCGAACAAGTACCGTGAGGGAAAGAT
'Galeropsis desertorum'          ATGGGTGGTAAATTCCATCTAAAGCTAAATATTGGCGAGAGACCGATAGCGAACAAGTACCGTAAGGGAAAGAT
'Agrocybe seimorbicularis'       ATGGGTGGTAAATTCCATCTAAAGCTAAATATTGGCGAGAGACCGATAGCGAACAAGTACCGTGAGGGAAAGAT
'Bolbitius vitellinus'           ATGGGTGGTAAATTCCATCTAAAGCTAAATATTGGCGAGAGACCGATAGCGAACAAGTACCGTGAGGGAAAGAT
'Yellow Gastrocybe'              ATGGGTGGTAAATTCCATCTAAAGCTAAATATTGGCGAGAGACCGATAGCGAACAAGTACCGTGAGGGAAAGAT
'Bolbitius lacteus'              ATGGGTGGTAAATTCCATCTAAAGCTAAATATTGGCGAGAGACCGATAGCGAACAAGTACCGTGAGGGAAAGAT
'Bolbitius variicolor'           ATGGGTGGTAAATTCCATCTAAAGCTAAATATTGGCGAGAGACCGATAGCGAACAAGTACCGTGAGGGAAAGAT
'Gastrocybe lateritia TYPE'      ATGGGTGGTAAATTCCATCTAAAGCTAAATATTGGCGAGAGACCGATAGCGAACAAGTACCGTGAGGGAAAGAT
'Intermediate 3'                 ATGGGTGGTAAATTCCATCTAAAGCTAAATATTGGCGAGAGACCGATAGCGAACAAGTACCGTGAGGGAAAGAT
'Intermediate 1'                 ATGGGTGGTAAATTCCATCTAAAGCTAAATATTGGCGAGAGACCGATAGCGAACAAGTACCGTGAGGGAAAGAT
'Intermediate 2'                 ATGGGTGGTAAATTCCATCTAAAGCTAAATATTGGCGAGAGACCGATAGCGAACAAGTACCGTGAGGGAAAGAT
'Gastrocybe lateritia 5'         ATGGGTGGTAAATTCCATCTAAAGCTAAATATTGGCGAGAGACCGATAGCGAACAAGTACCGTGAGGGAAAGAT
'Gastrocybe lateritia 3'         ATGGGTGGTAAATTCCATCTAAAGCTAAATATTGGCGAGAGACCGATAGCGAACAAGTACCGTGAGGGAAAGAT
'Gastrocybe lateritia 2'         ATGGGTGGTAAATTCCATCTAAAGCTAAATATTGGCGAGAGACCGATAGCGAACAAGTACCGTGAGGGAAAGAT
'Gastrocybe lateritia 4'         ATGGGTGGTAAATTCCATCTAAAGCTAAATATTGGCGAGAGACCGATAGCGAACAAGTACCGTGAGGGAAAGAT
'Bolbitius tener'                ATGGGTGGTAAATTCCATCTAAAGCTAAATATTGGCGAGAGACCGATAGCGAACAAGT-CCGTGAGGGAAAGAT
'Conocybe lactea UK'             ATGGGTGGTAAATTCCATCTAAAGCTAAATATTGGCGAGAGACCGATAGCGAACAAGTACCGTGAGGGAAAGAT
'Conocybe rickenii'              ATGGGTGGTAAATTCCATCTAAAGCTAAATATTGGCGAGAGACCGATAGCGAACAAGTACCGTGAGGGAAAGAT
'Conocybe subnuda'               ATGGGTGGTAAATTCCATCTAAAGCTAAATATTGGCGAGAGACCGATAGCGAACAAGTCCCGTGAGGGAAAGAT
'Conocybe lactea 5'              ATGGGTGGTAAATTCCATCTAAAGCTAAATATTGGCGAGAGACCGATAGCGAACAAGTACCGTGAGGGAAAGAT
'Conocybe lactea 3'              ATGGGTGGTAAATTCCATCTAAAGCTAAATATTGGCGAGAGACCGATAGCGAACAAGTACCGTGAGGGAAAGAT
'Conocybe lactea 2'              ATGGGTGGTAAATTCCATCTAAAGCTAAATATTGGCGAGAGACCGATAGCGAACAAGTACCGTGAGGGAAAGAT
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Appendix 6. Chapter 4 *Conocybe* 28S, cont.

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'Conocybe lactea Cheyenne'        ATGGGTGGTAAATTCCATCTAAAGCTAAATATTGGCGAGAGACCGATAGCGAACAAGTACCGTGAGGGAAAGAT
'Conocybe lactea Corvallis'       ATGGGTGGTAAATTCCATCTAAAGCTAAATATTGGCGAGAGACCGATAGCGAACAAGTACCGTGAGGGAAAGAT
'Conocybe lactea Eugene'          ATGGGTGGTRRATKSCATCTAAAGCTAAATATTGGCGAGAGACCGATAGCCAACARGTACCGTGAGGGAAAGAT
'Conocybe lactea 1'               ATGGGTGGTAAATTCCATCTAAAGCTAAATATTGGCGAGAGACCGATAGCGAACAAGTACCGTGAGGGAAAGAT
'Lactea-crispa intermediate'      ATGGGTGGTAAATTCCATCTAAAGCTAAATATTGGCGAGAGACCGATAGCGAACAAGTACCGTGAGGGAAAGAT
'Conocybe huijsmanii conica'      ATGGGTGGTAAATTCCATCTAAAGCTAAATATTGGCGAGAGACCGATTGCGAACAAGTACCGTGAGGGAAAGAT
'Conocybe crispa UK'              ATGGGTGGTAAATTCCATCTAAAGCTAAATATTGGCGAGAGACCGATAGCGAACAAGTACCGTGAGGGAAAGAT
'Conocybe crispa 1'               ATGGGTGGTAAATTCCATCTAAAGCTAAATATTGGCGAGAGACCGATAGCGAACAAGTACCGTGAGGGAAAGAT
'Conocybe crispa 2'               ATGGGTGGTAAATTCCATCTAAAGCTAAATATTGGCGAGAGACCGATAGCGAACAAGTACCGTGAGGGAAAGAT
'Conocybe crispa 3'               ATGGGTGGTAAATTCCATCTAAAGCTAAATATTGGCGAGAGACCGATAGCGAACAAGTACCGTGAGGGAAAGAT
'Conocybe crispa 4'               ATGGGTGGTAAATTCCATCTAAAGCTAAATATTGGCGAGAGACCGATAGCGAACAAGTACCGTGAGGGAAAGAT
'Conocybe crispa 5'               ATGGGTGGTAAATTCCATCTAAAGCTAAATATTGGCGAGAGACCGATAGCGAACAAGTACCGTGAGGGAAAGAT
'Conocybe subcrispa'              ATGGGTGGTAAATTCCATCTAAAGCTAAATATTGGCGAGAGACCGATAGCGAACAAGTACCGTGAGGGAAAGAT
'Conocybe filaris'                ATGGGTGGTAAATTCCATCTAAAGCTAAATATTGGCGAGAGACCGATAGCGAACAAGTACCGTGAGGGAAAGAT
'Cyttarophyllum besseyii'         ATGGGTGGTAAATTCCATCTAAAGCTAAATATTGGCGAGAGACCGATAGCGAACAAGTACCGTGAGGGAAAGAT
'Agrocybe praecox AF041545'       ATGGGTGGTAAATTCCATCTAAAGCTAAATATTGGCGAGAGACCGATAGCGAACAAGTACCGTGAGGGAAAGAT

'Weraroa cucullata'               GAAAAGAACTTTGGAAAGAGAGTTAAACAGTACGTGAAATTGCTGAAAGGGAAACGCTTGAAGTCAGTCGCGTTG
'Galeropsis desertorum'           GAAAAGAACTTTGGAAAGAGAGTTAAACAGTACGTGAAATTGCTGAAAGGGAAACGCTTGAAGTCAGTCGCGTCG
'Agrocybe seimorbicularis'        GAAAAGAACTTTGGAAaGAGAGT-AAACAGTACGTGAAATTGCTGAAaGGGGAACGCTTGAaGTCAGTCGCGTTG
'Bolbitius vitellinus'            GAAAAGAACTTTGGAAAGAGAGTTAAACAGTACGTGAAATTGCTGAAAGGGAAACGCTTGAAGTCAGTCGCGTCG
'Yellow Gastrocybe'               GAAAAGAACTTTGGAAAGAGAGTTAAACAGTACGTGAAATTGCTGAAAGGGAAACGCTTGAAGTCAGTCGCGTCG
'Bolbitius lacteus'               GAAAAGAACTTTGGAAAGAGAGTTAAACAGTACGTGAAATTGCTGAAAGGGAAACGCTTGAAGTCAGTCGCGTCG
'Bolbitius variicolor'            GAAAAGAACTTTGGAAAGAGAGTTAAACAGTACGTGAAATTGCTGAAAGGGAAACGCTTGAAGTCAGTCGCGTCG
'Gastrocybe lateritia TYPE'       GAAAAGAACTTTGGAAAGAGAGTTAAACAGTACGTGAAATTGCTGAAAGGGAAACGCTTGAAGTCAGTCGCGTTG
'Intermediate 3'                  GAAAAGAACTTTGGAAAGAGAGTTAAACAGTACGTGAAATTGCTGAAAGGGAAACGCTTGAAGTCAGTCGCGTTG
'Intermediate 1'                  GAAAAGAACTTTGGAAAGAGAGTTAAACAGTACGTGAAATTGCTGAAAGGGAAACGCTTGAAGTCAGTCGCGTTG
'Intermediate 2'                  GAAAAGAACTTTGGAAAGAGAGTTAAACAGTACGTGAAATTGCTGAAAGGGAAACGCTTGAAGTCAGTCGCGTTG
'Gastrocybe lateritia 5'          GAAAAGAACTTTGGAAAGAGAGTTAAACAGTACGTGAAATTGCTGAAAGGGAAACGCTTGAAGTCAGTCGCGTTG
'Gastrocybe lateritia 3'          GAAAAGAACTTTGGAAAGAGAGTTAAACAGTACGTGAAATTGCTGAAAGGGAAACGCTTGAAGTCAGTCGCGTTG
'Gastrocybe lateritia 2'          GAAAAGAACTTTGGAAAGAGAGTTAAACAGTACGTGAAATTGCTGAAAGGGAAACGCTTGAAGTCAGTCGCGTTG
'Gastrocybe lateritia 4'          GAAAAGAACTTTGGAAAGAGAGTTAAACAGTACGTGAAATTGCTGAAAGGGAAACGCTTGAAGTCAGTCGCGTTG
'Bolbitius tener'                 GAAAAGACCTTTGGAAAGAGAGTTAACCAGTACGTGAAATTGCTGAAAGGGAAACGCTTGAAGTCAGTCGCGTTG
'Conocybe lactea UK'              GAAAAGAACTTTGGAAAGAGAGTTAAACAGTACGTGAAATTGCTGAAAGGGAAACGCTTGAAGTCAGTCGCGTTG
'Conocybe rickenii'               GAAAAGAACTTTGGAAAGAGAGTTAAACAGTACGTGAAATTGCTGAAAGGGAAACGCTTGAAGTCAGTCACGTTG
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Appendix 6. Chapter 4 *Conocybe* 28S, cont.

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'Conocybe subnuda'              GAAAAGAACTTTGGAAAGAGAGTTAAACAGTACGTGAAATTGCTGAAAGGGAAACGCTTGAAGTCAGTCGCGTTG
'Conocybe lactea 5'             GAAAAGAACTTCGGAAAGAGAGTTAAACAGTACGTGAAATTGCTGAAAGGGAAACGCTTGAAGTCAGTCGCGTTG
'Conocybe lactea 3'             GAAAAGAACTTTGGAAAGAGAGTTAAACAGTACGTGAAATTGCTGAAAGGGAAACGCTTGAAGTCAGTCGCGTTG
'Conocybe lactea 2'             GAAAAGAACTTTGGAAAGAGAGTTAAACAGTACGTGAAATTGCTGAAAGGGAAACGCTTGAAGTCAGTCGCGTTG
'Conocybe lactea Cheyenne'      GAAAAGAACTTTGGAAAGAGAGTTAAACAGTACGTGAAATTGCTGAAAGGGAAACGCTTGAAGTCAGTCGCGTTG
'Conocybe lactea Corvallis'     GAAAAGAACTTTGGAAAGAGAGTTAAACAGTACGTGAAATTGCTGAAAGGGAAACGCTTGAAGTCAGTCGCGTTG
'Conocybe lactea Eugene'        GAAAAGAACTTTGGAAAGAGAGTTAAACAGTACGTGAAATTGCTGAAAGGGAAACGCTTGAAGTCAGTCGCGTTG
'Conocybe lactea 1'             GAAAAGAACTTTGGAAAGAGAGTTAAACAGTACGTGAAATTGCTGAAAGGGAAACGCTTGAAGTCAGTCGCGTTG
'Lactea-crispa intermediate'    GAAAAGAACTTTGGAAAGAGAGTTAAACAGTACGTGAAATTGCTGAAAGGGAAACGCTTGAAGTCAGTCGCGTTG
'Conocybe huijsmanii conica'    GAAAAGAACTTTGGAAAGAGAGTTAAACAGTACGTGAAATTGCTGAAAGGGAAACGCTTGAAGTCAGTCGCGTTG
'Conocybe crispa UK'            GAAAAGAACTTTGGAAAGAGAGTTAAACAGTACGTGAAATTGCTGAAAGGGAAACGCTTGAAGTCAGTCACGTTG
'Conocybe crispa 1'             GAAAAGAACTTTGGAAAGAGAGTTAAACAGTACGTGAAATTGCTGAAAGGGAAACGCTTGAAGTCAGTCGCGTTG
'Conocybe crispa 2'             GAAAAGAACTTTGGAAAGAGAGTTAAACAGTACGTGAAATTGCTGAAAGGGAAACGCTTGAAGTCAGTCGCGTTG
'Conocybe crispa 3'             GAAAAGAACTTTGGAAAGAGAGTTAAACAGTACGTGAAATTGCTGAAAGGGAAACGCTTGAAGTCAGTCGCGTTG
'Conocybe crispa 4'             GAAAAGAACTTTGGAAAGAGAGTTAAACAGTACGTGAAATTGCTGAAAGGGAAACGCTTGAAGTCAGTCGCGTTG
'Conocybe crispa 5'             GAAAAGGACTTTGGAAAGAGAGTTAAACAGTACGTGAAATTGCTGAAAGGGAAACGCTTGAAGTCAGTCGCGTTG
'Conocybe subcrispa'            GAAAAGAACTTTGGAAAGAGAGTTAAACAGTACGTGAAATTGCTGAAAGGGAAACGCTTGAAGTCAGTCGCGTTG
'Conocybe filaris'              GAAAAGAACTTTGGAAAGAGAGTTAAACAGTACGTGAAATTGCTGAAAGGGAAACGCTTGAAGTCAGTCGCGTTG
'Cyttarophyllum besseyii'       GAAAAGAACTTTGGAAAGAGAGTTAAACAGTACGTGAAATTGCTGAAAGGGAAACGCTTGAAGTCAGTCGCGTTG
'Agrocybe praecox AF041545'     GAAAAGAACTTTGGAAAGAGAGTTAAACAGTACGTGAAATTGCTGAAAGGGAAACGCTTGAAGTCAGTCGCGTTG

'Weraroa cucullata'             TCTGGGAATCAACCTTGCTTTTGCTGGGCTTACTTTCCAGATAATGGGTCAGCATCAATTTTGATCGTTGGATAA
'Galeropsis desertorum'         GCTGGGGATCAGCTTCGCATTTGCGTGGTGTACTTCCCAGTTGACGGGTCAGCATCAATTTTGACCGCTGGAAAA
'Agrocybe seimorbicularis'      GCCGGGgATCAaCCTTGCTTTtGCTGGgCGTACTTtCCGGTCGACGGGTCAGCATCAATTTTGACCGTTGGATAA
'Bolbitius vitellinus'          GCCAGGAATCAACCTTGCTTTTGCTGGGCGTACTTTCTGGTTGACGGGTCAGCATCAATTTTGACTGCTGGAAAA
'Yellow Gastrocybe'             GCCAGGAATCAACCTTGCTTTTGCTGGGCGTACTTTCTGGTTGACGGGTCAGCATCAATTTTGACTGCTGGAAAA
'Bolbitius lacteus'             GCCAGGAATCAACCTTGCTTTTGCTGGGCGTACTTTCTGGTTGACGGGTCAGCATCAATTTTGACTGCTGGAAAA
'Bolbitius variicolor'          GCCAGGAATCAACCTTGCTTTTGCTGGGCGTACTTTCTGGTTGACGGGTCAGCATCAATTTTGACTGCTGGAAAA
'Gastrocybe lateritia TYPE'     GCTGGAAATCAACCTTGCTTTTGCTGGGCGTACTTTCTAGTCAATGGGTCAGCATCAATTTTGACCATTGGAAAA
'Intermediate 3'                GCTGGAAATCAACCTTGCTTTTGCTGGGCGTACTTTCTAGTCAATGGGTCAGCATCAATTTTGACCATTGGAAAA
'Intermediate 1'                GCTGGAAATCAACCTTGCTTTTGCTGGGCGTACTTTCTAGTCAATGGGTCAGCATCAATTTTGACCATTGGAAAA
'Intermediate 2'                GCTGGAAATCAACCTTGCTTTTGCTGGGCGTACTTTCTAGTCAATGGGTCAGCATCAATTTTGACCATTGGAAAA
'Gastrocybe lateritia 5'        GCTGGAAATCAACCTTGCTTTTGCTGGGCGTACTTTCTAGTCAATGGGTCAGCATCAATTTTGACCATTGGAAAA
'Gastrocybe lateritia 3'        GCTGGAAATCAACCTTGCTTTTGCTGGGCGTACTTTCTAGTCAATGGGTCAGCATCAATTTTGACCATTGGAAAA
'Gastrocybe lateritia 2'        GCTGGAAATCAACCTTGCTTTTGCTGGGCGTACTTTCTAGTCAATGGGTCAGCATCAATTTTGACCATTGGAAAA
'Gastrocybe lateritia 4'        GCTGGAAATCAACCTTGCTTTTGCTGGGCGTACTTTCTAGTCAATGGGTCAGCATCAATTTTGACCATTGGAAAA
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Appendix 6. Chapter 4 *Conocybe* 28S, cont.

```
'Bolbitius tener'              GCTGGAAATCA?CCTTGCTTTTGCTGGGCGTACTTTCTAGTCAATGGGTCAGCATCAATTTTGACCATTGGAAAA
'Conocybe lactea UK'           GCTGGAAATCAACCTTGCTTTTGCTGGGCGTACTTTCTAGTCAATGGGTCAGCATCAATTTTGACCATTGGAAAA
'Conocybe rickenii'            GCTAGAAATCAACCTTGCTTTTGCTGGGCGTACTTTCTGGTCAACGGGTCAGCATCAATTTTGACCGTTGGAAAA
'Conocybe subnuda'             GCCAGAAATCAACCTTGCTTTTGCTGGGCGTACTTTCTGGTTGACGGGTCAGCATCAATTTTGACCGTTGGAAAA
'Conocybe lactea 5'            GCTGGAAATCAACCTTGCTTTTGCTGGGCGTACTTTCTAGTCAATGGGTCAGCATCAATTTTGACCATTGGAAAA
'Conocybe lactea 3'            GCTGGAAATCAACCTTGCTTTTGCTGGGCGTACTTTCTAGTCAATGGGTCAGCATCAATTTTGACCATTGGAAAA
'Conocybe lactea 2'            GCTGGAAATCAACCTTGCTTTTGCTGGGCGTACTTTCTAGTCAATGGGTCAGCATCAATTTTGACCATTGGAAAA
'Conocybe lactea Cheyenne'     GCTGGAAATCAACCTTGCTTTTGCTGGGCGTACTTTCTAGTCAATGGGTCAGCATCAATTTTGACCATTGGAAAA
'Conocybe lactea Corvallis'    GCTGGAAATCAACCTTGCTTTTGCTGGGCGTACTTTCTAGTCAATGGGTCAGCATCAATTTTGACCATTGGAAAA
'Conocybe lactea Eugene'       GCTGGAAATCAACCTTGCTTTTGCTGGGCGTACTTTCTAGTCAATGGGTCAGCATCAATTTTGACCATTGGAAAA
'Conocybe lactea 1'            GCTGGAAATCAACCTTGCTTTTGCTGGGCGTACTTTCTAGTCAATGGGTCAGCATCAATTTTGACCATTGGAAAA
'Lactea-crispa intermediate'   GCTGGAAATCAACCTTGCTTTTGCTGGGCGTACTTTCTAGTCAATGGGTCAGCATCAATTTTGACCATTGGAAAA
'Conocybe huijsmanii conica'   GCTGGAAATCAACCTTGCTTTTGCTGGGCGTACTTTCTAGTCAATGGGTCAGCATCAATTTTGAC??????????
'Conocybe crispa UK'           GCTAGAAATCAACCTTGCTTTTGCTGGGCGTACTTTCTGGTCAACGGGTCAGCATCAATTTTGACCGTTGGAAAA
'Conocybe crispa 1'            GCTGGAAATCAACCTTGCTTTTGCTGGGCGTACTTTCTAGTCAATGGGTCAGCATCAATTTTGACCATTGGAAAA
'Conocybe crispa 2'            GCTGGAAATCAACCTTGCTTTTGCTGGGCGTACTTTCTAGTCAATGGGTCAGCATCAATTTTGACCATTGGAAAA
'Conocybe crispa 3'            GCTGGAAATCAACCTTGCTTTTGCTGGGCGTACTTTCTAGTCAATGGGTCAGCATCAATTTTGACCATTGGAAAA
'Conocybe crispa 4'            GCTGGAAATCAACCTTGCTTTTGCTGGGCGTACTTTCTAGTCAATGGGTCAGCATCAATTTTGACCATTGGAAAA
'Conocybe crispa 5'            GCTGGAAATCAACCTTGCTTTTGCTGGGCGTACTTTCTAGTCAATGGGTCAGCATCAATTTTGACCATTGGAAAA
'Conocybe subcrispa'           GCTGGAAATCAACCTTGCTTTTGCTGGGCGTACTTTCTAGTTAATGGGTCAGCATCAATTTTGACCATTGGAAAA
'Conocybe filaris'             GCCAGGAATCAACCTTGCTTTTGCTGGGTGTACTTTCTGGTTGACGGGTCAGCATCAATTTTGACTGTTGGAAAA
'Cyttarophyllum besseyii'      GCCGGGGATCAACCTTGCTTTTGCTGGGCGTACTTTCCGGTCGACGGGTCAGCATCAATTTTGACCGTTGGATAA
'Agrocybe praecox AF041545'    GCCGGGAATCAACCTTGCTTTTGCTGGGCGTACTTTCCGGTCGACGGGTCAGCATCAATTTTGACCGTTGGATAA


'Weraroa cucullata'            AGTCGGGAGGAATGTGGCATCTTCGGGTGTGTTATAGCCTCTTGTCGCATACAACGGTTGGGATTGAGGAACTCA
'Galeropsis desertorum'        AGGTTTGGGGAATGTGGCAGCTTCGGCTGTGTTATAGCCCCTTGTCACATACAGCGGTTGGGATTGAGGAACTCA
'Agrocybe seimorbicularis'     AGTCTAGAGGAATGTGGCATCTTCGGGTGTGTTATAGCCTCTGGTCGCATACAACGGTTGGGATTGAGGAACTCA
'Bolbitius vitellinus'         AGTTCAAGGGAATGTGGCATCTTCGGATGTGTTATAGCCCTTGTTCGCATACAGCAGTTGGGATTGAGGTACTCA
'Yellow Gastrocybe'            AGCTCAAGGGAATGTGGCATCTTCGGATGTGTTATAGCCCTTGTTCGCATACAGCAGTTGGGATTGAGGTACTCA
'Bolbitius lacteus'            AGTTCAAGGGAATGTGGCATCTTCGGATGTGTTATAGCCCTTGTTCGCATACAGCAGTTGGGATTGAGGTACTCA
'Bolbitius variicolor'         AGTTCAAGGGAATGTGGCATCTTCGGATGTGTTATAGCCCTTGTTCGCATACAGCAATTGGGATTGAGGTACTCA
'Gastrocybe lateritia TYPE'    AGTGTAGGGGAATGTGGCATCTTCGGATGTGTTATAGCCCCTGCTCGCATACAATGGTTGGGATTGAGGAATTCA
'Intermediate 3'               AGTGTAGAGGAATGTGGCATCTTCGGATGTGTTATAGCCTTTGCTCGCATACAATGGTTGGGATTGAGGAATTCA
'Intermediate 1'               AGTGTAGGGGAATGTGGCATCTTCGGATGTGTTATAGCCCCTGCTCGCATACAATGGTTGGGATTGAGGAATTCA
'Intermediate 2'               AGTGTAGAGGAATGTGGCATCTTCGGATGTGTTATAGCCTTTGCTCGCATACAATGGTTGGGATTGAGGAATTCA
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Appendix 6. Chapter 4 *Conocybe* 28S, cont.

'Gastrocybe lateritia 5'	AGTGTAGGGGAATGTGGCATCTTCGGATGTGTTATAGCCCCTGCTCGCATACAATGGTTGGGATTGAGGAATTCA
'Gastrocybe lateritia 3'	AGTGTANGGGAATGTGGCATCTTCGGATGTGTTATAGCCCCTGCTCGCATACAATGGTTGGGATTGAGGAATTCA
'Gastrocybe lateritia 2'	AGTGTAGGGGAATGTGGCATCTTCGGATGTGTTATAGCCCCTGCTCGCATACAATGGTTGGGATTGAGGAATTCA
'Gastrocybe lateritia 4'	AGTGTAGGGGAATGTGGCATCTTCGGATGTGTTATAGCCCCTGCTCGCATACAATGGTTGGGATTGAGGAATTCA
'Bolbitius tener'	AGTGTAGGGGAATGTGGCATCTTCGGATGTGTTATAGCCCCTGCTCGCATCCAATGGTTGGGATTGAGGAATTCA
'Conocybe lactea UK'	AGTGTAGAGGAATGTGGCATCTTCGGATGTGTTATAGCCTTTGCTCGCATACAATGGTTGGGATTGAGGAATTCA
'Conocybe rickenii'	AGTTCAGAGGAATGTGGCATCTTCGGGTGTGTTATAGCCTCTGTTCGCATACAATGGTTGGGATTGAGGATCTCA
'Conocybe subnuda'	AGTTCAAGGGAATGTGGCATCTTCGGATGTGTTATAGCCCTGGTTCGCATACAGCGGTTGGGATTGAGGAACTCA
'Conocybe lactea 5'	AGTGTAGAGGAATGTGGCATCTTCGGATGTGTTATAGCCTTTGCTCGCATACAGTGGTTGGGATTGAGGAATTCA
'Conocybe lactea 3'	AGTGTAGAGGAATGTGGCATCTTCGGATGTGTTATAGCCTTTGCTCGCATACAGTGGTTGGGATTGAGGAATTCA
'Conocybe lactea 2'	AGTGTAGAGGAATGTGGCATCTTCGGATGTGTTATAGCCTTTGCTCGCATACAGTGGTTGGGATTGAGGAATTCA
'Conocybe lactea Cheyenne'	AGTGTAGAGGAATGTGGCATCTTCGGATGTGTTATAGCCTTTGCTCGCATACAATGGTTGGGATTGAGGAATTCA
'Conocybe lactea Corvallis'	AGTGTAGAGGAATGTGGCATCTTCGAATGTGTTATAGCCTTTGCTCGCATACAGTGGTTGGGATTGAGTAATTCA
'Conocybe lactea Eugene'	AGTGTAGAGGAATGTGGCATCTTCGGATGTGTTATAGCCTTTGCTCGCATACAGTGGTTGGGATTGAGGAATTCA
'Conocybe lactea 1'	AGTGTAGAGGAATGTGGCATCTTCGGATGTGTTATAGCCTTTGCTCGCAT-CAGTGGTTGGGATTGAGGAATTCA
'Lactea-crispa intermediate'	AGTGTAGAGGAATGTGGCATCTTCGGATGTGTTATAGCCTTTGCTCGCATACAGTGGTTGGGATTGAGGAATTCA
'Conocybe huijsmanii conica'	??
'Conocybe crispa UK'	AGTTCAGAGGAATGTGGCATCTTCGGGTGTGTTATAGCCTCTGTTCGCATACAATGGTTGGGATTGAGGATCTCA
'Conocybe crispa 1'	AGTGTAGAGGAATGTGGCATCTTCGGATGTGTTATAGCCTTTGCTCGCATACAGTGGTTGGGATTGAGGAATTCA
'Conocybe crispa 2'	AGTGTAGAGGAATGTGGCATCTTCGGATGTGTTATAGCCTTTGCTCGCATACAGTGGTTGGGATTGAGGAATTCA
'Conocybe crispa 3'	AGTGTAGAGGAATGTGGCATCTTCGGATGTGTTATAGCCTTTGCTCGCATACAGTGGTTGGGATTGAGGAATTCA
'Conocybe crispa 4'	AGTGTAGAGGAATGTGGCATCTTCGGATGTGTTATAGCCTTTGCTCGCATACAGTGGTTGGGATTGAGGAATTCA
'Conocybe crispa 5'	AGTGTAGAGGAATGTGGCATCTTCGGATGTGTTATAGCCTTTGCTCGCATACAGTGGTTGGGATTGAGGAATTCA
'Conocybe subcrispa'	AGTGTAGAGGAATGTGGCATCTTCGGATGTGTTATAGCCTTTGCTCGCAT-CAATGGTTGGGATTGAGGAATTCA
'Conocybe filaris'	AGTTCAAAGGAATGTGGCATCTTCGGATGTGTTATAGCCTTGGTTCGCATACAACAGTTGGGATTGAGGAACTCA
'Cyttarophyllum besseyii'	AGTCTAGAGGAATGTGGCATCTTCGGGTGTGTTATAGCCTCTGGTCGCATACAACGG?TGGGATTGAGGAACTCA
'Agrocybe praecox AF041545'	AGTCTAGAGGAATGTGGCATCTTCGGGTGTGTTATAGCCTCTGGTCGCATACAACGGTTGGGATTGAGGAACTCA

'Weraroa cucullata'	GCAggggaaaaaa
'Galeropsis desertorum'	GCAggggaaaaaa
'Agrocybe seimorbicularis'	GCAggggaaaaaa
'Bolbitius vitellinus'	GCAggggaaaaaa
'Yellow Gastrocybe'	GCA?gaaaaaaaa
'Bolbitius lacteus'	GCA??ggaaaaaa
'Bolbitius variicolor'	GCAgggaaaaaaa
'Gastrocybe lateritia TYPE'	GCAggggaaaaaa

Appendix 6. Chapter 4 *Conocybe* 28S, cont.

'Intermediate 3'	GCAggggaaaaaa
'Intermediate 1'	GCAggggaaaaaa
'Intermediate 2'	GCAggggaaaaaa
'Gastrocybe lateritia 5'	GCAggggaaaaaa
'Gastrocybe lateritia 3'	GCAggggaaaaaa
'Gastrocybe lateritia 2'	GCAggggaaaaaa
'Gastrocybe lateritia 4'	GCAggggaaaaaa
'Bolbitius tener'	GCAgaggggagga
'Conocybe lactea UK'	GCAggggaaaaaa
'Conocybe rickenii'	GCAggggaaaaaa
'Conocybe subnuda'	GCAggggaaaaaa
'Conocybe lactea 5'	GCAggggaaaaaa
'Conocybe lactea 3'	GCAggggaaaaaa
'Conocybe lactea 2'	GCAggggaaaaaa
'Conocybe lactea Cheyenne'	GCAagggaaaaaa
'Conocybe lactea Corvallis'	GCAggggaaaaaa
'Conocybe lactea Eugene'	GCA??????aaaa
'Conocybe lactea 1'	GCAggggaaaaag
'Lactea-crispa intermediate'	GCAggggaaaaaa
'Conocybe huijsmanii conica'	???????aagaa?
'Conocybe crispa UK'	GCAggggaaaaaa
'Conocybe crispa 1'	GCAggggaaaaaa
'Conocybe crispa 2'	GCAggggaaaaaa
'Conocybe crispa 3'	GCAggggaaaaaa
'Conocybe crispa 4'	GCAggggaaaaaa
'Conocybe crispa 5'	GCAggggaaaaaa
'Conocybe subcrispa'	GCAggggaaaaag
'Conocybe filaris'	GCAggggaaaaaa
'Cyttarophyllum besseyii'	GCAggggaaaaaa
'Agrocybe praecox AF041545'	GCAggggaaaaaa

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