PHYLOGENETIC RELATIONSHIPS OF ALPHA-AMANITIN PRODUCING *GALERINA* FROM BRITISH COLUMBIA

by

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B.Sc., Acadia University, 2016

A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF

THE REQUIREMENTS FOR THE DEGREE OF

MASTER OF SCIENCE

in

THE FACULTY OF GRADUATE AND POSTDOCTORAL STUDIES

(Botany)

THE UNIVERSITY OF BRITISH COLUMBIA

(Vancouver)

May 2019

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Phylogenetic Distribution of Alpha-Amanitin Producing *Galerina* from British Columbia

submitted by Brandon Landry in partial fulfillment of the requirements for

the degree of Master of Science

in Botany

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Abstract

Mushrooms of some *Galerina* species equal the most poisonous *Amanita* species in their concentrations of deadly amanitin toxins. Although individual *Galerina* mushrooms are small, eating about ten would risk delivering a lethal dose of amanitins to a child. Understanding which species of *Galerina* pose an acute poisoning risk requires a better understanding of species boundaries within the genus, as well broad sampling for the presence of amatoxins. I analyzed 61 *Galerina* and eight outgroup specimens for the presence of amatoxins using HPLC/LC-MS. I then used multi-locus DNA data (ITS, LSU and RPB2) from a broad sampling of *Galerina* and outgroup taxa to generate a constraint tree, to which 322 *Galerina* ITS sequences from herbarium specimens at UBC, from A.H. Smith's type material (University of Michigan) and from Genbank were added. I mapped toxin analysis data onto the resulting phylogeny, which indicated that amatoxin-production in BC *Galerina* is restricted to two species, *G. venenata* and *G. castaneipes*. These two species, along with two other reportedly toxic species (*G.* aff. *marginata* and *G. sulciceps*) and seven other species whose toxin production status remains unknown form a broad clade referred to as the *G. marginata* complex. Phylogenetic and toxin data suggest that the sister clade to the *G. marginata* complex (*G. badipes*) does not produce toxins, implying that the origin of amatoxin production in *Galerina* is somewhere within the *G. marginata* complex. Additionally, phylogenetic data also supports past evidence that members of the genus *Gymnopilus* are nested within the 'Mycenopsis' lineage of *Galerina*. The results provide the first comprehensive look at toxin production in *Galerina*, as well as the first report of additional toxin-producing species in North America. Using the molecular data from this study to update specimen names in herbarium collections and online databases will reduce downstream

confusion resulting from inaccurate identifications or misapplied names. Doing so will contribute to ongoing efforts to update of field guides and other resources that list poisonous and edible mushrooms, allowing amateur mycologists, foragers and healthcare professionals to gain a better understanding of which *Galerina* pose a poisoning risk.

Lay Summary

Galerina is a genus of unassuming small brown mushrooms found worldwide. Although seemingly innocuous, these mushrooms have been implicated in multiple poisoning cases across the globe. Unlike some other poisonous compounds, the toxic compounds in *Galerina* are not broken down by cooking or stomach acid. Exactly which species produce these toxins has been difficult to determine, largely because many species look the same. Using samples from the UBC herbarium and elsewhere, I was able to expand our understanding of which mushrooms contain toxins. I also obtained DNA sequences from these mushrooms, allowing me to identify the relationships between samples. I was then able to pinpoint which species contained toxin-producing mushrooms, giving me a better idea of which mushrooms could pose a poisoning risk. In doing so, I found that more species than previously thought are toxic. This work can help healthcare professionals identify which mushrooms should be of concern if patients report ingesting mushrooms.

Preface

The original idea to produce a large-scale phylogeny of *Galerina* was conceived by Dr. Mary Berbee. The DNA sequencing work in this study was a collaborative effort of many individuals from the Berbee lab: initial ITS sequencing was performed by Anna Bazzicalupo (section 2.2.1), whereas I collected additional ITS, LSU and RPB2 sequences with help from undergraduates Berni van der Meer and Julian Yu (section 2.2.2). I conducted the toxin-analysis portion of the study in Dr. Jonathan Walton's lab at Michigan State University with assistance from various members of his lab. I performed all data analyses presented throughout this study.

Preliminary results on the distribution of toxins in *Galerina* were presented in 2017 to the Mycological Society of America under the title:

Phylogenetic relationships of alpha-amanitin producing *Galerina* from British Columbia. Brandon R. Landry, Berni van der Meer, Mary Berbee. Mycological Society of America, July 2017.

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List of Abbreviations

AICc – Akaike information criterion (corrected).

EIC – Extracted Ion Chromatogram.

EtOH – Ethanol.

GI – Gastrointestinal.

HPLC – High performance liquid chromatography.

ITS – Internal transcribed spacer.

LD50 – Lethal dose 50%. Dosage of substance required to kill 50% of the tested samples.

LSU – Large ribosomal subunit.

MICH – University of Michigan herbarium.

MS – Mass spectrometry.

OTU – Operational taxonomic unit.

PCR – Polymerase chain reaction.

RFLP – Restriction length fragment polymorphism.

RPB2 – RNA polymerase II 2nd largest subunit.

TLC – Thin-layer chromatography.

UBC – University of British Columbia herbarium.

Acknowledgements

First and foremost, I would like to thank my academic supervisor, Dr. Mary Berbee for giving me the space, time and teachings needed to complete my graduate studies. Thank you as well to my co-supervisor and committee member Dr. Jeannette Whitton for all the immensely helpful feedback and advice during my time at UBC. Thank you to my other committee members, Dr. Jörg Bohlmann and Dr. Quentin Cronk, as well as my external examiner Dr. Rick Taylor, for the all the useful feedback and constructive criticism on my thesis.

Teaching was also an integral part of my experience as a graduate student here at UBC. Thank you to Kathy Nomme, Lynn Norman, Chin Sun, Brett Couch, Bernardita Germano, Dirk VandePol, Erica Jeffery and Maryam Moussavi for helping me be a better teacher. And of course, thank you to the dozens of students I have taught for keeping me on my toes and for allowing me to spread my love of biology.

Lastly, thank you to past and present members of the Berbee lab. Jackie Dee, Ben Auxier, Ludovic Le Renard, Anna Bazzicalupo, Berni van der Meer, Mika Miyamoto and Julian Yu: thank you all for sharing your knowledge and for helping me with various aspects of my project.

Dedication

I would like to dedicate my work to Jonathan D. Walton (1953-2018). Thank you for being immeasurably helpful in helping me settle in to East Lansing and Michigan State University. Our coffees, forages and brainstorms together were a highlight of my summer, and I learned so much from our many talks.

Chapter 1: Introduction

The history of poisonous mushrooms is rich and mysterious; various species have been implicated in the deaths of noteworthy individuals including Roman Emperor Claudius, Holy Roman Emperor Charles VI, Pope Clement VII and even Siddhartha Gautama, the Buddha (Walton, 2018). Despite their long-standing cultural prevalence, our understanding of poisonous mushroom species has grown immensely only with modern scientific advances. A multitude of toxins from a variety of species have now been identified, and this new information is used to update field guides and other sources of information for collectors. A noteworthy example of important updating is that of *Galerina marginata*: a publication by Agriculture Canada (Groves and Redhead, 1979) lists *Pholiota marginata* (= *G. marginata*) as edible but belonging to a toxic group, and Gulden et al. (2001) notes that pre-mid-20th century European field guides listed *G. marginata* as edible. As a result of chemical analyses following poisoning cases in Europe (Besl et al., 1984) and North America (Tyler and Smith, 1963), this species is now known to contain the deadly α-amanitin toxin. Other species in the genus have been reported as toxic, but the exact number of toxic species remains unknown.

Most of our current understanding of *Galerina* comes from Smith and Singer's 1964 monograph of the genus. Whereas past work by Kühner (1935) had focused primarily on a small number of European species, Smith and Singer included a greater number of collections primarily from North and South America. They present and discuss macro- and microscopic characteristics for close to 200 species, varieties and forms of *Galerina*. Currently, this monograph remains the primary source of information regarding *Galerina* in North America. However, Smith and Singer were limited by the technology of the time, and the monograph relied entirely on morphological characteristics to distinguish species. Due to difficulties in

breeding fungi, species boundaries in *Galerina* (and other genera) were therefore formed mostly based on the morphological species concept, with some consideration of habitat (i.e. ecological species concept) (Fig. 1.1) (Smith and Singer, 1964).

With increasingly accessible molecular data, past species concepts in fungi are being revisited as phylogenetic data reveal unexpected conspecificity and diversity among species. Simple and affordable DNA sequencing, combined with improvements in the field of analytical chemistry, have led to progress in identifying toxic mushroom species and their specialized metabolites. To better understand species boundaries and the distribution of toxins in *Galerina*, I applied these two methods to determine the identity mushrooms and test them for the presence of toxic compounds.

Herbaria house an invaluable source of data for mycologists. Dried mushroom specimens that have been properly stored retain valuable DNA data for decades after deposition and morphological data nearly indefinitely. Large collections of *Galerina* from both amateur and trained mycologists deposited in the University of British Columbia Herbarium (UBC) provide ample source material to begin investigating the phylogenetics of the genus. As previous studies on *Galerina* were based on DNA sequences primarily from European *Galerina*, the data collected from BC specimens would make a valuable supplement to existing data, allowing for a more complete *Galerina* phylogeny to be produced. Furthermore, where previous data focused mostly on single genetic loci, the addition of multiple genetic loci will allow for more in-depth analysis.

The stability of amanitins – the toxic components of *Galerina* – also facilitates observation of dated material. Due to the stable molecular configuration, these toxins have been detected in dried material nearly two decades old (Fig. 1.2). Furthermore, increased sensitivity of

analytical chemistry tools means that detection can be made from minute (<10 mg) quantities of dried material. As such, the same mushrooms used for DNA sequencing can also be used for toxin analysis, allowing two sources of data to be collected from a single mushroom sample. High-performance liquid chromatography (HPLC) and mass spectrometry (MS) would provide multiple lines of evidence for confirming the presence or absence of toxins, yielding reliable and accurate toxin data. The multi-locus phylogenetic data combined with the toxin presence/absence data can enable the origin of toxin production and the number of toxin-positive *Galerina* to be assessed, providing the first large-scale exploration of toxins in this genus.

Figure 1.1 - Six samples of *Galerina* from the UBC herbarium collection, highlighting 'typical' dried *Galerina* appearance. Samples A (F24586) and B (F29201) were identified by collectors as *G. mammillata*. Samples C (F26374) and D (F29592) were identified by collectors as *G. sideroides* but later revealed to share identical DNA sequences with samples of *G. mammillata*. Samples E (F27143) and F (27196) were also identified as *G. sideroides*. Smith and Singer (1964) used morphological characters to delimit most of their species: for these two taxa, the authors note that drying to white is a characteristic of *G. mammillata* whereas drying to brown is characteristic of *G. sideroides*.

UBC Herbarium
INCHES
METRIC
A
B
C
D
E
F

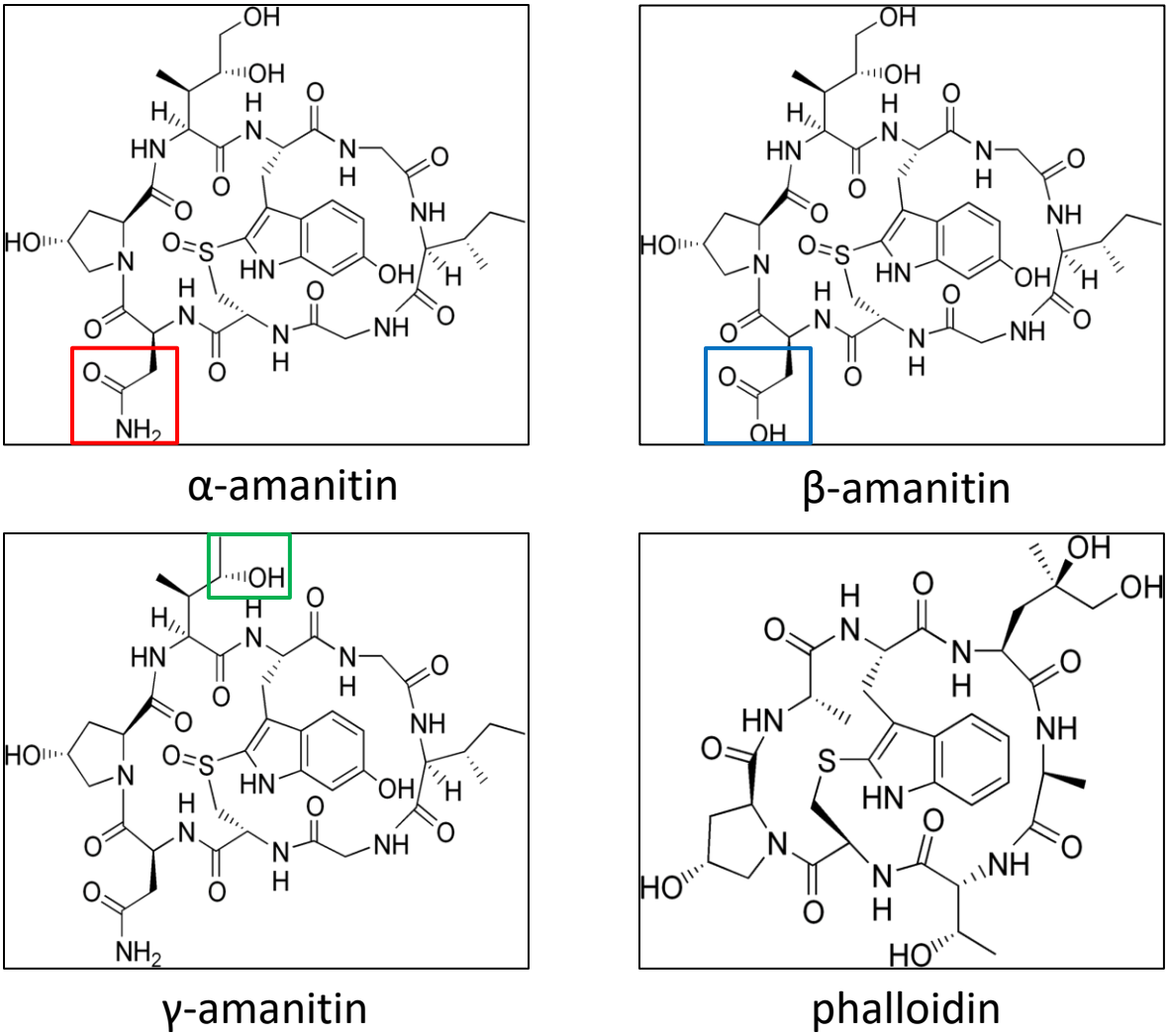


Figure 1.2 – Chemical structures of the three major amatoxins found in *Galerina*, with phalloidin (another cyclic peptide found in *Amanita* but not found in *Galerina*) for comparison. Colored squares represent major differences in structure between amatoxins.

Chapter 2: Phylogeny & Toxin Analysis of Galerina

2.1 Introduction

Galerina, a genus of little brown mushrooms, has been implicated in dozens of poisoning cases worldwide (Enjalbert et al., 2004). However, information about exactly which of the >300 species in the genus pose a poisoning risk is incomplete and confusing due to the lack of DNA sequences from specimen vouchers that have been tested for toxins, a poor understanding of species boundaries and relationships, and the absence of systematic studies to put the non-toxin producers into a phylogenetic context.

Deadly amatoxin production in *Galerina* has been known since the mid-20th century: in 1954, two patients consumed what was later identified as *G. venenata* and presented with symptoms mirroring *Amanita phalloides* poisoning (Grossman and Malbin, 1954). Prompted by these poisoning cases, Tyler and Smith (1963) performed a simple chromatographic analysis on other mushrooms identified as *G. venenata* and showed that α- and β-amanitin – two of the toxic peptides identified from and named for the genus *Amanita* – were present. Since then, *Lepiota* and a single sample of a mushroom identified as *Conocybe filaris* have also been reported to produce α-amanitin and other deadly amatoxins (Enjalbert et al., 2004). At higher taxonomic levels, the evolutionary history toxin production in these four genera is not well known, including whether this is the result of convergent evolution, descent from a common ancestor, or horizontal gene transfer (Luo et al., 2012).

Although individual mushrooms are small, quantification of amatoxins in *Galerina* suggests that given the LD50 of 0.1 mg/kg body weight, ten fruiting bodies of *Galerina* would be sufficient to poison a child weighing 20kg (Enjalbert et al., 2004). Arora (1986) suggests that poisoning cases may arise from mistaking *Galerina* species for other ‘little-brown mushrooms’

including hallucinogenic *Psilocybe* and *Gymnopilus* species. While this may be the case, reports often fail to address the underlying circumstances surrounding the ingestion of poisonous mushrooms.

Toxicology reports from the North American Mycological Association indicate that *Galerina* poisonings occur relatively infrequently in humans but remain a cause for concern for wild foragers. In addition to human poisoning cases, *Galerina* has also been implicated in multiple, sometimes-fatal animal poisoning cases in dogs (Beug, 2009, 2011, 2013, 2014) and cats (Beug et al., 2006). Because reporting mushroom poisonings can be arduous and is not legally required, under-reporting is the norm, thus making the true number of poisoning cases difficult to assess. In most instances, GI symptoms (nausea, vomiting, diarrhea) are the first to occur, manifesting as early as 6-hours post ingestion. However, symptoms and onset time can vary greatly, sometimes resulting in moderate to severe organ damage before treatment is sought (Table 2.1).

Table 2.1- Summary of recent (post-2000) human *Galerina* poisoning cases.

Species	Date	Symptom Onset	Symptoms/Notes	Fatal?
Galerina sp. (possibly *fasciculata*)[1]	2001, Japan	6-10 hrs; hospitalization at 36 hrs	Gastrointestinal (GI) distress (abdominal pain, nausea, vomiting, diarrhea), leading to dehydration. Liver failure diagnosed at 72 hrs post-ingestion. Recovery after day 18 following intense treatment.	N
Galerina cf. *marginata*[2]	<2006. 9 cases (8 adults + 1 child): AR, IL, KS, MI, OH, OR, WA	6-21 hrs; average 13 hrs	GI distress, bloody vomit/diarrhea, cramps, dehydration, disorientation, drowsiness, weakness, liver damage, inability to walk, dry heaving.	N
Galerina sp.[2]	<2006; OH	9 hrs	GI distress, liver failure.	N

Species	Date	Symptom Onset	Symptoms/Notes	Fatal?
Galerina sp.[3]	Nov. 2010, BC	?	Concern of possible liver damage. Reportedly seeking *Psilocybe* species and accidentally consumed *Galerina*.	N
Galerina sp.[4]	Oct. 2011, CA	?	Renal + later multi-system failure; death 48 hrs after going to emergency department.	Y
Galerina sp.[4]	Oct. 2011, CA	?	?	N
Galerina sp.[4]	2011, IL	?	?	N
G. marginata[5]	?	14 hrs	Dry heaves, diarrhea. Self-discharged from hospital after fighting with staff. Reported as possibly fabricated.	N
G. sulciceps[6]	Nov. 2013, China. 13 cases (males aged 19-56).	9-21 hrs	GI symptoms (nausea, vomiting, abdominal pain, diarrhea), fatigue, weakness, inertia, anorexia, palpitations, chest tightness. In the more severe cases, eye pain, blurred vision, leg cramps and low urine output also presented. All patients were discharged after 10 days, with normal hepatic function reported after 30 days.	N

1 : (Kaneko et al., 2001) 2 : (Beug et al., 2006), 3 : (Beug, 2011), 4 : (Beug, 2012), 5 : (Beug, 2013). 6: (Xiang et al., 2018)

Within *Galerina*, six amatoxin-producing species are reported in the literature: *G. marginata* (= *G. autumnalis, G. unicolor, G. venenata, G. oregonensis*), *G. badipes, G. beinrothii, G. fasciculata, G. helvoliceps,* and *G. sulciceps* (Enjalbert et al., 2004). Although progress in the field of analytical chemistry has greatly facilitated identification and quantification of mushroom toxins, amatoxin detection in minute quantities of mushroom tissue has been possible since the mid-20th century. Block et al. (1955) report an extraction procedure not unlike current methods, involving a simple methanol extraction, followed by drying and resuspension of the concentrate. A simple thin-layer chromatography (TLC) procedure follows, performed with a solution of methyl ethyl ketone (butanone), acetone, water and butanol and spraying with 1% cinnamaldehyde in methanol. This procedure, yielding violet or blue colored

spots on the paper, remained the primary method for detecting amanitins until the rise in popularity of HPLC.

The amount of information related to amatoxin production in purportedly toxic species is variable: for example, *G. marginata* is the best studied species, where part of the pathway for amatoxin production – including the gene coding for alpha-amanitin synthesis – has been successfully elucidated (Luo et al., 2012). It is important to note that all putatively toxic species except *G. marginata* have been identified on morphological characters alone: for this reason, the true number of toxic *Galerina* remains debatable. While none of the remaining species have been studied as intensively, TLC or HPLC results of samples identified as these various *Galerina* species have tested positive for the presence of amatoxins (Table 2.2).

Table 2.2 - Toxic *Galerina* species as reported in the literature. At the present time, all samples except *G. marginata* lack DNA sequence data and have been identified based on morphology alone.

Name	Chemical Data	Vouchered Collections
G. badipes	TLC[3]; HPLC[7]; Southern blotting[7]	No 3729, MTB 8544 (Institute of Botany, University of Regensburg)[3]; Centaalbureau voor Schemmelcultures (CBS) 268.50[7]
G. beinrothii	TLC[3]	MTB 7832 (Institute of Botany, University of Regensburg [REG])[3]
G. fasciculata	HPLC[4,5]	Strain GF-060 (The Mushroom Research Institute of Japan, Kiryu, Gunma)
G. helvoliceps	HPLC[5]	Strain GH-343 (The Mushroom Research Institute of Japan, Kiryu, Gunma)
G. marginata	TLC[1,3]; HPLC[6,7]; Southern Blotting[7]	CBS 339.88[7], CBS 924.72[7], MTB 6024 (REG)[3]
G. sulciceps	TLC[2,3]	MTB 7038 (REG)[3]

1 : Tyler and Smith, 1963, 2 : Besl, 1981, 3 : Besl et al., 1984, 4 : Muraoka et al., 1999, 5: Muraoka and Shinozawa, 2000, 6: Enjalbert et al., 2004, 7: Luo et al., 2012.

Available phylogenetic and toxin data for *Galerina* places all toxin-producers in the 'Naucoriopsis' lineage (Enjalbert et al., 2004; Gulden et al., 2005). Multiple attempts have been made by mycologists to classify infrageneric units within *Galerina*, largely based on macro- and

micromorphological characters. ‘Naucoriopsis’, originally defined formally as a section in *Galerina* by Kühner (1935), has varied in taxonomic rank between section (Bon, 1992; Smith and Singer, 1964; Watling et al., 1993) and subgenus (Gulden and Halgrimsson, 2000; Kühner, 1972). The most recent classification proposed by Gulden et al. (2005) using DNA sequence data for 36 *Galerina* species uses the term ‘lineage’ to refer to the unclear taxonomic level of ‘Naucoriopsis’, as well as lineages ‘Tubariopsis’, ‘Galerina’ and ‘Mycenopsis’.

Single locus internal transcribed spacer (ITS) and large ribosomal subunit (LSU) data from Gulden et al. (2005) more-or-less provided phylogenetic support for these lineages that were previously described based on morphology alone. The LSU phylogeny also suggested that *Galerina* may be polyphyletic: members of *Agrocybe, Phaeocollybia, Hebeloma* and other genera were nested within the various lineages of *Galerina*, albeit with no support. However, support was present for *Gymnopilus* being nested within the ‘Mycenopsis’ lineage of *Galerina*. The authors note that spore morphology of certain taxa from both *Galerina* and *Gymnopilus* can be similar, leading to difficult classification even with microscopic characters.

Matheny et al. (2015) noted the apparent polyphyly of *Galerina* in Gulden et al. (2005) and highlighted the importance of broad taxon sampling for addressing difficult taxonomic questions: in their phylogeny, the authors included samples identified as *Galerina clavus*, an unusual species whose status as a *Galerina* was ambiguous due to its unusual morphology (Matheny et al., 2015). Preliminary data showed a relationship between *G. clavus* and samples of *Pachylepyrium*, a distantly related genus (Matheny et al., 2015). Given the phylogenetic distance between these two genera, multi-locus data from a broad sampling of Agaricales was analyzed to resolve the uncertainty, ultimately placing samples of *G. clavus* as sister to *Pachylepyrium* and recategorizing these samples as a new genus, *Romagnesiella.* Additionally, although sampling of

both *Galerina* (5 species) and *Gymnopilus* (2 species) was limited, Matheny et al. (2015) also found that *Gymnopilus* sequences were nested within *Galerina,* albeit with low phylogenetic support.

The use of multi-locus phylogenetic data by Matheny et al. (2015) highlights the importance of using DNA sequence data to further explore species- and genus-level taxonomic issues. Until recently, most *Galerina* species have been described and delimited based on micro- and macromorphological differences. In Smith and Singer's (1964) monograph on the genus, 199 species of *Galerina* were described, not counting additional species discovered too late for inclusion in the publication. More recently, Horak (1994) suggested that more than 300 species of *Galerina* may exist. However, characters within species are often highly variable, making infrageneric identification challenging in the absence of molecular data. Questionable characters or combination of characters (as in the case of *Romagnesiella clavus* [= *G. clavus*]) merit further exploration with more modern tools.

In another example of phylogenetic data being used to explore taxonomic questions previously addressed using only morphology, Gulden et al. (2001) noted that species descriptions of the North American *G. autumnalis* and the European *G. marginata* were often indistinguishable. To better determine the relationships between these species and other *Galerina*, Gulden et al. (2001) produced phylogenies using ITS-2 sequence data and compared restriction fragment length polymorphisms (RFLP) profiles of the entire ITS region. Both lines of evidence showed that samples identified as *G. marginata* clustered with samples of *G. autumnalis, G. unicolor* and *G. venenata.* Together with *G. badipes*, these taxa also formed a supported 'Naucoriopsis' lineage as defined by Gulden and Hallgrimsson (2000). Ultimately, Gulden et al. (2001) proposed that *G. autumnalis, G. venenata, G. unicolor* and *G. oregonensis*

be synonymized with *Galerina marginata*. However, the authors noted that this delimitation of *G. marginata s.l,* comprised a high degree of variation (1.5% ± 1.2% mean sequence divergence).

As the shift from the morphological species concept towards sequence-based concepts (i.e. the monophyletic species concept [(Donoghue, 1985; Mishler, 1985)] takes place, genetic variation such as that noted by Gulden et al. (2001) presents new complications. Both morphological and monophyletic species concepts as applied to *Galerina* require difficult decisions in terms of defining species. De Queiroz (2007) stated that most traditional species concepts share a common theme, whereby 'species' often refers to independently evolving metapopulation lineages. De Queiroz (2007) also suggested that attempting to apply any single species concept is limiting, instead proposing a unification of all species concepts. Contemporary species concepts depend on one or more 'properties', whereby 'property' in this context refers to thresholds (e.g. reproductive isolation, sharing niches, unique evolutionary roles, shared derived characters, etc.) crossed by lineages, therefore differentiating them as separate species according to their respective concepts.

Multi-locus phylogenies of genera such as *Amanita* (Cai et al., 2014; Geml et al., 2008) and *Cantharellus* (Thorn et al., 2017) have revealed unexpected diversity in these genera, highlighting the benefits of heavy sampling and multi-locus (as opposed to single-locus) analyses. Despite its usefulness, multi-locus data may not serve as conclusive evidence for delimiting species: while DNA sequence differences are an additional line of evidence for species delimitation, they are still limiting in that they are only a single property potentially present in a given species. However, given the difficulties previously associated with species

delimitation in *Galerina*, it is highly probable that collecting multi-locus data from a variety of samples will provide new evidence for delimiting species.

For this study, 'species' is to be interpreted as a group of individuals whereby evidence of differences in their evolutionary history is present. Examples of properties which will be considered evidence of separately evolving metapopulations include reciprocal monophyly, concordance of multiple loci, comparison of patterns of branching (vs. substitution patterns expected within a species), lack evidence of shared genetic material between unique species, and differences in morphology. In this context, species concepts and species delimitation are separate issues. The properties previously associated with other species concepts (monophyly, morphological differences, ecological differences, etc.) simply provide evidence of evolutionary differences and support for delimitation (De Queiroz, 2007).

Due to the difficulty in accurately identifying *Galerina* species without sequence data, whether the previously proposed number of *Galerina* species (>300) is an overestimate or underestimate remains to be determined. No comprehensive *Galerina* phylogeny has been published since the single-locus ITS and LSU trees published by Gulden et al. (2005). The primary objective of this study is to create an improved *Galerina* phylogeny using *Galerina* sequence data from Gulden et. al (2005) and outgroup ITS, LSU and RPB2 (RNA Polymerase II 2nd largest subunit) data from Matheny et al. (2015). Multi-locus data from specimens deposited in the UBC herbarium will be collected and added to create a comprehensive multi-locus phylogeny of *Galerina* and related genera. This first step is critical in addressing other issues, particularly species boundaries within the genus, as well as broader genus-level boundaries. Additional information such as shared polymorphisms, morphological data and sequences from

Smith's type material will be used to supplement the phylogeny and provide additional evidence for establishing species boundaries.

With this improved phylogenetic framework, the number and phylogenetic placement of toxin-producing *Galerina* species can be revisited. Two studies (Cai et al., 2014; Hallen et al., 2002) have demonstrated that amatoxins are readily detected and quantified via liquid chromatography-mass spectrophotometry from as little as 8 mg dried *Amanita*, in specimens up to 17 years old. These standard protocols will be used to quantify α-amanitin concentrations from vouchered UBC *Galerina* specimens, with delimitations supported by the phylogenetic data. The distribution of toxins will then be mapped to the multi-locus phylogeny, creating the first large-scale toxin-analysis of *Galerina* specimens for which both toxin and sequence data are readily available. Together, these data will improve our understanding regarding which *Galerina* species pose a poisoning risk, allowing us to be more confident in our species identification, and helping to elucidate the evolution of toxins within the genus.

2.2 Materials and Methods

2.2.1 Sequencing using a 96-well Plate: DNA extraction and sequencing protocol

DNA from 148 UBC herbarium *Galerina* specimens was originally extracted and sent for sequencing by A. Bazzicalupo for Bazzicalupo et al. (2019; in press). All extractions were performed following the DNeasy 96-well Protocol from Qiagen (Hilden, Germany). Depending on the size of the sample, 5-20 mg of gill tissue from each sample was ground using a TissueLyser machine (Qiagen, Retsch MM301 Mixer Mill Pulverizer). To reduce the potential for contamination from neighboring wells, samples were extracted in duplicate in two separate plates. The chromatograms obtained from amplification and Sanger sequencing of the ITS region

(primers ITS1F and ITS4, [White et al., 1990]) were made available for use in this study by A. Bazzicalupo.

For each sample, two forward and two reverse sequence reads – representing the two replicates of the extraction – were expected. The number of useable sequences for a given sample ranged from 0/4 to 4/4. For samples in which two or more useable sequences were obtained, chromatograms (i.e. sequences) were concatenated using the 'de novo assembly' function in Geneious version 9.1 (Kearse et al., 2012). Ultimately, only samples containing at least one useable sequence from both PCR reactions were included for further analysis.

2.2.2 Sequencing of individual DNA extractions

Selected specimens from each operational taxonomic unit (OTU) were chosen from a preliminary maximum likelihood tree (not included) for manual re-extraction and re-sequencing to provide higher quality DNA for single-copy locus amplification, as well as for confirmation of ITS identity. Each OTU was roughly delimited based on monophyly. In cases where branch length & sequence divergence suggested minimal (<1%) difference within a clade, additional samples with small degrees of divergence were included where possible. This was done both to provide a third sequence for comparison with the consensus sequence obtained from the initial 96-well plate extraction, as well as to provide a greater quantity of DNA for amplification of subsequent loci. Where available, two representative samples were chosen from each OTU; otherwise, a single sample was used. In addition, DNA was extracted from 15 *Galerina* type specimens from A.H. Smith's collection (University of Michigan [MICH]) and included in analyses.

For each sample, 5-20 mg of gill tissue was placed in a 1.5 mL tube and ground manually using a pestle and a small quantity of sand. DNA was extracted using a Qiagen DNEasy Plant MiniKit, starting at step 2 (excluding RNase A) and skipping step 5 (Qiagen: Hilden, Germany). PCR amplification of individual loci was performed using 25 µL illustra PuReTaq Ready-To-Go PCR beads (GE Healthcare: Mississauga, ON, Canada) and locus-specific primer combinations as above (Table 2.3). Thermocycler settings were an initial denaturation at 95ºC for 5 min, followed by 30 cycles of 95ºC denaturation for 30 sec, 55ºC annealing for 30 sec, 72ºC elongation for 30 sec with 4 sec ramp up in each cycle, concluding with a final elongation at 72ºC for 7 minutes. For RPB2 samples in which no bands or weak bands were present using primers 6F and 7.1R, primers 6.1F (designed for this study) and 7R were used for a nested PCR. Primers 6F, 7R and 7.1R were modified from Matheny (2005) with B. van der Meer to reduce degeneracy (Table 2.3).

Table 2.3 - Primer combinations used for amplification of various *Galerina* loci.

Locus/Gene	Primer (forward)	Primer (reverse)
ITS	ITS1F: CTTGGTCATTTAGAGGAAGTAA[1]	ITS4: TCCTCCGCTTATTGATATGC[1]
ITS (type)	ITS1F: CTTGGTCATTTAGAGGAAGTAA[1]	ITS2: GCTGCGTTCTTCATCGATG[1]
RPB2	RPB2-6F: TGGGGAATGGTGTGCCCTGC[2,5]	RPB2-7.1R: CCCATAGCTTGCTTGCCCATRGC[2,5]
RPB2 (nested)	RPB2-6.1F: ATGGTGTGCCCTGCGGAAAC[4]	RPB2-7R: GACTGATTATGATCTGGGAATGG[2,5]
LSU	LR0R: ACCCGCTGAACTTAAGC[3]	LR5: TCCTGAGGGAAACTTCG[3]

1: White et al., 1990, 2: Modified from Matheny, 2005, 3: Vilgalys and Hester, 1990, 4: B. Landry, 2019, 5: Modified by B. Landry & B. van der Meer, 2019

PCR product was purified by mixing 2.6 µL 3M acetic acid sodium salt (NaOAc), 21 µL PCR product and 52.5 µL ice cold 95% ethanol (EtOH). The solution was centrifuged for 10 minutes at 12,000 rpm. The resulting supernatant was removed, and 500 µL ice cold 75% EtOH added. The mixture was then centrifuged for 5 minutes at 12,000 rpm and the resulting supernatant was removed. Tubes were left to dry in a laminar flow hood and once dried, the pellet was resuspended in 20 µL H_2O.

For the sequencing PCR reaction, 3 µL BigDye Terminator v3.1 (ThermoFisher Scientific: MA, USA), 5 µL of 1 µM primer and 2 µL template DNA (adjusted to a concentration of 15-20 ng/µL) were mixed. Cycling conditions were as follows: 1 cycle of 96ºC for 2 mins, 30 cycles of @ 96ºC for 10 secs, 50ºC for 5 secs and 60ºC for 4 mins, followed by a hold at 4ºC. For clean-up, 10 µL H_2O was added to the previous mixture. This mixture was pipetted over a tube containing 800 µL of Sephadex G-50 gel and spun for 3 minutes at 3,000 rpm. The cleaned sequencing product flow-through was submitted to the UBC Bioinformatics and Sequencing Consortium for Sanger Sequencing. Forward and reverse sequences from all loci were concatenated and trimmed as described above.

2.2.3 Phylogenetic analysis

Given past difficulties in resolving the relationship of *Galerina* and other Agaricales, 75 outgroup taxa from closely-related families (Hymenogastraceae, Strophariaceae, Crepidotaceae, Inocybaceae, Tubariaceae, Bolbitiaceae and Cortinariaceae) were included for analysis. This increased the likelihood that any unusual relationships (e.g. non-monophyly) between *Galerina* and outgroup taxa would be captured. Samples for which recent ITS and LSU and/or RPB2 data was available from these taxa (Matheny et al., 2015) were chosen to create a constraint tree. Sequences from 31 UBC *Galerina* + four *Gymnopilus* for which ITS + LSU and/or RPB2 data were available were also included in the constraint dataset. For the ITS-only dataset, only three outgroup genera (*Psilocybe*, *Hebeloma* and *Gymnopilus*) were included due to difficulties in aligning sequences from distantly-related taxa. These three taxa were chosen because preliminary trees from individual loci (not included) suggested that *Galerina* may be polyphyletic, with subsets of diversity nested in one or more of these genera.

Sequences from each locus were aligned using the MAFFT online server with the L-INS setting server (Katoh et al., 2017) and manually edited using Mesquite 3.5 (Maddison and Maddison, 2018). For the RPB2 dataset, introns were excluded in the final alignment. The LSU and RPB2 datasets were analyzed individually before concatenation. JModelTest 2 (Darriba et al., 2012) implemented on the CIPRES portal (Miller et al., 2010) selected as best models (AICc) GTR+I+G for the ITS and LSU datasets, TIM1+I+G for RPB2 codon position one and TVM+I+G for RPB2 codon positions two and three. No well-supported (>70% bootstrap) topological conflicts were observed among these loci (Figs. A.1-2), therefore a concatenated alignment was produced in Mesquite.

In addition to the 31 *Galerina* sequences used for the constraint tree, 117 sequences from UBC material, the 15 A.H. Smith type specimen sequences and 190 sequences from GenBank were added to the constraint tree. For each tree (individual locus trees, concatenated constraint tree and constraint + ITS tree), a 200 replicate best-tree search and 1000 replicate bootstrap maximum likelihood search was implemented in RAxML v.8.2.10 (Stamatakis, 2014) on the CIPRES portal (Miller et al., 2010). For the concatenated constraint dataset and final constraint + ITS dataset, the input alignments were partitioned by locus and for RPB2, by codon position.

2.2.4 Species delimitation

A total of 313 full-length (766bp) *Galerina* ITS sequences were used in the online version of Automatic Barcode Gap Detection (ABGD) with default settings (Puillandre et al., 2012). *Galerina* sequences containing only ITS1 or ITS2 data were excluded from the ABGD analysis as the software could not compute pairwise distances between sequences missing large numbers of nucleotides. This software recovered five ‘partitions’ (i.e. collections of groups) of

varying stringency, yielding between 47 and 68 ‘groups’ (i.e. candidate species). ABGD is intended only to provide possible delimitations, and the final choice of partition should be made in conjunction with other lines of evidence for the proposed species delimitations (Puillandre et al., 2012). The partition comprising 68 species of *Galerina* was chosen after comparing the proposed species boundaries with the constraint + ITS phylogeny, nucleotide polymorphism data and morphological data. The 68 species partition was the only partition that, along with the aforementioned data, supported multiple species delimitations in the *G. marginata* complex. This partition also maximized monophyletic species with the highest phylogenetic support, without over-grouping taxa together. One group containing only sample *G. marginata* uwodd6mo221929 was proposed by ABGD but was rejected as a unique species due to being nested in a well-supported clade and lacking additional evidence for its status as a unique species.

Additional support for species delimitation in the *G. marginata* complex came from ITS polymorphism data. ITS sequences were imported into Mesquite 3.5 (Maddison and Maddison, 2018) and were organized by species according to the partition above. Using the ‘remove invariant characters’ function, variable sites were analyzed for the presence of shared polymorphisms and fixed nucleotide characters. For all tentative species, multiple fixed characters were observed with no evidence of shared polymorphisms. These data supplemented ABGD groupings and phylogenetic support from multiple loci for delimiting candidate species.

Each delimited species was given a tentative name. Sequences from 15 of A.H. Smith’s type specimens fell into 11 of the groups in the 63 species partition: when a type fell within a delimited clade, the clade was assigned the name associated with the oldest type specimen in the

clade. The remaining clades were also given provisional names based largely on the identifications of mycologist G. Gulden (Table A.2).

2.2.5 Amanitin Detection

Sixty-nine specimens were selected for analysis: 61 *Galerina*, four *Gymnopilus*, three *Hebeloma* and one *Flammula.* For each sample, two ~5 mg tissue samples were removed and placed in individual 1.5 mL Eppendorf tubes (except 25 specimens from which only one 5 mg sample was removed due to lack of material). Four extraction methods were tested on a single *G. marginata* sample to compare and maximize amanitin extraction efficiency: (1) no tissue grinding, (2) grinding with a plastic pestle, (3) grinding with a wooden stir stick and (4) vortexing the tissue with a glass bead. Tissue grinding with a wooden stir stick yielded the most efficient extraction and was the method used for all subsequent samples. After grinding, 50% methanol was added to each tube at a ratio of 40 µL/mg starting tissue.

After 24 hours, samples were centrifuged at 13,300 rpm for 10 minutes in an accuSpin Micro 17 centrifuge (Thermo Fisher Scientific: MA, USA) and the supernatant was transferred to a new 1.5 mL tube. The solution was spun for 30-60 minutes in a SavantTM SPD111V SpeedVap (Thermo Fisher Scientific: MA, USA) to remove ≥50% of the 50% methanol solution. Autoclaved distilled H_2O was then added to reconstitute the solution to a final volume of 200 µL. Samples were once more centrifuged at 13,300 rpm for 10 minutes. Finally, 110 µL of the supernatant was loaded to individual 1.5 mL glass autosampler vials with 0.15 mL glass inserts. As a positive control, one vial containing 110 µL of 0.2 µg/µL α-amanitin standard (SIGMA A2263) dissolved in water was included. Injection volume for HPLC/MS analyses was 100 µL.

Chromatographic separation was performed using a Proto 300 C18 column (RS-2546-W185, Higgins Analytical: CA, USA) attached to an Agilent 1200 series HPLC, multi-wavelength detector, and Agilent 6120 Quadrupole MS (Agilent Technologies: CA, USA), with detection at 220, 280, 295 and 310 nm. Elution solution A was 20 mM ammonium acetate (adjusted to pH 5 with 6 M HCl) and solution B was 100% acetonitrile. The flow rate was 1 mL/min, with a gradient of 100% solution A to 100% solution B over 20 minutes. A column re-equilibration period of 10 minutes at 100% solution A was included at the end of each run.

Presence or absence of α-amanitin was first determined via HPLC and UV absorbance and confirmed by MS. The α-amanitin standard showed an absorption peak at 310 nm at 8.5-minute retention time, coupled with strong MS signals for an ion with a mass/charge ratio of 919 (M+H+). The chromatograms for each *Galerina* sample were first checked for 310 nm peaks at 8.5 minutes and extracted ion chromatogram (EIC) MS data were scanned for compounds at 8.5 minutes with a mass/charge ratio of 919. Where UV absorbance, retention time, and MS showed evidence of α-amanitin, samples were recorded as positive.

2.3 Results

Of the 61 *Galerina* samples assayed for the presence of amatoxins, all toxin-positive samples belonged to sect. Naucoriopsis: 24/25 samples from this group were unambiguously positive for the presence of α-amanitin, with 19 of these 24 also containing β-amanitin (Suppl. Table 1). Only one sample (*G. badipes*) from this subgenus was toxin-negative. *G. marginata s.l.* and *G. sulciceps* also belong to sect. Naucoriopsis and have been reported in the literature as containing amatoxins but were unconfirmed as toxin-producers in this study (Fig. 2.1)

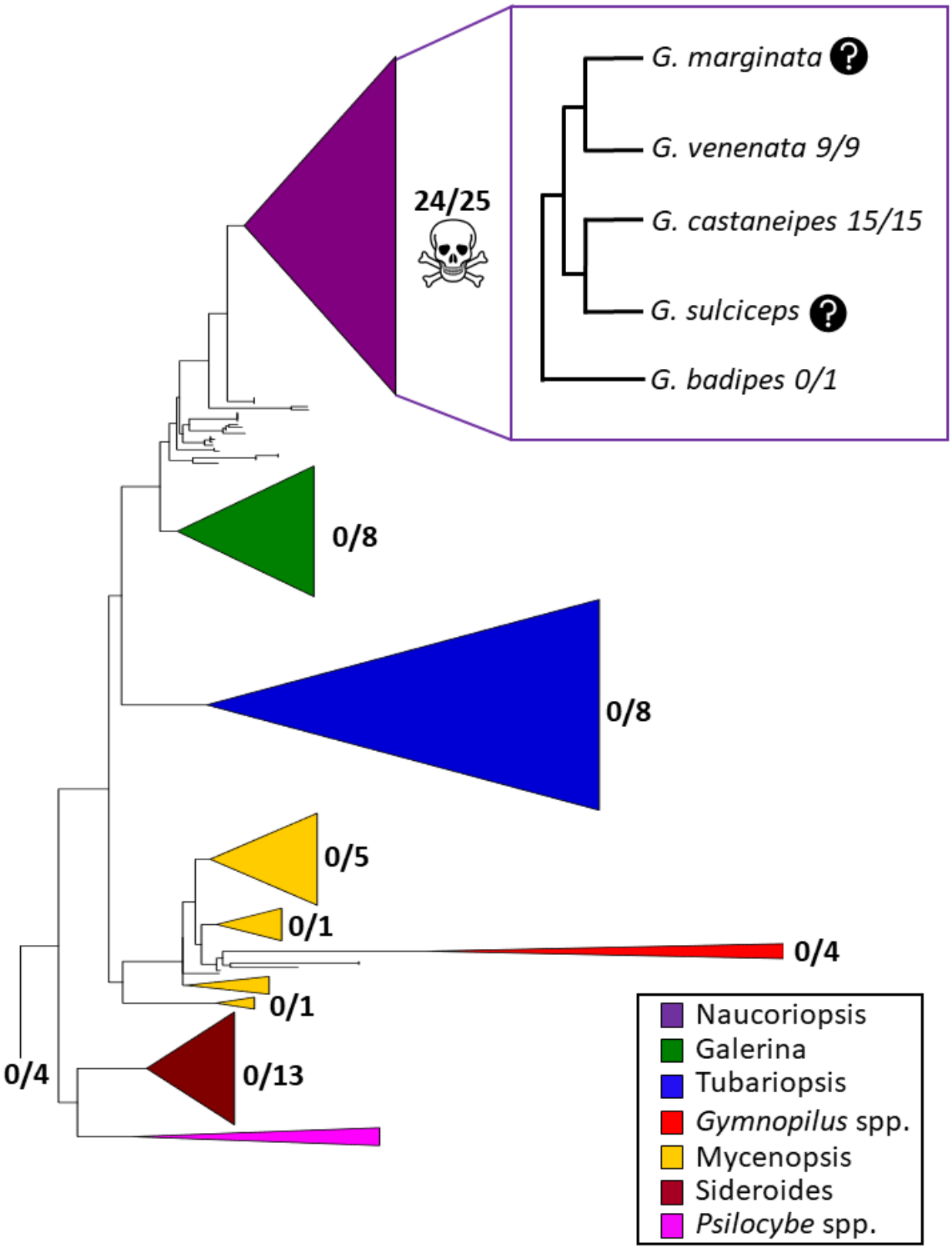

24/25
G. marginata ?
G. venenata 9/9
G. castaneipes 15/15
G. sulciceps ?
G. badipes 0/1
0/8
0/8
0/5
0/1
0/4
0/1
0/4
0/13
Naucoriopsis
Galerina
Tubariopsis
Gymnopilus spp.
Mycenopsis
Sideroides
Psilocybe spp.

Figure 2.1 - Maximum likelihood tree placing *Galerina* ITS sequences within a multi-locus (ITS, LSU, RPB2) constraint tree. Numbers on the right of each clade show the number of toxin-positive samples tested for the presence of toxins. Clade colors correspond to infrageneric units also recovered by Gulden (2005) (purple: sect. Naucoriopsis, green: sect. Galerina, blue: sect. Tubariopsis, yellow: sect. Mycenopsis). Three additional clades are indicated (red: *Gymnopilus* spp., magenta: *Psilocybe* spp., brown stirps Sideroides [Smith & Singer 1964]). Insert – cladogram showing relationships and toxin presence within sect. Naucoriopsis. Note that while other samples fall within this section, only those for which toxin data was collected or retrieved from the literature are shown. Taxa marked with (?) represent species for which toxin data is reported in the literature but without supporting DNA evidence confirming their identity

Using the approximate boundaries defined by Gulden et al. (2005) and the ABGD partition delimiting seven members of the *G. marginata* complex, sect. Naucoriopsis contained 14 species (*G. venenata* through *G. jaapii*). Where applicable, species were named according to the oldest type specimen in the clade. For species containing UBC samples, name justification was assisted by identifications from mycologist and *Galerina* scholar G. Gulden or using whichever name was present in the most samples (Table 2.4). For all remaining species, the name associated with the most samples was chosen.

Support for the 'Naucoriopsis' lineage was low-moderate in the LSU-only dataset (61% bootstrap support, Fig. A.2), moderate-high in the ITS-only dataset (85% bootstrap support, Fig. A.1) and high in the concatenated constraint tree dataset (99% bootstrap support, Fig. A.4). No RPB2 data was available for *G. jaapii*, limiting any inferences from this locus. Adding ITS sequences to the constraint tree yielded high support for most of the proposed 14 taxa in this subgenus, excluding instances where branch-length differences were minor (e.g. *G.* aff. *marginata* sp. 4) or large (e.g. *G. makereriensis*) (Fig. 2.2).

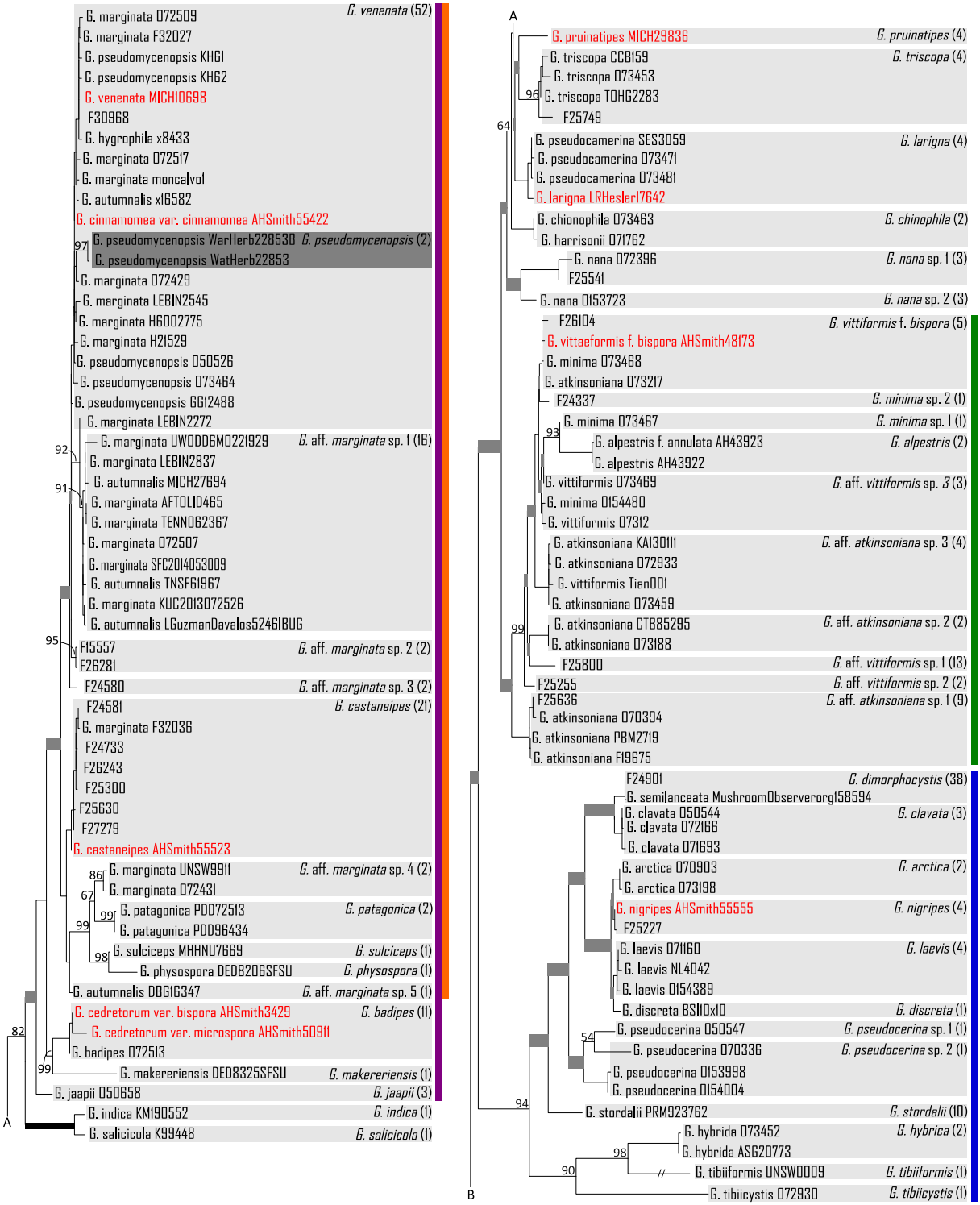
Without further sampling and analysis, the exact number of species in the *G. marginata* complex cannot be unambiguously determined due to poor resolution in the constrained ITS dataset (Fig. 2.2). The limits as shown were chosen based on ABGD data supplemented with support from individual locus analyses (Figs. A.1-3), as well as the apparent distinctness of the

G. patagonica-G. physospora clade (Fig. 2.2). The constrained ITS tree (Fig. 2.2) and each single-locus analysis plus the concatenated dataset (Figs. A.1-4) support the inclusion of toxin-producing *G. castaneipes* and *G. venenata* in the broader *G. marginata* complex. This complex appears sister to a clade containing *G. badipes* (toxin-negative) and *G. makeriensis* (untested), suggesting that toxin production is restricted to the *G. marginata* complex. Together with *G. jaapii*, the *G. marginata* complex and the sister *G. badipes/G. makereriensis* clade comprise the Naucoriopsis lineage as defined above (Fig. 2.2).

Table 2.4 – Name justification for delimited species where more than one sample name was present in the clade. For clades comprised entirely of samples with a single name, this name was used.

Species Name	Justification
G. aff. *atkinsoniana* sp. 1	Six *G. atkinsoniana* samples from Ceskas/Gulden/Matheny collections (+two *G. vittiformis* from Ceskas) – preference given to *G. atkinsoniana*.
G. aff. *atkinsoniana* sp. 2	Three *G. atkinsoniana*, one *G. vittiformis* – preference given to *G. atkinsoniana*.
G. badipes	Gulden lists synonymy of *G. cedretorum* and *G. badipes* (Gulden & Hallgrimsson 2000). Index Fungorum has *G. badipes* as current name for *G. cedretorum*.
G. castaneipes	Smith holotype sequence; possible that *G. oregonensis* is more fitting (spore measurements are closer to *G. oregonensis* for length ((*G. castaneipes* spores reported 7-9.5(10) and oregonensis 7-8.5; max spore size measured from our specimens = 8.5)), but *G. castaneipes* for width). However, *G. castaneipes* name predates by almost ten years.
G. chionophila	One *G. chionophila*, one *G. harrisonii* (Dennis) Bas & Vellinga. Another *G. harrisonii* specimen was given name preference, therefore Gulden's *G. chionophila* name used here.
G. dimorphocystis	*G. dimorphocystis* (Smith & Singer 1955) oldest name of G. heterocystis/*G. dimorphocystis*/*G. semilanceata*. *G. semilanceata* described exclusively from PNW, but *G. dimorphocystis* and *G. heterocystis* also described in PNW (+ other locales in Smith & Singer 1964).
G. fallax	Contained Gulden *G. fallax* samples. Odd mixture of names from Ceska collections, but DNA data not suggestive of contamination.
G. lubrica	Contained *G. lubrica* holotype.
G. larigna	Contained *G. larigna* specimen observed by Smith.
G. luteolosperma	Contained Gulden *G. luteolosperma*.
G. mammillata	Smith describes *G. mammillata* as drying to white (vs. *G. sideroides* drying to brown); all samples here dry white.
G. aff. *marginata* spp. 1-5	All contain various names once synonymized with *G. marginata* (Gulden et al. 2001), but the absence of a *G. marginata* type sequence renders identification of exact *G. marginata* clade impossible.
G. mimima sp. 1	Two *G. minima*, one *G. vittiformis* – preference given to *G. minima*.
G. mniophila	Abundant Gulden samples from both Europe and NA.
G. nana	Clade of only *G. nana* samples.

Species Name	Justification
G. nigripes	Smith monograph material sequence; also contained 2/21 *G. heterocystis* here - possible misidentifications.
G. aff. *pseudobadipes*	One *G. pseudobadipes*, one *G. stylifera* - Gulden *G. pseudobadipes* given preference.
G. pumila var. *subalpina*	Smith monograph material sequence.
G. aff. *sideroides* sp. 1	9/12 samples ID'd as *G. sideroides* (three *G. stylifera*). Smith suggests using Friesian name (*G. sideroides*) in data shows that *G. sideroides* and *G. stylifera* same. There are three or four *G. sideroides*/*G. stylifera* clades so it is unclear which name goes with which clade.
G. sphagnicola	One *G. sphagnicola*, one *G. calyptrata* – preference given to Gulden *G. sphagnicola*.
G. aff. *stylifera* sp. 1	One each of *G. sideroides*, *G. pseudobadipes* and *G. stylifera* – O'Dell name given precedence.
G. stylifera var. *badia*	Smith holotype sequences for *G. stylifera* var. *caespitosa* (Smith & Singer 1964) and *G. stylifera* var. *badia* (Smith & Singer 1958) fall here; *G. stylifera* var. *badia* older name.
G. subcerina var. *subcerina*	Smith monograph material sequence. Also contains Gulden's *G. calyptrata*, so may ultimately be changed.
G. triscopa	Contained Gulden *G. triscopa* samples.
G. venenata	Smith holotype sequence for *G. venenata* and *G. cinnamomea* var. *cinnamomea* – *G. venenata* older name (Smith 1952).
G. vexans	Smith paratype sequences (x2 samples).
G. aff. *vittiformis* sp. 1	Clade is exclusively *G. vittiformis* samples from Ceskas; closely related to *G. vittiformis* f. *bispora* (Smith sequence).
G. aff. *vittiformis* sp. 2	Clade is exclusively *G. vittiformis* samples from Ceska collection; closely related to *G. vittiformis* f. *bispora* (Smith sequence).
G. aff. *vittiformis* sp. 3	Two *G. vittiformis*, one *G. minima* – preference given to *G. vittiformis*.
G. vittiformis f. *bispora*	Smith monograph material sequence.


G. venenata (52)
G. marginata D72509
G. marginata F32027
G. pseudomycenopsis KH61
G. pseudomycenopsis KH62
G. venenata MICH10698
F30968
G. hygrophila x8433
G. marginata D72517
G. marginata moncalvo1
G. autumnalis x16582
G. cinnamomea var. cinnamomea AHSmith55422
G. pseudomycenopsis WarHerb228538 G. pseudomycenopsis (2)
G. pseudomycenopsis WatHerb22853
G. marginata D72429
G. marginata LEBIN2545
G. marginata H6002775
G. marginata H21529
G. pseudomycenopsis D50526
G. pseudomycenopsis D73464
G. pseudomycenopsis GG12488
G. marginata LEBIN2272
G. marginata UWOOD6M0221929 G. aff. marginata sp. 1 (16)
G. marginata LEBIN2837
G. autumnalis MICH27694
G. marginata AFTOLID465
G. marginata TENN062367
G. marginata D72507
G. marginata SFC20140530O9
G. autumnalis TNSF61967
G. marginata KUC20130725Z6
G. autumnalis LGuzmanDavalos524618UG
F15557 G. aff. marginata sp. 2 (2)
F26281
F24580 G. aff. marginata sp. 3 (2)
F24581 G. castaneipes (21)
G. marginata F32036
F24733
F26243
F25300
F25630
F27279
G. castaneipes AHSmith55523
G. marginata UNSW9911 G. aff. marginata sp. 4 (2)
G. marginata D72431
G. patagonica PDD72513 G. patagonica (2)
G. patagonica PDD96434
G. sulciceps MHHNU7669 G. sulciceps (1)
G. physospora DED8206SFSU G. physospora (1)
G. autumnalis DBG16347 G. aff. marginata sp. 5 (1)
G. cedretorum var. bispora AHSmith3429 G. badipes (11)
G. cedretorum var. microspora AHSmith5091I
G. badipes D72513
G. makereriensis DED8325SFSU G. makereriensis (1)
G. jaapii D50658 G. jaapii (3)
G. indica KMI90552 G. indica (1)
G. salicicola K99448 G. salicicola (1)
A
92
91
95
82
99
67
99
99
98
86
G. pruinatipes MICH29836 G. pruinatipes (4)
G. triscopa CCB159 G. triscopa (4)
G. triscopa D73453
G. triscopa TOHG2283
F25749
G. pseudocamerina SES3059 G. larigna (4)
G. pseudocamerina D73471
G. pseudocamerina D73481
G. larigna LRHesler17642
G. chionophila D73463 G. chinophila (2)
G. harrisonii D71762
G. nana D72396 G. nana sp. 1 (3)
F25541
G. nana D153723 G. nana sp. 2 (3)
F26104 G. vittiformis f. bispora (5)
G. vittaeformis f. bispora AHSmith48173
G. minima D73468
G. atkinsoniana D73217
F24337 G. minima sp. 2 (1)
G. minima D73467 G. minima sp. 1 (1)
G. alpestris f. annulata AH43923 G. alpestris (2)
G. alpestris AH43922
G. vittiformis D73469 G. aff. vittiformis sp. 3 (3)
G. minima D154480
G. vittiformis D7312
G. atkinsoniana KA130111 G. aff. atkinsoniana sp. 3 (4)
G. atkinsoniana D72933
G. vittiformis Tian001
G. atkinsoniana D73459
G. atkinsoniana CTB85295 G. aff. atkinsoniana sp. 2 (2)
G. atkinsoniana D73188
F25800 G. aff. vittiformis sp. 1 (13)
F25255 G. aff. vittiformis sp. 2 (2)
F25636 G. aff. atkinsoniana sp. 1 (9)
G. atkinsoniana D70394
G. atkinsoniana PBM2719
G. atkinsoniana F19675
F24901 G. dimorphocystis (38)
G. semilanceata MushroomObserverorg158594
G. clavata D50544 G. clavata (3)
G. clavata D72166
G. clavata D71693
G. arctica D70903 G. arctica (2)
G. arctica D73198
G. nigripes AHSmith55555 G. nigripes (4)
F25227
G. laevis D71160 G. laevis (4)
G. laevis NL4042
G. laevis D154389
G. discreta BSI10x10 G. discreta (1)
G. pseudocerina D50547 G. pseudocerina sp. 1 (1)
G. pseudocerina D70336 G. pseudocerina sp. 2 (1)
G. pseudocerina D153998
G. pseudocerina D154004
G. stordalii PRM923762 G. stordalii (10)
G. hybrida D73452 G. hybrica (2)
G. hybrida ASG20773
G. tibiiformis UNSW0009 G. tibiiformis (1)
G. tibiicystis D72930 G. tibiicystis (1)
A
B
64
96
94
54
98
90


B
C
Galerina arctica KH60 G. mniophila (10)
Galerina mniophila 050545
Galerina mniophila GG16088
Galerina mniophila MICH29880
Galerina mniophila 050679
Galerina mniophila 0154072
F27419
F25302
Galerina mniophila 073175
Galerina cephalotricha 0154146 G. cephalotrica (1)
Galerina pumila var. subalpina AHSmith56053 (6)
Galerina sphagnorum 070913 G. sphagnorum (2)
F27708 G. luteolosperma (2)
Galerina luteolosperma 0154076
F25365 G. fallax (9)
Galerina fallax 073450
F24816
Galerina fallax AK07KG1202R32Sp2
F28144
97
Galerina allospora m0289 G. allospora (2)
Galerina allospora 073460
Gymnopilus sp. F16466 Gymnopilus spp. (6)
Gymnopilus sapineus PBM1541WTU
Gymnopilus penetrans F23892
Gymnopilus spectabilis PBM2471CUW
Gymnopilus punctifolius F23761
Gymnopilus sp. F16467
Galerina sphagnicola 073441 G. sphagnicola (2)
Galerina harrisonii 050711 G. harrisonii (1)
Galerina fibrillosa LF1BA3A6 G. fibrillosa sp. 1 (1)
Galerina calyptrata MGW987 G. subcerina var. subcerina (6)
Galerina calyptrata m0280
Galerina calyptrata 073454
97
Galerina subcerina var. subcerina AHSmith61831
Galerina austrocalyptrata PDD97057 G. australocalyptrata (1)
Galerina hypnorum MICH46292 G. hypnorum (4)
Galerina hypnorum 073206
Galerina subcerina UNSW9931 G. subcerina (1)
Galerina fibrillosa MICH40850 G. fibrillosa sp. 2 (1)
Galerina lubrica AHSmith51977 G. lubrica (3)
Galerina paludosa 0153987 G. paludosa (4)
Galerina pumila 073440 G. vexans (4)
Galerina vexans AHSmith4371543719
Galerina pumila 073067 G. pumila (1)
F25633 G. mammillata (11)
F25750
F25762
Galerina pseudobadipes 0154252 G. aff. pseudobadipes (2)
Galerina stylifera x6920
Galerina sideroides CBS16246 G. stylifera var. badia (11)
Galerina stylifera var. badia AHSmith54112
Galerina stylifera var. caespitosa AHSmith41223
Galerina stylifera ODell4296 G. aff. stylifera sp. 1 (3)
F19775 G. aff. sideroides sp. 1 (12)
Psilocybe subaeruginosa TENN065481
Psilocybe cyanescens DNA1938TENN
Psilocybe cubensis DNA2052TENN
Psilocybe stuntzii VT1263
Psilocybe silvatica RV571989
Deconica montana PBM961WTU
Psilocybe caerulipes TENN064502
C
Anamika angustilamellata HKAS42927
Hebeloma affine TENN063921
Hebeloma aff remyi F23896
Hebeloma leucosarx F32044
Hebeloma velutipes PBM2277WTU
Hebeloma birrus F23894
Hebeloma olympianum BK21Nov9820UTC
undet. Hymenogastraceae PBM3420TENN
undet. Hymenogastraceae PBM3116TENN
Alnicola eschariodes PBM1719WTU
Phaeocollybia festiva PBM2366WTU
Flammula alnicola PBM2608CUW
Flammula alnicola F32022

Figure 2.2 - Maximum likelihood phylogeny placing *Galerina* ITS sequences within a multi-locus constraint tree. Species as delimited by ABGD were trimmed to include only samples with sequence variation. Light grey boxes indicate delimitation boundaries. Darker grey boxes indicate delimitation boundaries of species nested within another species. Names at the top right corner of boxes indicate the names given to the delimitations, whereas the number in parentheses represents the number of samples of a given species. Type specimens are indicated in red. Black thickened branches represent 100% bootstrap support for newly added sequences, whereas branches from the constraint tree are indicated in grey. Colored bars represent approximately the infrageneric units proposed by Gulden (2005) (purple: sect. Naucoriopsis, green: sect. Galerina, blue: sect. Turbariopsis, yellow: sect. Mycenopsis). Four additional clades are indicated (orange: *Galerina* marginata complex, red: *Gymnopilus* spp., magenta: *Psilocybe* spp., brown: stirps Sideroides [Smith & Singer 1964]).

Nucleotide polymorphism data also supports *G. marginata* as being multiple species. Although samples of this complex were found co-occurring in a very small (<1km^2) area (Observatory Hill, Victoria, BC), each locus shows fixed characters in multiple delimited species, with no characters showing evidence of shared polymorphisms that would indicate interbreeding among different lineages. In addition to *G. castaneipes* and *G. venenata*, support for two additional taxa in our samples – *G.* aff. *marginata* sp. 2 and *G.* aff. *marginata* sp. 3 – varied in the different datasets, ranging from no support in the LSU dataset to strong support in the RPB2 dataset (Fig. 2.2, Figs. A.1-3). Due to the small sampling of LSU and RPB2 sequences, only ITS data has been included (Fig. 2.3).

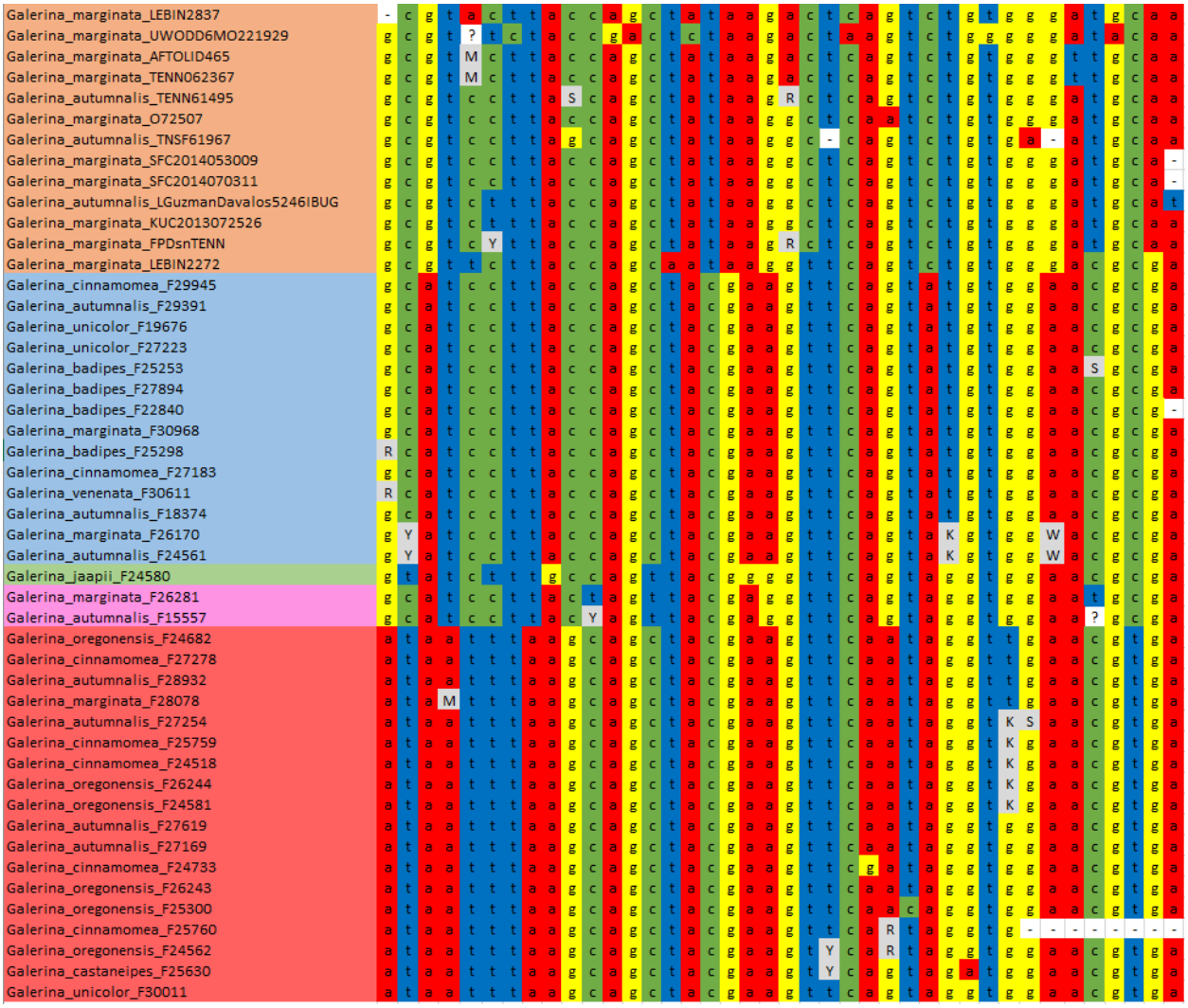


Figure 2.3 - ITS nucleotide character states among sympatric *Galerina* (taxon backgrounds: Blue: *G. venenata*, Green: *G.* aff. *marginata* sp. 3, Magenta: *G.* aff. *marginata* sp. 2, Red: *G. castaneipes*). A closely related species of *Galerina* (*G.* aff. *marginata* sp. 1, orange) for which no samples were found in BC is included for comparison.

All remaining samples yielded unambiguously negative results, suggesting that toxin production in *Galerina* is limited to certain members of lineage 'Naucoriopsis'. The other 36 *Galerina* samples assayed belonged to *Galerina* lineages 'Galerina' (three species, 0/8 toxin-positive samples), 'Tubariopsis' (two species, 0/8), stirps Sideroides (4 species, 0/13) and one unclassified sample (0/1). Four outgroup samples comprising three species of *Hebeloma* (0/3)

and one species of *Flammula* (0/1) were also assayed and tested negative for the presence of amatoxins (Fig. 2.1).

The remaining ten samples belonged to members of *Galerina* lineage 'Mycenopsis' (four species, 0/6 toxin-positive samples) and the genus *Gymnopilus* (four species, 0/4 toxin-positive samples). Originally included as an outgroup, members of *Gymnopilus* formed a monophyletic clade with members of *Galerina* lineage 'Mycenopsis' in the constrained ITS tree (Fig. 2.2). Although the level of support and phylogenetic relationship between 'Mycenopsis' and *Gymnopilus* spp. varied among loci, samples of both consistently grouped together: in the ITS phylogeny, 'Mycenopsis' was nonmonophyletic with members of 'Tubariopsis' and *Gymnopilus* spp. (Fig. A.1). In the LSU dataset, 'Mycenopsis' was nonmonophyletic, grouping with some members of this section forming a well-supported (99% bootstrap support) clade with *Gymnopilus* spp. (Fig. A.2). In the RPB2-only phylogeny, 'Mycenopsis' and *Gymnopilus sapineus* were monophyletic with moderate-low support (61% bootstrap support, Fig. A.3). Lastly, *Gymnopilus* spp. and 'Mycenopsis' formed a well-supported (85% bootstrap support) clade in the concatenated constraint tree dataset (Fig. A.4).

The phylogenetic relatedness of other *Galerina* infrageneric units varied among datasets. The constrained ITS topology (Fig. 2.2) places lineage 'Galerina' in a monophyletic group with 'Naucoriopsis' only if nine unclassified species (*G. indica* through *G. camarinoides*) are included in this latter subgenus. *Galerina* lineage 'Tubariopsis' appears as sister to the 'Naucoriopsis' + 'Galerina' clade, and this relationship is also supported in the RPB2-only and the concatenated constraint datasets (Fig. 2.1, Figs. A.3-4). 'Mycenopsis' + *Gymnopilus* spp. form a monophyletic clade that is sister to 'Naucoriopsis' + 'Galerina' + 'Tubariopsis' in the constrained ITS, RPB2 and concatenated constraint trees (Fig. 2.2, Figs. A.3-4). In the

constrained ITS, ITS-only, RPB2-only and concatenated constraint datasets, samples of *Psilocybe* were more closely related to *Galerina* than to the other outgroup taxa, although this relationship had no support in any dataset (Fig. 2.2, Figs. A.1, A.3-4).

2.4 Discussion

2.4.1 Species delimitations reveal both conspecificity and diversity among *Galerina* samples

Current understanding of most *Galerina* species comes largely from Smith & Singer's (1964) monograph. These descriptions – based primarily on morphological data with occasional habitat information – incorporate many sources of uncertainty. Many descriptions include notes regarding the visual similarities between different species, as well as the difficulties in distinguishing members of certain complexes. The data collected in my study highlight the additional insights gained by using molecular methods to supplement morphological data.

Although the proposed species delimitations are based on De Quieroz' (2007) idea that independently evolving metapopulation lineages ('species') show multiple evolutionary properties, it should be noted that not all delimitations were made based on the same criteria, and that species differ in the amount of evidence supporting their delimitation. Notably, Carstens et al. (2013) suggest that a minimum of 10 samples from each lineage are required to ensure a high (>90%) chance of capturing meaningful genetic variation is captured, particularly the deepest coalescence events in a given population. Of the species delimitated here, 10 contained 10 or more samples. Fewer samples were available for the remaining species. Nevertheless, the species delimited using ABGD were in many cases consistent with groups receiving phylogenetic support in the multi-locus and single-locus trees (Fig. 2.2; Figs. A1-A4).

The results of this study suggested that the some of Smith and Singer's (1964) species should be revisited. In three cases, synonymy of Smith and Singer's species may be appropriate: first, based on 38 samples from western North America (British Columbia to California), *G. dimorphocystis, G. heterocystis* and *G. semilanceata* shared nearly identical DNA sequences that differed by at most two nucleotides in their ITS regions. Second, a sample identified by Smith and Singer (1964) as *G. larigna* was part of a monophyletic group with all samples identified as *G. pseudocamerina*, suggesting possible conspecificity between these two taxa. Lastly, Gulden and Hallgrimsson (2000) synonymized *G. cedretorum* var. *bispora* with *G. badipes*. Type specimens of both *G. cedretorum* var. *bispora* and *G. cedretorum* var. *microspora* grouped together in a clade, along with other specimens identified as either *G. cedretorum* or *G. badipes,* supporting this synonymization.

In contrast, my phylogeny revealed many more cases of unexpected diversity. Samples identified by collectors under names including *G. marginata, G. atkinsoniana, G. vittiformis, G. sideroides, G. stylifera, G. pseudocerina* and *G. nana* each corresponded to multiple (two or more) delimited clades. For many of these taxa, Smith and Singer (1964) described various varieties and/or forms (e.g. three varieties and four forms of *G. vittiformis,* four varieties of *G. stylifera,* etc.). However, without sequences from type specimens for the species level and for the subspecific level, it has been impossible to verify which names apply to some clades. For example, a specimen identified by Smith and Singer (1964) as '*G. vittiformis* f. *bispora*' was sequenced, but no type specimen DNA sequence is available for *G. vittiformis*. It was therefore not possible to determine which of the three *G. vittiformis* clades represents the true *G. vittiformis*. Further complications arose in cases such as *G. pumila,* where the clades identified as

G. pumila and *G. pumila* var. *subalpina* both belonged to the 'Mycenopsis' lineage, but did not form a monophyletic group as expected from a species and its varieties.

2.4.2 *Gymnopilus* spp. is nested within *Galerina*

In a broad ITS-based phylogenetic study of the Agaricales, Moncalvo et al. (2002) reported weak phylogenetic support for a clade containing *Gymnopilus* and a single *Galerina paludosa* sample. In two subsequent phylogenetic studies *Gymnopilus* was found to be monophyletic: while both studies included *Galerina* samples that remained phylogenetically distinct from *Gymnopilus*, these studies did not include samples of *G. paludosa* or its close relatives (Guzmán-Dávalos et al., 2003; Rees et al., 2002). However, in a dataset consisting of LSU sequences from dark-spored agarics, members of the genus *Gymnopilus* were supported as sister to multiple samples of *Galerina paludosa*, and these taxa collectively were part of a monophyletic group with other members of *Galerina* lineage 'Mycenopsis' (Gulden et al., 2005). This relationship was further supported by Walther et al. (2005) who also found phylogenetic support for a clade including a sample of *G. paludosa* and samples of *Gymnopilus*.

The results of this study further strongly support including *Gymnopilus* spp. within *Galerina* lineage 'Mycenopsis' (Fig. 2.2; Figs. A.1-4). The underlying relationship of these genera is complex: certain members of *Gymnopilus* share similarities with *Galerina* in regards to their spore color, shape, size, ornamentation and in the presence of a plage (Gulden et al., 2005). While gross morphological characters can differ quite widely between members of these genera, some *Gymnopilus* species share 'little brown mushroom' characteristics and can be difficult to distinguish from certain *Galerina* species (Rees et al., 1999). The presence of styrylpyrone pigments in some small species of *Gymnopilus* was previously used to distinguish these from

Galerina, as 3 members of the latter genus were found to not contain these pigments (Rees et al., 1999). However, more recent research has revealed that certain *Gymnopilus* species do not contain this pigment, further complicating distinction between certain *Gymnopilus* and *Galerina* based on this trait.

Despite the apparent close relationship between these two genera, further phylogenetic and morphological studies are required before proposing any taxonomic changes. Notably, most studies – this one included – focused primarily on either *Galerina* or *Gymnopilus*, without including a variety of samples from both genera. The results of this study and others have provided enough evidence to merit additional investigation in to the relationship between these two mystifying genera.

2.4.3 *Galerina marginata* is a species complex

My study also gave special attention to the apparent diversity present in *G. marginata* and closely related species. Following the proposed synonymy of Gulden et al. (2001), *G. marginata* remained as the lone toxic *Galerina* occurring in North America. However, my results showed that *G. marginata* is likely a species complex. Using the aforementioned definition of species, the following are various 'properties' or lines of evidence suggesting a difference in evolutionary history between at least two species (*G. venenata* and *G. castaneipes*) in our collections: first, in the multi-locus tree (Fig. 2.2) and the ITS-only tree (Fig. A.1), *G. castaneipes* was monophyletic, albeit with no bootstrap support. Although *G. venenata* was not monophyletic in these trees, this may suggest recent speciation events within the *G. marginata* complex.

Second, DNA sequences of the ITS region showed that members of these clades collected in sympatry showed little evidence of sharing genetic information. Polymorphic/heterozygous sites were not shared between members of individual clades but were instead restricted to individual lineages. Additionally, multiple nucleotide characters were fixed within individual lineages (Fig. 2.3).

Finally, spore width and length measurements were both statistically significantly different ($p < 0.05$) between 6 samples (mean of 30 spores for each sample) of *G. castaneipes* and *G. venenata* (Figs. A.5, A.6; Table A.1).

These data suggested that the previous suggestion to merge *G. marginata, G. oregonensis, G. unicolor, G. venenata* and *G. autumnalis* into *G. marginata* (Gulden et al., 2001) into a single species merits revision. Gulden et al. (2001) recognized a high degree of variation among *G. marginata s.l.* samples in the form of 4 different ITS region restriction length fragment polymorphism (RFLP) profiles, as well as branching and branch length differences within the *G. marginata s.l.* ingroup. However, specimen names from expert identifiers did not correspond to clades in Gulden et al.'s (2001) phylogenies. This lack of correlation between morphologically based identifications and clade structure led to a decision to synonymize.

Our results also showed a high degree of variation in samples identified as *G. marginata*. Delimited species containing type specimens for *G. castaneipes* and *G. venenata* were given the names of these clades, whereas other species lacking type specimens were named *G.* aff. *marginata* 1-5. This is the first report of *G. castaneipes* belonging to the broader *G. marginata* complex, although Smith and Singer (1964) recognized some morphological similarities between members of this complex and *G. castaneipes*. Furthermore, a clade comprising two samples of

G. pseudomycenopsis from Scotland was also delimited by ABGD. Gulden et al. (2001) suggested that *G. pseudomycenopsis* may be distinct from *G. marginata*: however, G. Gulden's own *G. pseudomycenopsis* samples fell in various clades throughout the *G. marginata* complex, creating additional confusion regarding the relationship of these species.

Five more clades were tentatively named *G.* aff. *marginata* sp. 1-5 based on support from phylogenetic, nucleotide polymorphism and ABGD delimitation data. Samples of *G.* aff. *marginata* sp. 1 were collected from various locations across the globe (Ontario, Mexico, Central USA, Russia, South Korea, Japan) but were not present in BC collections. Species 2 through 4 contained only one or two samples with unique ITS sequences and their delimitations were supported by ABGD. Lastly, two samples identified as *G.* aff. *marginata* sp. 5 were closely related to Southern hemisphere *Galerina* species. Given the broad distribution of samples identified by collectors as *G. marginata*, additional sampling and DNA sequencing will be required to elucidate the relationship of these species.

2.4.4 Toxin production in the *G. marginata* complex

Considering the proposed revision of the *G. marginata* complex, revision of the number of toxic *Galerina* species will also be required. First, the clade comprising sequences from the types of both *G. venenata* and *G. cinnamomea* is of interest: although specimens of *G. cinnamomea* having never been reported as toxic, *G. venenata* has been reported as toxic since the mid-20th century (Grossman and Malbin, 1954). All members of these clades (*G. venenata* [n = 9] and *G. castaneipes* [n = 24) tested positive for alpha-amanitin (Fig. 2.1; Table A.2). This is, to the best of my knowledge, the first report of *G. castaneipes* containing toxins.

Based on the current phylogenetic placement of toxin-producing *Galerina* and previous reports of *G. marginata* toxicity, samples belonging to *G.* aff. *marginata* clades 1-4 would be the next logical place to seek toxin-producing *Galerina*. *G.* aff. *marginata* sp. 5 is also a plausible toxin producer given its close relatedness to *G. sulciceps*. In our phylogeny, *G. sulciceps* is the only additional taxon that has been reported to produce amatoxins (Besl, 1981; Besl et al., 1984). However, the lone sample present in this phylogeny does not have associated toxin data. To date, there are no reports of toxic *G. patagonica* or *G. physophora*.

It is unclear why the two samples identified as *G. marginata* (AF501564 and AF251168) in this clade are distinct from the other *G. marginata* samples. However, sample AF501564 was collected from Australia, and clustered with other southern hemisphere samples. Additionally, sample AF251168 had a unique sequence and RFLP profile relative to other *G. marginata* samples as indicated by Gulden et al. (2001).

Despite this gap in data, toxin production in *Galerina* is likely to have a single origin. Given the stability of amatoxins, chromatographic analyses of specimens belonging to *G.* aff. *marginata* sp. 1-5 is likely feasible. As most of these specimens are vouchered and have available genetic data, obtaining chromatographic data from these specimens would contribute greatly to our understanding of toxin-production within *Galerina*.

2.4.5 *Galerina badipes* may not contain α-Amanitin

At least two reports of potential toxin production in *G. badipes* exist: Besl et al. (1984) first reported the presence of γ-amanitin in *G. badipes*, but, consistent with the findings of this study, found no evidence of α- or β-amanitin. Luo et al. (2012) report Westerdijk Fungal Biodiversity Institute (CBS) strain 268.50 (*G. badipes*) as hybridizing with one copy of the

Galerina marginata α-amanitin gene (*GmAMA1*) and prolyloligopeptidase B (POPB - responsible for cleaving the leading proline in the propeptide sequence and activating the octapeptide toxin) but did not use HPLC/LC-MS to test for the presence of amatoxins.

Luo et al. (2012) also demonstrated that upregulation of *GmAMA1* on low-carbon media was observed in *G. badipes*, consistent with other amanitin-producing species showing upregulated production under these conditions (Muraoka and Shinozawa, 2000). However, the quantities of α-amanitin produced by *G. badipes* appeared much lower than in *G. marginata* (Luo et al., 2012). The low quantities of α-amanitin may be below detection limits and may explain the perceived absence of this toxin in both Besl et al. (1984) and this study. Gamma-amanitin is not genetically encoded but is an α-amanitin variant, resulting from post-translation hydroxylation differences. Since both α- and γ-amanitin share the same core amino acid sequence of IWGIGCNP (Walton, 2018), the positive *GmAMA1* hybridization result may suggest that large quantities of α-amanitin are being converted to γ-amanitin, explaining the results of both Besl et al. (1984) and Luo et al. (2012). Additional sampling and use of modern analytical chemistry techniques in the *G. badipes* clade are necessary to better understand toxin production in this species.

2.4.6 α-Amanitin toxin production is consistent within species

Of the *Galerina* tested for toxins, all specimens in toxin-producing clades were found to contain detectable quantities of amanitin and all specimens outside these clades were unambiguously toxin negative. In contrast, variability was observed regarding β-amanitin production in *Galerina* (Table A.2). Presence of β-amanitin is variable within clades, with some samples showing no β-amanitin whatsoever. Unlike the current study, Luo et al. (2012) did not

observe β-amanitin in North American *Galerina*. However, β-amanitin has been reported in North American samples since the initial discovery of amanitins in *Galerina* by Tyler and Smith (1963).

Sgambelluri et al. (2014) note the possibility that some toxin producing fungi may contain an enzyme (e.g. deaminase) that could convert the asparagine found in α-amanitin to the aspartic acid found in β-amanitin. While Walton (2018) suggests that very low levels of β-amanitin peaks may also be an artifactual deamidation product of alpha-amanitin breakdown, many samples contain levels of β-amanitin much too high to be explained by this phenomenon. In these cases, the levels of β-amanitin match or surpass the already-high levels of α-amanitin: as such, the aging mycelium or extraction procedure would not be expected to produce such large quantities of β-amanitin byproduct. Although the published genome of *G. marginata* does not contain a gene encoding for β-amanitin, this toxin appears to be genetically encoded in *Amanita* (Hallen et al., 2007; Pulman et al., 2016).

2.4.7 Future directions

In the last decade, there has been a growing interest in the use of amanitin as a possible anti-cancer/anti-tumor tool has been observed (Anderl et al., 2011; Kume et al., 2016; Moldenhauer et al., 2012; Moshnikova et al., 2013). However, the cost of high-purity α-amanitin is still high, owing to production methods: until recently, research-grade amanitin was obtained via extraction and purification of dried mushroom samples or cultures.

Unlike the obligately mycorrhizal *Amanita*, *Galerina* is saprobic and can be grown in culture. However, the mycelium is slow growing, and quantities of amanitin in the mycelium range from 0.5-1 mg amanitin/g dry weight (Luo et al., 2012). Identifying new species of

amanitin-producing *Galerina* may lead to the discovery of faster-growing species, thus enhancing our capability to produce these toxic compounds in a laboratory setting. Additionally, critical steps in amanitin synthesis are still not understood, and identifying new species may help further our understanding of the complete biosynthetic pathway.

Furthermore, the usefulness of most cycloamanides in medical research has yet to be explored, although some exhibit immunosuppressant activity (Wieczorek et al., 1993). Toxin-producing *Galerina*, *Lepiota* and *Amanita* contain an enzyme, prolyl-oligopeptidase B (POPB), which cleaves the leading proline in the propeptide sequence and activats the octapeptide toxin. POPB isolated from *G. marginata* and expressed in *Saccharomyces cerevisiae* has shown promise as a catalyst for cyclization of novel cycloamanides (Sgambelluri et al., 2018). The properties of some cyclic peptides are desirable for pharmaceutical products – in particular, the peptides have a relatively good ADME profile (absorption, distribution, metabolism and excretion) (Ward et al., 2013). Given that only toxin producing species of fungi have the POPB gene, the POPB enzymes from the newly-identified toxin-producing *G. castaneipes* and *G. venenata* can be explored in the context of producing new, potentially therapeutic cycloamanides.

2.4.8 Conclusion

This study provided a comprehensive look at *Galerina* phylogenetics and may be the first the first to combine multi-locus sequence data with HPLC-LC/MS toxin analysis data. Consistent with previous reports, toxin producing *Galerina* appeared to be restricted to section Naucoriopsis. However, the number of toxin producers – particularly those in the *G. marginata* complex – needs reassessing. The combined phylogenetic analyses, nucleotide

character/polymorphism data and species delimitation data supported splitting *G. marginata* into multiple species. Samples identified as *G. marginata* (and synonyms) were dispersed broadly throughout a large complex comprising nine species. Specimens in the two clades containing the type specimens for *G. venenata* and *G. castaneipes* produced both α- and β- amanitin. Furthermore, our results were also consistent with previous reports suggesting that some members of the genus *Gymnopilus* fall within *Galerina.*

Annotations of specimen identifications resulting from the study of UBC and MICH specimens will be reflected in updated UBC herbarium database entries, in GenBank records and for Smith's specimens in the University of Michigan herbarium. This may be especially beneficial in the cases of suspected mushroom poisonings: a promptly obtained sequence from mushroom tissue from stomach samples or from the collection locale could identify a toxin producer and speed patient admission for intensive medical care. The restricted and monophyletic distribution of toxins in *Galerina* will allow health care professionals to better assess the acute poisoning risk of patients by comparing macro- and microscopic characteristics of ingested mushrooms with the traits of known toxic species.

The proper identification of mushroom collections is paramount in providing a framework for further critical studies of species biology, ecology and evolution. Clarifying the phylogenetic relationships and application of names among toxic *Galerina* will reduce complications and uncertainties arising from inaccurate identifications. Future studies on speciation, biochemistry and ecology in *Galerina* and other fungi stand to benefit from a better understanding of exactly which species are being studied, highlighting the importance of revisiting taxonomically or systematically challenging groups.

Chapter 3: Conclusion

To explore the distribution of amatoxins in the genus *Galerina*, phylogenetic and toxin analysis data were combined to make a multi-locus phylogeny with toxin data mapped to the tree. In doing so, additional evidence for drawing species and lineage boundaries in *Galerina* was collected, and increased diversity of toxin-producing *Galerina* in North America was discovered. Knowing the morphology of known toxin-producers, health care professionals can better understand which *Galerina* species pose a poisoning risk.

Results of phylogenetic analyses showed that *Galerina marginata* is a species complex, comprising multiple species including the tentatively-named *G. venenata* and *G. castaneipes*. To the best of my knowledge, this research reports the first evidence that *G. castaneipes* is toxic. Furthermore, the phylogenetic data provided additional evidence for past claims that members of the genus *Gymnopilus* are nested within *Galerina* (Gulden et al., 2005; Matheny et al., 2015). While formal taxonomic changes are pending, this additional evidence will provide the framework for future researchers to explore these taxa in greater detail.

The combined phylogenetic and toxin data also supported past claims that toxin-production in *Galerina* appears to be restricted to the 'Naucoriopsis' lineage (Enjalbert et al., 2004; Gulden et al., 2005). All specimens testing positive for the presence of amatoxins belonged to two species, *G. venenata* and *G. castaneipes*. Given numerous reports of the European *G. marginata* also being toxic, it is likely that this species also contains toxins; however, no representatives were tested in this study. One other reportedly-toxic species (*G. sulciceps*) was not tested but is in a clade sister to the *G. marginata* complex: in order to paint a clear picture of the origin of amatoxins, testing other members of these two clades will be

required. Lastly, a single sample of *G. badipes* revealed no evidence of α- or β-amanitin. Previous reports of its toxicity (Besl, 1981; Besl et al., 1984) may be due to γ-amanitin, the presence of which was not tested in this study. Additional data collection is required to confirm or deny any toxicity, and foragers should therefore continue to avoid this species.

The findings of this study will allow names of the specimens from the UBC herbarium used in this study to be updated based on new molecular data. Additionally, newly generated DNA sequence data for these specimens will also be uploaded or updated in the Genbank database. The supporting molecular data will hopefully prevent downstream issues resulting from inaccurate identification of samples both in herbaria and in online databases. Although additional work on the morphological differences between species is still required, updated species boundaries will allow healthcare professionals and mycologists to identify *Galerina* species with greater confidence and precision, particularly those that pose a poisoning risk.

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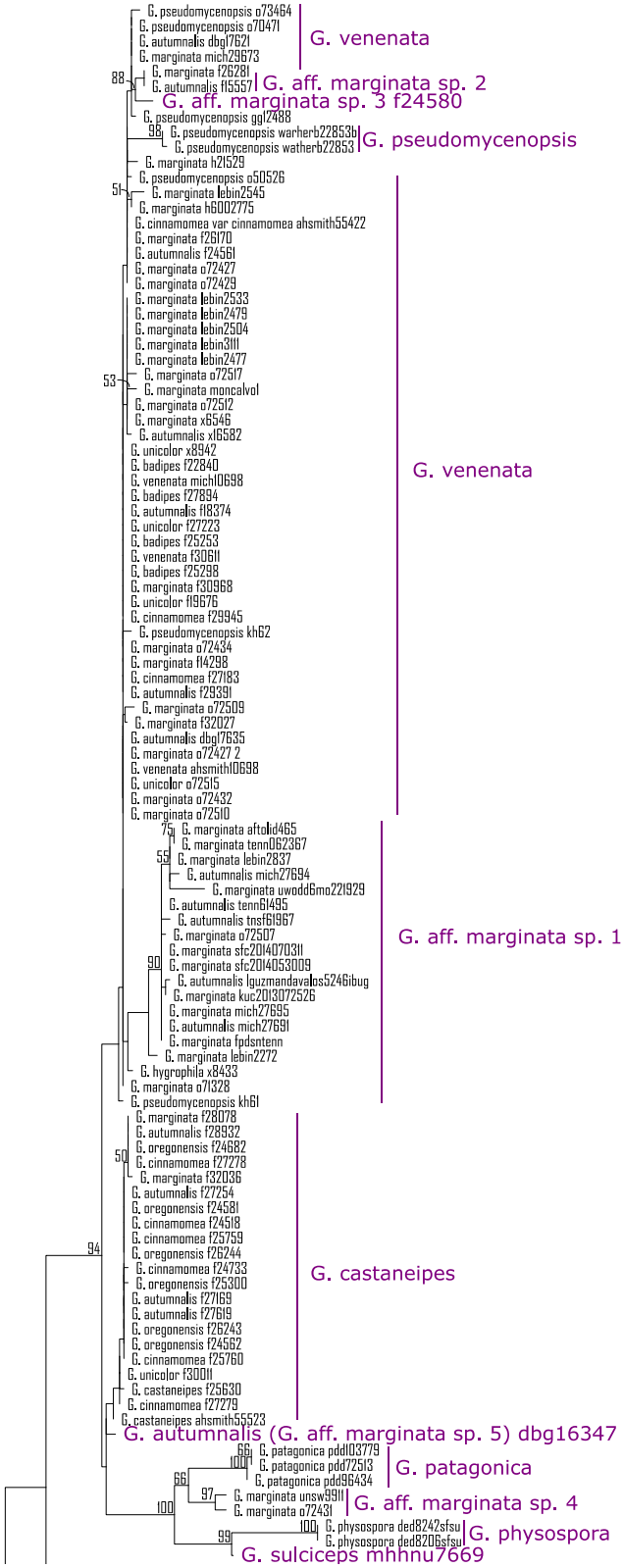
Appendix A: Supporting Figures and Tables for Chapter 2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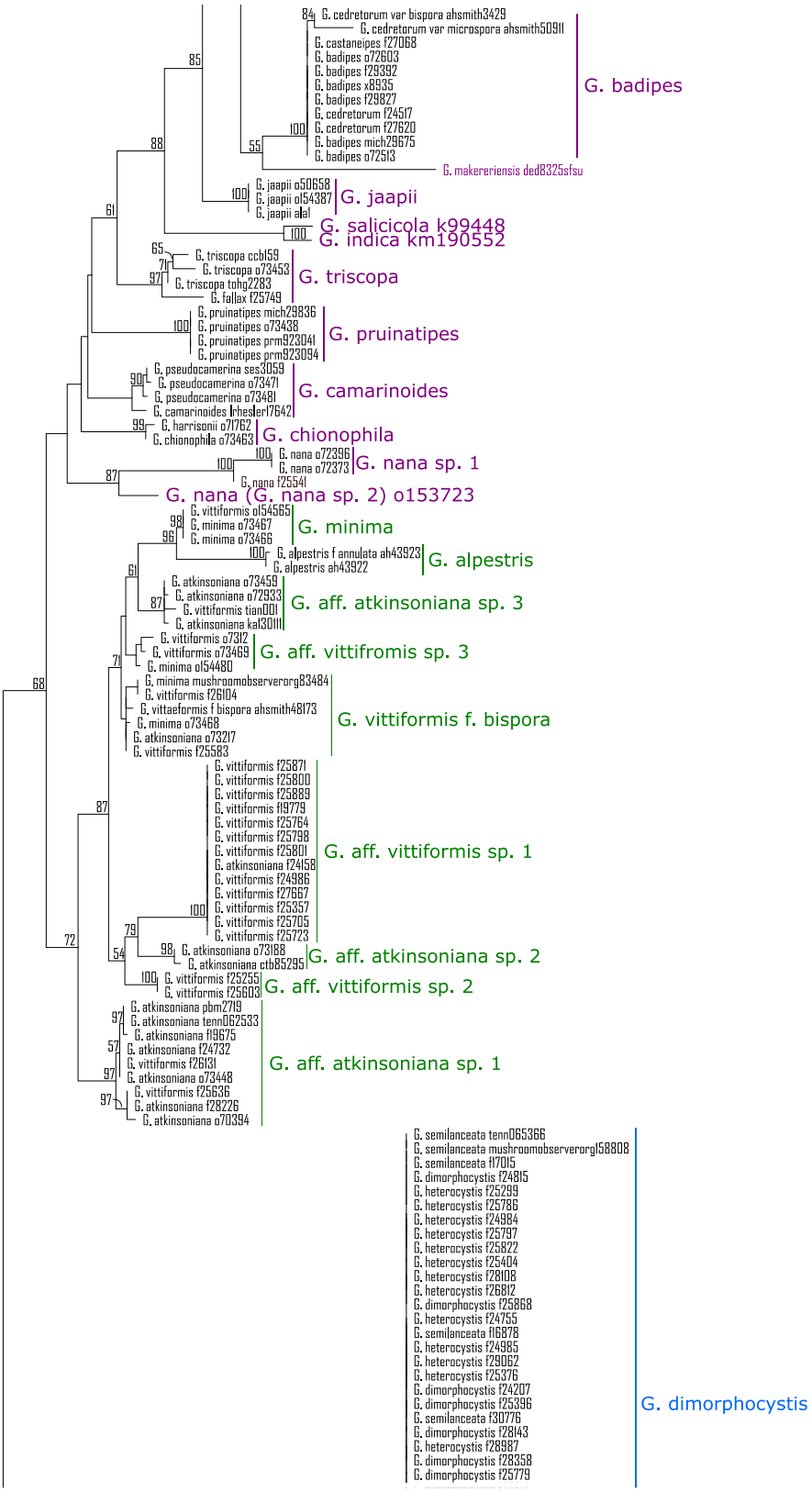
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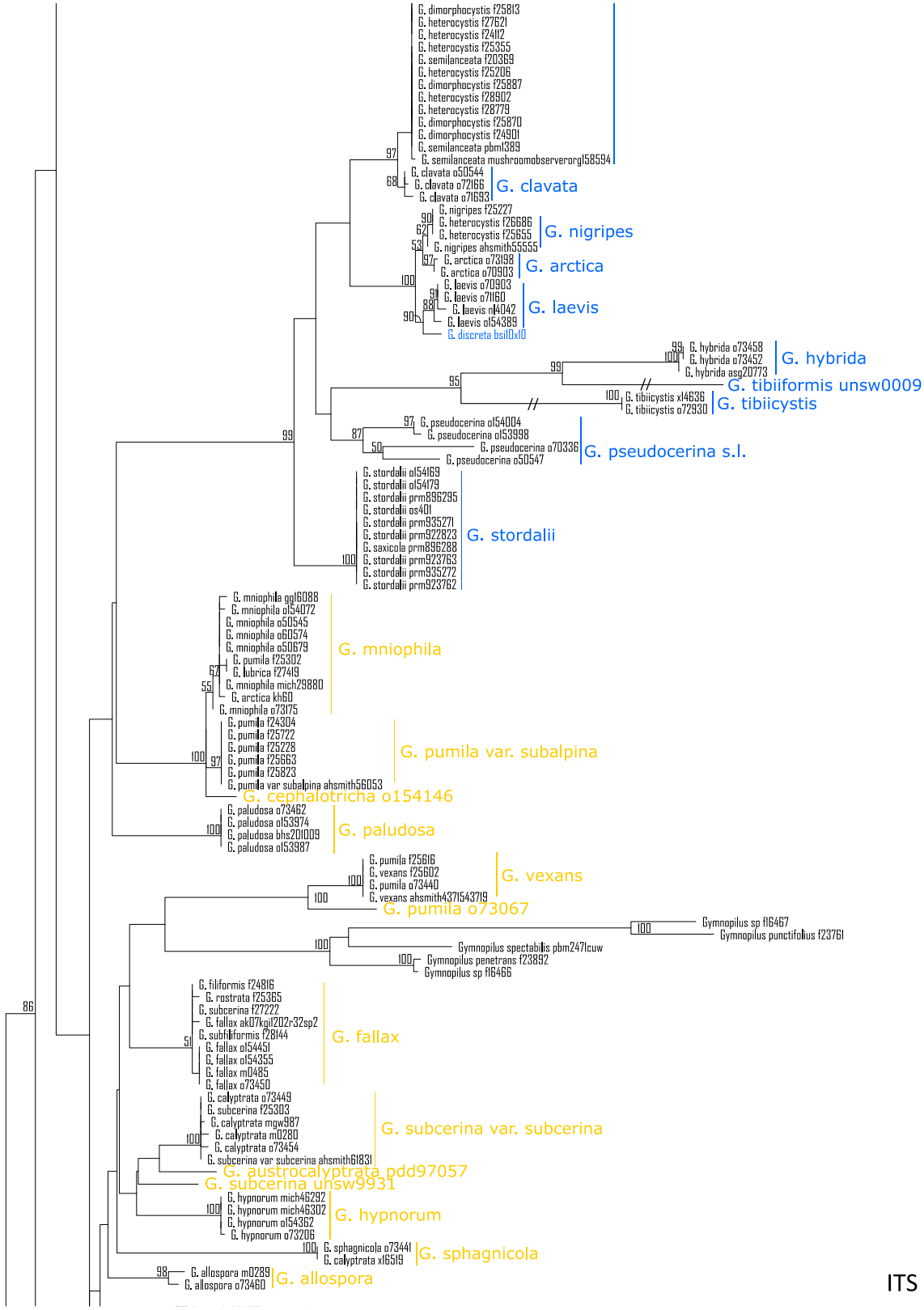
ITS Page 2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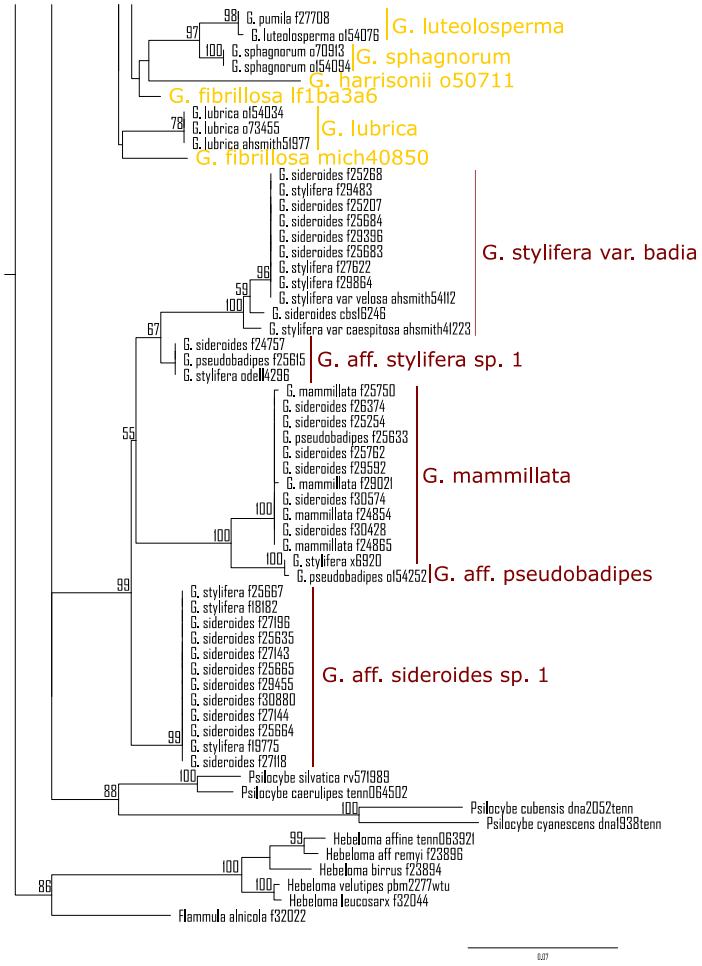
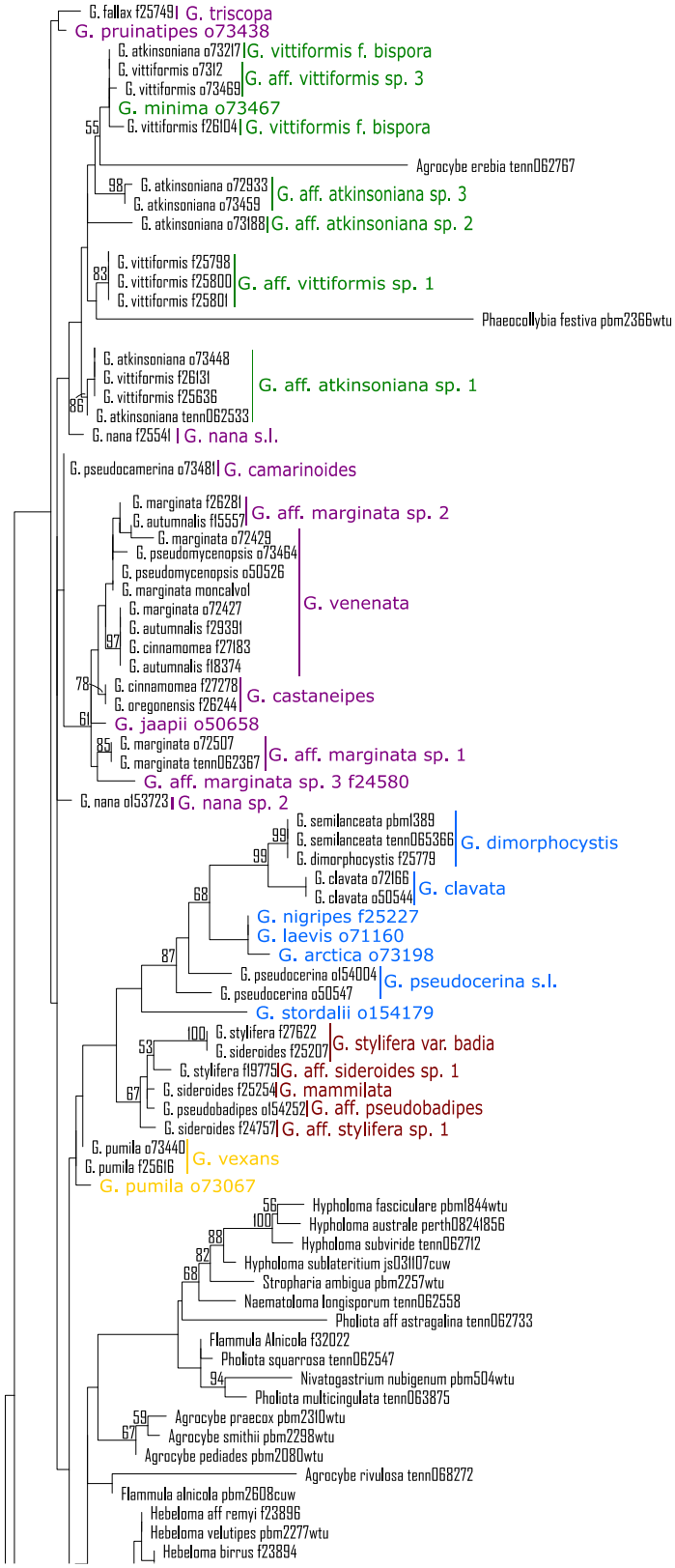


Figure A.1 - Maximum likelihood phylogeny of *Galerina* + outgroup ITS sequences. Data were aligned using MAFFT and analyzed with RAxML (200 ML tree searches, 1000 bootstrap replicates) using a GTR+G+I model. Only bootstrap values >50% are shown. Bars and species names represent delimitated species (Table A.2). Colored tip labels indicate a species delimitation in which the lone sample name matches the proposed delimited name. Colored bars represent approximately the infrageneric units proposed by Gulden (2005) (purple: sect. Naucoriopsis, green: sect. Galerina, blue: sect. Turbariopsis, yellow: sect. Mycenopsis). Two additional clades are indicated (red: *Gymnopilus* spp., brown: stirps Sideroides [Smith & Singer 1964]).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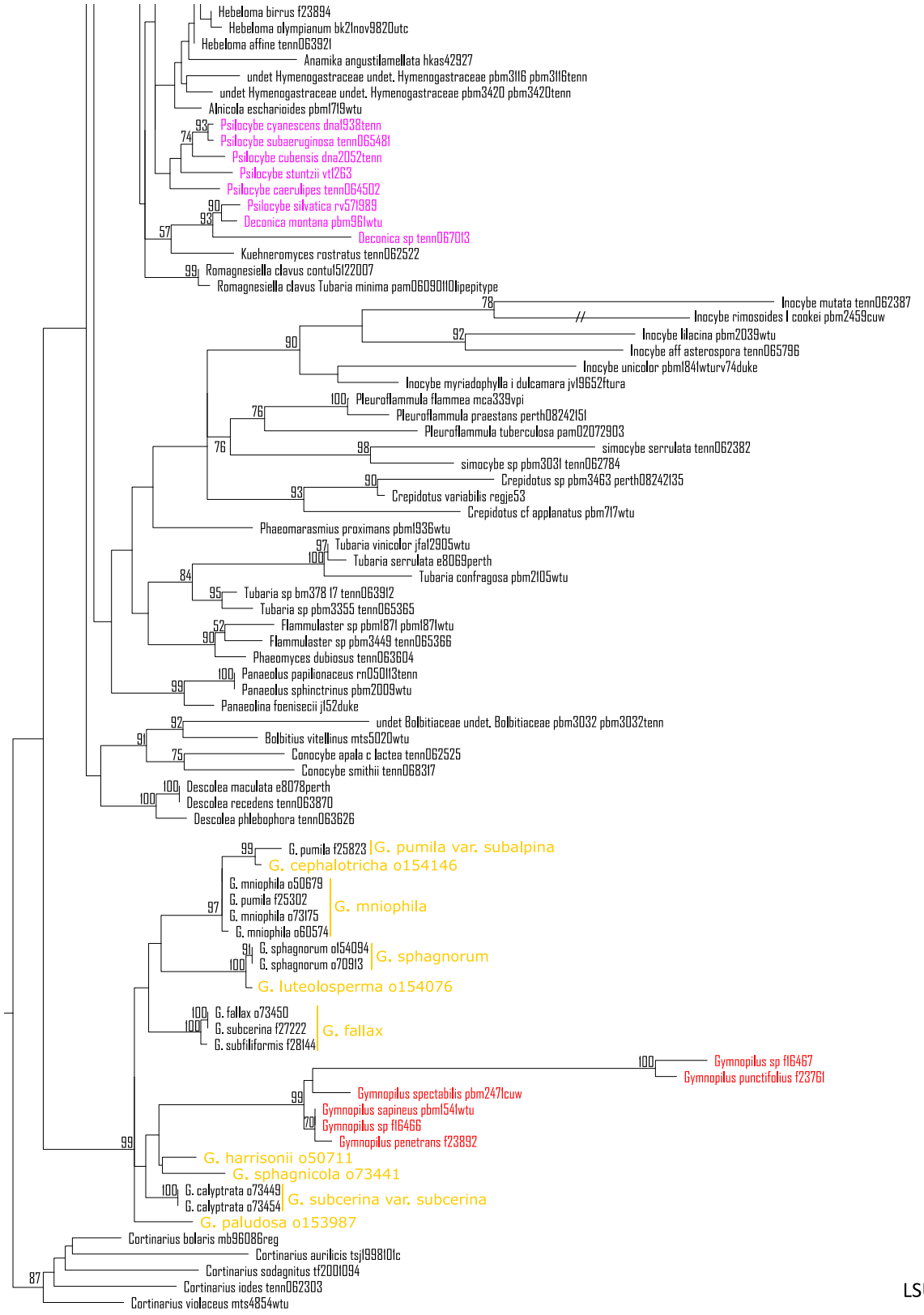
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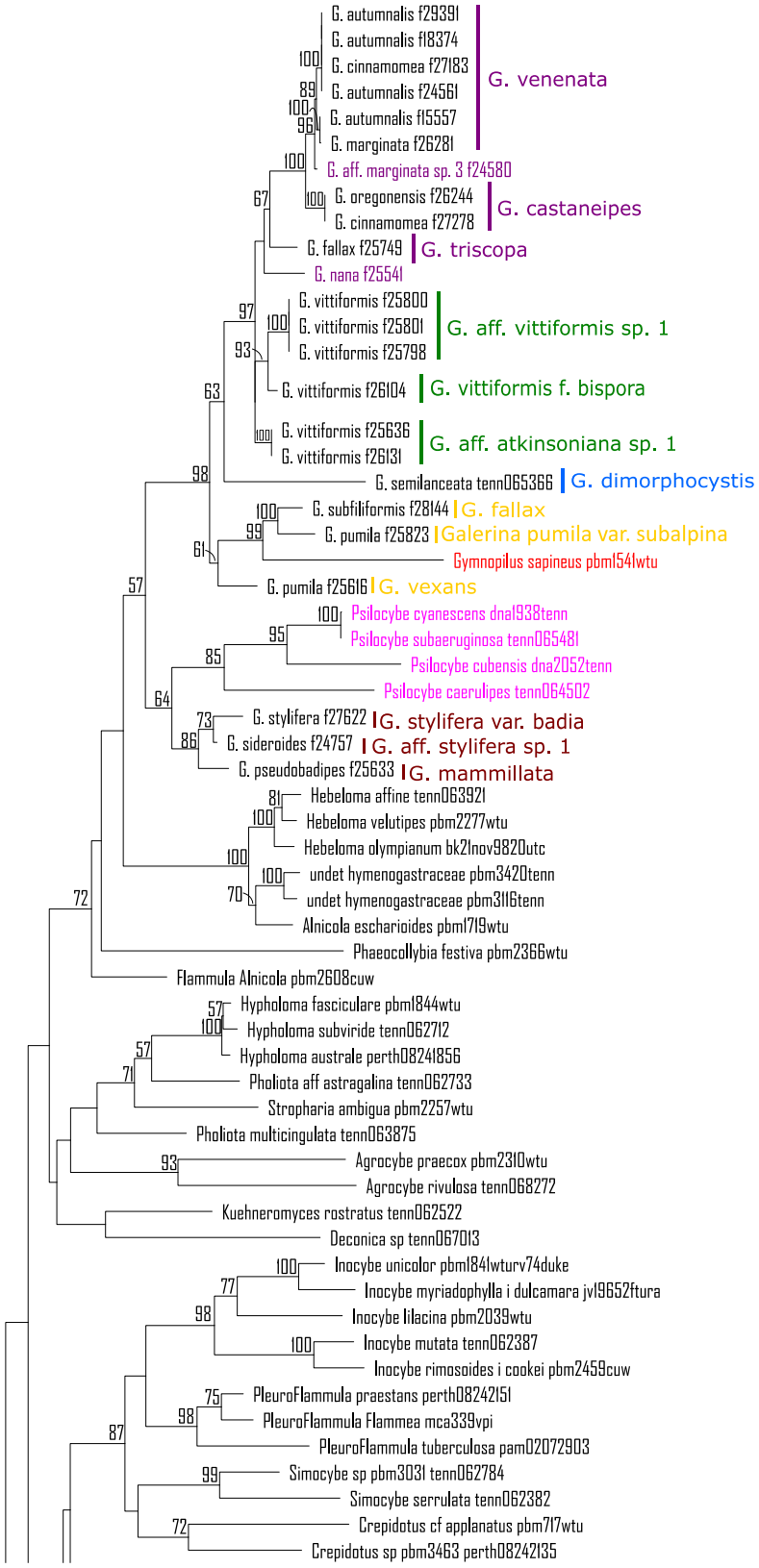
LSU Page 2

Figure A.2 - Maximum likelihood phylogeny of *Galerina* + outgroup LSU sequences. Data were aligned using MAFFT and analyzed with RAxML (200 ML tree searches, 1000 bootstrap replicates) using a GTR+G+I model. Only bootstrap values >50% are shown. Bars and species names represent delimitated species (Table A.2). Colored tip labels indicate a species delimitation in which the lone sample name matches the proposed delimited name. Colored bars represent approximately the infrageneric units proposed by Gulden (2005) (purple: sect. Naucoriopsis, green: sect. Galerina, blue: sect. Turbariopsis, yellow: sect. Mycenopsis). Three additional clades are indicated (red: *Gymnopilus* spp., magenta: *Psilocybe* spp., brown: stirps Sideroides [Smith & Singer 1964]).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G. venenata
G. aff. marginata sp. 3 f24580
G. oregonensis f26244
G. cinnamomea f27278
G. castaneipes
G. fallax f25749
G. triscopa
G. nana f25541
G. vittiformis f25800
G. vittiformis f25801
G. vittiformis f25798
G. aff. vittiformis sp. 1
G. vittiformis f26104
G. vittiformis f. bispora
G. vittiformis f25636
G. vittiformis f26131
G. aff. atkinsoniana sp. 1
G. semilanceata tenn065366
G. dimorphocystis
G. subfiliformis f28144
G. fallax
G. pumila f25823
Galerina pumila var. subalpina
Gymnopilus sapineus pbm1541wtu
G. pumila f25616
G. vexans
Psilocybe cyanescens dna1938tenn
Psilocybe subaeruginosa tenn065481
Psilocybe cubensis dna2052tenn
Psilocybe caerulipes tenn064502
G. stylifera f27622
G. stylifera var. badia
G. sideroides f24757
G. aff. stylifera sp. 1
G. pseudobadipes f25633
G. mammillata
Hebeloma affine tenn063921
Hebeloma velutipes pbm2277wtu
Hebeloma olympianum bk21nov9820utc
undet hymenogastraceae pbm3420tenn
undet hymenogastraceae pbm3116tenn
Alnicola escharioides pbm1719wtu
Phaeocollybia festiva pbm2366wtu
Flammula Alnicola pbm2608cuw
Hypholoma fasciculare pbm1844wtu
Hypholoma subviride tenn062712
Hypholoma australe perth08241856
Pholiota aff astragalina tenn062733
Stropharia ambigua pbm2257wtu
Pholiota multicingulata tenn063875
Agrocybe praecox pbm2310wtu
Agrocybe rivulosa tenn068272
Kuehneromyces rostratus tenn062522
Deconica sp tenn067013
Inocybe unicolor pbm1841wturv74duke
Inocybe myriadophylla i dulcamara jvl9652ftura
Inocybe lilacina pbm2039wtu
Inocybe mutata tenn062387
Inocybe rimosoides i cookei pbm2459cuw
PleuroFlammula praestans perth08242151
PleuroFlammula Flammea mca339vpi
PleuroFlammula tuberculosa pam02072903
Simocybe sp pbm3031 tenn062784
Simocybe serrulata tenn062382
Crepidotus cf applanatus pbm717wtu
Crepidotus sp pbm3463 perth08242135

RPB2 Page 1

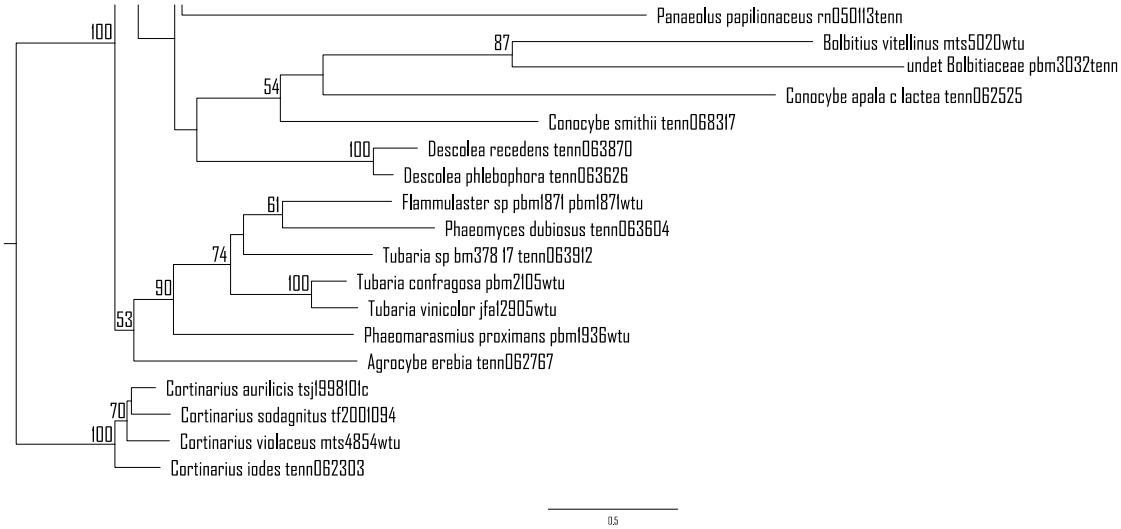
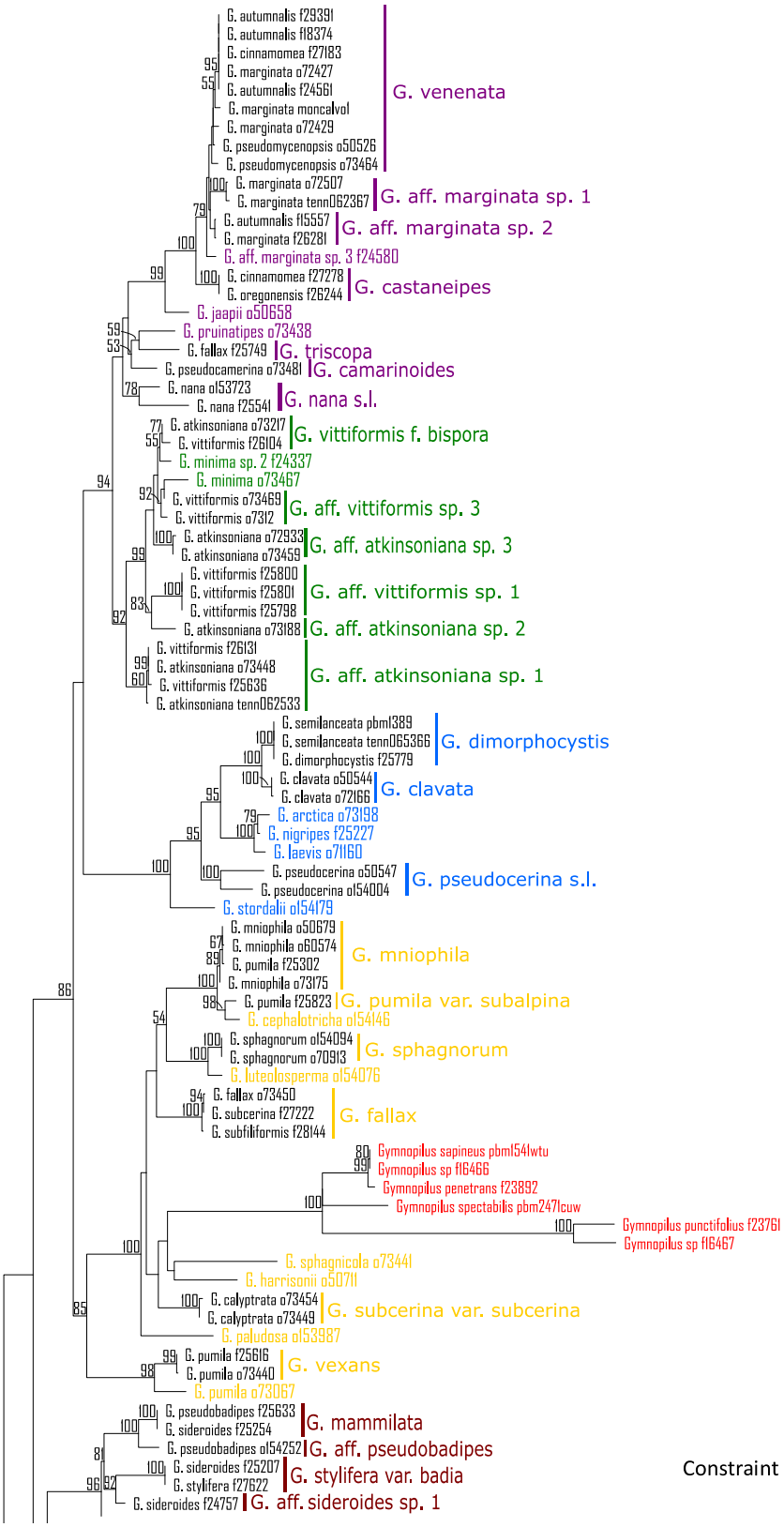
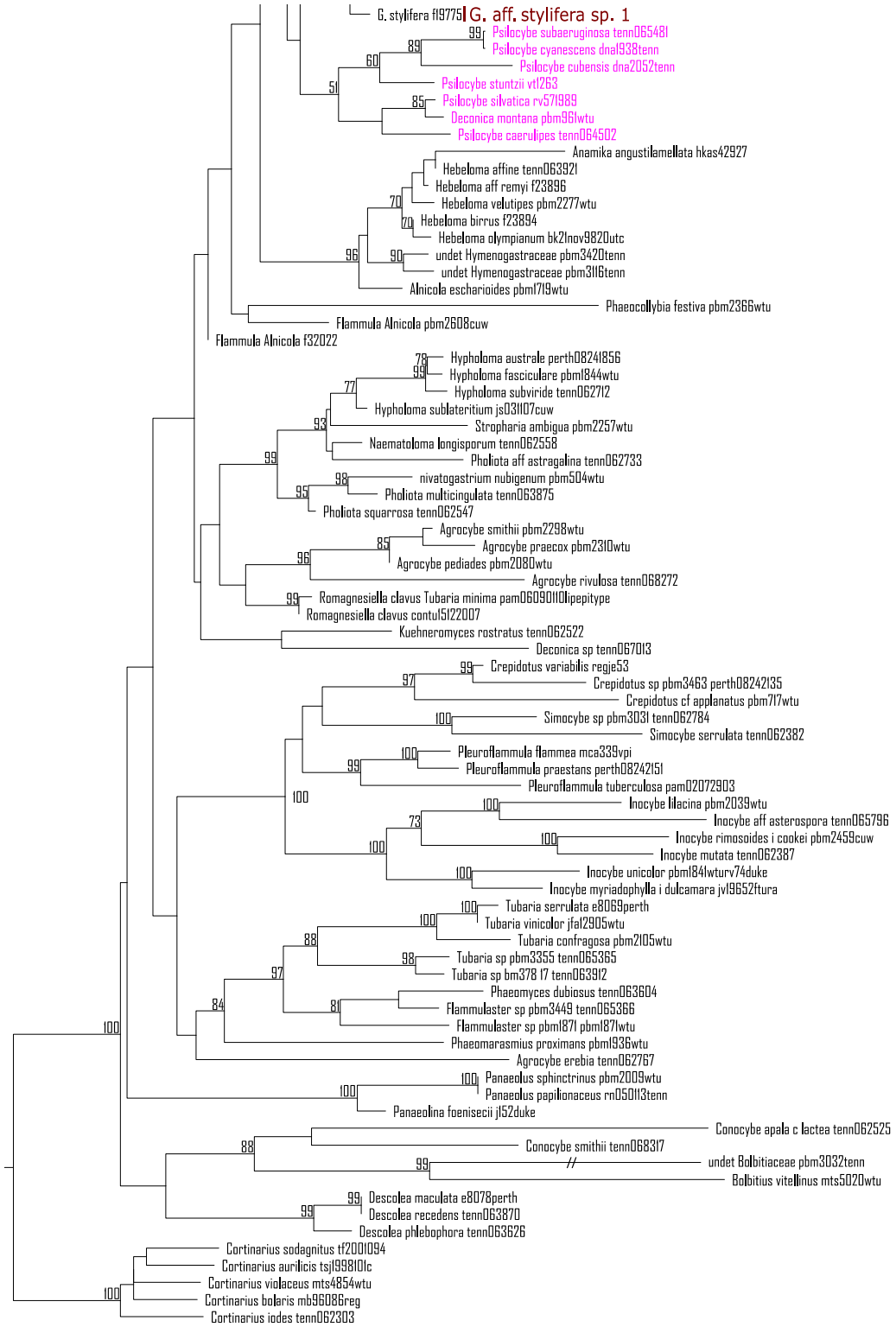


Figure A.3 - Maximum likelihood phylogeny of *Galerina* + outgroup LSU sequences. Data were aligned using MAFFT and analyzed with RAxML (200 ML tree searches, 1000 bootstrap replicates) using a GTR+G+I model for all three nucleotide positions. Only bootstrap values >50% are shown. Bars and species names represent delimitated species (Table A.2). Colored tip labels indicate a species delimitation in which the lone sample name matches the proposed delimited name. Colored bars represent approximately the infrageneric units proposed by Gulden (2005) (purple: sect. Naucoriopsis, green: sect. Galerina, blue: sect. Turbariopsis, yellow: sect. Mycenopsis). Three additional clades are indicated (red: *Gymnopilus* spp., magenta: *Psilocybe* spp., brown: stirps Sideroides [Smith & Singer 1964]).



Constraint Page 1



Constraint Page 2

Figure A.4 – Maximum likelihood phylogeny of concatenated *Galerina* + outgroup concatenated LSU and/or RPB2 (+ITS where available) sequences. Data were aligned using MAFFT and analyzed with RAxML (200 ML tree searches, 1000 bootstrap replicates) using a partitioned dataset (GTR+G+I model for each of ITS, LSU and all three RPB2 nucleotide positions). Only bootstrap values >50% are shown. Bars and species names represent delimitated species (Table A.2). Colored tip labels indicate a species delimitation in which the lone sample name matches the proposed delimited name. Colored bars represent approximately the infrageneric units proposed by Gulden (2005) (purple: sect. Naucoriopsis, green: sect. Galerina, blue: sect. Turbariopsis, yellow: sect. Mycenopsis). Three additional clades are indicated (red: *Gymnopilus* spp., magenta: *Psilocybe* spp., brown: stirps Sideroides [Smith & Singer 1964]).

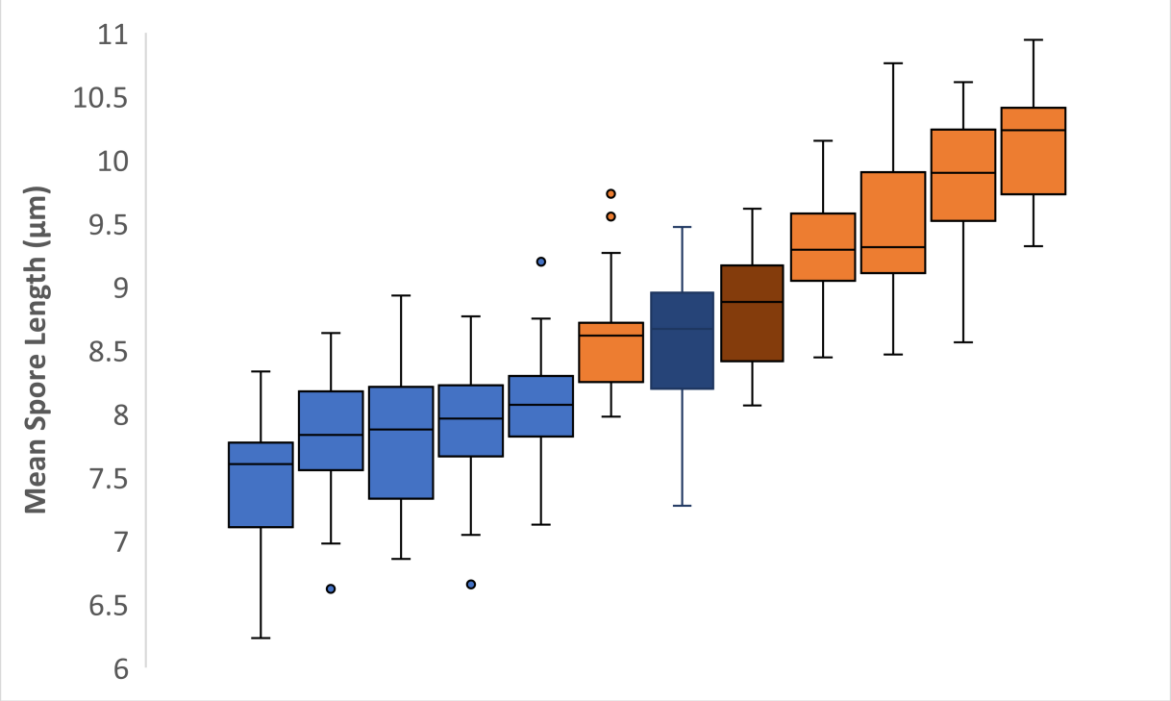


Figure A.5 : Box-and-whisker plot of mean spore length of 30 spores from *Galerina castaneipes* (n = 5, light blue), *G. castaneipes* type specimen (n = 1, dark blue), *G. venenata* (n = 5, light orange) and *G. venenata* type specimen (n = 1, dark orange). Solid lines in the box represent the median value (50th percentile), while the box represents the 25th to 75th percentiles of the dataset. The black whiskers mark the 5th and 95th percentiles, while colored circles represent outliers.

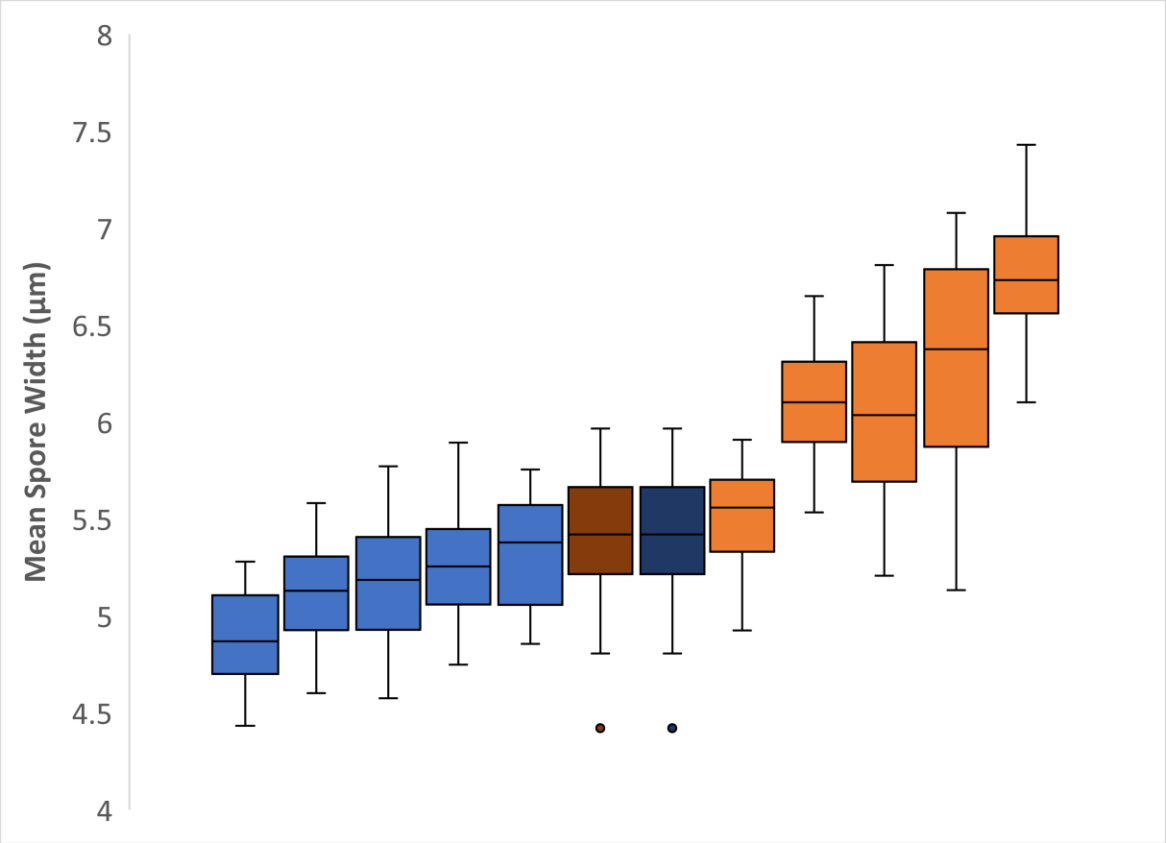


Figure A.6 : Box-and-whisker plot of mean spore length of 30 spores from *Galerina castaneipes* (n = 5, light blue), *G. castaneipes* type specimen (n = 1, dark blue), *G. venenata* (n = 5, light orange) and *G. venenata* type specimen (n = 1, dark orange). Solid lines in the box represent the median value (50th percentile), while the box represents the 25th to 75th percentiles of the dataset. The black whiskers mark the 5th and 95th percentiles, while colored circles represent outliers.

Table A.1 – Mean spore lengths & widths (+/- standard deviation) of *G. castaneipes* (n = 6, including Smith type specimen) and *G. venenata* (n = 6, including Smith type specimen). P-values calculated using a two-sample T-test.

	Length (µm)		Width (µm)	
	Mean	Range	Mean	Range
G. castaneipes	7.94 (± 0.367)	7.45 – 8.57	5.19 (± 0.186)	4.88 – 5.40
G. venenata	9.35 (± 0.571)	8.59 – 10.1	6.13 (± 0.402)	5.52 – 6.75
p-value	p = 0.0005		p = 0.0004	

Table A.2 – Sample collection information and Genbank accession numbers for all isolates used in the creation of phylogenetic trees.

Genus	Species	Variety	New Name	Toxin	Notes	Specimen ID	Country	City	ITS Accession	LSU Accession	RPB2 Accession
Agrocybe	erebia					TENN062767	USA	Massachusetts	DQ484056	DQ457663	DQ472712
Agrocybe	pediades					PBM2080WTU	USA	California	DQ484057	DQ110872	
Agrocybe	praecox					PBM2310WTU	USA	Washington	AY818348	AY646101	DQ385876
Agrocybe	rivulosa					TENN068272	USA	Tennessee	KF830098	KF830090	KF830069
Agrocybe	smithii					PBM2298WTU	USA	Washington	DQ484058	DQ110873	
Alnicola	escharioides					PBM1719WTU	USA	Washington	AY900086	AY380405	AY337411
Bolbitiaceae	PBM3032					PBM3032TENN	USA	Tennessee	HQ840656	HQ840657	HQ840658
Bolbitius	vitellinus					MTS5020WTU	USA	Washington	DQ200920	AY691807	DQ385878
Conocybe	apala					TENN062525	USA	Massachusetts	DQ486693	DQ457660	DQ470834
Conocybe	smithii					TENN068317	USA	Oregon	KF830097	KF830088	KF830068
Cortinarius	aurilicis					TSJ1998101C	France		DQ083772	AY684152	DQ083880
Cortinarius	bolaris					MB96086REG	Germany		AF389169	AY293173	
Cortinarius	iodes					TENN062303	USA	Massachusetts	AF389133	AY702013	AY536285
Cortinarius	sodagnitus					TF2001094	Denmark		DQ083812	AY684151	DQ083920
Cortinarius	violaceus					MTS4854WTU	USA	Washington	DQ486695	DQ457662	DQ470835
Crepidotus	cf_applanatus					PBM717WTU	USA	Washington	DQ202273	AY380406	AY333311
Crepidotus	sp_PBM3463					PERTH08242135	Australia	Western Australia	HQ728537	HQ728538	HQ728540
Crepidotus	variabilis					REGJE53	Unknown	Unknown		AY293174	
Deconica	montana					PBM961WTU	USA	Washington	DQ494692	DQ470823	
Deconica	sp.					TENN067013	Australia	Queensland	KC669314	KF830081	KF830064
Descolea	maculata					E8078PERTH	Australia	Western Australia	DQ192181	DQ457664	
Descolea	phlebophora					TENN063626	New Zealand		HQ728543	HQ728544	HQ728545
Descolea	recedens					TENN063870	Australia	Tasmania	HQ728546	HQ827174	HQ827175
Flammula	alnicola			No		F32022	Canada	Capilano River Regional Park, North Vancouver	KX236111	KX236111	
Flammula	alnicola					PBM2608CUW	USA	Tennessee	DQ486703	DQ457666	DQ472714
Flammulaster	sp_PBM1871					PBM1871WTU	USA	Washington		AY380408	AY333315
Flammulaster	sp_PBM3449					TENN065366	Australia	Tasmania	HQ827176	HQ827177	
Galerina	allospora		G. allospora			m0289	Russia	Northwest	MG597378		
Galerina	allospora		G. allospora			O73460	Scotland		AJ585452		
Galerina	alpestris		G. alpestris			AH43922	Italy	Sondrio, Lombardia	KC602410		
Galerina	alpestris	f. annulata	G. alpestris			AH43923	Italy	Sondrio, Lombardia	KC602411		
Galerina	arctica		G. mniophila			KH60			GU234123		
Galerina	arctica		G. nigripes			O70903	Norway		AJ585441		
Galerina	arctica		G. nigripes			O73198	Greenland		AJ585442	AJ871556	
Galerina	atkinsoniana		G. aff. atkinsoniana sp. 1			F19675	Canada	Vancouver, BC	HM240525		
Galerina	atkinsoniana		G. aff. atkinsoniana sp. 1			O70394	Iceland	S-Þingeyjarsýsla Co.	AF251183		
Galerina	atkinsoniana		G. aff. atkinsoniana sp. 1			O73448	Germany		AJ871572	AJ871534	
Galerina	atkinsoniana		G. aff. atkinsoniana sp. 1			PBM2719	USA	Colorado	DQ486705		
Galerina	atkinsoniana		G. aff. atkinsoniana sp. 2			CTB85295	Greenland		AJ585481		
Galerina	atkinsoniana		G. aff. atkinsoniana sp. 2			O73188	Greenland		AJ585480	AJ871533	
Galerina	atkinsoniana		G. aff. atkinsoniana sp. 3			KA130111	South Korea		KR673654		
Galerina	atkinsoniana		G. aff. atkinsoniana sp. 3			O72933	Norway		AJ585478	AJ871537	
Galerina	atkinsoniana		G. aff. atkinsoniana sp. 3			O73459	Scotland		AJ585479	AJ871536	
Galerina	atkinsoniana		G. vittiformis f. bispora			O73217	Greenland		AJ585482	AJ871543	
Galerina	atkinsoniana		G. aff. atkinsoniana sp. 1			TENN062533	USA	Colorado	DQ486705	DQ457668	
Galerina	atkinsoniana		G. aff. atkinsoniana sp. 1	No		F24732	Canada	Observatory Hill, BC	MF954828		
Galerina	atkinsoniana		G. aff. atkinsoniana sp. 1			F28226	Canada	Jordan River, BC	MF954831		
Galerina	atkinsoniana		G. aff. vittiformis sp. 1			F24158	Canada	Observatory Hill, BC	MF954862		
Galerina	austrocalyptrata		G. austrocalyptrata			PDD97057	New Zealand		KM975404		
Galerina	autumnalis		G. aff. marginata sp. 1			LGuzmanDavalos5246I BUG	Mexico		AY281020		
Galerina	autumnalis		G. aff. marginata sp. 1			MICH27691	USA	Oakland Co., CO	AF251171		
Galerina	autumnalis		G. aff. marginata sp. 1			MICH27694	USA	Washtenaw Co., MI	AF251172		
Galerina	autumnalis		G. aff. marginata sp. 1			TENN61495	USA	North Carolina	FJ596819		
Galerina	autumnalis		G. aff. marginata sp. 1			TNSF61967	Japan	Ishikawa, Kaga	KT368690		
Galerina	autumnalis		G. aff. marginata sp. 5			DBG16347	USA	Pitken Co., CO	AF251170		
Galerina	autumnalis		G. venenata			DBG17621	USA	Gilpin Co., CO	AF251169		

Genus	Species	Variety	New Name	Toxin	Notes	Specimen ID	Country	City	ITS Accession	LSU Accession	RPB2 Accession
Galerina	autumnalis		G. venenata			DBG17635	USA	Summit Co., CO	AF251173		
Galerina	autumnalis		G. venenata			x16582	Italy		JF908016		
Galerina	autumnalis		G. aff. marginata sp. 2	α, β		F15557	Canada	Manning Park, BC	MF954784	MH828255	MH829614
Galerina	autumnalis		G. castaneipes			F27169	Canada	Observatory Hill, BC	MF954808		
Galerina	autumnalis		G. castaneipes	α, β		F27254	Canada	Observatory Hill, BC	MF954811		
Galerina	autumnalis		G. castaneipes			F27619	Canada	Duncan, BC	MF954813		
Galerina	autumnalis		G. castaneipes			F28932	Canada	Observatory Hill, BC	MF954816		
Galerina	autumnalis		G. venenata	α, β		F18374	Canada	Whistler, BC	MF954785	MH828256	MH829615
Galerina	autumnalis		G. venenata	α		F24561	Canada	Observatory Hill, BC	MF954789		MH829616
Galerina	autumnalis		G. venenata	α, β		F29391	Canada	Observatory Hill, BC	MF954817	MH828257	MH829617
Galerina	badipes		G. badipes			MICH29675	USA	Idaho Co., ID	AF251175		
Galerina	badipes		G. badipes			O72513	Norway	Akershus Co.	AF251174		
Galerina	badipes		G. badipes			O72603	Norway		AJ585494		
Galerina	badipes		G. badipes			x8935	Italy		JF908012		
Galerina	badipes		G. badipes			F29392	Canada	Observatory Hill, BC	MF954826		
Galerina	badipes		G. badipes			F29827	Canada	Observatory Hill, BC	MF954827		
Galerina	badipes		G. venenata	α, β		F22840	Canada	Capilano River Park, North Vancouver, BC	MF954787		
Galerina	badipes		G. venenata			F25253	Canada	Observatory Hill, BC	MF954797		
Galerina	badipes		G. venenata			F25298	Canada	Observatory Hill, BC	MF954798		
Galerina	badipes		G. venenata	α, β		F27894	Canada	Metchosin, BC	MF954814		
Galerina	calyptrata		G. sphagnicola			x16519	Italy		JF908015		
Galerina	calyptrata		G. subcerina var. subcerina			m0280	Russia	Northwest	MG597379		
Galerina	calyptrata		G. subcerina var. subcerina			MGW987	USA	Tennessee, Great Smoky Mountains National Park, Cherokee Orchard area	MG663255		
Galerina	calyptrata		G. subcerina var. subcerina			O73449	Germany		AJ585465	AJ871503	
Galerina	calyptrata		G. subcerina var. subcerina			O73454	France		AJ585466	AJ871501	
Galerina	castaneipes		G. badipes			F27068	Canada	Observatory Hill, BC	MF954885		
Galerina	castaneipes		G. castaneipes	α, β		F25630	Canada	Observatory Hill, BC	MF954801		
Galerina	castaneipes		G. castaneipes		Holotype	AHSmith55523	USA	Oregon, Josephine: Grants Pass	MH827060		
Galerina	cedretorum	var. bispora	G. badipes		Paratype	AHSmith3429	USA	Oregon, Lane: Siltcoos Outlet Camp, Lake Tahkenitch	MH827061		
Galerina	cedretorum	var. microspora	G. badipes		Holotype	AHSmith50911	USA	Michigan, Emmet: Wilderness Point, Wilderness State Park	MH827062		
Galerina	cedretorum		G. badipes			F24517	Canada	Observatory Hill, BC	MF954823		
Galerina	cedretorum		G. badipes	No		F27620	Canada	Duncan, BC	MF954824		
Galerina	cephalotricha		G. cephalotrica			O154146	Norway		AJ585462	AJ871513	
Galerina	chionophila		G. chionophila			O73463	Switzerland		AJ585506		
Galerina	cinnamomea	var. cinnamomea	G. venenata		Holotype	AHSmith55422	USA	Oregon, Josephine: Grants Pass	MH827063		
Galerina	cinnamomea		G. castaneipes			F24518	Canada	Observatory Hill, BC	MF954788		
Galerina	cinnamomea		G. castaneipes	α, β		F24733	Canada	Observatory Hill, BC	MF954794		
Galerina	cinnamomea		G. castaneipes	α		F25759	Canada	Observatory Hill, BC	MF954803		
Galerina	cinnamomea		G. castaneipes	α, β		F25760	Canada	Observatory Hill, BC	MF954889		
Galerina	cinnamomea		G. castaneipes	α, β		F27278	Canada	Observatory Hill, BC	MF954812	MH828259	MH829619
Galerina	cinnamomea		G. castaneipes	α		F27279	Canada	Observatory Hill, BC	MF954880		
Galerina	cinnamomea		G. venenata	α, β		F27183	Canada	Observatory Hill, BC	MF954809	MH828258	MH829618
Galerina	cinnamomea		G. venenata			F29945	Canada	Observatory Hill, BC	MF954818		
Galerina	clavata		G. clavata			O50544	Svalbard		AJ585437	AJ871554	
Galerina	clavata		G. clavata			O71693	Iceland	N-Múlasýsla Co.	AF251181		
Galerina	clavata		G. clavata			O72166	Denmark		AJ585436	AJ871555	
Galerina	dimorphocystis		G. dimorphocystis			F24207	Canada	Observatory Hill, BC	MF954714		
Galerina	dimorphocystis		G. dimorphocystis			F24815	Canada	Observatory Hill, BC	MF954717		
Galerina	dimorphocystis		G. dimorphocystis			F24901	Canada	Observatory Hill, BC	MF954718		
Galerina	dimorphocystis		G. dimorphocystis			F25396	Canada	Observatory Hill, BC	MF954729		
Galerina	dimorphocystis		G. dimorphocystis			F25779	Canada	Observatory Hill, BC	MF954733	MH828260	
Galerina	dimorphocystis		G. dimorphocystis			F25813	Canada	Observatory Hill, BC	MF954736		
Galerina	dimorphocystis		G. dimorphocystis			F25868	Canada	Observatory Hill, BC	MF954738		

Genus	Species	Variety	New Name	Toxin	Notes	Specimen ID	Country	City	ITS Accession	LSU Accession	RPB2 Accession
Galerina	dimorphocystis		G. dimorphocystis	No		F25870	Canada	Observatory Hill, BC	MF954740		
Galerina	dimorphocystis		G. dimorphocystis			F25887	Canada	Observatory Hill, BC	MF954741		
Galerina	dimorphocystis		G. dimorphocystis			F28143	Canada	Saanich Peninsula, BC	MF954745		
Galerina	dimorphocystis		G. dimorphocystis			F28358	Canada	Observatory Hill, BC	MF954746		
Galerina	discreta		G. discreta			BSI10x10	Switzerland		KR606031		
Galerina	fallax		G. fallax			AK07KGI1202R32Sp2	Antarctica		MF692967		
Galerina	fallax		G. fallax			m0485	Russia	Northwest	MG597380		
Galerina	fallax		G. fallax			O154355	Norway		AJ585451		
Galerina	fallax		G. fallax			O154451	Norway		AJ585450		
Galerina	fallax		G. fallax			O73450	Germany		AJ585449	AJ871508	
Galerina	fallax		G. triscopa			F25749	Canada	Observatory Hill, BC	MF954861	MH828261	MH829620
Galerina	fibrillosa		G. fibrillosa sp. 1			LF1BA3A6	USA	Great Smoky Mountains	JQ272325		
Galerina	fibrillosa		G. fibrillosa sp. 2			MICH40850	USA		AJ585473		
Galerina	filiformis		G. fallax			F24816	Canada	Observatory Hill, BC	MF954752		
Galerina	harrisonii		G. chionophila			O71762	Iceland	S-Múlasýsla Co.	AF251179		
Galerina	harrisonii		G. harrisonii			O50711	Norway		AJ585463	AJ871506	
Galerina	heterocystis		G. dimorphocystis			F24112	Canada	Observatory Hill, BC	MF954713		
Galerina	heterocystis		G. dimorphocystis	No		F24755	Canada	Observatory Hill, BC	MF954715		
Galerina	heterocystis		G. dimorphocystis			F24984	Canada	Observatory Hill, BC	MF954720		
Galerina	heterocystis		G. dimorphocystis			F24985	Canada	Observatory Hill, BC	MF954721		
Galerina	heterocystis		G. dimorphocystis			F25206	Canada	Observatory Hill, BC	MF954722		
Galerina	heterocystis		G. dimorphocystis			F25299	Canada	Observatory Hill, BC	MF954724		
Galerina	heterocystis		G. dimorphocystis			F25355	Canada	Observatory Hill, BC	MF954726		
Galerina	heterocystis		G. dimorphocystis			F25376	Canada	Observatory Hill, BC	MF954728		
Galerina	heterocystis		G. dimorphocystis	No		F25404	Canada	Observatory Hill, BC	MF954731		
Galerina	heterocystis		G. dimorphocystis			F25786	Canada	Observatory Hill, BC	MF954734		
Galerina	heterocystis		G. dimorphocystis			F25797	Canada	Observatory Hill, BC	MF954735		
Galerina	heterocystis		G. dimorphocystis	No		F25822	Canada	Observatory Hill, BC	MF954737		
Galerina	heterocystis		G. dimorphocystis			F26812	Canada	Observatory Hill, BC	MF954742		
Galerina	heterocystis		G. dimorphocystis			F27621	Canada	Duncan, BC	MF954743		
Galerina	heterocystis		G. dimorphocystis			F28108	Canada	Observatory Hill, BC	MF954744		
Galerina	heterocystis		G. dimorphocystis			F28779	Canada	Observatory Hill, BC	MF954747		
Galerina	heterocystis		G. dimorphocystis			F28902	Canada	Cowichan Lake, BC	MF954748		
Galerina	heterocystis		G. dimorphocystis			F28987	Canada	Observatory Hill, BC	MF954749		
Galerina	heterocystis		G. dimorphocystis	No		F29062	Canada	Observatory Hill, BC	MF954750		
Galerina	heterocystis		G. nigripes	No		F25655	Canada	Observatory Hill, BC	MF954702		
Galerina	heterocystis		G. nigripes			F26686	Canada	Observatory Hill, BC	MF954703		
Galerina	hybrida		G. hybrida			ASG20773	USA	Washington County, Minnesota	KP757871		
Galerina	hybrida		G. hybrida			O73452	Germany		AJ585445		
Galerina	hybrida		G. hybrida			O73458	France		AJ585444		
Galerina	hygrophila		G. venenata			x8433	Italy		JF908011		
Galerina	hypnorum		G. hypnorum			MICH46292	USA	Michigan	AJ585469		
Galerina	hypnorum		G. hypnorum			MICH46302	USA	Michigan	AJ585470		
Galerina	hypnorum		G. hypnorum			O154362	Norway		AJ585468		
Galerina	hypnorum		G. hypnorum			O73206	Greenland		AJ585467	AJ871535	
Galerina	indica		G. indica			KM190552	India		KJ187768		
Galerina	jaapii		G. jaapii			Ala1	Germany		KY680667		
Galerina	jaapii		G. jaapii			O154387	Finland		AJ585505		
Galerina	jaapii		G. jaapii			O50658	Norway		AJ585504	AJ871520	
Galerina	jaapii		G. aff. marginata sp. 3			F24580	Canada	Observatory Hill, BC	MF954791	MH828262	MH829621
Galerina	laevis		G. laevis			NL4042	Hungary		KT591535		
Galerina	laevis		G. laevis			O154389	Norway		AJ585438		
Galerina	laevis		G. laevis			O70903	Norway		AJ585439		
Galerina	laevis		G. laevis			O71160	Norway		AJ585440	AJ871558	
Galerina	larigna		G. larigna		Monograph material	LRHesler17642			MH827059		
Galerina	lubrica		G. lubrica			O154034	Norway		AJ585471	AJ871525	
Galerina	lubrica		G. lubrica			O73455	France		AJ585472		
Galerina	lubrica		G. lubrica		Holotype	AHSmith51977	USA	Colorado, San Miguel: Ophir	MH827064		

Genus	Species	Variety	New Name	Toxin	Notes	Specimen ID	Country	City	ITS Accession	LSU Accession	RPB2 Accession
Galerina	lubrica		G. mniophila	No		F27419	Canada	Sooke Potholes Park, BC	MF954766		
Galerina	luteolosperma		G. lueteolosperma			O154076	Norway		AJ585453	AJ871509	
Galerina	makereriensis		G. makereriensis			DED8325SFSU	Africa	Sao Tome	KX017203		
Galerina	mammillata		G. mammillata			F24854	Canada	Observatory Hill, BC	MF954847		
Galerina	mammillata		G. mammillata	No		F24865	Canada	Observatory Hill, BC	MF954882		
Galerina	mammillata		G. mammillata			F25750	Canada	Observatory Hill, BC	MF954851		
Galerina	mammillata		G. mammillata	No		F29021	Canada	Observatory Hill, BC	MF954854		
Galerina	marginata		G. aff. marginata sp. 1			AFTOLID465			DQ192182		
Galerina	marginata		G. aff. marginata sp. 1			FPDsnTENN	USA	Tennesse, William Hastie Natural Area, south Knoxville	MF686493		
Galerina	marginata		G. aff. marginata sp. 1			KUC2013072526	South Korea		KJ713984		
Galerina	marginata		G. aff. marginata sp. 1			LEBIN2272	Russia	South Siberia, Altay Republic	KY327302		
Galerina	marginata		G. aff. marginata sp. 1			LEBIN2837	Russia	Far East, Kedrovaya Pad nature reserve	KY327295		
Galerina	marginata		G. aff. marginata sp. 1			MICH27695	USA	Oakland Co., MI	AF251166		
Galerina	marginata		G. aff. marginata sp. 1			O72507	USA	Michigan	AJ585496	AJ871521	
Galerina	marginata		G. aff. marginata sp. 1			SFC2014053009	Korea		KX773866		
Galerina	marginata		G. aff. marginata sp. 1			SFC2014070311	Korea		KX773867		
Galerina	marginata		G. aff. marginata sp. 1			UWODD6MO221929	Canada	Elgin Co., Ontario	KY706155		
Galerina	marginata		G. aff. marginata sp. 4			O72431	USA	Benton Co., OR	AF251168		
Galerina	marginata		G. aff. marginata sp. 4			UNSW9911			AF501564		
Galerina	marginata		G. castaneipes			F32036	Canada	Vancouver, BC	KX236132		
Galerina	marginata		G. venenata			F14298	Canada	Vancouver, BC	AY228347		
Galerina	marginata		G. venenata			F32027	Canada	Vancouver, BC	KX236118		
Galerina	marginata		G. venenata			H21529	Tunisia		KU973845		
Galerina	marginata		G. venenata			H6002775	Finland		GU373516		
Galerina	marginata		G. venenata			LEBIN2477	Russia	Western Caucasus, Caucasus State Nature Biosphere Researve	KY327299		
Galerina	marginata		G. venenata			LEBIN2479	Russia	Western Caucasus, Caucasus State Nature Biosphere Researve	KY327296		
Galerina	marginata		G. venenata			LEBIN2504	Russia	Western Caucasus, Teberda State Nature Biosphere Reserve	KY327298		
Galerina	marginata		G. venenata			LEBIN2533	Russia	Western Caucasus, Teberda State Nature Biosphere Reserve	KY327301		
Galerina	marginata		G. venenata			LEBIN2545	Russia	Western Caucasus, Teberda State Nature Biosphere Reserve	KY327297		
Galerina	marginata		G. venenata			LEBIN3111	Russia	Western Caucasus, Teberda State Nature Biosphere Reserve	KY327300		
Galerina	marginata		G. venenata			MICH29673	USA	Idaho Co., ID	AF251167		
Galerina	marginata		G. venenata			moncalvo1	Sweden		AF195590	AF195590	
Galerina	marginata		G. venenata			O71328	Norway		AJ585498		
Galerina	marginata		G. venenata			O72427	USA	Douglas Co., OR	AF251164		
Galerina	marginata		G. venenata			O72427	USA	Oregon	AJ585500	AJ871530	
Galerina	marginata		G. venenata			O72429	USA	Oregon	AJ585502	AJ871527	
Galerina	marginata		G. venenata			O72432	USA	Benton Co., OR	AF251165		
Galerina	marginata		G. venenata			O72434	USA	Oregon	AJ585499		
Galerina	marginata		G. venenata			O72509	Norway	Oslo	AF251163		
Galerina	marginata		G. venenata			O72510	Norway	Oslo	AF251162		
Galerina	marginata		G. venenata			O72512	Norway	Vestfold Co.	AF251161		
Galerina	marginata		G. venenata			O72517	Norway	Oslo	AF251160		
Galerina	marginata		G. venenata			x6546	Italy		JF908009		
Galerina	marginata		G. aff. marginata sp. 1			TENN062367	USA	Massachusetts	DQ192182	DQ457669	
Galerina	marginata		G. aff. marginata sp. 2	α, β		F26281	Canada	Observatory Hill, BC	MF954807	MH828263	MH829622
Galerina	marginata		G. castaneipes	α, β		F28078	Canada	Duncan, BC	MF954815		

Genus	Species	Variety	New Name	Toxin	Notes	Specimen ID	Country	City	ITS Accession	LSU Accession	RPB2 Accession
Galerina	marginata		G. venenata	α		F26170	Canada	Observatory Hill, BC	MF954804		
Galerina	marginata		G. venenata			F30968	Canada	Vancouver, BC	MF954821		
Galerina	minima		G. aff. vittiformis sp. 3			O154480			AJ585486		
Galerina	minima		G. minima sp. 1			O73466			AJ585488		
Galerina	minima		G. minima sp. 1			O73467	Greenland		AJ585489	AJ871540	
Galerina	minima		G. vittiformis f. bispora			MushroomObserverorg 83484	USA	California, Tahoe National Forest	MG966314		
Galerina	minima		G. vittiformis f. bispora			O73468	Greenland		AJ585483	AJ871514	
Galerina	minima		G. minima sp. 2	No		F24337	Canada	Observatory Hill, BC	MF954709	MH828264	MH829623
Galerina	mniophila		G. mniophila			GG16088			GU234050		
Galerina	mniophila		G. mniophila			MICH29880	USA	Idaho	AJ585461		
Galerina	mniophila		G. mniophila			O154072	Norway		AJ585456	AJ871538	
Galerina	mniophila		G. mniophila			O50545	Svalbard		AJ585458		
Galerina	mniophila		G. mniophila			O50679	Norway		AJ585457	AJ871516	
Galerina	mniophila		G. mniophila			O60574	Norway		AJ585459	AJ871515	
Galerina	mniophila		G. mniophila			O73175	Greenland		AJ585460	AJ871512	
Galerina	nana		G. nana sp. 1			O72373	USA	Benton Co., OR	AF251184		
Galerina	nana		G. nana sp. 1			O72396	USA	Benton Co., OR	AF251185		
Galerina	nana		G. nana sp. 2			O153723	Norway		AJ585490	AJ871518	
Galerina	nana		G. nana sp. 1			F25541	Canada	Observatory Hill, BC	MF954832	MH828265	MH829624
Galerina	nigripes		G. nigripes		Monograph material	AHSmith55555			MH827065		
Galerina	nigripes		G. nigripes	No		F25227	Canada	Observatory Hill, BC	MF954701	MH828266	
Galerina	oregonensis		G. castaneipes	α		F24562	Canada	Observatory Hill, BC	MF954790		
Galerina	oregonensis		G. castaneipes	α		F24581	Canada	Observatory Hill, BC	MF954792		
Galerina	oregonensis		G. castaneipes	α		F24682	Canada	Observatory Hill, BC	MF954793		
Galerina	oregonensis		G. castaneipes	α		F25300	Canada	Observatory Hill, BC	MF954799		
Galerina	oregonensis		G. castaneipes	α		F26243	Canada	Observatory Hill, BC	MF954805		
Galerina	oregonensis		G. castaneipes	α, β		F26244	Canada	Observatory Hill, BC	MF954806	MH828267	MH829625
Galerina	paludosa		G. paludosa			BHS201009	USA		HM856641		
Galerina	paludosa		G. paludosa			O153974	Norway		AJ585446		
Galerina	paludosa		G. paludosa			O153987	Norway		AJ585448	AJ871500	
Galerina	paludosa		G. paludosa			O73462	Estonia		AJ585447		
Galerina	patagonica		G. patagonica			PDD103779	New Zealand		KM975416		
Galerina	patagonica		G. patagonica			PDD72513	New Zealand		KM975403		
Galerina	patagonica		G. patagonica			PDD96434	New Zealand		KM975395		
Galerina	physospora		G. physospora			DED8206SFSU	Africa	Sao Tome	KX017204		
Galerina	physospora		G. physospora			DED8242SFSU	Africa	Sao Tome	KX017205		
Galerina	pruinatipes		G. pruinatipes			MICH29836	USA	Washington	AJ585509		
Galerina	pruinatipes		G. pruinatipes			O73438	France		AJ585510	AJ871531	
Galerina	pruinatipes		G. pruinatipes			PRM923041	Czech Republic		LT577697		
Galerina	pruinatipes		G. pruinatipes			PRM923094	Czech Republic		LT577698		
Galerina	pseudobadipes		G. aff. stylifera sp. 1			F25615	Canada	Observatory Hill, BC	MF954860		
Galerina	pseudobadipes		G. mammillata	No		F25633	Canada	Observatory Hill, BC	MF954850		MH829626
Galerina	pseudobadipes		G. aff. pseudobadipes			O154252	Norway		AJ585474	AJ871548	
Galerina	pseudocamerina		G. larigna			O73471	Germany		AJ585507		
Galerina	pseudocamerina		G. larigna			O73481	Germany		AJ585508	AJ871519	
Galerina	pseudocamerina		G. larigna			SES3059			KP100539		
Galerina	pseudocerina		G. pseudocerina sp. 1			O50547	Svalbard		AJ585432	AJ871552	
Galerina	pseudocerina		G. pseudocerina sp. 3			O153998	Norway		AJ585431		
Galerina	pseudocerina		G. pseudocerina sp. 3			O154004	Norway		AJ585433	AJ871553	
Galerina	pseudocerina		G. pseudocerina sp. 2			O70336	Iceland	Eyjafjarðarsýsla Co.	AF251182		
Galerina	pseudomycenopsis		G. pseudomycenopsis			WarHerb22853B	Scotland		AJ300157		
Galerina	pseudomycenopsis		G. pseudomycenopsis			WatHerb22853	Scotland		AJ300156		
Galerina	pseudomycenopsis		G. venenata			GG12488			GU234057		
Galerina	pseudomycenopsis		G. venenata			KH61			GU234132		
Galerina	pseudomycenopsis		G. venenata			KH62			GU234074		
Galerina	pseudomycenopsis		G. venenata			O50526	Svalbard		AJ585501	AJ871524	
Galerina	pseudomycenopsis		G. venenata			O70471	Iceland	S-Múlasýsla Co.	AF251177		
Galerina	pseudomycenopsis		G. venenata			O73464	USA	Alaska	AJ585503	AJ871523	
Galerina	pumila		G. pumila			O73067	Greenland		AJ585476	AJ871545	
Galerina	pumila		G. vexans			O73440	Germany		AJ585477	AJ871546	

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Galerina	pumila	var. subalpina	G. pumila var. subalpina		Monograph material	AHSmith56053			MH827066		
Galerina	pumila		G. luteolosperma			F27708	Canada	Metchosin, BC	MF954710		
Galerina	pumila		G. mniophila	No		F25302	Canada	Observatory Hill, BC	MF954762	MH828268	
Galerina	pumila		G. pumila var. subalpina	No		F24304	Canada	Observatory Hill, BC	MF954760		
Galerina	pumila		G. pumila var. subalpina			F25228	Canada	Observatory Hill, BC	MF954761		
Galerina	pumila		G. pumila var. subalpina	No		F25663	Canada	Observatory Hill, BC	MF954763		
Galerina	pumila		G. pumila var. subalpina			F25722	Canada	Observatory Hill, BC	MF954764		
Galerina	pumila		G. pumila var. subalpina			F25823	Canada	Observatory Hill, BC	MF954765	MH828270	MH829628
Galerina	pumila		G. vexans	No		F25616	Canada	Observatory Hill, BC	MF954774	MH828269	MH829627
Galerina	rostrata		G. fallax	No		F25365	Canada	Observatory Hill, BC	MF954800		
Galerina	salicicola		G. salicicola			K99448	England		AJ585493		
Galerina	saxicola		G. stordalii			PRM896288	Czech Republic		LT577691		
Galerina	semilanceata		G. dimorphocystis			F20369	Canada	Vancouver, BC	KC581355		
Galerina	semilanceata		G. dimorphocystis			MushroomObserverorg 158594	USA	California, Humboldt Co., Prairie Creek Redwoods State Park	MG966316		
Galerina	semilanceata		G. dimorphocystis			MushroomObserverorg 158808	USA	California, Humboldt Co., Dry Lagoon	MG966315		
Galerina	semilanceata		G. dimorphocystis			PBM1389	USA	Washington	DQ486706	AY038309	
Galerina	semilanceata		G. dimorphocystis			TENN065366	USA	Washington	DQ486706	AY038309	AY337357
Galerina	semilanceata		G. dimorphocystis	No		F16878	Canada	Graham Island, BC	MF954711		
Galerina	semilanceata		G. dimorphocystis			F17015	Canada	Vancouver, BC	MF954712		
Galerina	semilanceata		G. dimorphocystis			F30776	Canada	Graham Island, BC	MF954751		
Galerina	sideroides		G. stylifera var. badia			CBS16246	France		KT008365		
Galerina	sideroides		G. aff. sideroides sp. 1			F25635	Canada	Observatory Hill, BC	MF954836		
Galerina	sideroides		G. aff. sideroides sp. 1			F25664	Canada	Observatory Hill, BC	MF954837		
Galerina	sideroides		G. aff. sideroides sp. 1	No		F25665	Canada	Observatory Hill, BC	MF954838		
Galerina	sideroides		G. aff. sideroides sp. 1	No		F27118	Canada	Observatory Hill, BC	MF954840		
Galerina	sideroides		G. aff. sideroides sp. 1			F27143	Canada	Observatory Hill, BC	MF954841		
Galerina	sideroides		G. aff. sideroides sp. 1			F27144	Canada	Observatory Hill, BC	MF954842		
Galerina	sideroides		G. aff. sideroides sp. 1	No		F27196	Canada	Observatory Hill, BC	MF954843	MH828274	
Galerina	sideroides		G. aff. sideroides sp. 1			F29455	Canada	Observatory Hill, BC	MF954845		
Galerina	sideroides		G. aff. sideroides sp. 1			F30880	Canada	Graham Island, BC	MF954846		
Galerina	sideroides		G. aff. stylifera sp. 1	No		F24757	Canada	Observatory Hill, BC	MF954858	MH828271	MH829629
Galerina	sideroides		G. mammillata	No		F25254	Canada	Observatory Hill, BC	MF954849	MH828273	
Galerina	sideroides		G. mammillata			F25762	Canada	Observatory Hill, BC	MF954852		
Galerina	sideroides		G. mammillata	No		F26374	Canada	Observatory Hill, BC	MF954853		
Galerina	sideroides		G. mammillata	No		F29592	Canada	Observatory Hill, BC	MF954855		
Galerina	sideroides		G. mammillata			F30428	Canada	Graham Island, BC	MF954856		
Galerina	sideroides		G. mammillata			F30574	Canada	Moresby Island, BC	MF954857		
Galerina	sideroides		G. stylifera var. badia	No		F25207	Canada	Observatory Hill, BC	MF954775	MH828272	
Galerina	sideroides		G. stylifera var. badia			F25268	Canada	Observatory Hill, BC	MF954776		
Galerina	sideroides		G. stylifera var. badia			F25683	Canada	Observatory Hill, BC	MF954777		
Galerina	sideroides		G. stylifera var. badia	No		F25684	Canada	Observatory Hill, BC	MF954778		
Galerina	sideroides		G. stylifera var. badia			F29396	Canada	Observatory Hill, BC	MF954780		
Galerina	sphagnicola		G. sphagnicola			O73441	Estonia		AJ585464	AJ871505	
Galerina	sphagnorum		G. sphagnorum			O154094	Norway		AJ585455	AJ871510	
Galerina	sphagnorum		G. sphagnorum			O70913	Norway		AJ585454	AJ871511	
Galerina	stordalii		G. stordalii			O154169	Norway		AJ585435		
Galerina	stordalii		G. stordalii			O154179	Norway		AJ585434	AJ871551	
Galerina	stordalii		G. stordalii			OS401	Norway		KC842392		
Galerina	stordalii		G. stordalii			PRM896295	Czech Republic		LT577690		
Galerina	stordalii		G. stordalii			PRM922823	Czech Republic		LT577696		
Galerina	stordalii		G. stordalii			PRM923762	Czech Republic		LT577692		
Galerina	stordalii		G. stordalii			PRM923763	Czech Republic		LT577694		
Galerina	stordalii		G. stordalii			PRM935271	Czech Republic		LT577695		
Galerina	stordalii		G. stordalii			PRM935272	Czech Republic		LT577693		
Galerina	stylifera		G. aff pseudobadipes			x6920	Italy		JF908010		
Galerina	stylifera		G. aff. stylifera sp. 1			ODell4296	USA	Lane Co., OR	AF251180		
Galerina	stylifera	var. badia	G. stylifera var. badia		Holotype	AHSmith54112	USA	Idaho, Bonner: Granite Creek, Kaniksu National Forest	MH827068		

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Galerina	stylifera	var. caespitosa	G. stylifera var. badia		Holotype	AHSmith41223	USA	Michigan, Oakland: Haven Hill, Highland State Recreation Area	MH827067		
Galerina	stylifera		G. aff. sideroides sp. 1			F18182	Canada	Smithers Community Forest	MF954834		
Galerina	stylifera		G. aff. sideroides sp. 1	No		F19775	Canada	Pacific Spirit Park, Vancouver, BC	MF954835	MH828275	
Galerina	stylifera		G. aff. sideroides sp. 1			F25667	Canada	Observatory Hill, BC	MF954839		
Galerina	stylifera		G. stylifera var. badia	No		F27622	Canada	Duncan, BC	MF954779	MH828276	MH829630
Galerina	stylifera		G. stylifera var. badia			F29483	Canada	Observatory Hill, BC	MF954781		
Galerina	stylifera		G. stylifera var. badia			F29864	Canada	Observatory Hill, BC	MF954782		
Galerina	subcerina		G. subcerina			UNSW9931			AF501565		
Galerina	subcerina	var. subcerina	G. subcerina var. subcerina		Monograph material	AHSmith61831			MH827069		
Galerina	subcerina		G. fallax			F27222	Canada	Observatory Hill, BC	MF954755	MH828277	
Galerina	subcerina		G. subcerina var. subcerina	No		F25303	Canada	Observatory Hill, BC	MF954705		
Galerina	subfiliformis		G. fallax			F28144	Canada	Saanich Peninsula, BC	MF954756	MH828278	MH829631
Galerina	sulciceps		G. sulciceps			MHHNU7669	China	Liuyang county, Hunan province	KX214585		
Galerina	tibiicystis		G. tibiicystis			O72930	Norway		AJ585443		
Galerina	tibiicystis		G. tibiicystis			x14636	Italy		JF908014		
Galerina	tibiiformis		G. tibiiformis			UNSW0009			AF501566		
Galerina	triscopa		G. triscopa			CCB159	USA	Tennesse, Great Smoky Mountains National Park	KY744148		
Galerina	triscopa		G. triscopa			O73453	France		AJ585491		
Galerina	triscopa		G. triscopa			TOHG2283	Switzerland	Rodersdorf	KF826814		
Galerina	unicolor		G. venenata			O72515			AF251176		
Galerina	unicolor		G. venenata			x8942			JF908013		
Galerina	unicolor		G. castaneipes	α, β		F30011	Canada	Observatory Hill, BC	MF954819		
Galerina	unicolor		G. venenata			F19676	Canada	North Vancouver, BC	MF954786		
Galerina	unicolor		G. venenata			F27223	Canada	Observatory Hill, BC	MF954810		
Galerina	venenata		G. venenata			MICH10698	USA	Multnomah Co., OR	AF251178		
Galerina	venenata		G. venenata		Holotype	AHSmithMICH10698	USA	Multnomah Co., OR	MH827070		
Galerina	venenata		G. venenata			F30611	Canada	Graham Island, BC	MF954820		
Galerina	vexans		G. vexans			F25602	Canada	Observatory Hill, BC	MF954773		
Galerina	vexans		G. vexans		Paratype	AHSmith4371543719	USA	Michigan, Mackinac: Point Aux Chenes	MH827072		
Galerina	vittiformis	f. bispora	G. vittaeformis f. bispora		Monograph material	AHSmith48173			MH827073		
Galerina	vittiformis		G. aff. atkinsoniana sp. 3			Tian001			JF961372		
Galerina	vittiformis		G. aff. vittiformis sp. 1			F19779	Canada	Vancouver, BC	HQ604755		
Galerina	vittiformis		G. aff. vittiformis sp. 3			O7312	Greenland		AJ585484	AJ871544	
Galerina	vittiformis		G. aff. vittiformis sp. 3			O73469	Greenland		AJ585485	AJ871541	
Galerina	vittiformis		G. minima sp. 1			O154565	Norway		AJ585487		
Galerina	vittiformis		G. aff. atkinsoniana sp. 1	No		F25636	Canada	Observatory Hill, BC	MF954829	MH828279	MH829632
Galerina	vittiformis		G. aff. atkinsoniana sp. 1	No		F26131	Canada	Observatory Hill, BC	MF954830	MH828284	MH829637
Galerina	vittiformis		G. aff. vittiformis sp. 1			F24986	Canada	Observatory Hill, BC	MF954864		
Galerina	vittiformis		G. aff. vittiformis sp. 1			F25357	Canada	Observatory Hill, BC	MF954865		
Galerina	vittiformis		G. aff. vittiformis sp. 1			F25705	Canada	Observatory Hill, BC	MF954868		
Galerina	vittiformis		G. aff. vittiformis sp. 1			F25723	Canada	Observatory Hill, BC	MF954869		
Galerina	vittiformis		G. aff. vittiformis sp. 1			F25764	Canada	Observatory Hill, BC	MF954870		
Galerina	vittiformis		G. aff. vittiformis sp. 1	No		F25798	Canada	Observatory Hill, BC	MF954871	MH828280	MH829633
Galerina	vittiformis		G. aff. vittiformis sp. 1			F25800	Canada	Observatory Hill, BC	MF954872	MH828281	MH829634
Galerina	vittiformis		G. aff. vittiformis sp. 1			F25801	Canada	Observatory Hill, BC	MF954873	MH828282	MH829635
Galerina	vittiformis		G. aff. vittiformis sp. 1			F25871	Canada	Observatory Hill, BC	MF954875		
Galerina	vittiformis		G. aff. vittiformis sp. 1	No		F25889	Canada	Observatory Hill, BC	MF954876		
Galerina	vittiformis		G. aff. vittiformis sp. 1			F27667	Canada	Royal Roads University, BC	MF954879		
Galerina	vittiformis		G. aff. vittiformis sp. 2	No		F25255	Canada	Observatory Hill, BC	MF954769		
Galerina	vittiformis		G. aff. vittiformis sp. 2	No		F25603	Canada	Observatory Hill, BC	MF954771		
Galerina	vittiformis		G. vittiformis f. bispora			F25583	Canada	Observatory Hill, BC	MF954758		
Galerina	vittiformis		G. vittiformis f. bispora			F26104	Canada	Observatory Hill, BC	MF954759	MH828283	MH829636

Genus	Species	Variety	New Name	Toxin	Notes	Specimen ID	Country	City	ITS Accession	LSU Accession	RPB2 Accession
Gymnopilus	penetrans			No		F23892	Canada	Capilano River Regional Park, North Vancouver	KJ146708	KJ146708	
Gymnopilus	punctifolius			No		F23761	Canada	Capilano Regional Park	KC581324	KC581324	
Gymnopilus	sapineus					PBM1541WTU	USA	Wyoming		AY380362	AY337358
Gymnopilus	sp			No		F16466	Canada	McDonnell FSR, Smithers	FJ039687	FJ039687	
Gymnopilus	sp			No		F16467	Canada	McDonnell FSR, Smithers	FJ039688	FJ039688	
Gymnopilus	spectabilis					PBM2471CUW	USA	Massachusetts	DQ486707	AY700186	
Hebeloma	aff. remyi			No		F23896	Canada	Capilano River Regional Park, North Vancouver	KJ146712	KJ146712	
Hebeloma	affine					TENN063921	Canada	Ontario	FJ436320	EF561632	FJ436321
Hebeloma	angustilamellata					HKAS42927	China		AY575919	AY575919	
Hebeloma	birrus			No		F23894	Canada	Capilano River Regional Park, North Vancouver	KJ146710	KJ146710	
Hebeloma	leucosarx			No		F32044	Canada	Manning Park, BC	KX236127		
Hebeloma	olympianum					BK21Nov9820UTC	USA	Washington		AY038310	AY337359
Hebeloma	velutipes					PBM2277WTU	USA	California	AY818351	AY745703	DQ472718
Hymenogastraceae	PBM3116					PBM3116TENN	New Zealand		HQ840659	HQ840660	HQ840662
Hymenogastraceae	PBM3420					PBM3420TENN	Australia	Tasmania	HQ840663	HQ840664	HQ840666
Hypholoma	australe					PERTH08241856	Australia	Western Australia	HQ832446	HQ832456	HQ832434
Hypholoma	fasciculare					PBM1844WTU	USA	Washington		AY380409	AY337413
Hypholoma	sublateritium					JS031107CUW	USA	Massachusetts	AY818349	AY635774	
Hypholoma	subviride					TENN062712	USA	Tennessee	HQ222020	HQ832457	HQ832435
Inocybe	aff_asterospora					TENN065796	USA	New York	DQ404390	AY702015	
Inocybe	lilacina					PBM2039WTU	USA	Washington	HQ201357	AY380385	AY337388
Inocybe	mutata					TENN062387	USA	Massachusetts	JQ801410	AY732212	DQ472729
Inocybe	myriadophylla					JV19652FTURA	Finland	Finland	DQ221106	AY700196	AY803751
Inocybe	rimosoides					PBM2459CUW	USA	New York	DQ404391	AY702014	DQ385884
Inocybe	unicolor					PBM1841WTURV74D UKE	USA	Missouri	EU523554	AY380403	AY337409
Kuehneromyces	rostratus					TENN062522	USA	Massachusetts	DQ490638	DQ457684	DQ472730
Naematoloma	longisporum					TENN062558	USA	Massachusetts	DQ490634	DQ457681	
Nivatogastrium	nubigenum					PBM504WTU	USA	California	DQ494679	DQ470815	
Panaeolina	foenisecii					J152DUKE	Unknown	Unknown		AF041537	
Panaeolus	papilionaceus					RN050113TENN	Florida	Florida	KF830093	KF830082	KF830065
Panaeolus	sphinctrinus					PBM2009WTU	USA	Washington	DQ182503	DQ470817	
Phaeocollybia	festiva					PBM2366WTU	Norway		DQ494682	AY509119	AY509118
Phaeomarasmius	proximans					PBM1936WTU	USA	Vermont	DQ404381	AY380410	AY333314
Phaeomyces	dubiosus					TENN063604	France		KF830099	KF830089	KF830070
Pholiota	aff_astragalina					TENN062733	USA	Tennessee	HQ832448	HQ832462	HQ832439
Pholiota	multicingulata					TENN063875	New Zealand		HQ832449	HQ832463	HQ832440
Pholiota	squarrosa					TENN062547	USA	Colorado	DQ494683	DQ470818	
Pleuroflammula	Paludosa					MCA339VPI	Unknown		DQ494685	AF367962	DQ474124
Pleuroflammula	praestans					PERTH08242151	Australia	Western Australia	HQ832450	HQ832464	HQ832441
Pleuroflammula	tuberculosa					PAM02072903	France		HQ832452	HQ832465	HQ832442
Psilocybe	caerulipes					TENN064502	USA	Tennessee	KC669282	KF830084	KF830067
Psilocybe	cubensis					DNA2052TENN	Unknown		KF830094	KF830083	KF830066
Psilocybe	cyanescens					DNA1938TENN	Unknown		KJ137276	KJ137277	KJ137278
Psilocybe	silvatica					RV571989	Unknown		AY129362	AF042618	
Psilocybe	stuntzii					VT1263	Unknown			AF042567	
Psilocybe	subaeruginosa					TENN065481	Australia	Tasmania		KF830079	KF830062
Simocybe	serrulata					TENN062382	USA	Massachusetts	DQ494696	AY745706	DQ484053
Simocybe	sp_PBM3031					TENN062784	USA	Tennessee	GQ893023	GQ892979	HQ832444
Stropharia	ambigua					PBM2257WTU	USA	Washington	AY818350	AY646102	DQ484054
Tubaria	confragosa					PBM2105WTU	USA	Washington	DQ267126	AY700190	DQ408113
Tubaria	minima					Contu15122007	Italy		HQ832447	HQ832461	
Tubaria	minima					PAM06090110LIPepity pe	France		EF051060	EF051055	
Tubaria	serrulata					E8069PERTH	Australia	Western Australia	DQ182507	DQ156128	

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Tubaria	sp_BM378_17					TENN063912	USA	Washington	HQ832454	HQ832467	HQ839738
Tubaria	sp_PBM3355					TENN065365	Australia	Tasmania	HQ839739	HQ839740	
Tubaria	vinicolor					JFA12905WTU	USA	Washington	DQ536417	DQ536415	DQ536418