TAXONOMY, PHYLOGENY AND EVOLUTION OF HYPOGEOUS FUNGI IN THE SOUTHWEST CHINA

LIN LI

DOCTOR OF PHILOSOPHY
IN
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ABSTRACT

Hypogeous fungi represents a unique ecological type of macrofungi, characterized by the production of sequestrate (truffle-like) fruiting bodies that develop underground. Most species form ectomycorrhizal associations with plants and rely on animals for spore dispersal. In addition to their important ecological roles, many hypogeous fungi are edible or medicinal. In this study, an extensive survey of hypogeous fungi was conducted in southwestern China, encompassing 59 sampling sites, of which 209 specimens were collected. Based on combined morphological and molecular phylogenetic analyses, 21 new species were described, *viz. Hydnotrya oblongispora*, *H. zayuensis*, *Tuber albicavum*, *T. laojunshanense*, *T. umbilicicavatum*, *Truncocolumella pseudocolumella*, *Gautieria zixishanensis*, *Hymenogaster zayuensis*, *Chamonixia laojunshanensis*, *Rhizopogon laojunshanensis*, *R. melanocuticularis*, *R. zayuensis*, *R. weixiensis*, *Hysterangium alpinum*, *Hysterangium shibaoshanense*, *Protubera yunnanensis*, *Russula densiobtusispora*, *R. laojunshanensis*, *Jimgerdemannia cangshanensis*, *J. zixishanensis*, and *J. laojunshanensis*, Additionally, four new distribution records were documented: *Melanogaster panzhihuaensis* in Chuxiong, Yunnan; *Rhizopogon songmaodan* in Huili, Sichuan; *Rhizopogon jiyaozi* and *Rhizopogon sinoalbidus* in Linzhi, Xizang.

A comprehensive statistical survey of hypogeous fungal specimens from southwest China (including Yunnan, Sichuan, and Xizang provinces) revealed a total of 166 species in three phyla: Ascomycota (two orders, five families, eight genera, 91 species), Basidiomycota (five orders, 14 families, 20 genera, 72 species), and Mucoromycota (one order, one family, one genus, three species). Among these, 38 species are known to be

edible or medicinal. Each species was annotated with detailed specimen information, habitat and distribution data, molecular sequences, and known economic value.

Furthermore, this study analyzed the geographical distribution patterns of hypogeous fungi in southwestern China. The results show that these fungi are primarily concentrated in the Yarlung Zangbo River basins, Nujiang River, Lancang River, and Jinsha River basins, with the Jinsha River basin being the most prominent. This basin harbors the richest hypogeous fungal diversity, with 110 recorded species, including 92 endemics. The distribution pattern between river basins indicates that hypogeous fungi in this region exhibit high habitat specificity and strong basin-restricted distributions. Notably, 84.5% of species were confined to a single river basin, and cross-basin distributions were rare. The highly folded terrain and alternating ridges and valleys of the Hengduan Mountains are likely key drivers of the geographical isolation of hypogeous fungal species. The elevational range of these fungi spans from 860 m to 4005 m, with 96.1% of species occurring in alpine and subalpine forests between 1500 and 4000 m. Species diversity peaks between 2000 and 2500 m, and 40 species are found in alpine fir (*Abies*) forests at elevations of 3000–4000 m. It is noteworthy that 95% of hypogeous fungi in this region form ectomycorrhizal associations with trees of the families Pinaceae and Fagaceae. In addition, analysis of collection dates reveals a multimodal pattern in sporocarp formation, influenced by the complex interplay of monsoonal patterns and vertical climatic zonation in mountainous areas of southwestern China. The primary fruiting peak occurs from August to October, during the mid-to-late rainy season. A secondary peak is observed from November to January—the early dry season, which corresponds to the intensive harvesting of *Tuber* species. A minor spring peak, occurring between Day of Year (DOY) 100–160, is likely associated with increased soil moisture due to pre-monsoon rainfall.

The evolutionary history of hypogeous fungi in southwestern China was also investigated. Using a five-gene phylogenetic dataset, divergence times were estimated for 28 hypogeous *Boletales* species with valid molecular data. The results suggest that the evolution of hypogeous fruiting bodies within *Boletales* likely began in the late Eocene (ca. 50 Mya) and continued through the early Miocene (ca. 22 Mya). Within the southwestern China taxa, the earliest divergence was traced to the Eocene (e.g.,

Truncocolumella pseudocolumella, 50.09 Mya), and most diversification events occurred from the Oligocene (ca. 34 Mya) to the early Pliocene (ca. 5–3 Mya).

These findings highlight the unique climatic and geographic conditions that have fostered the rich diversity and distinct distribution patterns of hypogeous fungi in southwestern China.

Keywords: 21 New Species, Distribution Pattern, Evolutionary History, Hypogeous Fungi, Southwestern China, Taxonomy

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CHAPTER 1

INTRODUCTION

1.1 Background of the Research Problem

Hypogeous fungi are an exceptional group of mushrooms that grow perennially underground, except that in some genera parts of the fruit bodies grow out of the ground when they reach maturity. Fruit bodies are mostly spherical and grow underground or close to the ground without an obvious stipe and no ability to eject proactive spores as their important features (Bonito et al., 2013; Hawker, 1955; Zhang, 1986). Usually, hypogeous fungi are also called truffles, among which those belonging to the phylum Ascomycota are called true truffles, those belonging to the phylum Basidiomycota are called false truffles, and those belonging to the phylum Glomeromycota and Mucoromycota are called pea truffles. For example, the genus *Tuber* of the phylum Ascomycota, and the genus *Rhizopogon* of the phylum Basidiomycota.

Hypogeous fungi that grow underground are specially adapted to the environment. Hypogeous fungi cannot eject the spores, but they can attract small animals to eat them in the forest, and their spores are carried to other places by these consumers (Bonito et al., 2013; Hawker, 1955; Johnson, 1996; Ławrynowicz, 1990; Læssøe & Hansen, 2007). Furthermore, hypogeous fungi often form ectotrophic mycorrhizae with Pinaceae, Fagaceae, Betulaceae, and Salicaceae (Bonito et al., 2013; Hawker, 1955; Liu & Liu, 1984). Ectomycorrhizal associations are important for forest biomes, connecting and transferring nutrients through an intricate and complex system of underground hyphal networks to provide nutrients for plant growth (Smith & Read, 1997). In the process of adapting to the environment, the fungi-animal-plant association as a special association is in coevolution (Maser et al., 1978; Trappe et al., 2009).

Although they are often overlooked, hypogeous fungi have a very important ecological value.

Some hypogeous fungi are well-known edible fungi, such as *Tuber* melanosporum and *Tuber* magnatum. They are precious delicacies and an important aspect of the economics of edible fungi. They are precious delicacies and an important part of the edible fungus economy. Therefore, a large amount of research has been conducted on them.

In China, some species of hypogeous fungi are not only delicious but also have medicinal properties. They are considered important wild resources by local people, especially in southwest China, and harvesting and trading the fungi could be an important income for them. Hypogeous fungi of both ecological and economic value have not been studied much due to the difficulty of specimen collection, especially in southwestern China. And, because of the phenotypic plasticity and morphological similarities between species, many cannot be easily recognized based solely on morphological characteristics. This study aimed to conduct an extensive survey in southwest China and explore the characteristics of the hypogeous fungus resources.

1.2 Research Objectives

1.2.1 Extensive collection of hypogeous fungi specimens, clarifying the species diversity and resource distribution of hypogeous fungi in southwest China.

1.2.2 Refine and improve the taxonomic classification system of hypogeous fungi based on morphological and molecular evidence.

1.2.3 Elucidate the evolutionary relationships of hypogeous fungi to provide up-to-date basic information on hypogeous fungi in southwest China.

1.3 The Importance of Research

1.3.1 Insufficient specimen records and scientific data on hypogeous fungi in China. Current statistics indicate that the number of hypogeous fungal species reported from China is far from representing their actual species richness. Moreover, most of the reported taxa lack high-quality molecular data. Due to the overall deficiency of specimen collections and the limited accumulation of scientific data on hypogeous fungi, our understanding of their diversity remains extremely inadequate. This situation is particularly pronounced in southwestern China—a recognized biodiversity hotspot—where the scarcity of reliable data hampers studies on macrofungal evolution and forest ecology.

1.3.2 Rich but poorly understood diversity of hypogeous fungi in southwestern China. The hypogeous fungi in southwestern China are highly diverse and often unique; however, species concepts remain unclear, synonymy is widespread, and distribution patterns are poorly documented. This includes many edible and medicinal species, whose unclear taxonomy and distribution hinder both their sustainable utilization and the conservation of valuable edible and medicinal hypogeous fungi.

1.4 Research Hypothesis

1.4.1 The diversity of hypogeous fungus species is high in southwest China.

1.4.2 Southwest China has a unique geographical and climatic environment and is an ideal area to study hypogeous fungi, with unique resources of hypogeous fungal species.

1.5 Scope of Research

1.5.1 Extensive investigation and collection of hypogeous fungi specimens in southwest China. Hypogeous fungi specimens from southwest China were studied using a combination of molecular systematic analysis, morphological examination, and habitat information.

1.5.2 Revealing the evolutionary timeline of hypogeous fungi in southwestern China using molecular clock techniques.

1.5.3 Uncovering and illustrating the biogeographic distribution patterns of hypogeous fungi in southwestern China.

1.6 Research Limitations

1.6.1 Research on hypogeous fungi in China remains limited, with few preserved specimens available in herbaria. Many early-collected specimens have been lost or are unsuitable for molecular analysis due to improper preservation.

1.6.2 Due to the difficulty of locating and collecting hypogeous fungi, the sampling areas in this study were geographically restricted. As a result, the findings may not fully reflect the overall distribution and diversity of hypogeous fungi in southwest China.

CHAPTER 2

LITERATURE REVIEW

2.1 Current Status and Scientific Significance of Hypogeous Fungi Research

2.1.1 Introduction to Hypogeous Fungi

Hypogeous fungi, also known as sequestrate fungi, commonly referred to as hypogeous fungi, typically grow in the soil, forming large fruiting bodies that are fully or partially buried in the soil or leaf litter during growth or at maturity. A few species may fully emerge above the soil surface but lack a distinct stipe and do not actively release spores at maturity (Hawker, 1955; Cooke, 1979).

A prominent feature in the evolution of large fungi is convergent evolution (Oberwinkler, 2012), such as gasteromycetation in Basidiomycota and truffle syndrome in Ascomycota. Therefore, hypogeous fungi represent a polyphyletic group commonly found in Ascomycota, Basidiomycota, Glomeromycota, and Mucoromycota. Regardless of their taxonomic classification, hypogeous fungi form truffle-like fruiting bodies. These range in diameter from a few millimeters to nearly ten centimeters, varying from spherical to slightly lobed, and are composed of spore-bearing tissues (gleba) usually encased by an outer layer (peridium), although the peridium may disappear during development in some species.

Hypogeous fungi have significant ecological and economic value. Many have a high economic value as edible fungi, such as *Tuber* and *Rhizopogon*. In certain regions of Yunnan, various hypogeous fungi are traditional delicacies or ethnic medicines. They are also important ectomycorrhizal fungi, with numerous studies focus on their role in mycorrhization and cultivation. Moreover, animals serve as key spore dispersers, making these fungi an essential food source for wildlife (Trappe & Claridge, 2005; Komur et al., 2010). In forest communities, hypogeous fungi closely connect plants and animals, playing a crucial role in stabilizing biological communities. As more species of hypogeous fungi are discovered, they are increasingly featured in forest ecology research (Bruns et al., 1989; Claridge, 2002).

2.1.2 Research Status of Hypogeous Fungi in China and Abroad

2.1.2.1 Current Status of Classification and Systematics Research on Hypogeous Fungi

The unique morphology and habitat of hypogeous fungi have always been controversial in the classification and systematics of large fungi. With the accumulation of various types of hypogeous fungi specimens, their systematic position has gradually become clearer (Naranjo-Ortiz & Gabaldón, 2019, Komur et al., 2021). In 1800 and 1851, numerous publications by mycologists such as Fries, Tulasne, and Vittadini (Tulasne, 1851; Persoon, 1800, 1801; Fries, 1817, 1821, Vittadini, 1831, 1842; Hawker, 1968), including the famous “Monographia Tuberacearum,” “Hypogeous Ascomycetes from Idaho,” and “Fungi hypogæi,” laid the foundation for the modern classification and origin of hypogeous fungi.

In the twentieth century, European mycologists described numerous new species of hypogeous fungi, accumulating a wealth of specimens that supported classification and systematic research by mycologists like Haecker, Cooke, and Malen çon (Hawker, 1955; Cooke, 1979; Malen çon, 1938; Ramsbottom, 1953). There is now a clearer understanding of the morphological characteristics, individual development, ecology, and physiology of hypogeous fungi. Despite the overall similarity in mature fruiting bodies and habitat features, hypogeous fungi have been independently classified. However, scientists discovered early on that hypogeous fungi are closely related to epigean fungi from the perspectives of micro-characteristics and individual development (Malen çon, 1938; Fischer, 1943; Nannfeldt, 1946; Burdsall, 1968; Korf, 1973; Trappe, 1971, 1979).

In 1989, Bruns et al. used molecular systematics to explain that *Rhizopogon* is a result of the gasteromycetation of Suillus, first proposing the theory that hypogeous fungi evolved from epigean fungi (Bruns, 1989). Since then, with the discovery and description of numerous hypogeous fungal specimens, their systematic position has been constantly adjusted. Some hypogeous fungi are no longer classified solely as independent taxa. In 2007, Læss øe and Hansen (Læss øe & Hansen, 2007) provided new evidence for the systematic evolution of Pezizales through molecular and morphological analyses, suggesting that different types of hypogeous fruiting bodies independently evolved multiple times within the lineage. Albee-Scott’s 2007 analysis (Albee-Scott, 2007) of false-truffles within Basidiomycota revealed evolutionary relationships between hypogeous fungi and their epigean counterparts, suggesting that the evolution of some hypogeous fruiting bodies occurs at the species level. For instance, the genus *Lactarius*, which previously consisted entirely of epigean fungi, now includes the formerly independent genus *Zelleromyces* (Sang et al., 2016). Similarly, *Russula* now encompasses genera such as *Macowanites*, *Martellia*, and *Gymnomyces* (Albee-Scott, 2007). In 2020, Loizides et al., analyzed the genus *Chlorophyllum*, revealing that it contains both secotioid and hypogeous species (Loizides et al., 2020).

With the increasing recognition of hypogeous fungi, their ecological roles have garnered attention. In 2020, Van Dorp et al., highlighted the key role of Rhizopogon in the ecological restoration of Douglas-fir forests (Van et al., 2020). In 2023, Niego et al. emphasized the importance of hypogeous fungi in the forest ecosystem services provided by large fungi (Niego et al., 2023).

In China, the earliest record of hypogeous fungi might be a specimen (HM1372) collected by Austrian botanist Heinrich von Handel-Mazzetti on August 10, 1915, in Lijiang, Yunnan, China. In 1937, Keissler and Lohwag identified this specimen as Melanogaster variegatus (Vittad.) Tul. & C. Tul. 1851 (Keissler & Lohwag, 1937). In 1995, researcher Wang Yun re-examined this specimen and described it as a new species, Melanogaster ovoidisporus Y. Wang 1995 (Wang et al., 1995). In 1984, Eckblad and Ellingsen identified and reported hypogeous fungi specimens collected by Karl August Harald (Harry) Smith from China based on morphological examination (Eckblad & Ellingsen, 1984).

Since the 1980s, researchers such as Liu Bo, Tao Kai, Zhang Bincheng, Wang Yun, and Xu Asheng have collected numerous specimens, identified them based on morphological studies, and published many new species (including varieties) and new records of hypogeous fungi. According to Li Lin et al. (2014), there are more than 160 species (Li et al., 2014), although most lack molecular data. In recent years, many hypogeous fungi have been published with high-quality data. In 2022, Fan Li conducted a systematic study of the genus *Tuber* in China (Fan et al., 2022), and in 2023, Fan Li and Bau reported three new species of *Tuber* (Fu & Fan, 2023; Guo & Bau, 2023), bringing the total number of Tuber species in China to 68. From 2015 to 2022, Fan Li's team reported four new species of *Melanogaster* (Xu et al., 2022), four new species of *Hydnotrya* (Xu et al., 2018), four new species of *Hydnobolites* (Li et al., 2019a), two new species of *Pachyphloeus* (Li et al., 2019b), and four new species of hypogeous fungi in Russulaceae (Sang et al., 2016). In 2022, Wan Shanping et al. reported five new species of *Hydnobolites* (Wan et al., 2022) and one new species of *Choiromyces* (Wang et al., 2022). In 2016, Li Lin et al. reported three new species of *Rhizopogon* (Li et al., 2016), and in 2023, three new species of *Hydnotrya* (Li et al., 2016, 2023). Other researchers have occasionally reported hypogeous fungi specimens, and according to Fan Li (2022), 275 species of hypogeous fungi were recorded in China by 2022.

In the field of hypogeous fungi evolution research in China, in 2009, Yang et al., demonstrated in a phylogenetic and biogeographic study of the genus *Chroogomphus* (Li et al., 2009) that *Secotium albipes* Zeller is actually a gasteromycetes member of *Chroogomphus* (now named *Chroogomphus albipes*), though no related species have been reported in China. In 2016, Wu Gang et al. conducted a phylogenetic study of the *Boletaceae* (Wu et al., 2016a, 2016b), incorporating *Gastroboletus thibetanus* into the genus *Sutorius* (now named *Sutorius thibetanus*), suggesting that other species in *Gastroboletus* may not form a monophyletic group. Despite Lohwag establishing *Gastroboletus* based on specimens from China in 1937, with 21 recorded species in the genus, there are only six specimens and three molecular data entries for the Xizang Province *Gastroboletus* in China.

Comparing the classification and systematic research of hypogeous fungi domestically and internationally reveals that China has not paid sufficient attention to hypogeous fungi. The number of species discovered and described does not represent their richness, and high-quality specimen information and scientific data are lacking, hindering in-depth research in fungal evolution and forest ecology.

2.1.2.2 Cultivation and Application Research on Hypogeous Fungi: Domestic and International Perspectives

Exploring cultivation techniques for hypogeous fungi has led to the elucidation of scientific questions related to mycorrhizal synthesis, the physiology, ecology, and genetics of hypogeous fungi. The application of hypogeous fungi primarily falls into two areas: ecological value and symbiotic mechanisms, where they are studied for mycorrhization and ecological restoration (Allen et al., 1995). Though still in the exploratory stage, their ecological value is considerable. Secondly, based on their edible value and economic benefits, cultivation research and practice focus on edible genera such as *Tuber* and *Rhizopogon* (Čejka et al., 2023; Van et al., 2020; Boyno & Demir, 2022; Yamanaka et al., 2023). Currently, the artificial cultivation of truffles and *Rhizopogon* has been commercialized in Europe and the United States. Currently, the artificial cultivation of truffles and *Rhizopogon* has been commercialized in several countries in Europe, North America, and Australia. In China, some high-value truffle species have been successfully cultivated in Yunnan, Guizhou, and Sichuan in recent years (Wang et al., 2020). Apart from truffles, many hypogeous fungi in southwestern China are used and traded as edible and medicinal fungi by local people; however, they have not received sufficient attention.

2.2 Existing Issues in the Study of Hypogeous Fungi in China

2.2.1 Lack of Specimen Data and Scientific Information on Hypogeous Fungi in China

The reported species of hypogeous fungi in China significantly under represents their actual diversity. Additionally, more than half of the reported information lacks high-quality molecular data. In contrast, the extensive collection and accumulation of diverse hypogeous fungi specimens in Europe, North America, and Australia have provided ample specimen data and scientific information, facilitating in-depth research into fungal evolution and forest ecology. However, China lags considerably in terms of diversity and scientific data accumulation of hypogeous fungi, with a notable deficiency in specimen data. This gap is particularly pronounced in Southwest China, a biodiversity hotspot.

2.2.2 Taxonomic Uncertainty and Synonymy Issues in China’s Hypogeous Fungal Species

A common issue is that early identifications of Chinese hypogeous fungi specimens by both domestic and international scholars were primarily based on morphology, often assigning names of known European species. However, hypogeous fungi are sensitive to environmental heterogeneity, making such identifications questionable. For instance, a recent specimen of the genus *Chamonixia* collected in Yunnan was identified as *Chamonixia caespitosa* Rolland 1899 based on morphology. Despite morphological similarities, the ITS sequence similarity was less than 96%. Similar discrepancies are frequently observed, even in edible and medicinal hypogeous fungi, yet these misidentifications have not been corrected.

2.2.3 Unclear Species Concepts and Distribution of Hypogeous Fungi in Southwest China

Southwest China hosts a rich diversity of unique hypogeous fungi species, including many edible and medicinal types. However, species concepts are unclear, and distributions are poorly understood, hindering their development and utilization. As of 2023, 282 species of hypogeous fungi have been reported in China, with 122 species distributed in Yunnan, Sichuan, and Xizang Provinces. Many early reported species lack detailed specimen data, and numerous edible and medicinal species have been incorrectly named. Recent discoveries of new species, aside from those in the genus Tuber, are sparse. This scarcity is partly due to the difficulty of collecting hypogeous fungi and their tendency to be overlooked, but primarily because few researchers focus on them.

2.3 The Importance of Taxonomic and Systematic Studies on Hypogeous Fungi in Southwest China

2.2.2 Effective Accumulation of Basic Research Data on Hypogeous Fungi

Southwest China, located in the heartland of the country, is extremely rich in hypogeous fungi species (Feng & Yang, 2018). Extensive surveys and studies on the taxonomy and systematics of these fungi will result in the accumulation of numerous high-quality specimens and scientific data. This can provide significant data support for research on the systematics and evolutionary theory of large fungi, as well as forest ecosystem theories (Feng & Yang, 2018).

2.3.2 Southwest China as an Ideal Region for Hypogeous Fungi Research

Firstly, Southwest China features high mountains and deep valleys, with rivers creating natural barriers. For hypogeous fungi, which mainly rely on animals to disperse their spores, such geographic features increase the probability of gene flow interruption, leading to higher species diversification over long-term evolution and thus greater species diversity (Trappe & Claridge, 2005). Secondly, paleobotanical data indicate that the plant communities in the Himalayan-Hengduan Mountains of Southwest China are descendants of the Tethys tertiary flora (Sun et al., 2017). These plants adapted to arid conditions as the Mediterranean climate evolved and later to alpine environments as the Himalayas and Hengduan Mountains uplifted (Yao et al., 2010). This evolutionary adaptation led to the differentiation and development of plants in these regions, forming their diversification centers (Yao et al., 2010; Sun et al., 2017). Studies suggest that hypogeous fungi, common in Mediterranean climates, evolved as a strategy to conserve moisture under selective pressures such as warm, dry conditions (Trappe & Claridge, 2005). It can be inferred that hypogeous fungi evolved under Mediterranean climates were retained and developed alongside their host plants in the Himalayan-Hengduan Mountains, adapting to alpine environments and leading to rich hypogeous fungi resources in these areas. Thirdly, Southwest China has been a hotspot for large fungal surveys (Feng & Yang, 2018). In preliminary studies focusing on hypogeous fungi in this region, numerous specimens were collected, and many ideal sampling sites were identified, warranting further research.

2.3.3 Conservation and Utilization of Hypogeous Fungi Resources in Southwest China

Many hypogeous fungi species are rare wild edible and medicinal mushrooms, especially in Southwest China, such as the Jinsha River and Nu River valleys, known for truffle production, which provides substantial income for local residents annually. Preliminary surveys show that over 30 ethnic minorities have traditionally inhabited Southwest China (National Civil Affairs Commission (Ed.), 2018), with many hypogeous fungi serving as both delicious wild food and vital natural resources for poverty alleviation and economic development. Common market species include truffles (*Tuber* spp.), *Rhizopogon jiyaozi*, *R. sinoalbidium*, *R. songmaodan*, and *Scleroderma yunnanense* and so on (Li et al., 2016; Wang et al., 2020). Although hypogeous fungi are generally considered non-toxic, there have been multiple poisoning incidents annually due to their consumption (Li et al., 2020, 2022, 2023). Therefore, it is crucial to conduct taxonomic and systematic studies to clarify the species diversity and distribution characteristics of hypogeous fungi in Southwest China, an important distribution area for edible and medicinal hypogeous fungi.

CHAPTER 3

RESEARCH METHODOLOGY

3.1 Sample Collection

The studied specimens were collected from Southwest China, including Yunnan, Sichuan, and Xizang provinces. These specimens were deposited at the BMDU and HKAS, China. The information on hypogeous fungal species distributed in Southwest China is derived from publicly available literature and reports. The herbarium mentioned in the text is referenced with corresponding abbreviations, as detailed in Table 3.1.

Table 3.1 The abbreviations of the herbarium names

Abbreviations	Full name
BMDU	Biological Science Museum of Dali University, China
BJTC	Herbarium Biology Department, Capital Normal University, China
HMAS	Herbarium Mycologicum Academiae Sinicae, China
HMJAU	Herbarium of Mycology of Jilin Agricultural University, China
HKAS	Herbarium of Cryptogams Kunming Institute of Botany, Academia Sinica, China
HXZE	Herbarium of Xizang Institute of Ecology, China
KPM-NC	Kanagawa Prefectural Museum of Natural History, Japan
MHSU	Mycological Herbarium of Shanxi University, China
NIOHP	Herbarium of National Institute of Occupational Health and Poison Control, Chinese Center for Disease Control and Prevention.
YAAS	Herbarium of Yunnan Academy of Agricultural Sciences, China
YNAU	Herbarium of Yunnan Agricultural University, China

Samples collected in southwestern China, they were deposited in the herbarium of the herbarium of Cryptogams at the Kunming Institute of Botany, Chinese Academy of Sciences (HKAS) and Bioscience Museum of Dali University (BMDU), China. Additional collections were loaned from other herbaria and studied. Loans included those from the herbarium of Cryptogams at the Kunming Institute of Botany, Chinese Academy of Sciences (HKAS).

3.2 Morphological Studies

Descriptions of microscopic and macroscopic characters were based on specimens, following the methods of Kumar et al. (2017) and Truong et al. (2017). Macroscopic characters of ascomata and gleba were observed under a Nikon SMZ1000 stereo zoom microscope. The sections were made by hand with a razor blade, mounted in a 5% KOH solution or water. The sections were observed under a light microscope. The temporarily prepared microscope slides were placed under magnification up to 1000× using Nikon ECLIPSE80i (Nikon, Japan) compound stereomicroscope for observation and microscopic morphological photography. Measurements were made using the Image Frame work v.0.9.7. To represent variation in the size of basidiospores, 5% of measurements were excluded from each end of the range and extreme values were given in parentheses (Lin et al., 2024, 2025). In the taxonomic descriptions of species, ‘Q (L/I)’ refers to the length/width ratio of ascospores in side-view; ‘Qm’ refers to the average Q of all ascospores ± standard deviation; ‘n’ refers to the number of spores measured. Key colors were obtained from Kornerup and Wanscher (1978).

3.3 DNA Extraction, PCR Amplification, and Sequencing

Total genomic DNA was extracted from the specimen using the OMEGA Plant Genomic DNA Kit. The internal transcribed spacer (ITS) rDNA region was amplified with PCR primers ITS1F/ITS4 (Truong et al., 2017; White et al., 1990; Gardes & Bruns, 1993). The large subunit nuclear ribosomal DNA (LSU) region was amplified with the PCR primers LROR/LR5 (Vilgalys et al., 1990). The RNA Polymerase II Second Largest Subunit (*rpb2*) region was amplified with the PCR primers RPB2- 6F/RPB2-7R (Liu et al., 1999; Matheny, 2005). The ATP synthase F0 subunit 6 (*atp6*) region was amplified with the PCR primers ATP6-3/ATP6-2 (Kretzer & Bruns, 1999). The small subunit of the mitochondrial ribosomal DNA (mtSSU) region was amplified with the PCR primers MS1/MS2 (White et al., 1990), The translation elongation factor alpha (*tef1*) region was amplified with the PCR primers EF1-983F/EF1-1567R (Morehouse et al., 2003). Each 30 µL of PCR mixture contained 15 µL of 2 × Taq Plus Master Mix II (Sangon Biotechnology Co., Kunming, China), 13 µL of ddH2O, 0.5 µL and 10 µM of forward and reverse primers, 1 µL of DNA. PCR reactions were performed on a BIO-RAD C1000TM instrument. Thermal cycles with the following settings: initial denaturation for 5 min at 94°C, followed by 32 cycles of 40 s denaturation at 94°C, annealing at 56°C for 40 s for ITS, and 52°C for 30 s for LSU and mtSSU, extension for 1 min at 72°C, and final extension at 72°C for 10 min.; an initial denaturation at 95°C for 4 min, followed by 15 cycles of 94°C for 50 s, 65–50°C for 55 s (reduced by 1°C per cycle), 20 cycles of 94°C for 50 s, 50°C for 55 s, 72°C for 1 min, 72°C for 1 min, and a final extension at 72°C for 10 min for tef-1α. And Matheny et al. (2005) for rpb2, and Kretzer and Bruns (1999) for atp6. The PCR products were verified on 1% agarose electrophoresis gels stained with ethidium bromide. The purification and sequencing of the PCR products was conducted by Sangon Biotech Limited Company (Shanghai, China).

3.4 Sequence Alignment and Analyses

ITS was used for the analysis of species diversity in this study because it appears to be a useful locus for the delimitation of some genera. however, for some genera, we used polygenic analysis, such as LUS, mtSSU, atp6, *tef1*, *rpb1*, and *rpb2*. All sequences were extracted from fruiting bodies of specimens in this study. Sequences of all species generated in this study were submitted to the GenBank database. We first edited the sequences using BioEdit v. 7 (Hall, 2007), then used the basic local alignment search tool for the GenBank database to recheck whether the newly generated sequences were amplified DNA from contaminant or not and examined clusters with closely related sequences. DNA sequences were retrieved and assembled using SeqMan. Sequences were aligned using MAFFT version 7 (Katoh & Standley, 2013). Maximum likelihood (ML) analysis was performed using RAxML-HPC2 v. 8.2.12 (Stamatakis, 2014) as implemented on the Cipres portal (Miller et al., 2011), 1000 rapid bootstrap (BS) replicates for all genes. A reciprocal 70% bootstrap support approach was used to check for conflicts between the tree topologies from individual genes. As the topology of the ML tree and the Bayesian tree are similar, sequences were combined using SequenceMatrix (Vaidya et al., 2011), partitioned phylogenetic analyses. For Bayesian inference (BI), the best substitution model for each partition was determined by MrModeltest 2.2 (Nylander et al., 2004). The results suggested that ITS1: JC + I, 5.8S: GTR + G + I, ITS2: K80 + I + G. The best-fit models of the three genes were HKY+G (*atp6*), HKY+I (nLSU), and K80+I (*rpb1*/*rpb2*). Bayesian analysis was performed using MrBayes ver. 3.2.7a (Ronquist et al., 2024) on the Cipres (Miller et al., 2011); four parallel runs were performed for 10 million generations sampling every 100th generation for the single gene trees. Parameter convergence > 200 was verified in Tracer v. 1.7 (Rambaut et al., 2018). The phylogenetic clade was strongly supported if the bootstrap support value (BS) was ≥70% and/or a posterior probability (PP) < 0.01.

3.5 Biogeographic Analysis

All available distribution data of hypogeous fungal specimens collected from southwestern China were compiled, including collection localities (with GPS coordinates), habitat elevation, symbiotic host tree species, and associated vegetation types. Based on these data, spatial analyses were conducted using ArcGIS 10.8 (ESRI, Redlands, CA, USA) and QGIS 3.34 (Open Source Geospatial Foundation) to visualize the distribution patterns of hypogeous fungi across the region.

Two major environmental factors were used for spatial stratification: river basin systems and topographic elevation zones. River basin boundaries were delineated using hydrographic data from the HydroSHEDS database (https://www.hydrosheds.org/) and national geospatial datasets provided by the National Earth System Science Data Center (http://www.geodata.cn/). Elevation data were extracted from the SRTM (Shuttle Radar Topography Mission) 30 m digital elevation model, which was reclassified into standardized elevation bands to examine altitudinal distribution patterns.

To investigate the distribution patterns of hypogeous fungi across different river basins in southwestern China, we compiled a dataset comprising 447 specimens representing 161 distinct species, excluding records that lack collection locality information. The data were organized in an Excel spreadsheet and processed using the *pandas* library in Python 3.9 (McKinney, 2010). We quantified the number of species present in each basin and identified species shared among multiple basins. To explore the degree of overlap in species distributions across basins, we employed the set visualization tool *UpSet Plot*, which effectively illustrates the intersection structure of multiple sets (Lex et al.,, 2014). A binary species × basin matrix was then constructed, and a heatmap was generated using the *seaborn* library (Waskom, 2021) to visualize species occurrences across basins. Hierarchical clustering was applied to both species and basins to reveal potential ecological distribution patterns more intuitively.

We conducted three complementary analyses to characterize the elevational distribution of hypogeous fungi in southwestern China. First, we divided specimen elevations into 500 m strata (e.g., 500–1000 m, 1000–1500 m, etc.) and calculated the number of unique species in each stratum to assess species richness (McCain, 2005). Second, we performed a kernel density estimation on all specimen elevations to visualize changes in overall abundance along the elevational gradient (Silverman, 1986). Third, we constructed species by stratum abundance matrices to compute the Shannon diversity index for each 500 m band and then fitted a cubic B spline regression (a GAM equivalent approach) of the Shannon index against the stratum midpoint elevation. We derived 95 % confidence intervals for the spline fit to evaluate model precision (Wood, 2017).

This study analyzed the seasonal patterns of sporocarp maturation in 166 hypogeous fungal species distributed across southwestern China, based on specimen collection dates. Collection times were extracted from original herbarium records, and specimens lacking temporal information were excluded from the analysis. Data analysis was conducted using Python 3.9, employing the packages *pandas*, *matplotlib*, and *seaborn*. Kernel Density Estimation (KDE) was applied to identify peak periods of maturation and to assess seasonal distribution patterns, which were then visualized. For the purpose of this study, the rainy season in southwestern China was defined as May to October, and the dry season as November to April. Seasons were categorized as Spring (March–May), Summer (June–August), Autumn (September–November), and Winter (December–February), following Dong et al. (2018) and Xu et al. (2018).

These analytical approaches aim to reveal the biogeographic patterns of species diversity among hypogeous fungi in the montane ecosystems of southwestern China.

3.6 Calibration, Divergence Time, and Evolutionary Rate Estimations of Boletales

This study uses the order Boletales as an example, constructing a five-gene phylogenetic tree that includes hypogeous fungi from Southwest China and estimating the divergence times of hypogeous Boletales species distributed in this region.

Divergence time estimation within the order Boletales, including representative families, genera, and species, was conducted using BEAST v2.6.7 (Bouckaert et al., 2019) based on a concatenated five-locus dataset comprising ITS, LSU, TEF1, RPB1, and RPB2. Sequence alignments were partitioned by gene region, and substitution models for each partition were selected using jModelTest v2.1.10 (Darriba et al., 2012) based on the Akaike Information Criterion (AIC). The following models were applied in BEAST: GTR+G for ITS, GTR+G for LSU, HKY+G for TEF1, and GTR+G for both RPB1 and RPB2. Base frequencies were empirically estimated for each gene region, and substitution and clock models were unlinked across partitions to allow for model heterogeneity. Codon positions for protein-coding genes (TEF1, RPB1, and RPB2) were not partitioned separately. The use of gamma-distributed rate variation (+G) in the substitution models accounts for among-site heterogeneity, and the final models were chosen to balance goodness-of-fit with parameter complexity based on the AIC results from jModelTest.

An uncorrelated lognormal relaxed clock model (Drummond et al., 2006) was used for each gene region to accommodate rate variation among lineages. The tree prior was set to a Yule speciation process, assuming a constant speciation rate across lineages. Two calibration points were incorporated to estimate divergence times. A secondary calibration was applied at the stem node of Boletales, using a normal distribution with a mean of 188.82 million years ago (MYA) and a standard deviation of 15, corresponding to a 95% confidence interval of approximately 158–219 MYA, based on divergence estimates reported by Wu et al. (2023). Additionally, a fossil-based calibration was implemented for the crown node of the suborder Suillineae using ectomycorrhizal fossils attributed to *Rhizopogon*/*Suillus*, with a uniform distribution between 50 and 100 MYA (LePage et al., 1997).

BEAST analyses were performed for 100 million generations, with parameters sampled every 10,000 generations. The first 10% of samples were discarded as burn-in. Convergence of the Markov chain Monte Carlo (MCMC) analysis was assessed in Tracer v1.7.2 (Rambaut et al., 2018), ensuring that all effective sample sizes (ESS) exceeded 200. The maximum clade credibility (MCC) tree was summarized using TreeAnnotator v2.6.7 and visualized in FigTree v1.4.4 (Rambaut, 2014), with mean node ages and 95% highest posterior density (HPD) intervals displayed.

Among the hypogeous fungal species with estimated divergence times, 28 occur exclusively in Southwest China. Temporal patterns of biodiversity assembly were reconstructed using divergence time estimates from 28 endemic species. Kernel Density Estimation (bandwidth selection: Sheather-Jones method) identified speciation peaks, while histogram analysis (5-Mya bins) resolved event clustering. Geological epoch boundaries followed the International Chronostratigraphic Chart. All computations utilized R v4.3.0 (R Core Team, 2023).

CHAPTER 4

RESULTS

4.1 Survey and Specimen Collection

This study conducted extensive surveys in southwestern China, including Yunnan Province, Sichuan Province, and Xizang Province. A total of 59 sampling sites were investigated, covering both field plots representing the major forest types in the region and wild mushroom markets in ethnic minority areas. Over the course of four years, 209 hypogeous fungal specimens were collected. Based on the integration of morphological characteristics and molecular phylogenetic analyses, this study describes 21 new species.

4.2 Taxonomy

Ascomycota

4.2.1 *Hydnotrya*

4.2.1.1 Introduction

Hydnotrya Berk. & Broome is a genus of hypogeous fungi belonging to Pezizomycetes, Ascomycota, related to the genus *Gyromitra*. It was placed in the family Helvellaceae by Spooner (1992), but based on the recent molecular analysis, it has been shifted into the family Discinaceae (O’Donnell et al., 1997; Hansen & Pfister, 2006; Tedersoo et al., 2006; Læssøe & Hansen, 2007). Their ascomata are hollow to convoluted with simple or folded chambered, even nearly solid, lined with recognizable hymenium. *Hydnotrya* usually forms a symbiotic relationship with both conifer and broadleaf trees and is distributed throughout the northern hemisphere (Trappe, 1975; Spooner, 1992; Trappe & Castellano, 2000; Stielow et al., 2010; Yu et al., 2018; Slavova et al., 2021). There are 22 names listed in the Index Fungorum online database (http://www.indexfungorum.org /Names/Names.asp). However, among them, the species *H. jurana* Quél. and *H. carnea* (Corda) Zobel was merged into *H. tulasnei* (Berk.) Berk. & Broome (Gilkey, 1954; Trappe, 1969), *H. ploettneriana* (Henn.) Hawker, *H. yukonensis* Gilkey, and *H. dysodes* Kirschstein into *H. michaelis* (E. Fisch.) Trappe (Soehner, 1942; Trappe, 1975), and *H. convoluta* (McAlpine) McLennan was renamed as *Peziza jactata* Burds. & Korf (Burdsall, 1968), *H. ellipsospora* Gilkey named as *P. ellipsospora* (Gilkey) Trappe (Trappe, 1979). Up to now, there are 15 valid species remaining in the genus *Hydnotrya*.

Up to now, nine *Hydnotrya* species have been reported in China: *H. cerebriformis* in Shanxi and Xinjiang, *H. cubispora* in Xizang Province, *H. Michaelis*, *H. tulasnei* and *H. brunneospora* in Jilin (Tao & Liu, 1989; Zhang, 1991; Xu, 2000; Yu et al., 2018), *H. laojunshanensis* and *H. badia* in Yunnan (Li et al., 2013), *H. nigricans* in Sichuan, *H. puberula* in Yunnan and Jilin (Yu et al., 2018).

In the past two years, more *Hydnotrya* specimens have been collected in southwest China. Based on the morphological and molecular analyses, two new species were detected and described: *H. oblongispora* and *H. zayuensis*. Their relationships with other known *Hydnotrya* species were discussed, and a more detailed supplementary description was given to another species *H. laojunshanensis* found in Yunnan before. Additionally, the main morphological characteristics of 15 species of *Hydnotrya* were listed and a key to the species of the genus was provided.

4.2.1.2 Molecular Phylogeny

Total genomic DNA was extracted from the specimen using the OMEGA Plant Genomic DNA Kit. The internal transcribed spacer (ITS) rDNA region was amplified with PCR primers ITS1F, ITS4 (White et al., 1990; Gardes et al., 1993; Truong et al., 2017). The large subunit nuclear ribosomal DNA (LSU) region was amplified with the PCR primers LROR and LR5 (Vilgalys & Hester, 1990). PCR reactions were performed on a BIO-RAD C1000TM instrument. Thermal cycles with the following settings: initial denaturation for 5 min at 94°C, followed by 32 cycles of

40 s denaturation at 94°C, annealing at 56°C for 40 s for ITS, and 52°C for 30 s for LSU, extension for 1 min at 72°C, and final extension at 72°C for 10 min. The PCR products were verified on 1% agarose electrophoresis gels stained with ethidium bromide. The purification and sequencing of the PCR products were conducted by Sangon Biotech Limited Company (Shanghai, China).

ITS was used for the analysis of *Hydnotrya* species diversity in this study because ITS works well for the delimitation of *Hydnotrya* species. 46 ITS sequences from NCBI and this study representing 14 species of *Hydnotrya* (Table 4.1), including *Gyromitra infula* (Schaeff. Fr.) Quél. And *Gyromitra esculenta* Pers. ex Fr. as outgroups (Figure 4.1). All *Hydnotrya* ITS sequences were extracted from an ascoma. Sequences of *Hydnotrya* species generated in this study were submitted to the GenBank database. We first used the Basic Local Alignment Search Tool for the GenBank database to recheck whether the newly generated sequences were amplified DNA from a contaminant or not, and to examine clusters with closely related sequences. DNA sequences were retrieved and assembled using SeqMan. Sequence alignments were aligned using MAFFT version 7 (Katoh & Standley, 2013), ITS gene was analyzed using BioEdit v. 7 (Hall, 2007) Maximum Likelihood (ML) analysis was performed using RAxML-HPC2 v. 8.2.12 (Stamatakis, 2014) as implemented on the Cipres portal (Miller et al., 2011), with the GTR+G+I model and 1,000 rapid bootstrap (BS) replicates for all genes. A reciprocal 70 % bootstrap support approach was used to check for conflicts between the tree topologies from individual genes. As the topology of the ML tree and the Bayesian tree are similar, the ITS1, ITS2, and 5.8s sequences were combined using SequenceMatrix (Vaidya et al., 2011) and partitioned phylogenetic analyses. For Bayesian Inference (BI), the best substitution model for each partition was determined by MrModeltest 2.2 (Nylander et al., 2004). The result suggested that ITS1: JC+I, 5.8S: GTR+G+I, ITS2: K80+I+G. Bayesian analysis was performed using MrBayes version. 3.2.7a (Ronquist et al., 2011) on the Cipres (Miller et al., 2011), four parallel runs were performed for 10 million generations, sampling every 100th generation for the single gene trees. Parameter convergence > 200 was verified in Tracer v. 1.7 (Rambaut et al., 2018). The phylogenetic clade was strongly supported if the bootstrap support value (BS) was ≥ 70 % and/or a posterior probability (PP) <0.01.

Table 4.1 Taxa information and GenBank accession numbers of the sequences used in *Hydnotrya* study

Species name	Voucher	Origin	GenBank No.	Reference
Gyromitra esculenta	Gyr3	France	AJ544208	Kellner et al. (2007)
Gyromitra esculenta	m954	UK	AJ544209	Kellner et al. (2007)
Gyromitra infula	UBC herbarium F15196	Canada	DQ384573	GenBank
Gyromitra infula	Vellinga GLM	USA	AJ698480	Kellner et al. (2007)
Hydnotrya badia	BJTC:FAN270	China	NR_161070	Yu et al. (2018)
*Hydnotrya badi*a	BJTC:FAN270	China	MH445399	Yu et al. (2018)
Hydnotrya bailii	PRM 902032	Czech	AM261522	Stielow (2010)
Hydnotrya bailii	P.Reil_2	Germany	GQ140239	Stielow (2010)
Hydnotrya bailii	P.Reil	Germany	GQ140238	Stielow (2010)
Hydnotrya bailii	997	Germany	GQ149465	Stielow (2010)
Hydnotrya bailii	979	Germany	GQ149464	Stielow (2010)
Hydnotrya brunneospora	HMAS 97138	China	NR_161073	Yu et al. (2018)
Hydnotrya brunneospora	HMAS 97138	China	MH445404	Yu et al. (2018)
Hydnotrya cerebriformis	89_A12_Stielow	Germany	GQ140236	Stielow (2010)
Hydnotrya cerebriformis	87_G11_Stielow	Germany	GQ140235	Stielow (2010)
Hydnotrya cerebriformis	BJTC:FAN647	China	MH430537	Yu et al. (2018)
Hydnotrya cerebriformis	GO-2010-097	Mexico	KC152120	Piña-Páez et al. (2017)
Hydnotrya cerebriformis	GO-2009-455	Mexico	KC152118	Piña-Páez et al. (2017)
Hydnotrya cerebriformis	GO-2009-242	Mexico	KC152119	Piña-Páez et al., (2017)
Hydnotrya cubispora	SAT-13-273-01	USA	MZ054357	GenBank
Hydnotrya cubispora	RBG Kew K(M)104976	UK	EU784273	Brock et al. (2009)
Hydnotrya laojunshanensis	YAAS L2425	China	NR_132886	Li et al. (2013)
Hydnotrya laojunshanensis	**BMDU L21211**	**China**	**ON982580**	**This study**

Table 4.1 (continued)

Species name	Voucher	Origin	GenBank No.	Reference
Hydnotrya laojunshanensis	**BMDU L21212**	**China**	**ON982593**	**This study**
Hydnotrya laojunshanensis	**BMDU L21215**	**China**	**ON982594**	**This study**
Hydnotrya laojunshanensis	**BMDU L21197**	**China**	**ON982592**	**This study**
Hydnotrya laojunshanensis	**HKAS95802**	**China**	**OP908303**	**This study**
Hydnotrya michaelis	RBG Kew K(M)61643	UK	EU784275	Brock et al. (2009)
Hydnotrya michaelis	RBG Kew K(M)38647	UK	EU784274	Brock et al. (2009)
Hydnotrya michaelis	6463-307EMC	Germany	HM146816	Cox et al. (2010)
Hydnotrya nigricans	BJTC:FAN349	China	NR_161071	Yu et al. (2018)
Hydnotrya nigricans	BJTC:FAN349	China	MH445400	Yu et al. (2018)
Hydnotrya oblongispora	**BMDU L20067**	**China**	**OM232075**	**This study**
Hydnotrya oblongispora	**BMDU L20069(Holotype)**	**China**	**OM232079**	**This study**
Hydnotrya oblongispora	**BMDU L21217**	**China**	**OM232084**	**This study**
Hydnotrya puberula	BJTC:FAN721	China	NR_161072	Yu et al. (2018)
Hydnotrya puberula	BJTC:FAN721	China	MH445401	Yu et al. (2018)
Hydnotrya puberula	HMAS96758	China	MH445402	Yu et al. (2018)
Hydnotrya tulasnei	RBG Kew K(M)99871	UK	EU784276	Brock et al. (2009)
Hydnotrya tulasnei	Berk. & Broome C34659 (Copenhagen Botanical Museum)	Denmark	AJ969621	Tedersoo et al. (2006)
Hydnotrya tulasnei	IT8	Germany	GQ140240	Stielow (2010)
Hydnotrya tulasnei	605040	Russia	KY401249	GenBank
Hydnotrya variiformis	TK1615	USA	AY558770	Izzo et al. (2005)
Hydnotrya zayuensis	**BMDU L22024**	**China**	**OP908304**	**This study**
Hydnotrya zayuensis	**BMDU L22027 (Holotype)**	**China**	**OP908305**	**This study**
Hydnotrya sp1.	SNF160	USA	AY558768	Izzo et al. (2005)
Hydnotrya sp2.	SNF82	USA	AY558769	Izzo et al. (2005)

Table 4.1 (continued)

Species name	Voucher	Origin	GenBank No.	Reference
Hydnotrya sp3.	JT19176	USA	MN653030	GenBank
Hydnotrya sp4.	JT19085	USA	MN653044	GenBank
Hydnotrya sp5.	JLF2015	USA	MH220061	GenBank

Note The newly generated sequences are in bold.

The ML and Bayes analyses of the 50 ITS sequences are shown in Figure 4.1 with associated bootstrap supports for branches.

In the phylogenetic tree, the 46 ITS sequences from *Hydnotrya* ascomata revealed the phylogenetic relationship of 14 species there are: Clade 1 includes 5 sequences of *H. bailli* from Europe. Clade 2 includes 2 sequences of *H. brunneospora* from China. Clade 3 includes 4 sequences of *H. tulasnei* from Europe. Clade 4 includes 3 sequences of *H. puberula* from China. Clade 5 includes 2 sequences of *H. badia* from China. Clade 6 includes 2 sequences of *H. nigricans* from China. Clade 7 includes 6 sequences of *H. cerebriformis* from Germany, China, and Mexico; it includes two distinct clades were revealed, one comprising Eurasian specimens, and the other comprising specimens from Mexico, which is probably because the specimens are from Holarctic and Neotropical regions, respectively. Clade 8 includes 3 sequences of *H. variiformis* from the USA. Clade 9 includes 2 sequences of *H. cubispora* from the UK and USA. Clade 10 includes 3 sequences of *H. michaelis* from Europe. Clade 11 includes 3 sequences of new species, *H. oblongispora* from China. Among them, *H. oblongispora* and *H. michaelis* are closely related, but they have independent phylogenetic branches, which have strong bootstrap support (BS = 100%; PP = 1.0). Moreover, *H. oblongispora* shares less than 88.3% (90/772) similarity in the ITS region with its closest relative. So, it is distinct from other *Hydnotrya* species. Clade 12 includes 3 sequences of *Hydnotrya sp.* from the USA. They may be new species from North America that have not yet been reported. Clade 13 includes 6 sequences of *H. laojunshanensis* from China. When the species *H. laojunshanensis* was reported, only one specimen was found, and many more were collected over the past few years, so the DNA sequences of *H. laojunshanensis* were supplemented. Clade 14 includes 2 sequences of a new species, *H. zayuensis* from China. This new species *H. zayuensis* and *H. laojunshanensis* are closely related, but they have independent phylogenetic branches, which have strong bootstrap support (BS = 100%; PP = 0.99). *Hydnotrya zayuensis* shares less than 96.3% (28/755) similarity in the ITS region with its closest relative. The phylogenetic analysis shows that the new species is distinct from other *Hydnotrya* species. In addition to the ITS sequences used in this phylogenetic analysis, the LSU sequences were amplified from the newly supplemented specimens in this study and uploaded to NCBI for future study.

From the phylogenetic tree, two major phylogenetic branches are presented, showing a strong sister relationship (BS=100%; PP = 1.0). They are Clade A (including Clade 1-9) and Clade B (including Clade 10-14) respectively. Species included in the two phylogenetic clades have commonalities and uniqueness in macroscopic and microscopic morphology. Clade A and Clade B are monophyletic groups.

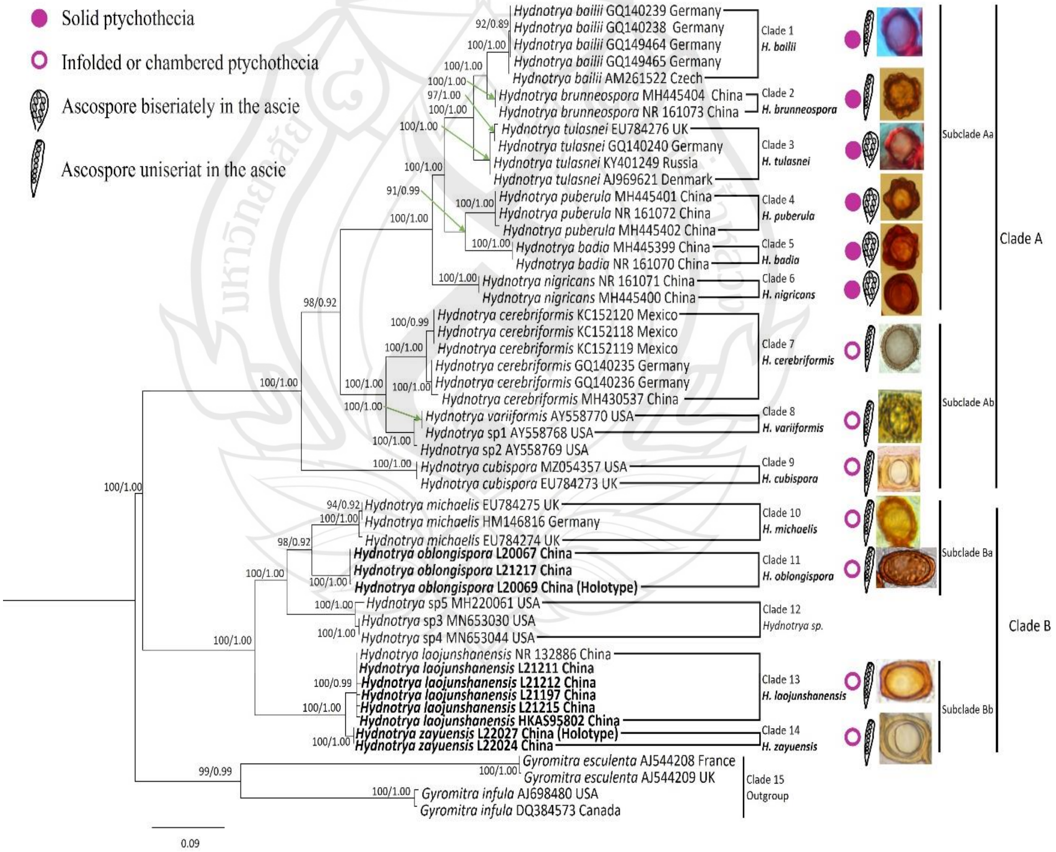


Figure 4.1 Phylogeny derived from a maximum likelihood (ML) analysis of the nrDNA-ITS sequences from *Hydnotrya* species, using *Gyromitra esculenta* and *Gyromitra infula* as the outgroup

Figure 4.1 Values next to nodes reflect, left maximum likelihood bootstrap support values (BS) and right Bayesian posterior probabilities (PP). Names of novel species and samples with newly generated sequences in bold. Symbols by taxon names indicate specific fruiting body types, the arrangement of the ascospores in the ascus, and the Ascospore looks of *Hydnotrya*.

Hydnotrya oblongispora L. Li & S.H. Li **sp. nov.** Figure 2

Mycobank number: MB 846735

Diagnosis: Differs from other species in the genus *Hydnotrya* by its nearly single-chambered ascomata and long ellipsoid ascospores.

Etymology: oblongispora from Latin, refers to the long ellipsoid ascospores of this fungus.

Holotype: CHINA, Yunnan, Lijiang (26 °37N 99 °42E), alt. 3737.4 m, in the forest of *Abies forrestii* Coltm.-Rog, 12 August 2020, Lin Li, BMDU L20069.

Ascomata irregularly globose, 1.0–2.5cm in diameter when fresh, smooth, sometimes gently folded inward, surface light khaki (4C5) to reddish brown (8D8); nearly single-chambered with a primary apical opening up to 0.2–0.8 cm in diameter, sometimes the opening is just an almost closed seam, white fluffy inside cavity. Elastic and crisp. No special smell.

Peridium two-layered, 280–340 µm thick, outer layer 80–100 µm thick, composed of light brown (6D8) ellipsoidal or irregular cells, red brown (6E8) pigment deposited on the outermost cells; inner layer, 200–240 µm thick, consists of hyaline interwoven hyphae. Gleba chamber hollow, lined with milky white (4B2) hymenium, hymenial surface fluffy. *Asci* cylindrical, 102.5–138.5 × 13.0–25.5 µm, 8-spored, strictly uniseriate, thin wall, narrowed into a long stalk (20–35 µm) at the base, arranged in a palisade. *Ascospore* long ellipsoid, hyaline when immature, golden yellow (5B7) when mature, with the thickening exosporium, surface pitted, (20.0–) 26.5–39.0 ×(9.5–) 11.0–21.5 µm, Q = 2.0±0.03. *Paraphyses* hyaline, straight stick-shaped, 2.5–5 µm in diam, septate, exceeding the asci by 60–70 µm.

Ecology & Distribution: Hypogeous, solitary, or in groups in soils under the forest of *A. forrestii* mixed with trees of *Rhododendron* spp., fruiting from late summer to early autumn. Known only from Yunnan Province, China.

Additional specimen examined: China, Yunnan Province, in the forest of *A. forrestii*, 12.Aug.2020, Lin Li (BMDU L20067. GenBank: ITS = OM232075, LSU = ON982626); 19.Sept.2021, Lin Li (BMDU L21217. GenBank: ITS = OM232084, LSU = ON982625).

Notes: *H. oblongispora* is characterized by its mostly simple-chambered ascomata and golden yellow long-ellipsoid ascospores, especially with pitted surfaces, which differ from all other species of *Hydnotrya*. Molecular analysis also shows that *H. oblongispora* is distinct from other *Hydnotrya* species, although it is closely related to *H. michaelis*. However, *H. michaelis* has convoluted, lobed ascomata and broadly ellipsoid spores with warty ascospores, which differ from this new species.

Figure 4.2 *Hydnotrya oblongispora* morphological characteristics

Figure 4.2 A. Young sarcomata. B. Mature ascomata with different openings; C. A piece of section of the ascomata in lactophenol cotton blue; D. A peridium section in lactophenol cotton blue; E. A section of hymenium in lactophenol cotton blue; F. A piece of paraphyses in lactophenol cotton blue; G. Ascospores released from the ascus; H. Asci in lactophenol cotton blue; I. An ascus with 8 ascospores; J - L. Ascospores under SEM. Scale bars: A, B = 1 cm; C = 100 µm; D = 50 µm; E, G–I = 10 µm; F, J, L = 5 µm; K = 2 µm.

Hydnotrya zayuensis L. Li & S.H. Li **sp. nov.** Figure 4.3

Mycobank number: MB 846736

Diagnosis: Differs from all other species in *Hydnotrya* by its almost single-chambered ascomata, light golden yellow a ellipsoid ascospores, and is only found in Zayu, Tibert, China.

Etymology: zayuensis from Latin, referring to the type locality.

Holotype: CHINA, Xizang Province, Zayu (28 °35N 98 °06E), alt. 3770 m, in the forest of *Abies* sp., 11 August 2022, Lin Li BMDU L22027.

Ascomata irregularly globose, 1.5–2.5cm in diameter when fresh, smooth, convoluted, almost single-chambered with a primary apical opening, sometimes the opening nearly closed like a seam, white fluffy inside, surface cinnamon (5E8); shrinked, becoming fuzzy when dried, although there are no protruding hyphae cells from the outermost layer of the peridium. Elastic to crisp. No special smell.

Peridium two-layered, 180–250 µm thick, outer layer 40–80 µm thick, composed of ellipsoid or irregular cells, which grow larger toward the surface, yellow brown (4C5) pigment deposited on the outermost cells; inner layer, 110–160 µm thick, consisting of hyaline parallel interwoven hyphae. Gleba chamber hollow, lined with off-white (1A2) hymenium when immature; two-layered when mature, the outer layer golden brown (5C7), the inner layer yellowish to whitish (4A2), hymenial surface fluffy. *Asci* cylindrical, 118.5–130.5×15.0–22.5 µm, 8-spored, strictly uniseriate, thin wall, narrowed into a long stalk (20–40 µm) at the base, arranged in a palisade. *Ascospore* ellipsoid, hyaline, exosporium thin when immature, ellipsoid (including thickening exosporium), surface roughness, and looks crumbly, golden yellow (4B8) when mature, (17–)20–30.5 × 15.5–18.0 µm, Q = 1.5 ± 0.16. *Paraphyses* hyaline, straight stick-shaped, 1.5–2.5 µm in diam, septate, apical slightly inflated, exceeding the asci by 120–160 µm.

Ecology & Distribution: Hypogeous, solitary in the humus under the forest of *Abies* sp. mixed with trees of *Rhododendron* spp. fruiting from July to September. Known only from Zayu, Tibert, China.

Additional specimens examined: China, Xizang Province, Zayu in the forest of *Abies* sp., 15.July.2022, Shucheng He (BMDU L22024. GenBank: ITS = OP908304, LSU = OP908301).

Notes: Morphologically, *H. zayuensis* is similar to *H. laojunshanensis.* However, *H. zayuensis* has much smaller spores, and thinner peridium, and lighter colored ascomata than *H. laojunshanensis*. Molecular analysis showed that *H. zayuensis* is distinct from *H. laojunshanensis* and other species of *Hydnotrya*.

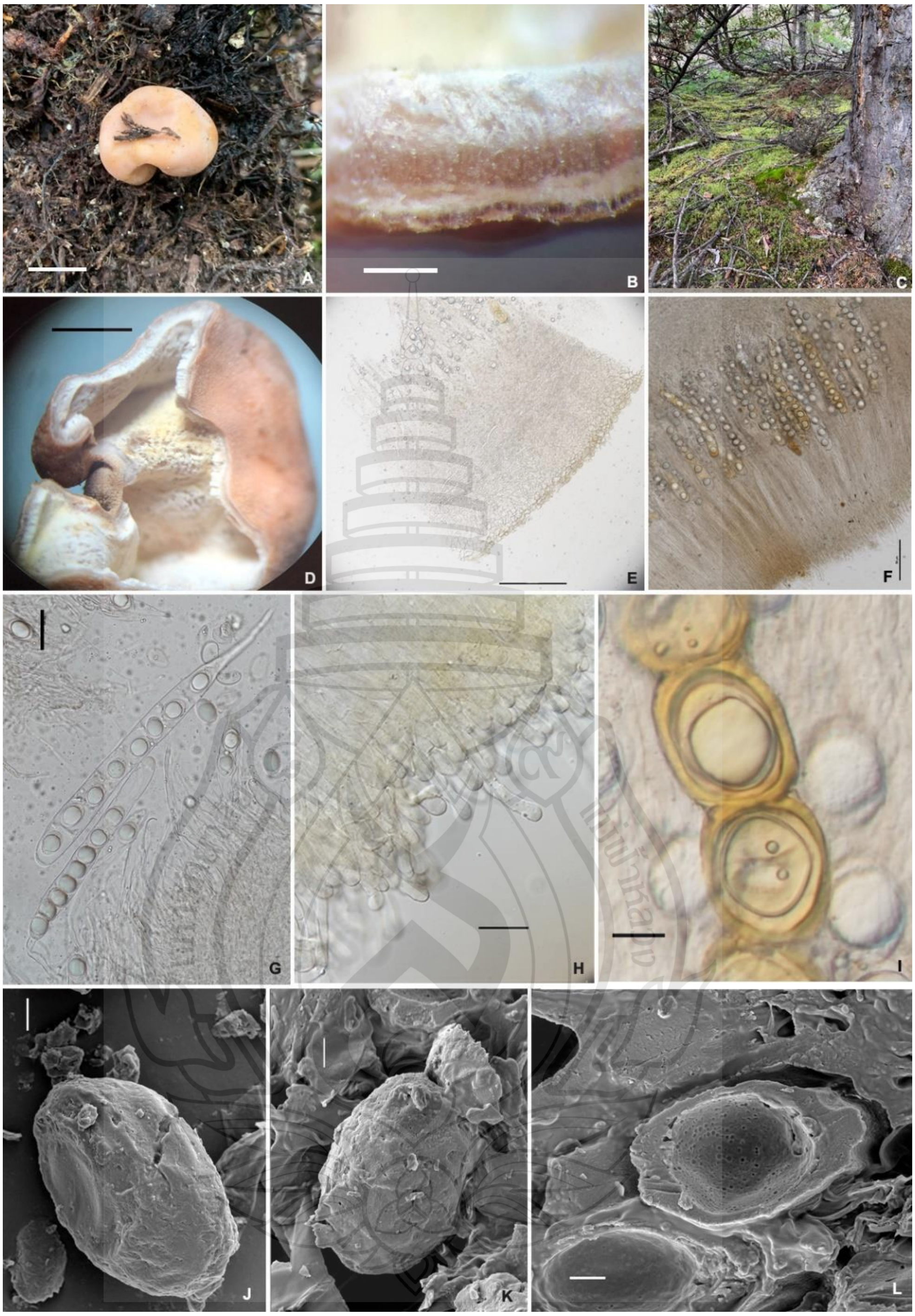


Figure 4.3 *Hydnotrya zayuensis* morphological characteristics

Figure 4.3 A. Ascomata. B. Section of ascomata, has hymenium-lined chambers. C. Habitat; D. Inner surface of ascomata. E. Peridium in 5% KOH; F. Hymenium. G. Asci in 5% KOH. H. Paraphyses. I. Ascospores in 5% KOH. J. K. L. Ascospores under SEM (L. SEM of a single ascospore cut in half). Scale bars: A =1cm;

B = 1 mm; D = 0.5cm; E = 100 µm; F = 50 µm; G = 20 µm; H = 10 µm; I = 10 µm; J, K, L = 5 µm.

Hydnotrya laojunshanensis L. Li, D.Q. Zhou & Y.C. Zhao 2013 Figure 4

Mycobank number: MB 803968

Ascomata irregularly globose, 1.0–3.0 cm in diameter when fresh, brownish orange (6C8), smooth, mostly single-chambered with a primary apical opening to 0.1–0.5 cm in diameter, the opening narrowing rarely into a slit, sometimes folded, forming a few channels, lined with white fluffy hymenium. Elastic to crisp. No special smell.

Peridium two-layered, 350–570 µm thick, outer layer 160–200 µm thick, composed of light brown (6E8) angular or irregular cells, inner layer, 220–350 µm thick, consisting of hyaline interwoven hyphae. Gleba chamber hollow, lined with hymenium with orange (6B8) asci and yellowish to whitish (4A2) paraphyses. *Asci* cylindrical, 331.5–390.5 × 25.5–35.5 µm, 8-spored, strictly uniseriate, thin wall, narrowed at the base into a long stalk (30–50 µm), arranged in a palisade. *Ascospore* ellipsoid (excluding thickening exosporium), rectangular (including thickening exosporium), surface rough, reddish orange to golden (6B8) when mature, (26.5–)33.0–50.5 × (15.5–)20.5–35.5(–38.0) µm Q = 1.35±0.02. *Paraphyses* hyaline, straight stick-shaped, 2.0–6 µm in diam, apical slightly inflated, septate, exceeding the asci by 180–300 µm.

Ecology & Distribution: Hypogeous, solitary, or in groups in soils under forests of *Abies* spp., fruiting from late summer to early autumn. Known only from Yunnan Province, China.

Specimen examined: China, Yunnan Province, Laojun mountains, in the forest of *A. forrestii* var. *smithii*, 30.Aug.2012, Lin Li (Holotype, YAAS L2425; GenBank KC878618); Shangri-La, in forest of *Abies* sp., 19.Aug.2014, Shanping Wan (HKAS95802 GenBank: ITS = OP908303), in the forest of *A. forrestii*, 12.Sept.2019, Lin Li (BMDU L21197 GenBank: ITS = ON982592, LSU = ON982620); Lijiang, in the forest of *A. forrestii*, 21.Sept.2021, Lin Li (BMDU L21211 GenBank: ITS = ON982580, LSU = ON982621, BMDU L21212 GenBank: ITS = ON982593, LSU = ON982622, BMDU L21215 GenBank: ITS = ON982594, LSU = ON982623).

Notes — When the species was described in 2013, only based on one collection from Mt. Laojun in Yunnan Province, China. More specimens of *H. laojunshanensis* have been found at other places in Yunnan since then. It was discovered that this species not only had a simple chambered acomata but also folded, chambered ascomata. This species has large, rectangular ascospores (including thickening exsporium) with a rough surface differing from other species in *Hydnodrya*.

Figure 4.4 *Hydnotrya laojunshanensis* morphological characteristics

Figure 4.4 A. Young sarcomata cut in half; B. Mature ascomata with one cut in half; C. Infolded and chambered ascoma; D. Section of hymenium in 5% KOH; E. A peridium section in 5% KOH; F. Ascospores released from asci in 5% KOH; G - I. Ascospores under SEM (I. SEM of a single ascospore cut in half). Scale bars: A, B = 1 cm; D – E = 50 μm; F = 20 μm; G, H, I = 5 μm.

4.2.1.3 Discussion

So far, 17 valid species in *Hydnotrya* (including these two new species) are accepted worldwide (Kirk et al., 2008; Stielow et al., 2010; Li et al.,2013; Yu et al., 2018). The main macroscopic and microscopic characteristics of 17 species were provided and discussed based on published literature (Table 4.2).

The ascospore morphology is highly variable among different species in *Hydnotrya*, which is useful for distinguishing species. Abbott and Currah (1997) once divided the genus *Hydnotrya* into two subgenera: Subg. *Hydnotrya* and *Cerebriformae*, *according to* the characteristics of their ornamentation. The Subg. *Hydnotrya* had four species of *H. tulasnei*, *H. michaelis*, *H. cubispora,* and *H. variiformis* , having ascospores with rounded or irregular warts. The Subg.*Cerebriformae* has only one species of *H. cerebriformi* differs from Subg. *Hydnotrya* in ascospores with short, rounded aculei. However, the current phylogenetic analysis showed that ascospore characteristics were not reliable for differentiating the genus into subgenus (Figure 4.1).

Based on ITS analyses, 14 species in *Hydnotrya* were divided into two clades A and B, on the phylogenetic tree. The species in the clade A mostly have nearly solid gleba (6 out of 9) and globose, warty ascospores, either uniseriatly or biscriately arranged in asci. The clade A is divided into two subclades: the subclade Aa (clade 1-6) and Ab(clade 7-9). The species in the subclade Aa have solid ascomata, which have two groups: group 1 (clades 1 and 2) and group 2 (clades 3-6), found both in China and Europe. Group 1 has two species with ascospores uniseriatly arranged in asci; the Group 2 has four species with ascospores biscriately arranged in asci. Species in the Subclade Ab have a hollow ascoma and ascospores uniseriatly arranged in asci distributed in China, Europe, and America. The species in the clade B has a hollow to chambered gleba and ellipsoid ascospores(without thickening exosporium), biscriately arranged in asci. The clade B is divided into two groups: Ba and Bb. The group Ba (clade 10-11) has 2 species with ellipsoid ascospores, surface pitted, distributed in China and Europe.

The group Bb (clade 13-14) has two species with rectangular and ellipsoid ascospores (with thickening exosporium), surface rough, only found in China. (Figure 4.1).

Based on the morphological and molecular phylogenetic analyses there seems to be a trend in morphologyical revolution among the species within the genus *Hydnotrya,* that is, the gleba evolved from hollow or chambered to nearly solid; the ascus becoming shorter and wider, and the ascospores arranged from uniseriate to biseriate; ascospores from ellipsoid to globose, and the ornamentation from smooth to rough as well. This evolutionary trend in the morphological changes between species in the genus *Hydnotrya* is related to their hypogeous habits, that is, the gleba of the ascoma has more chambers, more ascospores the ascoma will hold, and the more chances of ascospores being dispersed by its mycological animals (Hawker, 1955; Ławrynowicz, 1990; Læssøe & Hansen, 2007; Bonito et al., 2013). All of this improves their survival and reproduction better. Of course, more collections would be needed for comprehensive morphological and molecular analyses to provide more evidence to support the hypothesis of the evolution trend of *Hydnotrya*.

In China, 9 species were recorded before this study (Yu et al., 2018). In this study, two new species are described. In total, 11 species have now been discovered in China, among which 7 species are distributed in southwest China. China has good diversity in *Hydnotrya*, particularly in the southwest region.

4.2.1.4 Key to species of *Hydnotrya*

1. Ascomata hollow, gleba chamber simple or infolded……………………..……..……..2
1’. Ascomata solid, gleba labyrinthine chambered……………………….…………11
2. Ascospores rectangular or cubical*….…………………….………......…………...…3
2’. Ascospores ellipsoid or globose*……..…………….………………….……..……4
3. Ascospores cubical*…………………..…………...…….......…...……*H. cubispora*
3’. Ascospores rectangular*……………….…………………...….. *H. laojunshanensis*
4. Odor special smell……………….……………..…….……………………….……5
4. Odor no special smell……………………………..………………………..6
5. Odor and taste strong garlic…………………………….……………….. *H. subnix*
5’. Odor strong pungent and persistent……………………….……………*H. michaelis*
6. Ascospores mostly globose* ………………………………….………………………7
6’. Ascospores ellipsoid or long ellipsolid* …………………….…………………....…....8

7. Ascospores globose*, with prominent echinate…………….………. *H. cerebriformis*

7'. Ascospore mostly globose*, with aggregated, irregular flexuous spines………. *H. inordinata*

8. Ascospores long ellipsoid*, surface pitted, ascomata mostly single chambered…*H. oblongispora*

8'. Ascospores ellipsoid, aspect ratio less than 2….………..………….…………….9

9. Ascospores incompletely biseriate at immaturity, strictly uniseriate at maturity in asci……………………………………………………………………..……...….…10

9'. Ascospores strictly uniseriate arranged in asci from immature to mature……………………………………………………………….……….. *H. zayuensis*

10. Ascospores broad ellipsoid*, vertically grooved, forming irregular warts…………………………………………………………..……......…*H. confusa*

10'. Ascospores ellipsoid*, surface appearing punctate and with small irregular nodules……………………………………………………….………….... *H. variiformis*

11. Ascospores mostly uniseriate arranged in asci……………….…….…………12

11'. Ascospores mostly biseriate arranged in asci……………….………………..13

12. Ascospores less than 35 µm*, reddish brown………..……………………...…*H. bailii*

12'. Ascospores up to 46 µm*, brown to golden brown……......…… *H. brunneospora*

13. Odor light fragrance…………………………..…………………….………*H. soehneri*

13'. Odor no smell……………………………………………………..…………14

14. Ascoma surface tomentulose, purple tints when fresh…..…………….. *H. puberula*

14'. Ascoma not tomentulose………………………………………...……..……..…15

15. Ascospores without prominent protuberances, trigonal outline in cross section, ascomata blackish……………………………………..…………..*H. nigricans*

15'. Ascospores with recognizable protuberances………………...…………………16

16. Ascospores, 20–30 µm diam.*, ochre-reddish, with conspicuous, irregular warts..…………………………………………………………….……*H. tulasnei*

16'. Ascospores, 25–40 µm in diam.*, red brown to reddish, with regular large protuberances.…………………………………………………………....*H. badia*

*Including ornamentation

Table 4.2 List of main characteristics of *Hydnotrya*

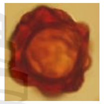
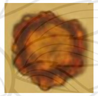
Species	Ascomata	Gleba	Ascospore	Asci	Host Plants	Distribution	References
Hydnotrya badia L. Fan, Y.W. Wang & Y.Y. Xu 2018	Irregularly subglobose, 7–15 × 14–19 mm diam., surface even, brown to earth brown.	Gleba solid, with numerous variably compacted canals and chambers (usually without empty space).	Roughly globose, 25–40 μm in diam. including ornamentation, 17.5–27.5 μm in diam. excluding ornamentation, red-brown to reddish, thickened exisporium with regular large protuberances 	Asci broadly clavate to somewhat saccate, sessile, or narrowed at the base into a short stalk, 125–172.5 × 65–75 μm, randomly immersed in paraphyses, 8-spored, spores mostly biseriate.	*Pinus* sp.	Huize, China Asia 2000-2900m	Yu et al. (2018)
Hydnotrya bailii Soehner 1959	Irregularly subglobose, 10–20(−25) mm diam., dark brown, with deep furrows often with multiple lobes, with one or many openings at the apex, with pleasant aromatic smell.	Gleba solid, dark brown, strongly convoluted cavities.	Globose, (27.5-) 30–34 (−37.5) μm in diam, brown reddish, exosporium thickening with blistered warts 	Asci cylindrical, 250–300×30–40 μm, 8-spored, spores mostly uniseriate.	*Picea abies*	Europe	Stielow et al. (2010)
Hydnotrya brunneospora L. Fan, Y.W. Wang & Y.Y. Xu 2018	Irregularly globose, 20–23 mm diam., dark brown when dry, surface smooth.	Gleba solid, scattered with some small, isolated, and irregularly shaped chambers.	Roughly globose, 26.25–46.25 μm diam., brown to golden brown at maturity (never reddish), exosporium thickening with small protuberances. 	Asci cylindrical to clavate, narrowed at the base into a short stalk, 162.5–237.5 × 30–47.5 μm, randomly immersed in paraphyses, 8-spored, spores mostly uniseriate.	*Betula platyphylla*	Jilin, China Asia	Yu et al. (2018)

Table 4.2 (continued)

Species	Ascomata	Gleba	Ascospore	Asci	Host Plants	Distribution	References
Hydnotrya cerebriformis Harkn. 1899	Irregular spherical, lobulated, 10-35 × 10-20 mm diam., reddish-brown, cerebriform, with cavities that communicate with the gleba.	Gleba with labyrinthine chambers composed of invagination and fusion from the walls of the ascoma.	Globose ascospores 20-25µm diam. (x = 22.85 µm), excluding ornamentation, amber-brown, walls 1µm wide. Finely warty ornamentation, warts up to 4µm long. 	Asci cylindrical, 175–200 × 25–35 µm, 8-spored, spores mostly uniseriate.	*Pinus* sp. *Abies* sp.	Europe North America 3100-4000m	Harkness (1899), Abbott and Currah (1997), Piña-Páez et al. (2017)
Hydnotrya confusa Spooner 1992	Ovoid or irregular, size from ca. 20 × 20 × 15 cm up to 40 × 40 × 20 mm, greyish-brown or red-brown, with a primary apical opening and sometimes some smaller secondary openings.	Gleba hollow, with single chambered but mostly cerebriform folded.	Ovoid or very broad ellipsoid, 38–50 × 28–32 µm, golden brown, exosporium much thickened, vertically grooved, forming irregular warts. 	Asci cylindrical, 290–320 × 38–43 µm, 8-spored, spores mostly uniseriate. clavate at immature, with irregular or biseriate, cylindric at maturity, strictly uniseriate.	*Picea* sp.	Europe 361m	Spooner (1992), Bemmann and Bandini (2011)
Hydnotrya cubispora (E.A. Bessey & B.E. Thomps.) Gilkey 1939	Irregularly globose, 5-10 mm diam., Isabella color, with somewhat cerebriform folds radiating distinctly from central opening	Gleba with cavity simple, but somewhat irregular due to surface lobing.	Cubical, 47–50 × 23–32 µm, including thickening exosporium, brownish, with maturity. 	Asci cylindrical, 100-120 µm long, 8-spored, spores mostly uniseriate.	Coniferous forest	Europe North America	Gilkey (1939), Bryan (2003), K(M)189248

Table 4.2 (continued)

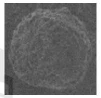
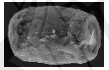
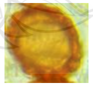
Species	Ascomata	Gleba	Ascospore	Asci	Host Plants	Distribution	References
Hydnotrya inordinata Trappe & Castellano 2000	Irregular globose, 8-30mm diam., dark red-brown, convolute and infolded ptychothecia with one or a few openings from the interior	Gleba complex, of infolded tramal plates forming canals and chambers 0.5-3mm broad.	Globose to ellipsoid, 20-30×20-28µm excluding ornamentation, brown-yellow, with aggregated , irregular flexuous spines 	Asci cylindrical, ±300 ×25–33 µm, (6–) 8-spored, spores mostly uniseriate.	*Abies amabilis* *Tsuga mertensiana*	North America 1800m	Trappe and Castellano (2000)
Hydnotrya laojunshanensis Lin Li, D.Q. Zhou & Y.C. Zhao 2013	Irregularly globose, 10–30 mm diam., brownish orange, smooth, mostly single-chambered with a primary apical opening, rare the opening narrowing into a slit, sometimes folded forming a few channels, lined with white fluffy hymenium. No special smell.	Gleba hollow, single-chambered, sometimes infolded and chambered, lined with hymenium with orange asci and whitish to yellowish paraphyses.	Ellipsoid without thickened exosporium, rectangle (including exosporium), (42.5–) 50.0–57.2(–60.3) × (27.5–)30.4–36.9(–38.2) µm, reddish orange, thickening exosporium with rough surface.  	Asci cylindrical, 331.5–390.5 ×25.5–35.5 µm, 8-spored, spores strictly uniseriate	*Abies* spp.	Yunnan, China Asia 3500-3800m	Li et al. (2013), This study
Hydnotrya michaelis (E. Fisch.) Trappe 1975	Irregular or subspherical, up to 60 mm across, with rounded opening, wrinkled, lobulate, with numerous invaginations, odor very strong, somewhat pungent, rather persistent.	Gleba labyrinthoid, with large, sinuous cavities, separated by folded inwards portions of ascoma wall.	Broadly ellipsoid, (21.2–)24.9–29.6(–32.2) ×(18.8–)19.8–22.4 (–24.9) µm; ornamentation excluded), honey-yellow, exosporium thickened, with conspicuous, irregular, often interconnected warts 	Asci cylindrical, 200–220 × 30–35 µm, 8-spored, spores strictly uniseriate	Pinaceae	Europe North America	Trappe (1975), Slavova et al. (2021)

Table 4.2 (continued)

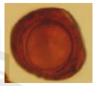
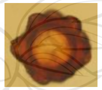
Species	Ascomata	Gleba	Ascospore	Asci	Host Plants	Distribution	References
Hydnotrya nigricans L. Fan, Y.W. Wang & Y.Y. Xu 2018	Irregular globose, 13 × 9mm, black brown to blackish	Gleba solid, brown, red to dark reddish, with some irregularly shaped and isolated small chambers lined with pale whitish hymenium.	Irregularly globose, 25.0–37.5 µm in diam., red brown, exosporium unevenly thickened, and usually of trigonal outline in cross section 	Asci broadly clavate to saccate, sessile or narrowed at the base into a short stalk, 87.5–190 × 25–62.5 µm, scattered between paraphyses in a hymenium,8-spored,with spores mostly biseriate.	*Pinus* sp.	Sichuan, China Asia	Yu et al. (2018)
Hydnotrya oblongispora sp. nov.	Irregularly globose, 10–25mm in diam. when fresh, light khaki to reddish brown, smooth, mostly single-chambered with a primary apical opening up to 02–08 mm in diam., sometimes infolded.	Gleba hollow, single-chambered lined with milky white hymenium, hymenium surface fluffy.	Long-ellipsoid (20.0–) 26.5–39.0 × (9.5–) 11.0–21.5 µm, golden brown, thickened exporium with pitted surface. 	Asci cylindrical, 102.5–138.5 × 13.0–25.5 µm, narrowed at the base into a long stalk (20–30 µm), 8-spored, spores strictly uniseriate	*Abies forrestii*	Yunnan, China Asia 3500-4000m	This study
Hydnotrya puberula L. Fan, Y.W. Wang & Y.Y. Xu 2018	Irregularly subglobose, 11–20 × 8–19 mm, brown to dark brown, sometimes with purple tints when fresh, much convoluted with deep furrows, ascoma surface tomentulose	Gleba solid, compact, dark brown to purple reddish at maturity, with numerous small chambers.	Roughly globose, 22.5–42.5 µm in diam., red brown to reddish, exosporium unevenly thickened by irregularly large protuberances. 	Asci clavate to saccate, 125–190 × 55–80 µm, sessile or with a short stalk, borne among palisade-like paraphyses in the hymenium, 8-spored, with spores mostly biseriate.	*Pinus* sp.	Yunnan, China Asia	Yu et al. (2018)

Table 4.2 (continued)

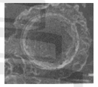
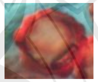
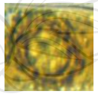
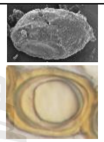
Species	Ascomata	Gleba	Ascospore	Asci	Host Plants	Distribution	References
Hydnotrya soehneri Svrček 1955	Irregularly subglobose, tuberous, 10-40 mm wide, reddish and reddish-gray to reddish brown, odor light fragrance	Gleba solid, whitish to yellowish gray, at maturity is colored reddish-brown corridors (from mature spores).	Spherical, 25 – 36 (– 42), red brown, exosporium thickened, coarsely warty.	Asci mostly cylindrical to saccate, 150–300 × 35–70 µm, 8-spored, mostly incompletely arranged biseriate.	Mixed woods	Europe	Svrček (1955)
Hydnotrya subnix Trappe & Castellano 2000	Irregular subglobose, 50-65mm in diam, dark red-brown, glabrous to minutely roughened. Odor and taste strongly of spicy garlic.	Gleba variable, deeply convoluted and infolded lacking openings from the interior, forming canals and locules 1-10mm broad.	Globose to rarely ellipsoid, 23-30µm in diam. excluding ornamentation, brown, coarsely warty. 	Asci mostly cylindrical, 300–340 × 25–40 µm, 8-spored, mostly incompletely arranged uniseriate	*Abies amabilis*	North America 950m	Trappe and Castellano (2000)
Hydnotrya tulasnei (Berk.) Berk. & Broome 1846	Irregularly spherical or lobed, sometimes with inward folds, 20–70 mm diam., ochre-reddish to brick red	Gleba solid, later yellow brown, with labyrinthic chambers.	Globose, 20–30 (–33) µm diam.(including ornamentation), ochre-reddish, with conspicuous, irregular warts. 	Asci broadly clavate or cylindrical, 175–210 × 30–62.5 µm, (4–) 8-spored, spores biseriate.	Coniferous forest	Europe North America 1600m	Dimitrova and Gyosheva (2008) Stielow et al. (2010)
Hydnotrya variiformis Gilkey 1947	Globose to subglobose to flattened, somewhat depressed, 7-40 mm broad, cinnamon-buff to cream-buff	Gleba variable, from a simple cavity to extremely lobed with numerous small chambers the interior, usually opening to the exterior at one or more points.	Ellipsoid, 24-28×36-36 µm, yellow-brown, thickened exosporium wall, surface appearing punctate and with small irregular nodules 	Asci 240–280 × 24 µm, 8-spored, clavate at immaturity, spores incompletely biseriate; cylindrical at maturity, spores strictly uniseriate.	Coniferous forest	North America 1200-2400m	Gilkey (1947), Abbott and Currah (1997), Beug et al. (2014)

Table 4.2 (continued)

Species	Ascomata	Gleba	Ascospore	Asci	Host Plants	Distribution	References
Hydnotrya zayuensis sp. nov.	Irregularly globose, 15–20 mm in diameter when fresh, smooth, gentle inward folds, surface cinnamon. Mostly single-chambered with a primary apical opening, the opening is just an almost closed seam, white fluffy inside cavity. Elastic and crisp. No special smell.	Gleba hollow, single-chambered with a primary apical opening, sometimes the opening is just an almost closed seam.	Ellipsoid, (17–)20–30.5 × 15.5–18.0 µm, (including thickened exosporium), golden yellow, surface rough, looking like crumbly. 	Asci cylindrical, 118.5–130.5×15.0–22.5 µm, 8-spored, spores strictly uniseriate	*Abies* sp.	Zayu, China Asia 3770m	This study

4.2.2 *Tuber*

4.2.2.1 Introduction

The genus *Tuber* (Tuberaceae, Pezizales, Pezizomycotina, Ascomycota) is an important group of fungi both economically and ecologically. *Tuber* species are mainly distributed throughout the northern hemisphere (Hall et al., 2007; Trappe et al., 2009; Deng et al., 2014; Fan et al., 2022; Rennick et al., 2023). They form mutually beneficial symbiotic associations with dominant trees, such as *Corylus* spp. *Fagus* spp., *Larix* spp., *Pinus* spp., *Picea* spp., *Populus* spp., *Pseudotsuga* spp., and *Quercus* spp., and play an important role in these forest ecosystems (Pacioni, 1989; Talou et al., 1990; Maser et al., 2008; Courty et al., 2010; Trappe & Claridge, 2010; Allen & Bennett, 2021; Caiafa et al., 2021; Money, 2021). *Tuber* species have a unique aroma, which not only attracts small animals to disperse their spores but is also favored by humans. Some *Tuber* species, such as *T. melanosporum* and *T. magnatum* are the most expensive delicacies on earth. Species such as *T. sinense*, *T. sinoaestivum,* and *T. pseudohimalayense* produced in China have become one of the worldwide commercial truffles since 1990'. In southwest China, hunting and trading these truffle species have become one of their important sources of cash income. In recent years, new species of *Tuber* have been continuously discovered in southwest China, and some of them have also appeared in the trading market. All the above have given *Tuber* a lot of attention.

Since the first Chinese species of *Tuber* of *T. taiyuanense* was published in 1985 more than 60 truffle species have been reported in China (Liu, 1985; Fan et al., 2022; Guo & Bau, 2023; Fu & Fan, 2023). More new *Tuber* species will be discovered and described from China. Three new *Tuber* species were found under the alpine fir forests in Northwest Yunnan, China, recently and described in this paper.

4.2.2.2 Materials and Methods

The specimens were collected from the alpine *Abies forrestii* var. *smithii* Viguié & Gaussen forests in Northwest Yunnan, China. These specimens were included with other studied specimens and were deposited at the BMDU (Biological Science

Museum of Dali University) and KUN-HKAS (Herbarium of Cryptogams Kunming Institute of Botany, Academia Sinica), China.

Morphological Study

Descriptions of microscopic and macroscopic characters were based on specimens (BMDU L20065, L20066, L21218a, HKAS131251, 131252, 131253, 131254, 131255, 131256, 131257, 131258), following the methods of Kumar et al. (2017) and Truong et al. (2017), and mycorrhizal specimens (HKAS131253-ECM) following the methods of Agerer (1987–2008) and Janowski and Leski (2023). Macroscopic characters of ascomata and gleba were observed under a Nikon SMZ1000 stereo zoom microscope. The sections were made with a razor blade by hand, mounted in a 5% KOH solution or water. The sections were observed under a light microscope. The temporarily prepared microscope slides were placed under magnification up to 1000× using Nikon ECLIPSE80i (Nikon, Japan) compound stereomicroscope for observation and microscopic morphological photography. Measurements were made using the Image Frame work v.0.9.7. To represent variation in the size of basidiospores, 5% of measurements were excluded from each end of the range, and extreme values were given in parentheses. In the taxonomic descriptions of species, ‘Q (L/I)’ refers to the length/width ratio of ascospores in side-view; ‘Q_m’ refers to the average Q of all ascospores ± standard deviation; ‘n’ refers to the number of spores measured. Key colors were obtained from Kornerup and Wanscher (1978).

DNA Extraction, PCR Amplification, and Sequencing

Total genomic DNA was extracted from the specimen using the OMEGA Plant Genomic DNA Kit. The internal transcribed spacer (ITS) rDNA region was amplified with PCR primers ITS1F and ITS4 (White et al., 1990; Gardes & Bruns, 1993; Truong et al., 2017). The large subunit nuclear ribosomal DNA (LSU) region was amplified with the PCR primers LROR and LR5 (Vilgalys & Hester, 1990). PCR reactions were performed on a BIO-RAD C1000TM instrument. Thermal cycles with the following settings: initial denaturation for 5 min at 94°C, followed by 32 cycles of

40 s denaturation at 94°C, annealing at 56°C for 40 s for ITS, and 52°C for 30 s for LSU, extension for 1 min at 72°C, and final extension at 72°C for 10 min. The PCR products were verified on 1% agarose electrophoresis gels stained with ethidium bromide. The purification and sequencing of the PCR products were conducted by Sangon Biotech Limited Company (Shanghai, China).

Sequence Alignment and Analysis

ITS was used for the analysis of *Tuber* species diversity in this study because it appears as a useful locus for the delimitation of *Tuber* species. 99 ITS sequences from NCBI and this study representing 54 species of *Tuber* (Table 4.3), including *Labyrinthomyces* sp., *Choiromyces alveolatus,* and *Choiromyces meandriformis* as outgroups. All *Tuber* ITS sequences were extracted from an ascoma, except one extracted with ECM. Sequences of *Tuber* species generated in this study were submitted to the GenBank database. We first used the Basic Local Alignment Search Tool for the GenBank database to recheck whether the newly generated sequences were amplified DNA from a contaminant or not, and examine clusters with closely related sequences. DNA sequences were retrieved and assembled using SeqMan. Sequence alignments were aligned using MAFFT version 7 (Katoh & Standley, 2013), ITS gene was analyzed using BioEdit v. 7 (Hall, 2007) Maximum Likelihood (ML) analysis was performed using RAxML-HPC2 v. 8.2.12 (Stamatakis, 2014) as implemented on the Cipres portal (Miller et al., 2011), with the GTR+G+I model and 1,000 rapid bootstrap (BS) replicates for all genes. A reciprocal 70 % bootstrap support approach was used to check for conflicts between the tree topologies from individual genes. As the topology of the ML tree and the Bayesian tree are similar, the ITS1, ITS2, and 5.8s sequences were combined using SequenceMatrix (Vaidya et al., 2011), and partitioned phylogenetic analyses. For Bayesian Inference (BI), the best substitution model for each partition was determined by MrModeltest 2.2 (Nylander et al., 2004). The result suggested that ITS1: JC+I, 5.8S: GTR+G+I, ITS2: K80+I+G. Bayesian analysis was performed using MrBayes version. 3.2.7a (Ronquist et al., 2011) on the Cipres (Miller et al., 2011), four parallel runs were performed for 10 million generations, sampling every 100th generation for the single gene trees. Parameter convergence > 200 was verified in Tracer v. 1.7 (Rambaut et al., 2018). The phylogenetic clade was strongly supported if the bootstrap support value (BS) was ≥ 70 % and/or a posterior probability (PP) <0.01.

Table 4.3 Taxa information and GenBank accession numbers of the sequences used in *Tuber* study

Species Name	Voucher	Origin	GenBank No.
Choiromyces alveolatus	MES97	USA	HM485332
Choiromyces meandriformis	RH691	USA	HM485330
Labyrinthomyces sp	JT27750	Australia	HM485335
Tuber aestivum	JT30500	Japan	HM485340
Tuber albicavum	HKAS131256 *	China	PP151577
Tuber albicavum	HKAS131255	China	PP151578
Tuber anniae	JT13209	Japan	HM485338
Tuber anniae	JT22695	Japan	HM485339
Tuber badium	HKAS 88789	China	NR_155922
Tuber badium	BMDU-L3152	China	PP151584
Tuber borchii	GB1/GB32	Italy	FJ809852
Tuber caoi	BJTC FAN271	China	KP276183
Tuber caoi	BJTC FAN293	China	KP276182
Tuber crassitunicatum	BJTC FAN465	China	MH115295
Tuber excelsum-reticulatum	BJTC FAN863	China	NR_182412
Tuber excelsum-reticulatum	BJTC FAN864	China	OM265273
Tuber formosanum	BJTC FAN107	China	MF621549
Tuber formosanum	BJTC FAN356	China	MF627986
Tuber gibbosum	JT30580	USA	FJ809868
Tuber gibbosum	JT26632	USA	FJ809862
Tuber huiliense	BJTC FAN288	China	NR_182569
Tuber huizeanum	BJTC FAN314	China	KT067685
Tuber huizeanum	BJTC FAN186	China	JQ910651
Tuber humilireticulatum	BJTC FAN189	China	OM286867
Tuber humilireticulatum	BJTC FAN174	China	OM286866
Tuber jinshajiangense	BJTC FAN406	China	KX575841
Tuber jinshajiangense	BJTC FAN407	China	KX575842
Tuber laojunshanense	HKAS131253 *	China	PP151583
Tuber laojunshanense	BMDU-L20065	China	PP151573
Tuber laojunshanense	BMDU-L20066	China	PP151574
Tuber laojunshanense	HKAS131251	China	PP151579
Tuber laojunshanense	BMDU-L22070ECM	China	PP124613

Table 4.3 (continued)

Species Name	Voucher	Origin	GenBank No.
Tuber laojunshanense	HKAS131252	China	PP151580
Tuber laojunshanense	HKAS131254	China	PP151581
Tuber laojunshanense	BMDU-L21218a	China	PP151582
Tuber latisporum	BJTC FAN125	China	KT067676
Tuber latisporum	BJTC FAN126	China	KP276189
Tuber liaotongense	BJTC FAN550	China	MH115302
Tuber lijiangense	BJTC FAN307	China	KP276188
Tuber lishanense	BJTC FAN683	China	MH115305
Tuber lishanense	BJTC FAN718	China	MH115303
Tuber liui	HXZE 984	China	DQ478660
Tuber liui	HXZE 984	China	DQ478636
Tuber liyuanum	BJTC FAN162	China	JQ771191
Tuber luyashanense	BJTC FAN846	China	OM256828
Tuber luyashanense	BJTC FAN1031	China	NR_182568
Tuber maculatum	RBG Kew K(M)17936	UK	EU784428
Tuber magnameshanum	BJTC FAN537	China	OM256767
Tuber malacodermum	JT32319	Spain	FJ809889
Tuber melosporum	AH31737	Spain	JN392144
Tuber microcarpum	BJTC FAN880	China	OM256832
Tuber microcarpum	BJTC FAN866	China	OM256770
Tuber neoexcavatum	BJTC FAN316	China	OM256741
Tuber neoexcavatum	BJTC FAN184	China	JX458715
Tuber oligospermum	AH38984	USA	JN392261
Tuber parvomurphium	BJTC FAN298	China	KP276186
Tuber piceatum	HMAS 97125	China	MH115318
Tuber piceatum	HMAS 97124	China	MH115320
Tuber pseudobrumale	BJTC FAN322	China	OM287839
Tuber pseudobrumale	BJTC FAN306	China	OM287838
Tuber pseudofulgens	BJTC FAN399	China	NR_182567
Tuber pseudofulgens	BJTC FAN399	China	OM256757
Tuber pseudomaganatum	BJTC FAN391	China	OM265244
Tuber shidianense	HKAS 88770	China	KT444595
Tuber shidianense	HKAS 88771	China	KT444596
Tuber shii	BJTC FAN405	China	KX555453
Tuber shii	BJTC FAN409	China	KX555454
Tuber sinense	BJTC FAN108	China	MF627968
Tuber sinense	BJTC FAN110	China	MF627970
Tuber sinoaestivum	BJTC FAN522	China	OM256774
Tuber sinoaestivum	BJTC FAN487	China	OM256773

Table 4.3 (continued)

Species Name	Voucher	Origin	GenBank No.
Tuber sinoborchii	BJTC FAN169	China	OM286800
Tuber sinoborchii	BJTC FAN171	China	OM286802
Tuber sinoexcavatum	BJTC FAN166	China	JX458718
Tuber sinoexcavatum	BJTC FAN130	China	JX458717
Tuber sinoniveum	HKAS 88792	China	KX904882
Tuber sinosphaerosporum	BJTC FAN136	China	JX092087
Tuber sinosphaerosporum	BJTC FAN135	China	JX092086
Tuber sp	GGPI1	China	LC193461
Tuber sp	GGPC2	China	LC193455
Tuber sp	GGPC3A	China	LC193456
Tuber sphaerospermum	AH39184	USA	JN392247
Tuber sphaerosporum	JT12487	USA	FJ809853
Tuber taiyuanense	T42_HM75888	China	GU979033
Tuber tomentellum	BJTC FAN1330	China	OP852126
Tuber tomentellum	BJTC FAN1340	China	OP852127
Tuber tomentellum	BJTC FAN1346	China	OP852128
Tuber turmericum	BJTC FAN471	China	KT758835
Tuber umbilicicavatum	HKAS131257	China	PP151576
Tuber umbilicicavatum	HKAS131258 *	China	PP151575
Tuber variabilisporum	BJTC FAN330	China	OM287841
Tuber variabilisporum	BJTC FAN362	China	OM287845
Tuber wanglangense	HMAS60220	China	DQ478637
Tuber wumengense	BJTC FAN218A	China	KT067682
Tuber wumengense	BJTC FAN292	China	KT067683
Tuber xuanhuaense	HMAS 60213	China	KP276179
Tuber xuanhuaense	BJTC FAN618	China	MK045627
Tuber zhongdianense	HKAS:Wang-0299	China	NR_119621
Tuber zhongdianense	BJTC FAN178	China	KT067679

Note The newly generated sequences are in bold.

* indicates Holotype.

Figure 4.5 Phylogeny derived from a maximum likelihood (ML) analysis of the nrDNA-ITS sequences from Tuber species, using Choiromyces alveolatus, C. meandriformis, and Labyrinthomyces sp. as outgroup

Figure 4.5 Values next to nodes reflect maximum likelihood bootstrap support values (BS), left, and Bayesian posterior probabilities (PP), right. Names of novel species and samples with newly generated sequences are in bold.

4.2.2.3 Results

Phylogenetic Analysis

The ML and Bayesian analyses of the 99 ITS sequences are shown in Figure 1 with associated bootstrap supports for branches.

In the phylogenetic tree, the 99 ITS sequences from *Tuber* ascomata revealed the phylogenetic relationship of 54 species: Clade 1 includes seven sequences of new species *T. laojunshanense* ascomata and one sequence of ectomycorrhizae formed by *T. laojunshanense* and *Abies forrestii* var. *smithii* from China. Clade 2 includes two sequences of the new species *T. umbilicicavatum* from China. Clade 3 includes three sequences of the new species *T. albicavum* from China. They belong to the *Puberulum* phylogroup. We selected the sequences of similar species of the genus *Tuber* distributed in China, and the sequences of species belonging to the *Puberulum* phylogenetic group for phylogenetic analysis with our collected specimens. The phylogenetic analysis showed that the new species are distinct from other *Tuber* species. In addition to the ITS sequences used in this phylogenetic analysis, the LSU sequences were amplified from the newly supplemented specimens in this study and uploaded to NCBI for future study.

Taxonomy

Tuber albicavum Y Wang, S.H. Li & L. Li sp. nov **Figure 6.**

MycoBank MB 851760

Diagnosis: Differs from other *Tuber* spp. by its almost single chamber ascomata, 0.5–1.8 cm diam., with an apical opening of 0.2–0.6 cm in diam., and light golden-brown alveolate reticulate ascospores up to 30 µm length.

Etymology: albicavum, refers to the ascomata having a white interior chamber.

Holotype: China, Yunnan, Lijiang, Jiuhe Town, 26°38′ N 99°43′ E, alt. 3753.4 m, in a forest of *Abies forrestii* var. *smithii*, 19 September 2021, Lin Li, HKAS 131256 (GenBank: ITS = PP151577 LSU = PP151587).

Ascomata subglobose, 2.0–3.5 cm diam., surface even and finely tomentose, light cinnamon to light khaki (6C7) when fresh; with a single chamber formed by the base depression, 0.5–1.8 cm diam., with an apical opening of 0.2–0.6 cm in diam., a white fluffy inner surface of the chamber; a little elastic and crisp. Gleba white (4B1) when immature, becoming khaki (6D4) at maturity, marbled with a few whitish veins. Odor: pleasant.

Peridium 80–140 µm thick, composed of two layers: outer layer pseudoparenchymatous, 27.0–62.5 µm thick, composed of subglobose to subangular cells of 6.5–14.0(–18.0) µm wide, hyaline, thin-walled; the cells in the outermost layer expand into bristle-like outer hyphae, 0.5–1.0 µm diam. at the broadest part of the base, needle-like heads, some are perpendicular to the surface, some are intertwined and prostrate, and occasionally with yellow-brown (5B4) pigment; the inner layer consists of hyaline interwoven hyphae, 29.6–74.4 µm thick, the boundary between the inner and outer layers gradually transitions, with the cells of the outer layer becoming smaller. The interior of the chamber is composed of hyaline interwoven hyphae, 50.2–80.6 µm thick, many hyphae extend beyond the surface, giving it a white, fluffy appearance, outer hyphae stubby, blunt head, with septa, occasionally forked, 45.2–76.2 µm long, 1.0–1.5 µm diam.

Asci pyriform, broadly clavate or subglobose, sometimes with short stalk, thin-walled, 45.9–61.0 × 27.5–34.6 µm, 1–4(–5)-spored.

Ascospores broadly ellipsoid, at first hyaline, becoming light golden-brown (6C8) at maturity, reticulate, thin walled 1–1.6 µm thick; dimension ranges (excluding ornamentation) 22.0–30.0 × 14.5–17.5 µm (in 1-spored asci), Q (L/I) = 1.49–2.03 Q_m = 1.66 ± 0.19 (n = 30), 18.5–22.5 (–24.5) × 12.0–13.5 µm (in 2-spored asci), Q (L/I) = 1.52–1.68 Q_m = 1.63 ± 0.06 (n = 30), (17.5–) 19.5–21.0 × 11.5–14.0 µm (in 3-spored asci), Q (L/I) = 1.50–1.67 Q_m = 1.60 ± 0.06 (n = 30), 18.5–20.0 (–22.5) × 11.0–13.0 µm (in 4-spored asci), Q (L/I) = 1.60– 1.69 Q_m = 1.65 ± 0.02 (n = 30), 15.5–18.0 (–20.0) × 9.0–11.0 µm (in 5-spored asci), Q (L/I) = 1.37–1.84 Q_m = 1.66 ± 0.15 (n = 10);

ornamentation consists of irregular quadrilateral or pentagonal or hexagonal alveolate reticulum, the mesh 3–5 ×1–3 µm, 1–2 µm deep, 3–4 meshes across the spore width.

Ecology and distribution: Hypogeous, solitary, or in groups in the soils under the forest of *Abies forrestii* var. *smithii*, alt. 3700–3800 m, fruiting from autumn. Known only from Yunnan Province, China.

Additional specimen examined: China, Yunnan Province, Lijing, Jiuhe Town, 26°29′ N, 99°39′ E, alt. 3846 m, 12 September 2020, Lin Li (GenBank: HKAS131255 ITS = PP151578 LSU = PP151588).

Edibility: fragrant, edible.

Notes: The phylogenetic tree shows that *Tuber albicavum* is closely related to the known species *T. tomentellum*, *T.liui*, and a new species reported in this study, *T. umbilicicavatum*, forming the same clade. Compared to them, firstly, in terms of macroscopic characteristics, *T. albicavum* has a basal depression that forms a cavity, while *T. umbilicicavatum* only presents a navel-like depression. The ascomata of *T. tomentellum* are merely described as having 'an indistinctly basal depression' (Xu, 1999; Guo & Bau, 2023), and ascomata of *T. liui* have grooves and very small pores, with white soft hairs within the grooves (Xu, 1999). Secondly, the ascomata surface of *T. albicavum* is even and finely tomentose, which is similar to *T. tomentellum*, but *T.liui* and *T. umbilicicavatum* have smooth ascocarp surfaces. Additionally, both *T. tomentellum* and *T. albicavum* are found in Yunnan Province, China, but *T. tomentellum* is distributed in *Pinus* forests at altitudes not exceeding 2000 m (Guo & Bau, 2023), while *T. albicavum* is found in *Abies forrestii* var. *smithii* forests at altitudes of 3800–3900 m. *T. liui*, which also occurs in high-altitude regions (3100 m), is found in the alpine *Quercus aquifolioides* Rehder & E. H. Wilson forests (Xu, 1999). Molecular analysis also shows that *T. albicavum* is separated from other *Tuber* species, they were divided into different species with a high support rate.

Figure 4.6 *Tuber albicavum* morphological characteristics

Figure 4.6 (**A**) ascomata; (**B**) cross-section of dried ascomata showing gleba and cavity; (**C**) a piece of section of the peridium in 5%KOH, the blue arrows indicating bristle-like hyphae; (**D**) a cavity section in 5%KOH, red arrow indicating hyphae extending beyond the chamber surface; (**E**) asci in 5%KOH; (**F**–**M**) ascospores and ascus. Scale bars: A = 1 cm; B = 0.2 cm; C = 50 µm; D = 10 µm; E–G = 30; H–K = 20; L–M = 30 µm.

Tuber laojunshanense Y Wang, S.H. Li & L. Li sp. nov Figure 4.8

Mycobank number: MB 851752

Diagnosis: Differs from other *Tuber* spp. by its ascomata, which have a slightly tomentose surface, sometimes with a white navel, a relatively thick peridium up to 280 µm, and yellow-brown spores with alveolate reticulum patterns, up to 34 µm in length.

Etymology: laojunshanense, refers to the type locality of the Mt. laojunshan.

Holotype: China, Yunnan, Lijiang, Juhe Town, 26°37′ N 99°43′ E, alt. 3856 m, in the forest of *Abies forrestii* var. *smithii*. 16 September 2022, Lin Li, HKAS 131253 (GenBank: ITS = PP151583 LSU = PP151593).

Ascomata subglobose or irregular in form, occasionally irregularly lobed with furrows on the surface, slightly tomentose, 1.0–3.5 cm diam, sometimes with a white navel, light khaki (4C6) when fresh. Gleba white (4B1) when immature, becoming brown (4D4) at maturity, marbled with whitish veins. Odor light scent.

Peridium 160–280 µm thick, composed of two layers: outer layer pseudoparenchymatous, 50–110 µm thick, composed of subglobose to subangular cells of 3.5–18.0(–20.0) µm wide, hyaline, thin-walled, the cells in the outermost layer expanding into bristle-like outer hyphae, 0.5–1.0 µm diam., irregularly arranged, either interwoven or prostrate, stubby, blunt head, with septa, occasionally with yellow-brown (4E8) pigment; inner layer consists of hyaline interwoven hyphae, 80–160 µm thick, the boundary between the inner and outer layers is gradually transitioned by the cells of the outer layer becoming smaller.

Asci pyriform, broadly clavate or subglobose, sometimes with short stalk, thin-walled, 30.5–50.0 × 29.5–39.5 µm, 1–3(-4)-spored.

Ascospores ellipsoid or broadly ellipsoid, at first hyaline, becoming yellow brown (5C8) at maturity, reticulate, thin walled 1–2 µm thick; dimension ranges (excluding ornamentation) are 28.0–34.0 × 19.5–21.0 μm (in 1-spored asci), Q (L/I) = 1.34–1.85 Q_m = 1.64 ± 0.05 (n = 30), 26.0–29.5 × 17.0–20.5 μm (in 2-spored asci), Q (L/I) = 1.25–1.88 Q_m = 1.59 ± 0.03 (n = 30), (19.5–) 24.0–28.5 × (10.5–) 15.0–18.0 μm (in 3-spored asci), Q (L/I) = 1.27–1.84 Q_m = 1.56 ± 0.04 (n = 30), 11.5–16.5 × 11.5–8.0 μm (in 4-spored asci), Q (L/I) = 1.30– 1.76 Q_m = 1.48 ± 0.09 (n = 11); ornamentation consists of regular pentagonal or hexagonal alveolate reticulum, reticulum 1–3 μm high, mostly 3–5 meshes across the spore width.

Ecology and distribution: Hypogeous, solitary, or groups in the soil under the forest of *Abies forrestii* var. *smithii*, alt. 3600–3900 m, fruiting in autumn. It forms ectomycorrhizae (ECM) with *A. forrestii* var. *smithii* (Figure 4.7). ECMs have simple ramified systems, in a monopodial-pinnate pattern; up to 8.0 mm long, 2.0 mm wide, light yellow–ocher (5C8-7E7), unramified ends up to 3.0 mm long, 0.4–1.0 mm in diam. Mantle 10–35 μm thick, three to five layers with an interlocked pseudoparenchymatous surface. Cystidia needle-like, smooth, colorless, nonseptated or monoseptated. Hartig nets palmetii and single hyphal rows.

Found only in Yunnan Province, southwestern China.

Additional specimen examined: China, Yunnan Province, Lijing, Jiuhe Town, 26°27′ N 99°37′ E, alt. 3645 m, 11. Aug. 2020, Lin Li (BMDU L20065 GenBank: ITS = PP151573, L20066 GenBank: ITS = PP151574). China, Yunnan Province, Lijing, Jiuhe Town, 26°29′ N 99°27′ E, alt. 3875 m, 19 September 2021, Lin Li (GenBank: HKAS131251 ITS = PP151579 LSU = PP151589, HKAS131252 ITS = PP151580 LSU = PP151590, HKAS131254 ITS = PP151581 LSU = PP151591, BMDU-L21218a ITS = PP151582 LSU = PP151592). China, Yunnan Province, Lijing, Jiuhe Town, 26°37′ N 99°43′ E, alt. 3856 m, at the root tips of *Abies forrestii* var. *smithii*, 16 September 2022, Lin Li (BMDU L22070 ECM isolate GenBank: ITS = PP124613).

Edibility: fragrant, edible.

Notes: The phylogenetic tree shows that *Tuber. laojunshanense* is closely related to *T. liyuanum* and a potential *Tuber* species found in Taiwan, forming the same clade. When comparing the two, *T. liyuanum* (Fan & Cao, 2012) and *T. laojunshanense* have similar colored ascomata, which are light brown or light khaki, with a similar surface characterized by shallow irregular fissures and slight tomentose cover. They also have a similar peridium thickness and a two-layered structure. However, the obvious differences are that *T. liyuanum* has larger ascospores reaching 60 µm in length, while *T. laojunshanense* has smaller ascospores, only up to 34 µm in length. Furthermore, *T. liyuanum* has a strong or pungent but pleasant scent when fresh, whereas *T. laojunshanense* has a light, pleasant scent when fresh. Additionally, both *T. liyuanum* and *T. laojunshanense* are found in Yunnan Province, China, but *T. liyuanum* is distributed in *Pinus yunnanensis* Franch. Forests at altitudes not exceeding 2000 m (Fan & Cao, 2012), while *T. laojunshanense* is found in *Abies forrestii* var. *smithii* forests at altitudes of 3600–3800 m. Molecular analysis also shows that *T. laojunshanense* is separated from other *Tuber* species; they were divided into different species with a high support rate.

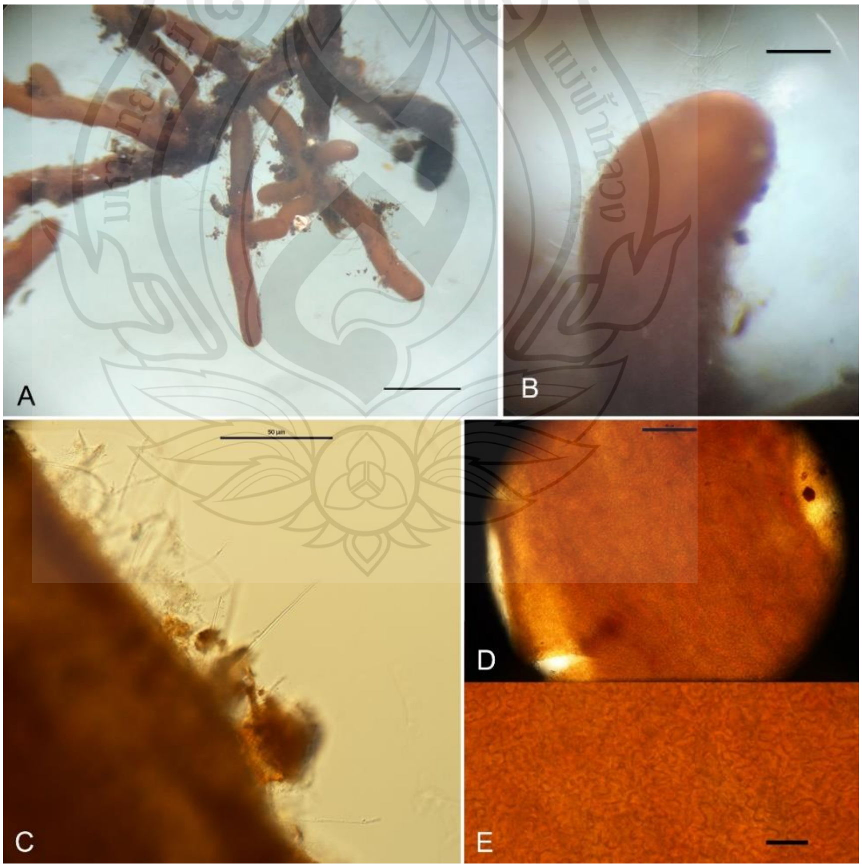


Figure 4.7 Ectomycorrhizae of *T. laojunshanense* with *Abies forrestii* var. *smithii*

Figure 4.7 (**A**) Mycorrhizal clusters; (**B**) a mycorrhizal tip with spiky cystidia; (**C**) spiky cystidia arising from the cells of the outer mantle layer; (**D**,**E**) mantle surface structure. Scale bars: A = 0.5 cm; B = 1 mm; C,D = 50 μm; E = 20 μm.

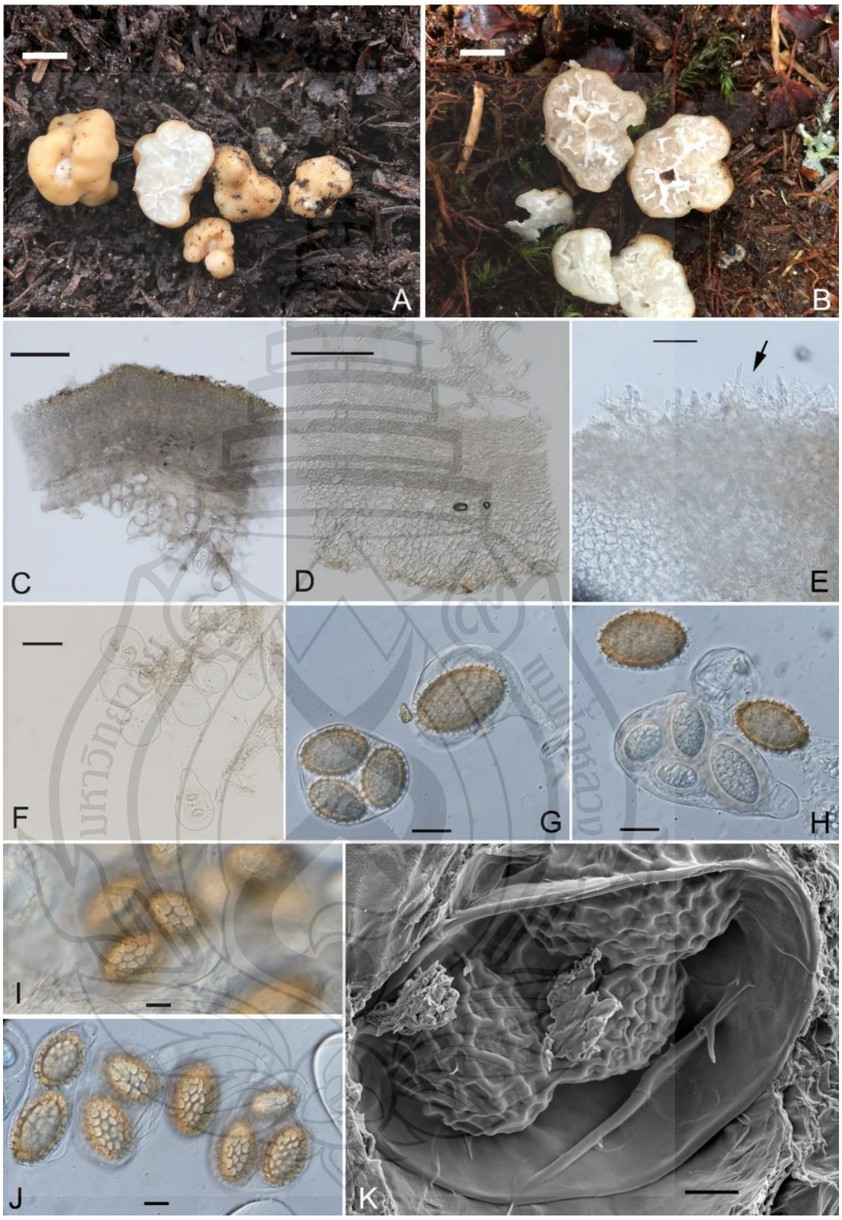


Figure 4.8 *Tuber laojunshanense* morphological characteristics

Figure 4.8 (**A**,**B**) ascomata and gleba; (**C**,**D**) a piece of the section of peridium in 5% KOH; (**E**) peridium outer layer in 5% KOH, the black arrows indicating hyphae extending beyond the surface; (**F**) asci with immature ascospores in 5%KOH;

(**G**) ascus contains 1 spore or 3 spores when mature; (**H**) released ascospores and asci; (**I,J**) asci and ascospores; (**K**) SEM ascospores (dry sample). Scale bars: A,B = 1 cm; C,D = 100 µm; E = 10 µm; F = 50 µm; G–K = 10 µm.

Tuber umbilicicavatum Y Wang, S.H. Li & L. Li sp. nov Figure 4.9

MycoBank MB 851759

Diagnosis: Differs from other *Tuber* spp. by its smooth ascomata with a distinct white navel, a relatively thin peridium up to 110 µm, and golden or golden-brown alveolate reticulate ascospores up to 40 µm length.

Etymology: umbilicicavatum, refers to the ascomata having a white navel.

Holotype: China, Yunnan, Lijing, Jiuhe Town, 26°37′ N 99°43′ E, alt. 3870.7 m, in a forest of *Abies forrestii* var. *smithii*, 19 September 2021, Lin Li, HKAS 131258 (GenBank: ITS = PP151575 LSU = PP151585).

Ascomata subglobose or irregular in form, 0.5–1.5 cm diam., with a distinct white navel, light cinnamon (5D8) when fresh, poor elasticity, brittle, easy to crack when pressed by hand, smooth on the surface. Gleba white (4B1) when immature, lighter brown (4D6) at maturity, marbled with a few whitish veins. Odor is pleasant.

Peridium 80–110 µm thick, composed of two layers: outer layer pseudoparenchymatous, 29.5–47.5 µm thick, composed of subglobose to subangular cells of 8.5–30 µm wide, hyaline, thin-walled, occasionally cells of the outermost layer with light brown (4B6) pigment; inner layer of hyaline interwoven hyphae, 27.7–52.2 µm thick, the boundary between the inner and outer layers is gradually transitioned by the cells of the outer layer becoming smaller.

Asci pyriform, broadly clavate or subglobose, sometimes with short stalk, tubular links were not observed, thin-walled, 52.0–67.5 × 31.6–40.4 µm, 1–3(–4)-spored.

Ascospores ellipsoid or broadly ellipsoid, at first hyaline, becoming golden or golden-brown (5B8) at maturity, reticulate, thin walled 0.9–1.7 µm thick; excluding ornamentations, dimension ranges (excluding ornamentation) are (34.0–)38.5–40.5 ×

20.0–26.5 µm (in 1-spored asci), Q (L/I) = 1.54–1.67 Q_m = 1.63 ± 0.05 (n = 30), 35.0–38.5(–40.0) × 19.0–22.5 µm (in 2-spored asci), Q (L/I) = 1.56–1.74 Q_m = 1.68 ± 0.06 (n = 30), (27.5–) 31.0–35.5 × 19.0–24.0 µm (in 3-spored asci), Q (L/I) = 1.57–1.66 Q_m = 1.59 ± 0.18 (n = 30), 28.5–32.5 × 18.0–20.5 µm (in 4-spored asci), Q (L/I) = 1.54–1.69 Q_m = 1.67 ± 0.14 (n = 11); ornamentation consists of regular quadrilateral or pentagonal alveolate reticulum, the mesh 2.5–6 × 1.5–3.5 µm, 2–3 µm deep, 3–5 meshes across the spore width.

Ecology and distribution: Hypogeous, solitary, or in groups in the soils under the forest of *Abies forrestii* var. *smithii*, alt. 3800–3900 m fruiting in autumn. Known only from Yunnan Province, China.

Additional specimen examined: China, Yunnan Province, Lijing, Jiuhe Town, 26°29′ N, 99°39′ E, alt. 3916 m, 19 September 2021, Lin Li (GenBank: HKAS131257 ITS = PP151576 LSU = PP151586).

Edibility: fragrant, edible.

Notes: The phylogenetic tree shows that *Tuber umbilicicavatum* is closely related to *T. liui* and *T. tomentellum*, forming the same clade. Comparing the three, all have small ascomata not exceeding 3 cm, but the distinguishing feature of *T. umbilicicavatum* is its smooth surface with a distinct navel-like depression. In contrast, *T. liui* also has a smooth surface but with grooves and very small pores, and the grooves contain white soft hairs (Xu, 1999), *T. tomentellum* has a tomentose surface with an indistinct navel (Fu & Fan, 2023). Additionally, the ascospores of *T. umbilicicavatum* are smaller, up to 40 µm in length, whereas *T. tomentellum* ascospores can be 70 µm in length (Bonito & Smith, 2016), and *T. liui* ascospores can reach 78 (–94) µm in length (Xu, 1999). Furthermore, *T. tomentellum* is distributed in *Pinus* forests at an altitude of around 2000 m in central Yunnan (Fu & Fan, 2023), *T. liui* is found in *Quercus aquifolioides* forests at an altitude of 3100 m, and *T. umbilicicavatum* is distributed in *Abies forrestii* var. *smithii* forests at altitudes of 3800–3900 m. Molecular analysis also shows that *T. umbilicicavatum* is separated from other *Tuber* species; they were divided into different species with a high support rate.

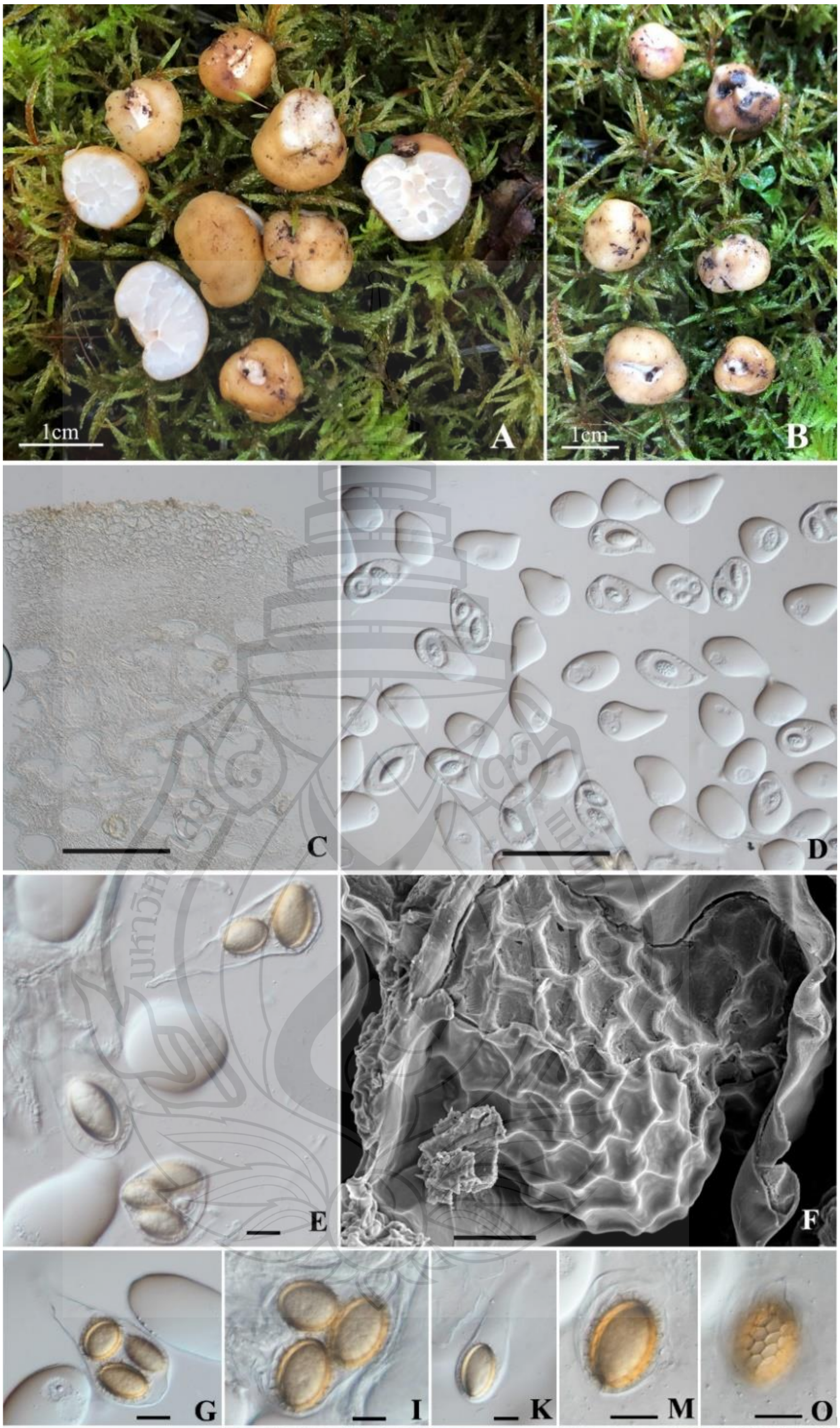


Figure 4.9 *Tuber umbilicicavatum* morphological characteristics

Figure 4.9 A,B. ascomata; C. a piece of the section of the ascomata in 5%KOH; D. asci in lactophenol; E.1-spored, 2-spored, and 4-spored asci; F. SEM ascospores (dry sample); G–P. ascospores and ascus; Scale bars: A,B = 1 cm; C,D = 100 µm; E,G–P = 20 µm; F = 10 µm.

4.2.2.4 Discussion

Since the first *Tuber* species was documented in China in 1985, more than sixty species have been reported, with half of these being newly identified by science (Fan et al., 2014; Guo & Bau, 2023; Fu & Fan, 2023). The great majority of these species are found in southwest China (Yunnan, Sichuan, and Xizang Province), implying southwest China might be one of the epicenters for the evolution of *Tuber* species. Quite a few alpine *Tuber* species have been found in southwest China, which further supports this speculation. *T. liui* was the first alpine species found in the *Quercus aquifolioides* forest at an altitude. 3100 m, Xizang of China (Xu, 1999). *T. zhongdianense* was the second one discovered at 3400 m in *Quercus monimotricha* bush in Yunnan, China (He et al., 2004). *T. albicavum*, *T. laojunshanense*, and *T. umbilicicavatum* were recently found under the alpine fir forests in northwest Yunnan, growing with *Abies forrestii* var. *smithii* at even higher altitudes between 3600 and 4000 m. All five species belong to the *Puberulum* phylogroup, sharing the same morphological features: smaller light-colored ascomata, double-layer peridium, and alveolate reticulum ascospores. Phylogenetic analysis showed all the alpine species were grouped, indicating they are closely related in phylogeny. The unique climate of the alpine zone forests in southwest China nurtured these truffles and made them different from other *Tuber* species.

In a molecular-based study on the symbiotic tree partners of *Tuber* species (Wilgan, 2023), 16 European *Tuber* species were analyzed, including 156 ECM symbionts formed by *Tuber* species. None of the *Tuber* species were found to be exclusively associated with trees of the Pinaceae family, reflecting the diversity of the mycorrhizal tree partners among the *Tuber* species. The study also revealed that, for species within the *Puberulum* phylogroup (specifically *T. borchii* and *T. anniae*), approximately 30% of ECM symbionts were formed with coniferous trees (Pinaceae), while about 70% were formed with broad-leaved trees (Wilgan, 2023).

The three new species reported in this paper also belong to the *Puberulum* phylogroup, and the habitats of the studied specimens are similar. Although these species were confirmed to be associated with *Abies forrestii* var. *smithii*, it is known that the same *Tuber* species can form symbiotic mycorrhizae with different trees in various habitats, including both coniferous and broad-leaved trees. Therefore, it cannot be conclusively stated that these species form mycorrhizal associations exclusively with *Abies forrestii* var. *smithii*. Nevertheless, these truffles play an important role in the alpine forest ecosystems, symbiotically associated with their trees, and provide food to animals dwelling in these forests.

4.2.2.5 Conclusions

Based on morphological and DNA sequence evidence, this study describes three new species of white truffles, *T. albicavum*, *T. laojunshanense*, and *T. umbilicicavatum*, collected from alpine fir forests in Yunnan, China, as belonging to the *Puberulum* phylogroup. These species represent new scientific records of *Tuber* species distributed at elevations of 3600–4000 m.

Basidiomycota

4.2.3 *Chamonixia*

4.2.3.1 Introduction

The genus *Chamonixia* Rolland (Boletaceae, Boletales) was established in the late 19th century to accommodate a single species, *C. caespitosa* Rolland, which was originally discovered in Chamonix, France (Rolland, 1899). Since its establishment, this species has been sporadically reported from Europe (Soehner, 1922; Montecchi & Sarasini, 2000; Mleczko et al., 2009), North America (Smith & Singer, 1959), China (Eckblad & Ellingsen, 1984), and Japan (Orihara et al., 2016). *Chamonixia* is characterized by gasteroid, spongy basidiomata that often exhibit bluish discoloration, and basidiospores with 6–12 large, blunt, longitudinal ridges.

To date, aside from reports in Europe and North America, *Chamonixia* has only been recorded in Sichuan Province of China and on Honshu Island of Japan, with both reports referring to the species *Chamonixia caespitosa*. The Chinese record was based on morphological examination of a specimen collected by Dr. Harry Smith in 1922 (Eckblad & Ellingsen, 1984). In 2016, Orihara et al., reported specimens collected from central Honshu, Japan, and confirmed their identity as *C. caespitosa* based on both morphological characteristics and molecular phylogenetic analyses (Orihara et al., 2016). In the present study, specimens of *Chamonixia* collected from Yunnan Province, China, are described as a new species, *Chamonixia laojunshanensis*, based on detailed morphological observations and molecular phylogenetic evidence.

4.2.3.2 Sequence Alignment and Analysis

The internal transcribed spacer (ITS) region was used in this study to analyze species diversity within the genus *Chamonixia*, as it has proven to be a useful locus for delimiting species in this group. A total of 33 ITS sequences, including those generated in this study and those retrieved from NCBI, were analyzed. These sequences represent two species of *Chamonixia*, nine species of *Octaviania*, three species of *Rossbeevera*, two species of *Leccinum*, two species of *Leccinellum*, and one species of *Retiboletus* (Table 4.4). The analytical methods followed those described in the Materials and Methods section. Phylogenetic clades were considered strongly supported if they exhibited a bootstrap support (BS) value ≥ 70% and/or a posterior probability (PP) ≥ 0.90.

Table 4.4 Taxa information and GenBank accession numbers of the sequences used in *Chamonixia* study

Species Name	Voucher	Origin	GenBank No.
Chamonixia caespitosa	KPM-NC 18070	Japan	KP222907
Chamonixia caespitosa	KPM-NC 18071	Japan	KP222908
Chamonixia caespitosa	KRA F-2013-38	Poland	KT001255
Chamonixia caespitosa	KRA F-2014-113	Poland	KT001257
Chamonixia caespitosa	KRA F-2013-60	Poland	KT001258
Chamonixia caespitosa	KRA F-2014-110	Poland	KT001259
Chamonixia laojunshanensis	**L22053**	**China**	**This study**
Chamonixia laojunshanensis	**L22054**	**China**	**This study**

Table 4.4 (continued)

Species Name	Voucher	Origin	GenBank No.
Chamonixia laojunshanensis	**L22055**	**China**	**This study**
Chamonixia laojunshanensis	**L22056**	**China**	**This study**
Chamonixia laojunshanensis	**L22057**	**China**	**This study**
Chamonixia laojunshanensis	**L22058**	**China**	**This study**
Chamonixia laojunshanensis	**L22059**	**China**	**This study**
Chamonixia laojunshanensis	**L22060**	**China**	**This study**
Chamonixia laojunshanensis	**L21208**	**China**	**This study**
Chamonixia laojunshanensis	**L21209**	**China**	**This study**
Octaviania tasmanica	OSC 132097	Australia	KP222909
Octaviania nonae	KPM-NC-0017748	Japan	JN257985
Octaviania mortae	KPM-NC-0017771	Japan	JN257995
Octaviania asterosperma	Trappe23377	Spain	JN257998
Octaviania celatifilia	KPM-NC-0017776	Japan	JN257997
Octaviania cyanescens	PNW FUNGI 5603	USA	KC552006
Octaviania kobayasii	KPM-NC-0017783	Japan	JQ619171
Octaviania japonimontana	KPM-NC-0017797	Japan	JQ619174
Octaviania etchuensis	KPM-NC-0017822	Japan	JQ619182
Rossbeevera vittatispora	TO-AUS-72	Australia	KC551977
Rossbeevera westraliensis	MEL2219011	Australia	KC551980
Rossbeevera pachydermis	KPM-NC0023347	New Zealand	KJ001089
Leccinum vulpinum	KPM-NC-0017834	UK	KC552013
Leccinum scabrum	KPM-NC-0017840	UK	KC552012
Leccinellum quercophilum	M Kuo 07120801 (holotype, NY)	USA	KC691207
Leccinellum crocipodium		Netherlands	AF454590
Retiboletus aff. Griseus	HKAS59460	China	JQ928613

Note The newly generated sequences are in bold.

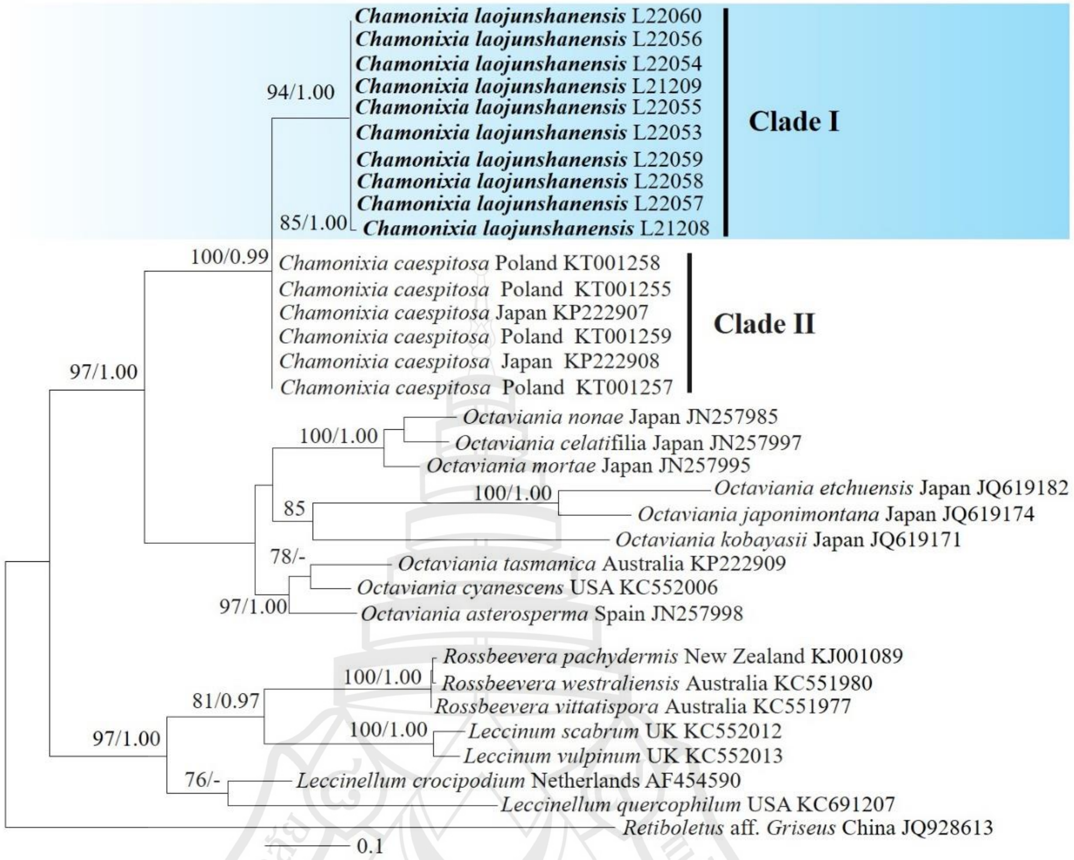


Figure 4.10 Phylogeny derived from a maximum likelihood (ML) analysis of the nrDNA-ITS sequences from *Chamonixia* species

Figure 4.10 Values next to nodes reflect maximum likelihood bootstrap support values (BS), left, and Bayesian posterior probabilities (PP), right. Names of novel species and samples with newly generated sequences are in bold.

4.2.3.3 Results

Phylogenetic Analysis

The ML and Bayesian analyses of the 33 ITS sequences are shown in Figure 10 with associated bootstrap supports for branches.

In the phylogenetic tree, the 16 ITS sequences from *Chamonixia* revealed the phylogenetic relationship of 2 species: Clade 1 includes ten sequences of the new species *C. laojunshanense*. Clade 2 includes six sequences of the species *C. caespitosa* from Japan and Poland. For phylogenetic analysis with our collected specimens, we selected sequences from 11 species belonging to the closely related genera *Octaviania* and *Rossbeevera*, and used representatives of *Leccinum*, *Leccinellum*, and *Retiboletus* as outgroups. The phylogenetic analysis showed that the new species is distinct from *C. caespitosa*. In addition to the ITS sequences used in this phylogenetic analysis, the LSU sequences were amplified from the newly supplemented specimens in this study and uploaded to NCBI for future study.

Taxonomy

Chamonixia laojunshanensis L. Li, K.D. Hyde & S.H. Li, sp. nov. Figure 4.11

Mycobank number: MB 860402

Diagnosis: Differs from other *Chamonixia* spp. by feature smaller golden-brown basidiospores up to 9.5 µm length, and with thinner peridium up to 100 µm thickness.

Etymology: laojunshanensis, referring to the type locality of the Mt. Laojunshan.

Holotype: CHINA, Yunnan, Mt. laojunshan (26º48N 99º42E), alt. 3946 m, in the forest of *Abies forrestii* var. *smithii*, 15 Sep. 2022, Lin Li, BMDU L22053 (GenBank: ITS=PX105425, LSU=PX096422, SSU=PX097606).

Basidiomata subglobose to depressed-globose, 0.5–2.5 cm diam., surface felty to wooly or slightly lobed, ivory white(1B2), but rapidly turn indigo blue(20D8) when touched or scratched, and do not fade when dried, and turns yellow(3B8) when exposed to 5% KOH, fragile, base is inwardly concave and connected to stipe. Stipe rod-shaped and short, 0.2-0.6 cm in length, 0.1-0.2 cm in diam., sometimes slightly swollen at the base, showing the same discoloration as surface of basidiomata. Basal rhizomorphs are white, commonly extend from the base of the stipe, showing the same color and discoloration as the basidiomata. Odor not distinctive.

Gleba spongy, white when immature, becoming blackish brown at maturity, composed of irregular locules up to 1.0 mm in diam., columella often present, unbranched, penetrating the gleba from the point of stipe attachment to the top, and the peridium section, columella section, and some trama sections rapidly turning indigo blue(20D8) when cut, and the immature dried gleba section turns yellow(3B8) when exposed to 5% KOH.

Peridium 80–100 μm thick, composed of two layers: inner layer 48–79.4 μm thick, consists of subglobose to subangular or columnar cells of 3.5–10 μm wide, hyaline, thin-walled; outer layer 18.0–42.5 μm thick, consists of hyaline interwoven hyphae of 1–2 μm wide, showing yellow brown (4C7) pigment in 5% KOH, partially arranging perpendicularly to surface.

Basidia clavate, 18.5–26.0 × 10.5–16.0 μm, slightly enlarged at the apex, colorless, 2-4-spored, sterigmata distinct, 4.0–6.0 (–7.0) μm long, basidia collapsed and disappeared at maturity. Basidioles broad clavate, 7.0–9.0 × 5.5–7.5 μm, colorless, arranged in a palisade.

Basidiospores broadly ellipsoid, at first hyaline, becoming golden-brown (5C8) at maturity, dimension ranges (5.0–)7.5–9.5 (–11.5) × 5.5–7.5 (–9.0) μm, Q (L/I) = 1.27–1.52, Qm = 1.24 ± 0.08 (n = 45) excluding exosporium, (10.5–)12.5–16.0 (–18.0) × 7.5–10.5 (–12.0) μm, Q (L/I) = 1.15–1.34, Qm = 1.09 ± 0.11 (n = 45) including exosporium, walls 0.5–1.5 μm thick. The hilar appendage at the base of the basidiospore, remaining after detachment from the sterigma, is cylindric-truncate to conico-truncate in shape, 1–2 μm long, 1 μm wide, sometimes retaining a portion of the sterigma. Exosporium long, remaining hyaline, finally golden-brown when the spores mature, irregularly wrinkled to form longitudinal 6–10 large, salient, often furcate ridges up to 2–2.5 mm high. From the lateral view of the spore, the surface of the exosporium exhibits parallel, fine transverse striations, making the ridges appear as if they are layered from the base to the apex of the spore. The ridges converge at the spore apex, presenting a sharply pointed, 4-6-lobed petal-like structure in scanning electron microscope (SEM) images.

Ecology & Distribution: Hypogeous, solitary, or in groups in the soils under the forest of *Abies forrestii* var. *smithii*, alt. 3700–3900m fruiting in autumn. The mature basidiomata are soft and spongy, and often riddled with wormholes left by insect feeding, which penetrate the entire basidiomata, but no insect bodies have been observed. Known only from northwestern Yunnan Province, China.

Additional specimen examined: China, Yunnan Province, Mt. laojunshan, alt. 3715 m, 28 Sep. 2021, Lin Li (GenBank: BMDU L21208-L21209 ITS = XXXX LSU = XXXX), alt. 3946 m, 15 Sep. 2022, Lin Li, BMDU L21208 (GenBank: ITS = PX105426), BMDU L21209 (GenBank: ITS = PX105427), alt. 3946 m, 15 Sep. 2022, Lin Li, BMDU L22054 (GenBank: ITS = PX105428, LSU = PX096423, SSU=PX097607), BMDU L22055 (GenBank: ITS = PX105429, LSU = PX096424, SSU= PX097608), BMDU L22057 (GenBank: ITS = PX105431, LSU = PX096426, SSU= PX097610), BMDU L22059(GenBank: ITS = PX105433, LSU = PX096428, SSU= PX097612), BMDU L22060 (GenBank: ITS = PX105434, LSU = PX096429, SSU= PX097613).

Notes: Besides *Chamonixia laojunshanensis* reported in this paper, there are only four other valid species in the genus *Chamonixia*: *C. albida* (Massee & Rodway) Y.S. Chang and Kantvilas 1993, *C. brevicolumna* A.H. Sm. & Singer 1959, *C. caespitosa* Rolland 1899, and *C. caudata* (Zeller & C.W. Dodge) A.H. Sm. & Singer 1959. Species of the genus *Chamonixia* have been recorded only once in China, when Dr. Harry Smith collected a specimen in Sichuan Province in 1922. This specimen was later studied by Eckblad and Ellingsen in 1984, who reported spore dimensions of 18–23 × 10–14 μm, with hyaline pedicels 1–3 μm long. The authors also noted that H. Smith observed that the specimens became intensely blue when bruised, which directly points to *C. caespitosa* (Eckblad & Ellingsen, 1984). Based on these characteristics, Eckblad and Ellingsen (1984) identified this specimen from Sichuan, China, as *C. caespitosa*. Although the species described in this paper, *C. laojunshanensis*, and the specimen of *C. caespitosa* from Sichuan were both found in high-altitude coniferous forests (alt. 3500–3900 m) and share similar macroscopic features, the basidiospores of *C. laojunshanensis* are noticeably smaller.

Currently, molecular data are available only for *C. caespitosa* within the genus *Chamonixia*, and phylogenetic analysis supports the distinction of *C. caespitosa* from *C. laojunshanensis*. A comparison of the main morphological characteristics of five species in *Chamonixia* (Table 4.5) shows that *C. caespitosa* and *C. laojunshanensis* are morphologically similar due to the strong bruising blue coloration of the basidiomata. However, we note that the spore size range for specimens of *C. caespitosa* collected from different regions of Europe is (13.0–) 16–24 (–28) × (8.0–) 10.5–15 (–17) µm (Lange & Hawker, 1951; Capellano, 1967; Kotlaba, 1971; Groß et al., 1980; Hagara, 1985; Kers, 1985; Breitenbach & Kränzlin, 1986; Hæggström, 1987; Cheype, 1990; Montecchi & Sarasini, 2000; Mleczko et al., 2009; Hobart, 2016), while spores from specimens collected in Japan measure 18.7 × 11.7 µm (Takamichi, 2020). The spores of *C. laojunshanensis* described in this paper are 12.5–16.0 × 7.5–10.5 µm, which are significantly smaller than *C. caespitosa*.

Table 4.5 Comparison of characteristics of four species in the genus *Chamonixia*

Species	peridium	Basidia (µm)	Basidiospores (µm)	Distribution	Host	Reference
Chamonixia albida	Initially dirty white, eventually becoming yellow.	2-spored	Yellowish-brown 21–28×14–18	no data	no data	Chang and Kantvilas (1993), Massee and Rodway (1900)
Chamonixia brevicolumna	At first near olive-ochre or bluish-olive but soon stained blue when bruised.	2-4-spored 27–41 ×12–17	Rusty cinnamon or fulvous 15–18 × 10–13	North America (August)	spruce-fir	Smith and Singer (1959)
Chamonixia caespitosa	Became intensively blue when bruished, greenish to olive on drying.	4-spored 37–45 (–51) × 16–18.5(–20)	Yellow to reddish-brown 18–20.5×12.7–15.2	North America, Central Europe, East Asia	*Picea abies*, *Abies amabilis*, *Tsuga* spp., *Pseudotsuga menziesii*	Smith and Singer (1959) Mleczko et al. (2009) Hobart (2016)
Chamonixia caudata	Light brown to dark yellowish brown or maroon, not stain blue.	2-4-spored No basidia data	Dull brown 9–15 × 9–11	North America (November)	*Quercus* spp.	Smith and Singer (1959)
Chamonixia laojunshanensis	Initially ivory white, became intensively blue when bruished.	2-4-spored 14.5–18.0 × 5.5–7.0	Golden-brown 12.5–16.0 × 7.5–10.5	China, Yunnan (September)	*Abies forrestii* var. *smithii*	This study

Figure 4.11 *Chamonixia laojunshanensis* morphological characteristics

Figure 4.11 A.B. Basidiomata and basidiomata cross-section; C. Mature basidiomata riddled with wormholes; D.E. The color change of the peridium after excavating the basidiomata and exposing it to air; F. Immature basidiomata cross-section; G. Dry peridium turns yellow when 5%KOH is encountered; H. Dry gleba turns yellow when 5% KOH is encountered; I. Locules composing the gleba; J. The section of the peridium in 5% KOH; K. A piece of the section of the hymenium in 5% KOH; L.M.N.O. hymenium and basidia in 5% KOH (M. 4-spored basidia); P.Q. Top view of basidiospore in 5% KOH (P. Immature basidiospore). R.S.T. Lateral view of the basidiospore in 5% KOH (S. Immature basidiospore). U.V.W.X.Y. SEM basidiomata (dry sample). Scale bars: A-F = 1 cm; G-I = 1mm; J = 50 μm; K.L.U = 20 μm; M.N.Y = 10 μm; O-R.V= 5 μm; W.V= 2 μm.

4.2.4 *Gautieria*

4.2.4.1 Introduction

The genus *Gautieria* Vittad. 1831 (*Gomphaceae*, *Gomphales*, *Agaricomycotina*, *Basidiomycota*, He et al., 2024) was established by Vittadini in 1831, with the initial description of two species from Italy, *G. morchelliformis* (type species) and *G. graveolens* (Vittadini, 1831). Over the next nearly two centuries, additional species within this genus were described and reported across the Northern Hemisphere, South America, and Oceania (Soehner, 1951; Pilát, 1958; Rauschert, 1975, Gross et al., 1980; Halling, 1981; Calonge et al., 1985b, 1996; Montecchi & Lazzari, 1988, 1993; Calonge & Pasabán, 1993; Vidal, 1994; Bougher & Lebel, 2001; Bau & Liu, 2013; Yang et al., 2023; Vidal et al., 2023). Currently, there are 77 valid names listed in MycoBank for this genus (https://www.mycobank.org/). Vidal et al. (2023) conducted a morphological and phylogenetic revision of the genus *Gautieria* in Europe and the Mediterranean Basin, which clarified the four sections within the genus: sect. *Gautieria* Vittad. 1831, sect. *Hymenogastroides* J.M. Vidal 2023, sect. *Parvicellae* J.M. Vidal and States, 2023, and sect. *Glutinosiglebae* J.M. Vidal 2023. *Gautieria* is a genus of hypogeous fungi, typically forming ectomycorrhizal associations with symbiotic tree partners, such as Pinaceae, Betulaceae, and Fagaceae (Liu, 1998; Vidal et al., 2023). Upon maturation, their basidiomata emit odors that attract small animals, which facilitate spore dispersal. The distribution of *Gautieria* species is diverse, reflecting variations in both geographic range and symbiotic tree associations. For example, species found in Mediterranean sclerophyllous forests differ significantly from those in boreal forests (Linares, 2011; Tsuda et al., 2016; Vidal et al., 2023).

Since the description of the first Chinese *Gautieria* species, *Gautieria chengdensis* J.Z. Ying, in 1984, eleven species have been reported (Ying, 1984, 1995; Liu et al., 1996; Tao et al., 1996; Liu 1998; Bau & Liu, 2013; Yang et al., 2023). This paper describes a new *Gautieria* species recently discovered in mixed forests in Central Yunnan, China.

4.2.4.2 Sequence Alignment and Analysis

Fifty-four ITS and forty-one nrLSU sequences from NCBI and this study represent 31 species of *Gautieria* (Table 4.6), including two sequences of *Ramaria abietina* as outgroups. All ITS and nrLSU sequences were extracted from basidiomata of *Gautieria* specimens. Sequences of *Gautieria* species generated in this study were submitted to the GenBank database. We first edited the sequences using BioEdit v. 7 (Hall, 2007), then used the basic local alignment search tool for the GenBank database to recheck whether the newly generated sequences were amplified DNA from a contaminant or not. DNA sequences were retrieved and assembled using Sequence Matrix (Vaidya et al., 2011).

Sequences were aligned using MAFFT version 7 (Katoh & Standley, 2013). Phylogenies and node support were first inferred by Maximum Likelihood (ML) from the two single-locus alignments separately, using RAxML-HPC2 version 8.2.12 (Stamatakis, 2014) with 1,000 rapid bootstraps, as implemented on the Cipres portal (Miller et al., 2010). Since no supported conflict (bootstrap support value (BS) ≥ 70%) was detected among the topologies, the two single-gene alignments were concatenated using Sequence Matrix (Vaidya, 2011). Partitioned Maximum likelihood (ML) analysis was performed on the concatenated data set, as described above. For Bayesian Inference

(BI), the best substitution model for each data set was determined with the program MrModeltest 2.3 (Nylander et al., 2004) on CIPRES. The results suggested that the best substitution models are as follows: nrLSU: GTR+I+G, ITS1: JC + I, 5.8S: GTR + G + I, ITS2: K80 + I + G. Bayesian analysis was performed using MrBayes version 3.2.7a (Ronquist, 2011) as implemented on the Cipres portal (Miller et al., 2010); four parallel runs were performed for 10 million generations, sampling every 100th generation for the single gene trees. Parameter convergence > 200 was verified in Tracer v. 1.7 (Rambaut et al., 2018). The phylogenetic clade was strongly supported if the bootstrap support value (BS) was ≥70% and/or the posterior probability (PP) was < 0.01.

Table 4.6 Taxa information and GenBank accession numbers of the sequences used in *Gautieria* study

Species Name	**Voucher**	**Origin**	**GenBank No.**	
			ITS	**nrLSU**
Gautieria chilensis	MES-1977	Argentina	KY462630	(a)
Gautieria chilensis	Halling5818	Chile	AF377069	(b)
Gautieria cistophila	JMV960527-1 (**H**)	Spain		OL342777
Gautieria confusa	SOMF30317	Bulgaria	OL311045	OL311043
Gautieria confusa	ZB1732	Hungary	OL467206	
Gautieria convoluta var. *convoluta*	ELG950600	Italy	OL304003	OL304004
Gautieria convoluta var. *convoluta*	TPN-19-0476	Poland	OL304005	OL304002
Gautieria convoluta var. *petrakii*	M126216 (**H**)	Czech	OL304009	
Gautieria fenestrata	SOMF30331	Bulgaria	OL314643	OL314644
Gautieria fenestrata	GK6445	Greece	OL467120	OL415542
Gautieria fusella	SOMF30328	Bulgaria	OL311143	
Gautieria fusella	SOMF30329	Bulgaria	OL467196	

Table 4.6 (continued)

Species Name	Voucher	Origin	GenBank No.	
			ITS	nrLSU
Gautieria graveolens	GK1228	Greece	OL331103	
Gautieria graveolens	ZB2321	Hungary	OL331102	
Gautieria hellenica	GK5594	Greece	OL304014	
Gautieria hellenica	GK5608	Greece	OL304013	OL304015
Gautieria hymenogastroides	JC160320NR	Spain	OL314650	OL314647
Gautieria hymenogastroides	JMV20110811(**H**)	Spain	OL314648	
Gautieria iberica	JMV800472	Spain	OL304008	
Gautieria iberica	JMV800473	Spain	OL304011	
Gautieria macrocoilia	PRM678964	Czech	OK669118	OK669119
Gautieria macrocoilia	M126222	Germany	OL467181	OL415561
Gautieria mianjin	HKAS126926 (**H**)	China	NR187094	NG229098(c)
Gautieria mianjin	HTBM1288	China	PP542496	PP542495(d)
Gautieria monticola	SNF136CA	USA	AF377079	(b)
Gautieria morchelliformis var. *dubia*	KRA F-2010-60	Poland	OL467177	OL415553
Gautieria morchelliformis var. *dubia*	KRA F-2013-1	Poland	OL467178	OL415556
Gautieria morchelliformis var. *intermedia*	JMV20021110-1	Spain	OK663116	
Gautieria morchelliformis var. *intermedia*	JMV20180726-3 (**H**)		OK663108	OK663115
Gautieria morchelliformis var. *morchelliformis*	SOMF30306	Bulgaria	OL467192	OL415564
Gautieria morchelliformis var. *morchelliformis*	GK6781	Bulgaria	OK669113	OK663097

Table 4.6 (continued)

Species Name	Voucher	Origin	GenBank No.	
			ITS	nrLSU
Gautieria obtexta	VK1376	Greece	OL311145	OL311139
Gautieria obtexta	VK1395	Greece	OL467200	OL415573
Gautieria otthii	ZB443	Hungary	OL467225	
Gautieria otthii	ZB2286	Hungary	OL467210	
Gautieria persimilis	JC100613BT	Spain	OL467126	
Gautieria persimilis	JC110724BT	Spain	OL467127	
Gautieria pervestita	JMV800556 (**H**)	Greece	OL310906	OL310907
Gautieria pervestita	VK880	Greece	OL310904	OL310908
Gautieria pityophila	GK6359	Greece	OL467119	OL415541
Gautieria pityophila	VK2980	Greece	OL310919	OL310916
Gautieria queletii	KRAF-2011-77	Poland		OL415554
Gautieria queletii	JMV20180823-1 (**H**)	Spain		OL342774
Gautieria subglobispora	O152350	Norway	OL304062	OL304061
Gautieria subglobispora	JMV990626-12	Spain	OL304059	
Gautieria trabutii	JC70812BT	Spain		OL342780
Gautieria trabutii	JMV20020523	Spain		OL342778
Gautieria villosa var. *inflata*	SOMF30321	Bulgaria	OL467195	OL415569
Gautieria villosa var. *inflata*	TPN-19-0123	Poland	OL036691	OL036693
Gautieria villosa var. *pilifera*	MG109	Greece	OL036695	OL036690
Gautieria villosa var. *pilifera*	JMV800561 (**H**)	Greece	OL036694	OL036692
Gautieria villosa var. *villosa*	IB1985254a	Austria	OL467123	OL415544
Gautieria villosa var. *villosa*	SOMF30318	Bulgaria	OL467194	OL415568
Gautieria violascens	JC120715BT	Spain	OL342571	OL342573
Gautieria xinjiangensis	HMJAU6009 (**H**)	China	NR153450	(e)
Gautieria zixishanensis	**BMDU L23106 (H)**	**China**	**PQ672295**	**PQ672297**

Table 4.6 (continued)

Species Name	Voucher	Origin	GenBank No.	
			ITS	nrLSU
Gautieria zixishanensis	**BMDU L23107**	**China**	**PQ672296**	**PQ672298**
Ramaria abietina	OSC 134649	USA	JX310378	JX287478 (f)
Ramaria abietina	OSC 140661	USA	JX310379	JX287479 (f)

Note The newly generated sequences are in bold. (**H**) = holotype; Sequence references: (a) Truong et al. (2017), (b) Bidartondo and Bruns (2002), (c) Yang et al. (2023), (d) Yang (2024), (e) Bau and Liu (2013), (f) Gordon, M. (Unpublished); unannotated sequences are from Vidal et al. (2023).

4.2.4.3 Results

Phylogenetic Analysis

The maximum likelihood (ML) and Bayesian analyses of the 54 ITS and 41 nrLSU sequences are shown in Figure 4.12, with associated bootstrap support for branches, and reveal the phylogenetic relationship of 31 species: Clade 1 includes 21 species and variants belonging to sect. *Gautieria*. Clade 2 consists of five species belonging to sect. *Glutinosiglebae*. Clade 3 includes three species that belong to sect. *Parvicellae*. Clade 4 comprises *Gautieria hymenogastroides* J.M. Vidal, Fern. Rodr., Cabero, Sáinz and Pasabán 2023, and a newly described species in this study, *Gautieria zixishanensis*, both of which belong to sect. *Hymenogastroides*. The phylogenetic analysis showed that the new species is distinct from other *Gautieria* species.

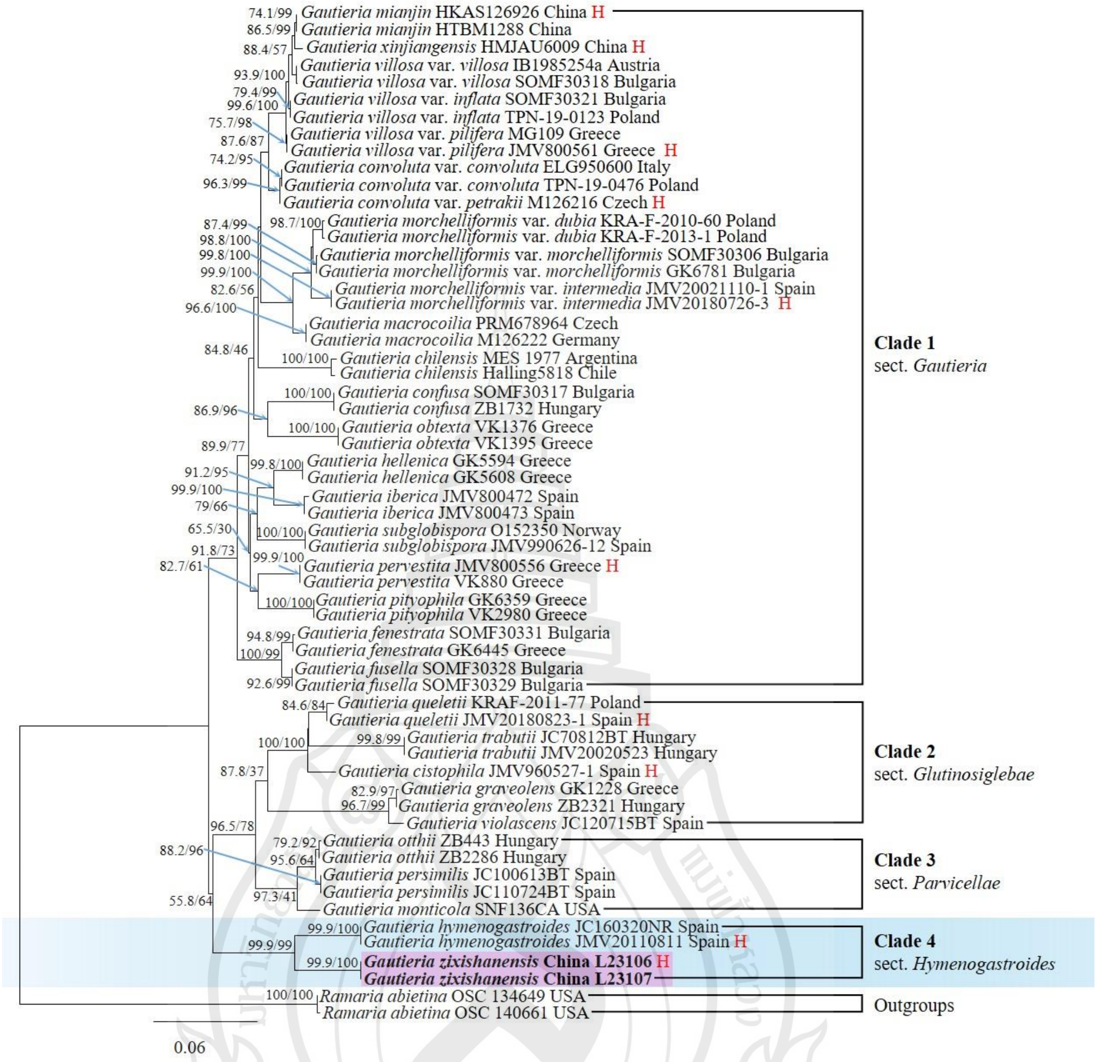


Figure 4.12 Phylogeny derived from a maximum likelihood (ML) analysis of the ITS and nrLSU sequences from *Gautieria* species, using two samples of *Ramaria abietina* as outgroups

Figure 4.12 Values next to nodes are maximum likelihood bootstrap support values (BS) on the left, and Bayesian posterior probabilities (PP) on the right. Samples of novel species with newly generated sequences are in bold.

Gautieria zixishanensis L. Li, K.D. Hyde & S.H. Li, sp. nov. Figure 4.13.

MycoBank MB 856847

Diagnosis: Differs from other Gautieria species by its basidiomata have an obvious base attached with a white basal rhizomorph; basidiomata surface tomentose, pseudoperidium intact and not easy to fall off when mature, without foveate-porate, ridges invisible; and light golden-brown longitudinal ridges basidiospore up to 17–22 μm length.

Etymology: zixishanensis, refers to the type locality of the Zixishan Mountain.

Holotype: China, Yunnan, Chuxiong, Zixi Mountain, 25°00′ N 101°23′ E, alt. 2053.7 m, in a mixed forest of Pinus yunnanensis Franch. and Quercus sp., 2 November 2023, Lin Li, BMDU L23106 (GenBank: ITS = PQ672295 nrLSU = PQ672297).

Basidiomata pseudoperidial, 1–2 cm in diameter, subglobose to reniform, with a basal depression connected to abundant white basal rhizomorphs, 0.5–1.5 mm diameter thick, surface tomentose, with hairs easily detached upon friction, pseudoperidium completely covered a basidioma and not easy to fall off when mature, not foveate or porate, pale yellowish white (4A2) when freshly unearthed, with brownish (7D5) patches gradually appearing after exposure to air or rubbed, becoming wrinkled and darker (4D7) after drying, becoming black when treated with $FeSO_4$. Ridges invisible. Gleba fleshy brown (8C7) to golden brown (5D7), composed of crowded, irregularly shaped to radially elongated, narrow, sinuous, inordinate, small locules sized 0.2–1 × 0.1–0.5 mm covered by yellow-brown to yellow-tan hymenium. Tramal plates white to almost transparent. Columella dendroid, widest at the base, up to 2–3 mm, branching towards the top, consists of hyaline interwoven hyphae 0.5–1.0 μm in diameter, white to hyaline, gelatinous, hard, and brittle. Odour pleasant.

Pseudoperidium 110–230 μm thick, composed of two layers: outer layer a trichotomentocutis, 60–140 μm thick, composed of light reddish brown (4B4) interwoven hyphae of 5–10 µm in diameter, thin-walled, septate; inner layer 45.5–90 µm thick, composed of hyaline, nearly parallel interwoven hyphae 0.5–1.5 µm in diameter, dense, and seeming gelatinous. The boundary between the inner and outer layers indistinct.

Hymenophoral trama 64–110 µm thick, composed of slightly gelatinized, hyaline interwoven hyphae, without a color change in contact with FeSO4.

Basidia clavate, thin-walled, 31.5–45.0 ×7.5–14.0 µm, 2-spored. Paraphysoid cells abundant, 15–40 × 3–9 µm, with polymorphic terminal cell, cylindrical, clavate, narrow pyriform.

Basidiospore ovate to broadly ovate (shape including the thickened exosporium), light golden yellow (4B8), cyanophilous, non-amyloid, measured (16.0–) 17.5–22.0 (–24.0) × 11.0–14.5 µm (including exosporium), Q (L/I) = 1.30–1.81, Qm = 1.56 ± 0.15 (n = 50). Exosporium thin and almost transparent when immature, thickening and forming 7–11 longitudinal ridges 2.2–2.9 µm high following maturity. Apical ring prominent, sometimes exhibiting irregular protrusions, with a central depression approximately 1 µm in diameter. The ends of the ridges merged seamlessly with the apical ring. Hilar appendix 2–3 µm long, conico-truncate, with a hilum 0.5–1 µm in diameter, without or with very short remnants of sterigma.

Ecology and distribution: Hypogeous, solitary or gregarious, in a mixed forest of Pinus yunnanensis and Quercus sp., alt. 2020–2050 m, fruiting in winter. Known only from Yunnan Province, China.

Additional specimen examined: China, Yunnan Province, Chuxiong, Zixi Mountain, 25°00′ N 101°23′ E, alt. 2038 m, 2 November 2023, Lin Li (GenBank: BMDU L23107 GenBank: ITS = PQ672296 nrLSU = PQ672298).

Notes: The phylogenetic tree indicates that the newly described species, Gautieria zixishanensis, is closely related to the known species G. hymenogastroides, with both species forming a single clade within sect. Hymenogastroides. Comparatively, in terms of macroscopic characteristics, although both species have pseudoperidial basidiomata, G. hymenogastroides exhibits a tomentose surface with lemon-yellow hues and a foveate-porate pseudoperidium that shows purplish oxidation when rubbed (Vidal et al., 2023). In contrast, the pseudoperidium surface of G. zixishanensis completely covers the basidiomata, does not present foveate-porate structures, and is pale yellowish-white, turning brownish when rubbed, with invisible ridges. Moreover, at the microscopic level, the spores of both species are similarly colored, but the spores of G. hymenogastroides are larger. Additionally, G. hymenogastroides has more diverse paraphysoid cell morphologies, including pyriform, papillate, or attenuate terminal cells, which are absent in G. zixishanensis. Furthermore, G. hymenogastroides is distributed in the Mediterranean region, found in Northern Spain at altitudes between 500–1100 m in montane broadleaf forests (Castanea, Fagus, Quercus), while G. zixishanensis is distributed in the subtropical low-latitude plateau with a monsoon climate, found in central Yunnan, China, at altitudes between 2020–2050 m in mixed forests (Pinus, Quercus). Molecular analysis also shows that G. zixishanensis is separated from other Gautieria species, and they were divided into different species generally with high statistical support.

4.2.4.4 Discussion

The detailed and comprehensive study by Vidal et al. (2023) on the morphology and phylogenetic classification of the genus *Gautieria* has been recognized and well-received. Based on this work, the newly described species *Gautieria zixishanensis* is classified within the sect. *Hymenogastroides*, represents the second species identified in this section. As noted earlier, *G. zixishanensis* shares some characteristics with the type species of sect. *Hymenogastroides*, *G. hymenogastroides*, but there are also some distinct differences. As more species within this group are discovered and described, the defining features of this section are expected to become clearer.

Interestingly, while *G. hymenogastroides* is distributed in the Mediterranean climate zone of Spain, *G. zixishanensis* is found in the subtropical, low-latitude plateau with a monsoon climate of southwestern China. Although these two species appear to be geographically distant and grow under different climatic conditions, it is important to note their shared climatic origin. On one hand, paleobotanical data suggest that the flora of the Himalaya-Hengduan Mountain region in southwestern China is a descendant of the Tethyan tertiary flora, which gradually adapted to arid conditions after experiencing a Mediterranean climate (Hang, 2002). On the other hand, it has been suggested that subterranean fungi are common in Mediterranean climate zones, where the evolution of subterranean fruiting bodies is an adaptive strategy to withstand selective pressures such as warm and dry conditions (Vogt-Schilb et al., 2022). It can be inferred that as the Himalaya-Hengduan Mountain region uplifted and the alpine environment evolved, the flora underwent a transition from a Mediterranean to an alpine climate, and the subterranean fungi associated with these plants adapted and diversified accordingly. Vidal et al. (2023) noted that fungal taxa with trichotomentocutis structures are typical of Mediterranean forests, though not all Mediterranean species within the genus *Gautieria* exhibit this feature. Our descriptions and detailed images of the pseudoperidium also reveal the presence of such structures in both *G. zixishanensis* and *G. hymenogastroides*.

Since the first species of the genus *Gautieria* was reported in China in 1984, a total of 12 species have now been identified in this region (Table 4.7). According to descriptions in the original literature (Ying, 1984, 1995; Liu et al., 1996; Tao et al., 1996; Liu, 1998; Bau & Liu, 2013; Yang et al., 2023), five species exhibit a stable pseudoperidium structure during the development of their basidiomata. Among these, four species are distributed in southwestern China: *G. pallida* Harkn. 1934 is found in Sichuan Province, *G. shennongjiaensis* K. Tao, Ming C. Chang & B. Liu 1996 is distributed in Hubei and Xizang Provinces, *G. sinensis* J.Z. Ying 1995 is found in Guizhou Province, and *G. zixishanensis* is distributed in Yunnan Province. The pseudoperidia of these species share a similar trichotomentocutis microstructure. In contrast, species found in northern China generally lack a pseudoperidium, or if present, it disappears as the basidiomata mature. Regrettably, only three of the species distributed in China have molecular data available, highlighting the need for more comprehensive data to better understand their evolutionary history.

Table 4.7 Distribution, habitats and pseudoperidium structure of 12 *Gautieria* species known in China

Species	Herbarium voucher	Pseudoperidia structure	Symbiotic tree partner	Collection location/altitude	Collecting date
G. chengdensis	HMAS 44096-**H**	Pseudoperidia absent.	*Corylus heterophylla*	Hebei, Chengde county	31 August 1981
	MHSU 2243		*Castanea mollissima*	Hubei, Shennongjia	7 October 1995
G. gautierioides	MHSU 1479	Pseudoperidium whitish, thin, composed of interwoven hyphae with 4 to 6 μm diam., with inflated cells.	*Quercus* sp.	Shanxi, Yuanqu County	24 July 1987
G. globispora	MHSU 2109-**H**	Pseudoperidia absent.	*Picea wilsonii*	Shanxi, Guancenshan	18 July 1993
G. hubeiensis	MHSU 2258-**H**	Pseudoperidia absent.	*Castanea mollissima*	Hubei, Shennongjia	7 October 1995
G. macrospra	MHSU 2262	Pseudoperidium white when fresh, turning yellowish-brown upon drying, and disappears upon maturity.	*Quercus liaotungensis*	Shanxi	16 July 1987
	MHSU 2261		*Lonicera japonica*	Shanxi	7 September 1987
	MHSU 2265		*Quercus variabilis, Ulmus* sp.	Shanxi	29 May 1988
	MHSU 2264		*Sabina chinensis*	Shanxi	29 May 1988
G. mianjin	HKAS 126926-**H**	Pseudoperidium practically absent..	Mixed forest of *Larix* and *Picea*	Gansu, Gannan city (2600m)	11 October 2022 **(a)**
	HTBM 1288		Ditto	Ditto	23 August 2023 **(b)**
G. morchelliformis	MHSU 1480	Pseudoperidia no observed.	*Quercus mongolica*	Jilin, Dunhua city	15 September 1987

Table 4.7 (continued)

Species	Herbarium voucher	Pseudoperidia structure	Symbiotic tree partner	Collection location/altitude	Collecting date
*G. pallida**	R.P.Farges (FH$^{1)}$ 1566)	Pseudoperidia two layers: an outer layer approximately 160 µm thick and an inner layer about 40 µm thick. The outer layer is composed of loosely interwoven hyphal bundles formed by moderately thin-walled hyphae, with large intercellular spaces. The inner layer is formed by tightly packed hyphal bundles composed of thick-walled hyphae and is brown in color.	-	Sichuan, Chengkou county	-
G. shennongjiaensis	MHSU 2259-**H**	Pseudoperidia thin, composed of hyaline to pale yellow interwoven hyphae of 2.5–10 µm diameter, thin-walled.	*Castanea mollissima*	Hubei, Shennongjia	7 October 1995
	MHSU 2232		*Quercus aquifolioides*	Xizang, Sejilashan (3000m)	24 August 1995
	MHSU 2235		*Pinus densata*	Xizang, Sejilashan (3050m)	10 September 1995
	MHSU 2236		*Quercus aquifolioides, Pinus densata*	Xizang, Bomi county (2700m)	14 September 1995
	MHSU 2238		*Pinus yunnanensis*	Xizang, Xiachayu town (2200m)	16 September 1995

Table 4.7 (continued)

Species	Herbarium voucher	Pseudoperidia structure	Symbiotic tree partner	Collection location/altitude	Collecting date
G. sinensis	HMAS 61236-**H**	Pseudoperidia thick, 184–366 µm, composed of hyaline and gelatinized interwoven hyphae of 7.5–9 µm diameter, with granular inclusions.	Broadleaf forest	Guizhou, Daozhen county	19 July 1988
G. xinjiangensis	HMJAU 6009	Pseudoperidia practically absent, or barely visible.	*Picea schrenkiana*	Xinjiang, Tianshan	5 September 2005 **(c)**
G. zixishanensis	L23106-**H**	Pseudoperidium, 110–230 µm thick, two layers: outer layer trichotomentocutis, composed of light reddish brown interwoven hyphae of 5–10 µm diameter, thin-walled, septate. Inner layer consists of hyaline nearly parallel interwoven hyphae of 0.5–1.5 µm diameter, dense.	*Pinus, Quercus*	Yunnan, Chuxiong City (2050m)	2 November 2023 **(d)**
	L23107		Ditto	Ditto	Ditto **(d)**

Note **H** = holotype. ***** = the habitat information for the species is not mentioned in the literature. References: **(a)** Yang et al. (2023), **(b)** Yang (2024), **(c)** Bau and Liu (2013), **(d)** this study; unannotated sequences are from Liu (1998).

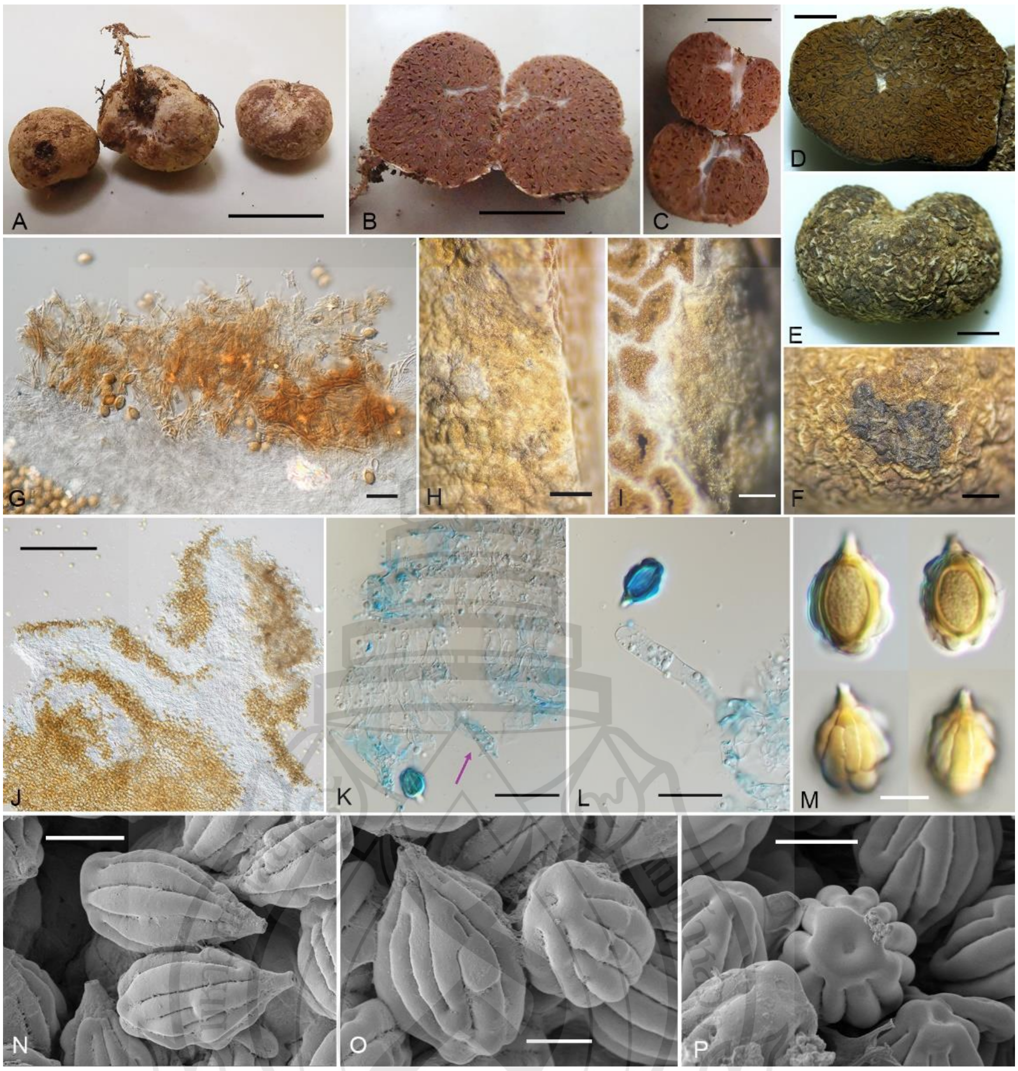


Figure 4.13 *Gautieria zixishanensis* morphological characteristics

Figure 4.13 A. fresh basidiomata; B–C. fresh gleba; D. dried gleba with locules; E. wrinkled surface of a dried basidioma; F. the surface of a basidioma reacted in contact with $FeSO_4$ solution. G. the trichotomentocutis structure on the surface of a basidioma; H. I. detail of the tomentose surface; J. a piece of the section of trama in 5% KOH; K. hymenium in lactophenol cotton blue, the purple arrow indicates a basidium; L. a paraphysoid cell stained in lactophenol cotton blue; M. basidiospores and exosporium in 5% KOH; N–P. basidiospores under SEM. Scale bars: A–C = 1 cm; D–E = 2mm; F, H–I = 1 mm, G = 50 μm; J = 100 μm; K = 50 μm; L = 20 μm; M–P = 5 μm.

4.2.5 *Hymenogaster*

4.2.5.1 Introduction

The genus *Hymenogaster* is the globally distributed group that is a species-rich genus of false truffles. As of now, according to the records from the MycoBank database, about 170 species of this group have been reported globally. *Hymenogaster* is characterized by basidiomata with some degree of columella development from almost none to having a narrow percurrent one but never a stipe-columella, spores mostly under 20 μm long, ellipsoid to ovoid in shape (some species lacking a pronounced beak), ornamentation in the form of a warty-wrinkled mostly adnate perisporium (Soehner, 1922; Smith, 1966, Stielow et al., 2011). They form ectomycorrhizae with the plant.

According to statistics, there are currently 40 species and variants are now known in China, among which 7 species are distributed in the southwest region: *Hymenogaster zunhuaensis*, *H. gilkeyae, H. niveus*, *H. latifusisporus* and in Yunnan, *H. tener* in Yunnan and Xizang, *H. xizangensis* in Xizang, *H. subnanus* in Sichuan province (Liu et al., 1996; Liu 1998; Liu et al., 2002; Fan, 2023; Li et al., 2024a; Li et al., 2024b). In the present study, specimens of *Hymenogaster* collected from Xizang Province, China, are described as a new species, *Hymenogaster zayuensis*, based on detailed morphological observations and molecular phylogenetic evidence.

4.2.5.2 Sequence Alignment and Analysis

The internal transcribed spacer (ITS) region was used in this study to analyze species diversity within the genus *Hymenogaster*, as it has proven to be a useful locus for delimiting species in this group. A total of 50 ITS sequences, including those generated in this study and those retrieved from NCBI, were analyzed. These sequences represent 27 species of *Hymenogaster* (Table 4.8). The analytical methods followed those described in the Materials and Methods section. Phylogenetic clades were considered strongly supported if they exhibited a bootstrap support (BS) value ≥ 70% and/or a posterior probability (PP) ≥ 0.90.

Table 4.8 Taxa information and GenBank accession numbers of the sequences used in *Hymenogaster* study

Species Name	**Voucher**	**Origin**	**GenBank No.**
Hebeloma lactariolens	taxon:301353		AY818352
Hebeloma lactariolens	HC 88/95		NR_119524
Hymenogaster arenarius	BJTC FAN786	China	PP467413
Hymenogaster arenarius	BJTC FAN856	China	PP467414
Hymenogaster arenarius	it5_2	Germany	GU479272
Hymenogaster bulliardii	it20_4	Germany	GU479261
Hymenogaster rehsteineri	KR-M-0044423	Germany	MT005990
Hymenogaster citrinus	BJTC FAN915	China	PP467411
Hymenogaster glacialis	GP 5302		AF325634
Hymenogaster griseus	Trappe 12841	USA	AF325636
Hymenogaster knappii	it9_2	Germany	GU479287
Hymenogaster latisporus	BJTC FAN1134, holotype	China	PP467404
Hymenogaster luteus	zb1457	Hungary	GU479306
Hymenogaster luteus	zb2603	Hungary	GU479341
Hymenogaster luteus	zb235	Hungary	GU479334
Hymenogaster lycoperdineus	zb3533	Hungary	GU479353
Hymenogaster megasporus	it12_1	Germany	GU479239
Hymenogaster megasporus	it8_5_1	Germany	GU479286
Hymenogaster minisporus	BJTC FAN1244, holotype	China	PP467407
Hymenogaster niveus	it17_3	Germany	GU479255
Hymenogaster niveus	zb28	Hungary	GU479344
Hymenogaster olivaceus	dt8293	Belgium	GU479292
Hymenogaster olivaceus	zb2300	Hungary	GU479332
Hymenogaster papilliformis	BJTC FAN1074, holotype	China	PP467400
Hymenogaster papilliformis	BJTC FAN891	China	PP467388
Hymenogaster parksii	Trappe 13296	USA	AF325638
Hymenogaster parksii	SOC1643	USA	JN022510
Hymenogaster gardneri	Trappe 7192	USA	AF325639
Hymenogaster perisporius	BJTC FAN606	China	PP467379
Hymenogaster perisporius	BJTC FAN651, holotype	China	PP467380
Hymenogaster populetorum	it16_1_1	Germany	GU479252
Hymenogaster populetorum	zb1436	Hungary	GU479304
Hymenogaster huthii	it12_3_1	Germany	GU479242
Hymenogaster huthii	zb95	Hungary	GU479366

Table 4.8 (continued)

Species Name	Voucher	Origin	GenBank No.
Hymenogaster rehsteineri	it2_4_1	Germany	GU479259
Hymenogaster rehsteineri	dt8455	Luxembourg	GU479293
Hymenogaster rehsteineri	KR-M-0044018	Germany	MT005953
Hymenogaster raphanodorus	Fogel 2698（H）	USA	AY945303
Hymenogaster subalpinus	Trappe 22752	USA	AF325640
Hymenogaster intermedius	it16_2, holotype	Germany	GU479253
Hymenogaster thwaitesii	it2_2	Germany	GU479258
Hymenogaster thwaitesii	it3_2	Germany	GU479264
Hymenogaster variabilis	BJTC FAN1141	China	PP467405
Hymenogaster variabilis	BJTC FAN656, holotype	China	PP467382
Hymenogaster vulgaris	RBG Kew K(M)27363		EU784365
Hymenogaster vulgaris	GN_4d_I	Sweden	JQ724028
Hymenogaster minisporus	QL054	China	HM105539
Hymenogaster minisporus	taxon:522720	China	LT980461
Hymenogaster zayuensis	**L22039**	**China**	**This study**
Hymenogaster zayuensis	**L22039-1**	**China**	**This study**

Note The newly generated sequences are in bold.

4.2.5.3 Results

Phylogenetic Analysis

The ML and Bayesian analyses of the 50 ITS sequences are shown in Figure 14 with associated bootstrap supports for branches. In the phylogenetic tree, the 48 ITS sequences from *Hymenogaster* revealed the phylogenetic relationship of 27 species, and used representatives of *Hebeloma* as outgroups. The phylogenetic analysis showed that the new species is clearly distinct from other species of the genus *Hymenogaster*. In addition to the ITS sequences used in this phylogenetic analysis, the LSU sequences were amplified from the newly supplemented specimens in this study and uploaded to NCBI for future study.

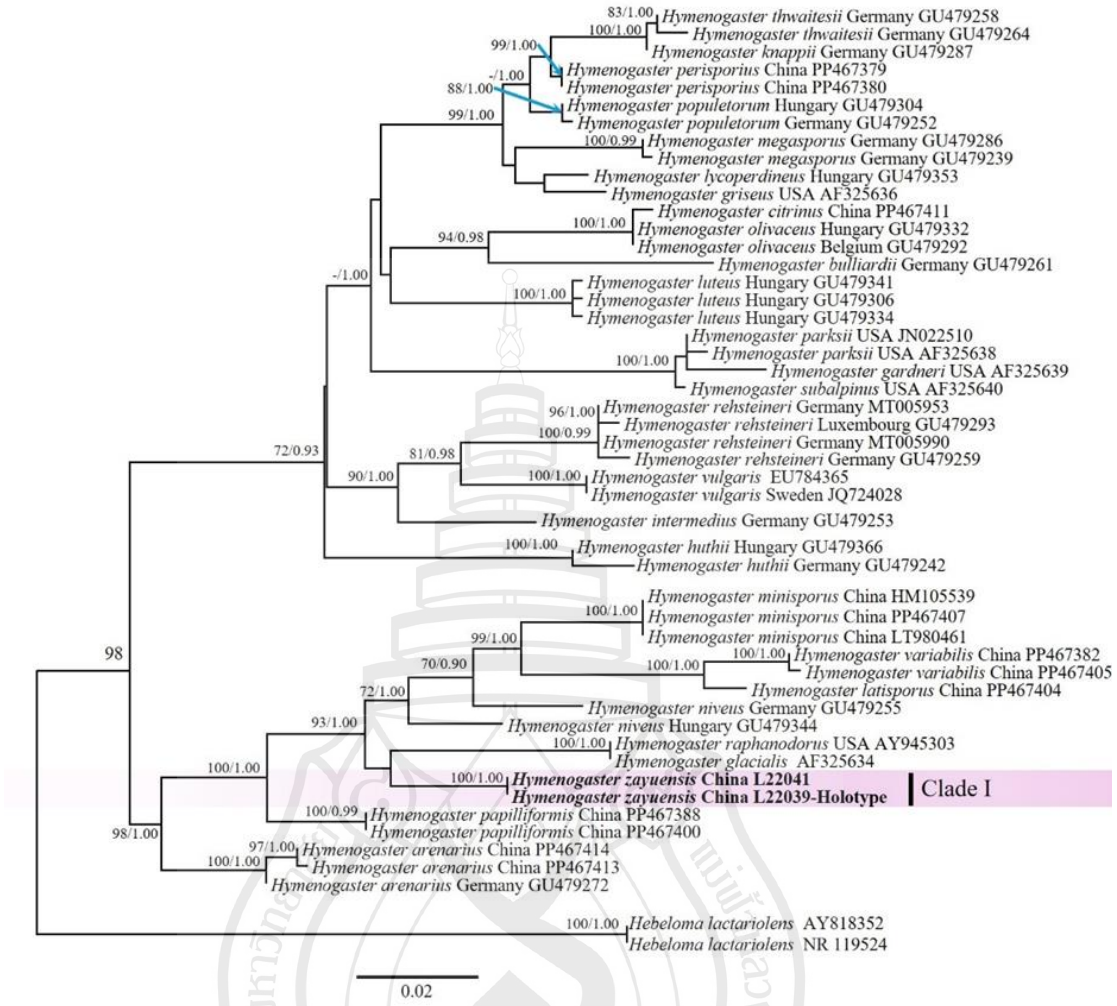


Figure 4.14 Phylogeny derived from a maximum likelihood (ML) analysis of the nrDNA-ITS sequences from *Hymenogaster* species

Figure 4.14 Values next to nodes reflect maximum likelihood bootstrap support values (BS), left, and Bayesian posterior probabilities (PP), right. Names of novel species and samples with newly generated sequences are in bold.

Taxonomy

Hymenogaster zayuensis L. Li, K.D. Hyde & S.H. Li, sp. nov. Figure 4.15

Mycobank number: MB 860400

Diagnosis: Differs from other *Hymenogaster* spp. by basidiomata with a distinct sterile base, and features smaller golden-brown fusiform basidiospores up to 12 μm in length, with conspicuous interwoven ridges ornamentation, with a prominent apical rostrum of 1–1.5 μm in height and 1–1.8 μm in width, obtuse, papillary, and smooth.

Etymology: Zayuensis, refers to the type locality of the Zayu.

Holotype: CHINA, Xizang, Zayu (28°43N 97°28E), alt. 3410 m, in the forest of *Quercus pannosa* Hand.-Mazz., 11 August 2022, Lin Li, BMDU L22039 (GenBank: ITS=PX096319 LSU=PX096419).

Basidiomata fusiform or elongated, elliptical, surface smooth and glabrous, 1–1.8 cm in diam., soft and elastic, dirty white(4A2) when fresh, occasionally with furrows on the surface. Gleba white (6D1) when immature, becoming brown (6D6) at maturity. Trama white to pale brown. Columella absent but with a distinct sterile base. Odor: not obvious.

Peridium is 80–100 μm thick, single layer, consists of hyaline interwoven hyphae, thin-walled, with septa, light yellow-brown(4B3) to pale yellow in 5% KOH; the outer surface of the peridium locally exhibits a layer of more-or-less parallel interwoven hyphae of 2–3 μm broad, hyaline singly and light yellow-brown in mass.

Hymenium is 15–28 μm broad, hymenial cystidia are cylindrical, 8–12 μm long, and only present when young occasionally, not observed when mature. Basidia clavate, 11.5–14.0 × 3.5–5.5 μm, slightly enlarged at the apex, 2-spored, occasionally 4-spored, sterigmata distinct, 1.5–2.5 μm long, basidia collapsed and disappeared at maturity.

The basidiospores ellipsoidal or fusiform, (4.5–)6.0–9.5 (–11.5) × 3.5–5.0 (–6.0) μm Q (L/I) = 1.30–1.95 Qm = 1.63 ± 0.15 (n = 40), including ornamentation but not sterigmal attachment, golden-brown (5C8) in KOH and water, both singly and in mass; with conspicuous interwoven ridges ornamentation, with a prominent apical rostrum of 1–1.5 μm in height and 1–1.8 μm in width, obtuse, papillary, and smooth, there is a distinct boundary between the ridged ornamentation and the smooth apical rostrum; without gelatinous perisporium, the sterigmal attachment is gradually tapering and truncate, 1–2 µm tall × 1–2 µm broad at base, 0.5–1 µm at truncation.

Ecology & Distribution: Hypogeous, solitary, or in groups in the soils under the forest of *Quercus pannosa*, alt. 3400m fruiting in autumn. Known only from southeastern Xizang Province, China.

Additional specimen examined: China, Xizang Province, Zayu, 28°43N 97°28E, alt. 3400 m, 11 August 2022, Lin Li BMDU L22041 (GenBank: ITS = PX096320 LSU = PX096420).

Notes: *Hymenogaster zayuensis* and *H. xizangensis* have similar habitats, both distributed in the southeastern part of Xizang province, influenced by warm and humid air currents from the Indian Ocean, growing under alpine sclerophyllous broad-leaved forests. *H. xizangensis* (Liu et al., 1996) was collected at alt. 3000m under *Quercus aquifolioides* Rehder & E. H. Wilson in Sarg., while *H. zayuensis* was collected at an altitude. 3400m under *Quercus pannosa*. Unfortunately, molecular data for *H. xizangensis* are not available, but there are significant morphological differences between the two. For example, the spores of *H. zayuensis* are smaller, measuring 6-9.5 µm in length, golden brown, with conspicuous interwoven ridges ornamentation. In contrast, the spores of *H. xizangensis* are larger, measuring 10-13 µm in length, light brown to brown, with a smooth outer wall and an inner wall featuring irregular warts and short ridges, covered by a colorless spore sheath. Molecular analysis also shows that *Hymenogaster zayuensis* is separated from other *Hymenogaster* species, they were divided into different species with a high support rate.

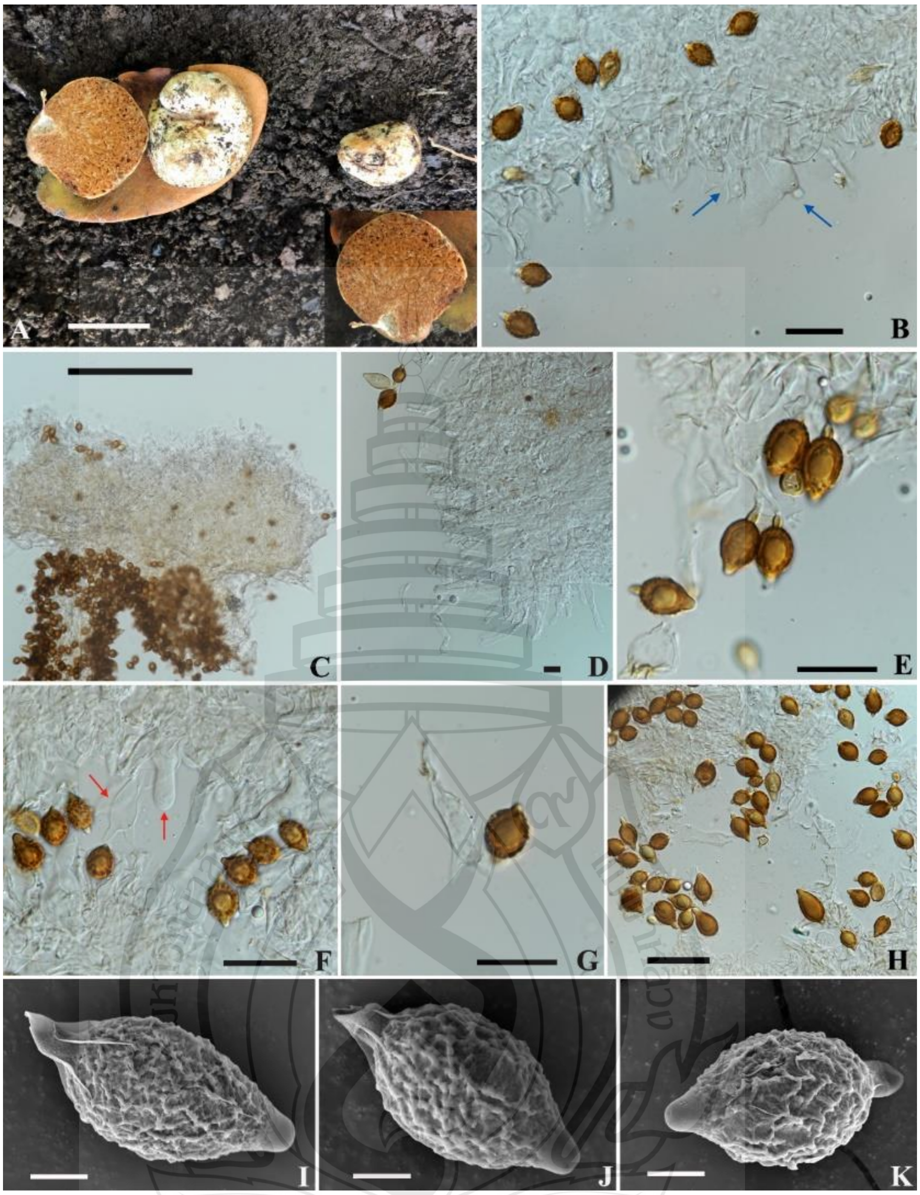


Figure 4.15 *Hymenogaster zayuensis* morphological characteristics

Figure 4.15 A. Basidiomata; B. A piece of the section of the hymenium in 5% KOH; C. A piece of the section of the basidiomata in 5% KOH; D. Hyphae composing the peridium; E.F.G. Basidia in 5% KOH; H. Basidiospores in 5% KOH; I.J.K. SEM basidiomata (dry sample). Scale bars: A = 1cm; B = 10µm; C = 100µm; D = 5µm; E-G. = 10µm; I-K = 2µm.

4.2.6 *Hysterangium*

4.2.6.1 Introduction

Hysterangium was established by Vittadini (1831) to accommodate a hypogeous species with smooth basidiospores and a peridium that dissolves or splits at maturity. The generic concept has since been revised, and the diagnostic features of *Hysterangium* are enclosed basidiomata, an irregularly developed columella, a cartilaginous gleba, and narrowly ellipsoid to fusoid basidiospores that are smooth to rugose and covered by a membranous utricle or perisporium (Zeller & Dodge, 1929; Hosaka et al., 2006). Recent molecular phylogenetic studies have demonstrated that *Hysterangium* belongs to a distinct order, Hysterangiales Hosaka & Castellano, which, together with Phallales E. Fisch., Gomphales Jülich, and Geastrales Hosaka & Castellano, is placed within the subclass Phallomycetidae Hosaka, Castellano & Spatafora (Hosaka et al., 2006; Davoodian et al., 2021, He et al., 2024, Hyde et al., 2024). The genus *Hysterangium* is species-rich and globally distributed (Zeller & Dodge, 1929; Liu et al., 2005; Guevara et al., 2008; Elliott et al., 2020). Seventy-four species epithets for *Hysterangium* are listed in Index Fungorum (https://www.indexfungorum.org/names/Names.asp), all of which produce hypogeous basidiomata. Members of *Hysterangium* are known to form ectomycorrhizal associations with hosts in Fagaceae, Myrtaceae, Nothofagaceae, and Pinaceae, and they exhibit a wide geographical distribution (Malajczuk & Bougher, 1987; Castellano, 1988, 1999; Sulzbacher et al., 2019).

In China, 15 species of *Hysterangium* are currently recognized (Liu et al., 2005): *H. album* Zeller & C.W. Dodge 1929, distributed in Shanxi Province and Nei Mongol Autonomous Region; *H. clathroides* Vittad. 1831, *H. harknessii* Zeller & C.W. Dodge 1929, *H. neglectum* Massee & Rodway 1899, *H. occidentale* Harkn. 1899, and *H. thwaitesii* Berk. & Broome 1848, all reported from Shanxi Province; *H. calcareum* R. Hesse 1891, *H. cistophilum* (Tul. & C. Tul.) Zeller & C.W. Dodge 1929, and *H. hautu* G. Cunn. 1938, recorded from Guangdong Province; *H. microsporum* Liu & Tao 1996, reported from Xinjiang Uygur Autonomous Region and Shanxi Province (Tao et al., 1996); *H. stoloniferum* Tul. & C. Tul. 1851, distributed in Shanxi, Jilin, and

Hubei Province; *H. strobilus* Zeller & C.W. Dodge 1929, collected by Dr. Harry Smith in Sichuan Province in 1922, representing the first record of the genus *Hysterangium* in China (Eckblad & Ellingsen, 1984); *H. obtusum* Rodway 1920 and *H. fuscum* Harkn. 1899 from Yunnan Province; and *H. latiappendiculatum* A.-S. Xu & B. Liu 2003 from Xizang Autonomous Regio (Xu & Liu, 2003). In this study, two new species, *Hysterangium alpinum* collected from high-elevation fir forests, and *Hysterangium shibaoshanense* collected from broadleaf forests of Shibaoshan Mountain, all in Yunnan, is described. With the addition of molecular data, it is likely that many new species will be discovered worldwide (Phukhamsakda et al., 2022).

4.2.6.2 Sequence Alignment and Analysis

In this study, ITS, nrLSU, *atp6*, *rpb2*, and *tef1* were used to analyze species diversity within the genus *Hysterangium*. A total of 50 ITS, 42 nrLSU, 36 *atp6*, 34 *rpb2*, and 32 *tef1* sequences, obtained from NCBI and this study, represent 31 described species of *Hysterangium* and 22 undescribed *Hysterangium* species, as well as four known and three undescribed species of the genus *Aroramyces*. Two sequences of *Trappea* and four sequences of *Gallacea* were included as outgroups (Table 4.9).

Sequences of *Hysterangium* species generated in this study were submitted to the GenBank database. We first edited the sequences using BioEdit v. 7 (Hall, 2007), then used the basic local alignment search tool for the GenBank database to recheck whether the newly generated sequences were amplified DNA from a contaminant or not, and examined clusters with closely related sequences. DNA sequences were retrieved and assembled using SeqMan.

Sequences were aligned using MAFFT version 7 (Katoh & Standley, 2013). Phylogenies and node support were first inferred by Maximum Likelihood (ML) from the two single-gene alignments separately, using RAxML-HPC2 version 8.2.12 (Stamatakis, 2014) with 1,000 rapid bootstraps, as implemented on the Cipres portal (Miller et al., 2010). Since no supported conflict (bootstrap support value (BS) $\geq$ 70%) was detected among the topologies, the two single-gene alignments were concatenated using Sequence Matrix (Vaidya, 2011). Partitioned Maximum likelihood (ML) analysis was performed on the concatenated data set, as described above. For Bayesian Inference (BI), the best substitution model for each character set was determined with the program MrModeltest 2.3 (Nylander 2004) on CIPRES. The results suggested that nrLSU: GTR+I+G, ITS1: JC + I, 5.8S: GTR + G + I, ITS2: K80 + I + G, *atp6*: TPM2u+F+R3, *rpb2*: K2P+I+G4, *tef1*: TIM2e+G4. Bayesian analysis was performed using MrBayes version 3.2.7a (Ronquist, 2011) as implemented on the Cipres portal (Miller et al., 2010); four parallel runs were performed for 10 million generations, sampling every 100th generation for the single gene trees. Parameter convergence > 200 was verified in Tracer v. 1.7 (Rambaut et al., 2018). The phylogenetic clade was strongly supported if the bootstrap support value (BS) was $\geq 70\%$ and/or a posterior probability (PP) < 0.01.

Table 4.9 Taxa information and GenBank accession numbers of the sequences used in *Hysterangium* study

Species Name	Voucher	Origin	GenBank No.					References
			ITS	nrLSU	atp6	rpb2	tef1	
Aroramyces gelatinosporus	strain H4010	-	-	DQ218524	DQ218809	DQ218941	DQ219118	Hosaka et al. (2008)
Aroramyces guanajuatensis	ITCV 1613 holotype	Mexico	NR_174045	MK811036	-	-	-	Peña-Ramírez et al. (2019)
Aroramyces guanajuatensis	ITCV 1689	Mexico	MN392935	-	-	-	-	Peña-Ramírez et al. (2019)
Aroramyces herrerae	ITCV 1225	Mexico	MZ343612	-	-	-	-	Peña-Ramírez et al. (2019)
Aroramyces herrerae	ITCV 1211 holotype	Mexico	MZ343611	MK811032	-	-	-	Guevara-Guerrero et al. (2019)
Aroramyces radiatus	Verbeken99062	-	-	DQ218525	DQ218810	DQ218942	DQ219119	Hosaka et al. (2008)
Aroramyces sp	Verbeken 99-148	-	OQ566910	-	-	-	-	GenBank
Aroramyces sp	OSC122590	-	-	DQ218529	DQ218814	DQ218946	DQ219123	Hosaka et al. (2008)
Aroramyces sp	OSC122858	-	-	DQ218528	DQ218813	DQ218945	DQ219122	Hosaka et al. (2008)
Gallacea scleroderma	OSC59621	-	-	AY574645	AY574787	DQ218961	DQ219139	Giachini et al., 2010
Gallacea sp	OSC122728	-	-	DQ218542	DQ218828	DQ218965	DQ219143	Hosaka et al. (2008)
Gallacea sp	REB2364	-	-	DQ218540	DQ218825	DQ218962	DQ219140	Hosaka et al. (2008)
Gallacea subalpina	MEL 2236389 holotype	Australia	NR_173471	NG_077392	-	-	-	Davoodian et al. (2020)
Hysterangium affine	24B S0174	Australia	KY697570	-	-	-	-	Nuske et al. (2018)
Hysterangium affine	24C S0333	Australia	KY697569	-	-	-	-	Nuske et al. (2018)
Hysterangium affine	OSC76884	-	-	DQ218546	DQ218831	DQ218970	-	Hosaka et al. (2006)
Hysterangium aggregatum	OSC114262	-	-	DQ218489	DQ218773	DQ218971	DQ219146	Hosaka et al. (2006)
Hysterangium aggregatum	25B S0278	Australia	KY697567	-	-	-	-	Nuske et al. (2018)

Table 4.9 (continued)

Species Name	Voucher	Origin	GenBank No.					References
			ITS	nrLSU	atp6	rpb2	tef1	
Hysterangium aggregatum	25B S0279	Australia	KY697566	-	-	-	-	Nuske et al. (2018)
Hysterangium album	OSC115139	-	-	DQ218490	DQ218774	DQ218972	DQ219147	Hosaka et al. (2006)
Hysterangium atlanticum	UFRN:Fungos 2115 holotype	Brazil	NR_165211	-	-	-	-	Sulzbacher et al. (2019)
Hysterangium atlanticum	UFRN-Fungos 2112	Brazil	LT623204	-	-	-	-	Sulzbacher et al. (2019)
Hysterangium atlanticum	UFRN-Fungos 1750	Brazil	LT623206	-	-	-	-	Sulzbacher et al. (2019)
Hysterangium aureum	OSC56988 strain	-	-	DQ218491	DQ218775	DQ218973	DQ219148	Hosaka et al. (2006)
Hysterangium cistophilum	OSC11088	-	-	DQ218493	DQ218777	DQ218975	DQ219150	Hosaka et al. (2006)
Hysterangium clathroides	MPUSzemere 11-SEPT-1955	-	-	DQ218547	DQ218832	DQ218976	DQ219151	Hosaka et al. (2006)
Hysterangium clathroides	SZEMORE	-	-	AF213121	-	-	-	Humpert et al. (2001)
Hysterangium coriaceum	OSC64939	-	-	AY574686	AY574826	DQ218977	DQ219152	Hosaka et al. (2006)
Hysterangium crassirhachis	OSC58056	-	-	DQ218494	DQ218778	DQ218978	DQ219153	Hosaka et al. (2006)
Hysterangium crassirhachis	HAD1187 iNat#203532541	USA	PV832617	PV832629	-	-	-	Dawson and Dawson (2024)
Hysterangium crassirhachis	OSC 4860	USA	MN809540	-	-	-	-	GenBank
Hysterangium crassirhachis	OSC 58067	USA	DQ365631	-	-	-	-	Dunham et al. (2007)
Hysterangium crassum	OSC110447	-	-	AY574687	AY574827	DQ218979	DQ219154	Giachini et al. (2010)

Table 4.9 (continued)

Species Name	Voucher	Origin	GenBank No.					References
			ITS	nrLSU	atp6	rpb2	tef1	
Hysterangium epiroticum	OSC16116	-	-	DQ218495	DQ218779	DQ218980	DQ219155	Hosaka et al. (2008)
Hysterangium fragile	OSCKers 3971	-	-	DQ218496	DQ218780	DQ218981	DQ219156	Hosaka et al. (2008)
Hysterangium gardneri	OSC16950	-	-	DQ218548	DQ218835	DQ218982	DQ219157	Hosaka et al. (2008)
Hysterangium hallingii	OSCHalling 5741	-	-	DQ218497	DQ218781	DQ218983	DQ219158	Hosaka et al. (2008)
Hysterangium inflatum	OSC144035	-	-	DQ218549	DQ218836	DQ218984	DQ219159	Hosaka et al. (2008)
Hysterangium membranaceum	OSC112836	-	-	DQ218498	DQ218782	DQ218985	DQ219160	Hosaka et al. (2008)
Hysterangium neotunicatum	OSC115545	-	-	DQ218550	DQ218837	DQ218986	DQ219161	Hosaka et al. (2008)
Hysterangium nephriticum	RBG Kew K(M)136962	-	EU784366	-	-	-	-	Brock et al. (2009)
Hysterangium occidentale	OSC47048	-	-	AY574685	AY574825	DQ218987	DQ219162	Hosaka et al. (2006)
Hysterangium pompholyx	17032	Italy	JF908086	-	-	-	-	Osmundson et al. (2013)
Hysterangium pterosporum	14171	Italy	JF908085	-	-	-	-	Osmundson et al. (2013)
Hysterangium rugisphorum	OSC59662	-	-	DQ218500	DQ218784	DQ218988	DQ219164	Hosaka et al. (2006)
Hysterangium rupticutis	OSC59667	-	-	DQ218551	DQ218838			Hosaka et al. (2006)
Hysterangium salmonaceum	KBeaton 33	-	-	DQ218501	DQ218785	DQ218989	DQ219165	Hosaka et al. (2006)
Hysterangium separabile	OSC69030	-	-	DQ218502	DQ218786	DQ218990	DQ219166	Hosaka et al. (2006)
Hysterangium separabile	UBCOFS14	Canada	EU563921	-	-	-	-	Twieg (2006)
Hysterangium setchellii	OSC58071	USA	DQ365630	DQ218552	DQ218839	DQ218991	DQ219167	Hosaka et al. (2006)
Hysterangium setchellii	mc5	USA	DQ365635	-	-	-	-	Dunham et al. (2007)
Hysterangium setchellii	29.3	USA	DQ365634	-	-	-	-	Dunham et al. (2007)

Table 4.9 (continued)

Species Name	Voucher	Origin	GenBank No.					References
			ITS	nrLSU	atp6	rpb2	tef1	
Hysterangium shibaoshanense	BMDU L24099 holotype	Yunnan, China	XXXX	XXXX	XXXX	XXXX	XXXX	This study
Hysterangium shibaoshanense	BMDU L24104	Yunnan, China	XXXX	XXXX	XXXX	XXXX	XXXX	This study
Hysterangium sp	BMDU L21199	Yunnan, China	PX108631	PX105441	-	-	-	Unpublished
Hysterangium sp	GO2010-018	Mexico	KC152127	-	-	-	-	GenBank
Hysterangium sp	GO2009-491	Mexico	KC152128	-	-	-	-	GenBank
Hysterangium sp	GO2009-214	Mexico	KC152130	-	-	-	-	GenBank
Hysterangium sp	GO2010-014	Mexico	KC152129	-	-	-	-	GenBank
Hysterangium sp	PDD103520	New Zealand	KP191954	-	-	-	-	GenBank
Hysterangium sp	MHY2012	Poland	JX559776	-	-	-	-	GenBank
Hysterangium sp	src642	USA	DQ974736	-	-	-	-	Smith et al. (2007)
Hysterangium sp	MCA972	Guyana	KC155392	-	-	-	-	Smith et al. (2013)
Hysterangium sp	LM5580B	Hungary	KM576428	-	-	-	-	Suz et al. (2014)
Hysterangium sp	GO2009-205	Mexico	KC152126	-	-	-	-	GenBank
Hysterangium sp	GO2010-096	Mexico	KC152124	-	-	-	-	GenBank
Hysterangium sp	GO2009-244	Mexico	KC152125	-	-	-	-	GenBank
Hysterangium sp	PDD67598	New Zealand	KP191953	-	-	-	-	GenBank
Hysterangium sp	SOC1070	USA	FJ789605	-	-	-	-	Gladish et al. (2010)
Hysterangium sp	PDD69213	New Zealand	KP191952	-	-	-	-	GenBank
Hysterangium sp	PDD95779	New Zealand	HQ533033	-	-	-	-	GenBank
Hysterangium sp	PDD90683	New Zealand	KP191957	-	-	-	-	GenBank
Hysterangium sp	SOC788	USA	DQ453697	-	-	-	-	Frank et al. (2006)
Hysterangium sp	SOC1410	USA	JN022512	-	-	-	-	GenBank

Table 4.9 (continued)

Species Name	Voucher	Origin	GenBank No.					References
			ITS	nrLSU	atp6	rpb2	tef1	
Hysterangium sp	TH8517	Guyana	KC155391	-	-	-	-	Smith et al. (2013)
Hysterangium sp	GO2010-155	Mexico	KC152131	-	-	-	-	GenBank
Hysterangium sp	SOC1306	USA	JN022511	-	-	-	-	GenBank
Hysterangium spegazzinii	Singer3426	-	-	DQ218503	DQ218787	-	-	Hosaka et al. (2006)
Hysterangium stoloniferum	17033	Italy	JF908087	-	-	-	-	Osmundson et al. (2013)
Hysterangium strobilus	OSCT5285	-	-	DQ218504	DQ218788	DQ218992	DQ219168	Hosaka et al. (2006)
Hysterangium thwaitesii	RBG Kew K(M)136967	-	EU784367	-	-	-	-	Brock et al. (2009)
Hysterangium youngii	OSC59645	-	-	DQ218505	DQ218789	DQ218993	DQ219169	Hosaka et al. (2006)
Trappea darkeri	OSC65085	-	-	DQ218651	DQ218938	DQ219116	DQ219292	Hosaka et al. (2006)
Trappea pinyonensis	OSC AHF530	-	-	NG_064311	DQ218884	DQ219043	DQ219221	Hosaka et al.(2008)

Note The newly generated sequences are in bold

4.2.6.3 Results

Phylogenetic Analysis

To reconstruct the phylogeny of *Hysterangium* and determine the taxonomic position of the new hypogeous species, five gene regions were included in the analysis, as single-gene datasets were poorly represented in nucleotide databases. Consequently, a concatenated dataset comprising ITS, nrLSU, *atp6*, *rpb2*, and *tef1* sequences was prepared. Maximum likelihood (ML) and Bayesian analyses of the 50 ITS, 42 nrLSU, 36 *atp6*, 34 *rpb2*, and 32 *tef1* sequences are presented in Figure 1, showing bootstrap support values for branches and the phylogenetic relationships among *Hysterangium* species.

The concatenated matrix included sequences from 31 described and 22 undescribed *Hysterangium* species, four described and three undescribed *Aroramyces* species, as well as outgroups, totaling 3,528 nucleotide positions (Figure 16). The tree topologies indicate that the new species forms a well-supported terminal clade (Clade II; MPbs = 97.2; MLbs = 90), which is sister to a clade (Clade I) composed of species distributed in the Americas and Europe, including *H. affine*, *H. aureum*, *H. coriaceum*, *H. crassirhachi*, *H. pterosporum*, *H. separabile*, together with ten undescribed *Hysterangium* sequences. Clade II contains the newly described species in this study, *Hysterangium shibaoshanense*. Clade III contains the newly described species in this study, *Hysterangium alpinum*. Phylogenetic analysis demonstrates that this new species is distinct from all other known *Hysterangium* species.

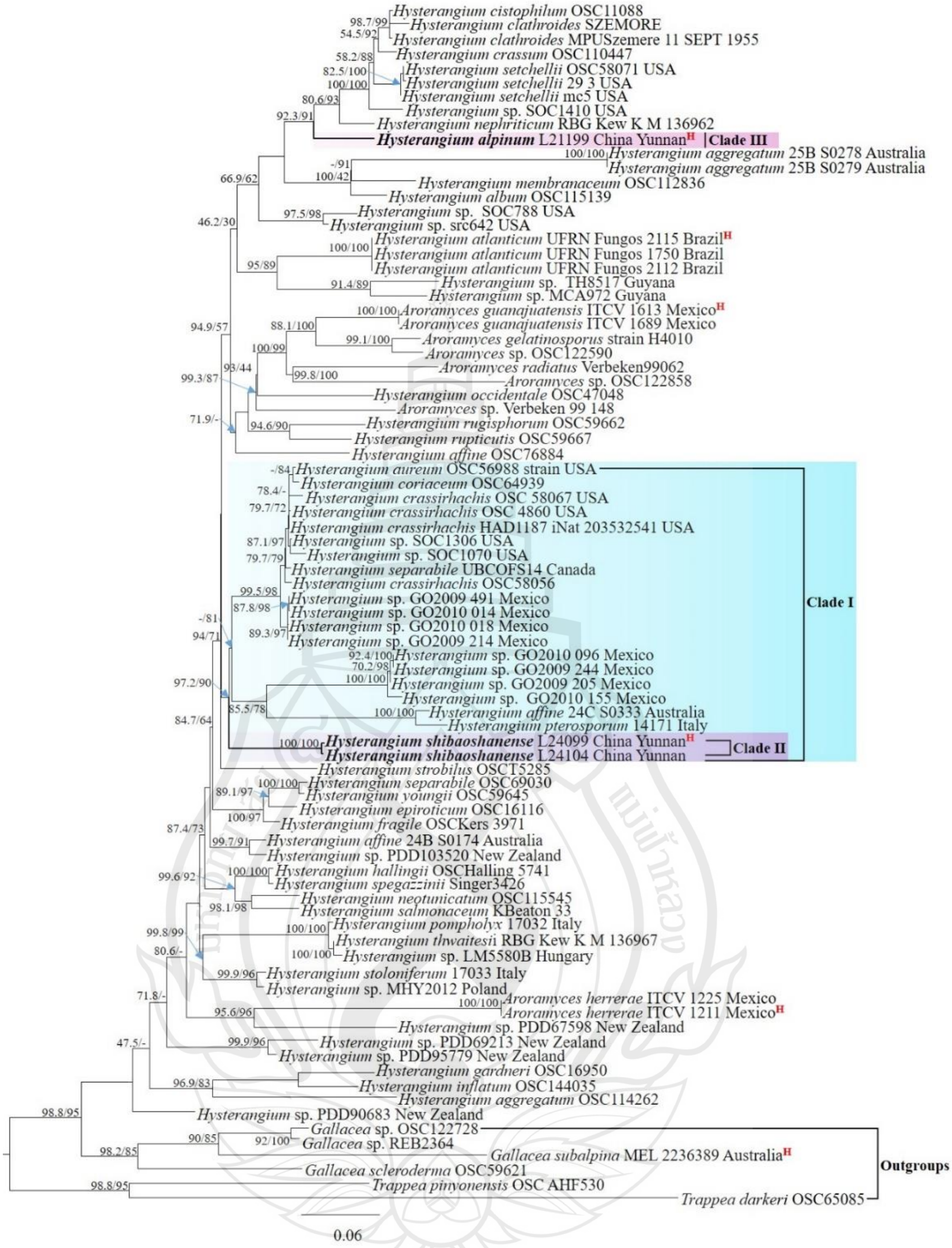


Figure 4.16 Phylogeny derived from a maximum likelihood (ML) analysis of the nrDNA-ITS sequences from *Hysterangium* species

Figure 4.16 Values next to nodes reflect maximum likelihood bootstrap support values (BS), left, and Bayesian posterior probabilities (PP), right. Names of novel species and samples with newly generated sequences are in bold.

Taxonomy

Hysterangium alpinum L. Li, K.D. Hyde & S.H. Li, sp. nov. Figure 4.17

Mycobank number: MB 860407

Diagnosis: Differs from other *Hysterangium* spp. by feature smaller golden-brown basidiospores up to 10.0 μm length, and with thinner peridium up to 90 μm thickness. Additionally, this species is distributed in alpine environments, at elevations reaching up to 3800 meters.

Etymology: alpinum, referring to the type specimens are found in alpine environments, at elevations reaching up to 3800 meters.

Holotype: CHINA, Yunnan, Mt. laojunshan, 26º37′ N 99º43′ E, alt. 3875.7 m, in the forest of *Abies forrestii* var. *smithii*, 19 Sep. 2021, Lin Li, BMDU L21199 (GenBank: ITS=PX108631 LSU=PX105441).

Basidiomata 5 mm diam., globose to reniform, yellowish grey (2B2) to yellowish brown (2C3), smooth surface, with a distinct rhizomorphic base. Gleba finely loculate, gelatinized, compacted, olive brown (4F4), with rounded to irregular locules (<0.5 mm diam.) radially arranged. Columella irregular in shape, distinctly gelatinous, translucent, scattered in the gleba.

Rhizomorphs 0.1–1.0 mm diam., white (1A1) to yellowish grey (3B2), long and numerous going into the ground, at the base of the basidiomata, reaching lengths of up to 5 cm, constituted by light brown (4A2), thin-walled hyphae of 1.0– 1.5 μm diam., nearly parallelly intertwined, clamp connection can be observed.

Peridium 80 – 100 μm thick, easily separable from gleba, 2-layered: external layer 45–60 μm thick, composed of intertwined hyphae of 1.5-2.5 μm diam. that are hyaline or yellow brown; the internal layer, 20–30 μm thick, primarily consists of nearly intertwined hyaline hyphae, sometimes arranged almost parallel, with interspersed yellow-brown hyphae. Clamp connections present.

Tramal plates of 30–60 μm thick, constituted by hyaline, mostly collapsed at maturity. Basidia 12–19.5 × 4–6 μm, cylindrical to clavate, 2–4 spored, hyaline.

Basidiospores broadly fusiform, yellowish-brown (2C5) at maturity, dimension ranges (ornamentation and sterigmal attachment base excluded) 7.5–9.5 (–10.0) × 2.0–3.5 μm Q (L/I) = 2.55–4.02 Qm = 3.12 ± 0.37 (n = 40), surface covered with brunt, parallel or furcate, sinuate transverse ridges up to 1.0–1.5 μm high, walls

1.0–1.5 µm thick, with a sterigmal attachment base (up to 2.5 µm high).

Habitat and distribution: Hypogeous, solitary under organic soil and forest debris, under the forest of *Abies forrestii* var. *smithii*, alt. 3875m, fruiting in autumn. Known only from the type locality, Yunnan, China.

Notes: The phylogenetic tree shows that *Hysterangium alpinum* clusters with three sequences of *H. setchellii* from the USA and one unknown species of the genus *Hysterangium*, and with the sequence of *H. nephriticum* from England. According to the descriptions in the literature (Castellano, 1988), they have distinct differences. *H. nephriticum*'s spore-producing tissue, Gleba, is pale blue or gray with a green or pinkish cast, the columella is dendroid, the peridium is relatively thick, reaching up to 350 µm, and the basidiospores are minutely verrucose in age, measuring 13-18 µm long. *H. setchellii*'s columella is distinct in youth, thick, opaque, with a few branches reaching the peridium, soon becoming indistinct and translucent. The peridium is thicker, reaching up to 450 µm, and the basidiospores are minutely verrucose in age, measuring 14-19 µm long. In comparison, *H. alpinum*'s columella is irregular in shape, distinctly gelatinous, translucent, and scattered in the gleba, the peridium is thinner, measuring only 100 µm, the basidiospores are smaller, reaching only up to 10 µm, and the basidiospore surface is covered with blunt, parallel or furcate, sinuate transverse ridges up to 1.0–1.5 µm high, with walls 1.0–1.5 µm thick.

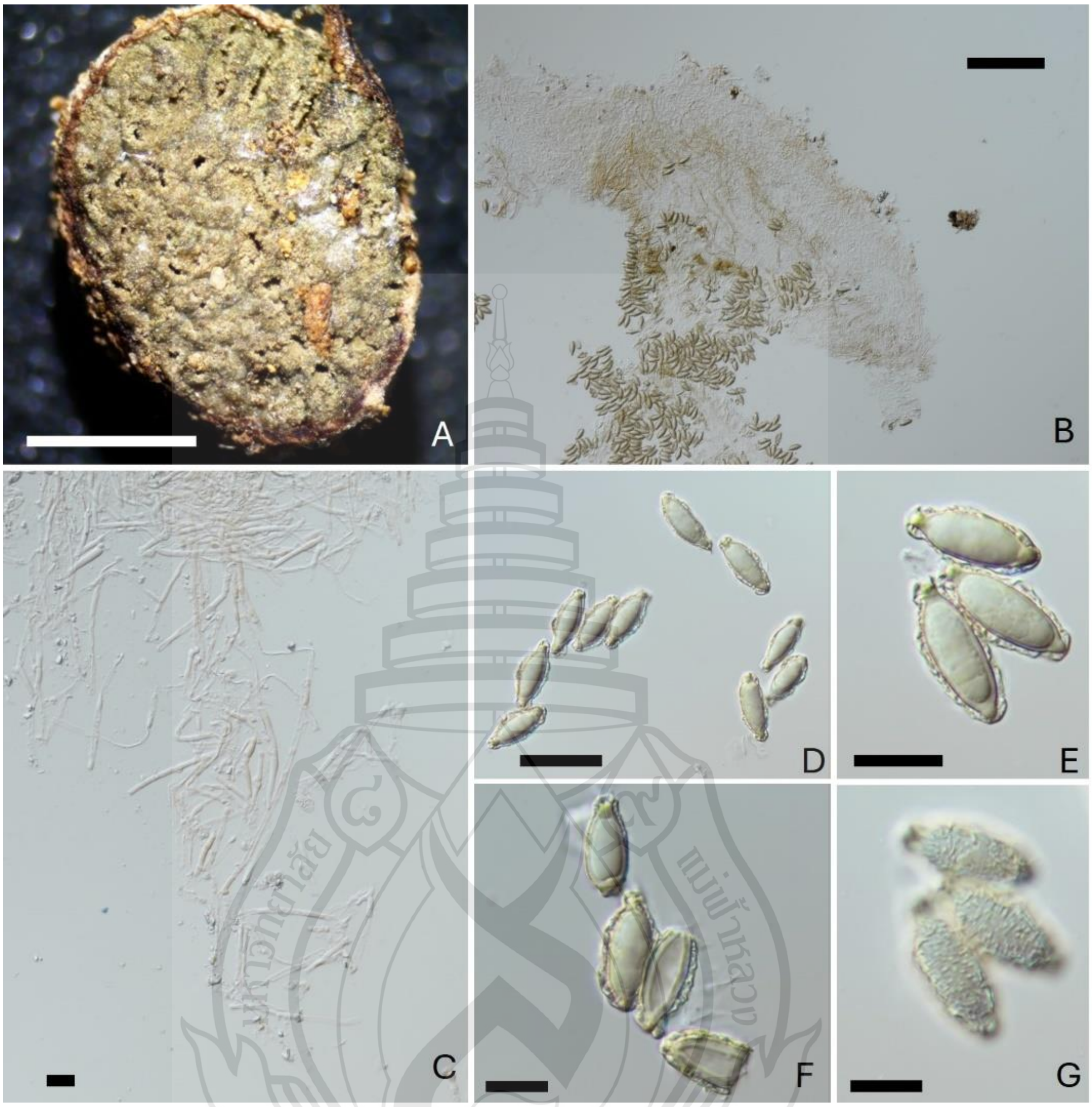


Figure 4.17 *Hysterangium alpinum* morphological characteristics

Figure 4.17 A. Basidiomata; B. A piece of the section of the basidiomata in 5% KOH; C.Hyphae composing the rhizomorphs; D.E.F.G. Basidiospores in 5% KOH. Scale bars: A = 2mm; B = 50μm; C-D = 10μm; E-G. = 5μm.

Hysterangium shibaoshanense L. Li & K.D. Hyde sp. nov Figure 4.18

MycoBank MB 860942

Diagnosis: Differs from other *Hysterangium* species by its non-discoloring peridium; comparatively smaller, light golden-yellow basidiospores, measuring only 2–4 × 0.8–1.5 μm without the utricle; in 5% KOH or water, the utricle expands asymmetrically, inflated up to 0.5–1.5 μm from the spore wall.

Etymology: *shibaoshanense*, referring to the type locality, Shibaoshan Mountain, Dali Bai Autonomous Prefecture, Yunnan Province, China.

Holotype: China, Yunnan, Dali Bai Autonomous Prefecture, Shibaoshan Mountain, 26°43′ N, 99°50′ E, alt. 2508.8 m, hypogeous in soil of broad-leaved forest dominated by *Alnus* sp., 8 Sept. 2024, Songming Tang, BMDU L24099 (GenBank: ITS = PX560236 nrLSU = PX560238).

Basidiomata 0.5–1.5 cm, globose, subglobose, to irregular. Peridial surface pale khaki to white (4A2), pale brown (5B3), slightly tomentose, often with cotton-like patches of white hyphae encompassing soil debris; with a few white, thin, rhizomorph-like strands, 0.2–0.5 mm in diam. Basidiomata elastic when fresh, hard when dried, without distinctive odor.

Gleba pale grayish green (27A3); trama gelatinized; locules irregular in shape; columella radiating outward from the center in a dendroid manner, up to 2–3 mm wide at the broadest central portion, gelatinous, translucent gray (26B2). Upon drying, the central gelatinous columella shrinks into a transparent membrane, leaving the gleba hollow.

Peridium mostly hyaline, the outer portion pale brown, with a conspicuous dark ring adjacent to the gleba. Peridium three-layered, 300–550 μm thick; outer layer 20–52 μm thick, composed of hyaline to yellowish brown (6D8), thin-walled, interwoven to repent or erect hyphae, 3–5 μm wide, densely interwoven and nearly parallel; middle layer 250–400 μm thick, consisting of abundant hyaline, globose to subglobose, angular pseudoparenchymatous cells, 16–27 μm wide, occasionally irregularly shaped; inner layer 10–30 μm thick, formed of flattened yellow-brown (5D8) cells, connecting the middle layer with the gleba, gradually transitioning from the pseudoparenchymatous cells of the middle layer, which become progressively smaller.

Trama of hyaline, interwoven hyphae, 2–3 µm wide, embedded in a gelatinized matrix, 50–110 µm wide, with clamp connections present. Basidia fusoid to clavate, hyaline, 15.5–33.5 × 6.0–10.5 µm, thin-walled, with clamp connections present. Cystidia lageniform to pyriform, with a swollen basal portion and a distinctly narrowed neck, hyaline, 16.5–28.0 × 6.5–11.0 µm, thin-walled.

Basidiospores ellipsoid to broadly ellipsoid, symmetrical, light golden yellow (2C6) individually, golden brown (5D8) in mass, excluding utricle 2–4 × 0.8–1.5 µm, Q (L/W) = 1.84–3.02, Qm = 2.35 ± 0.42 (n = 60); ornamentation consisting of irregular crests contained within an inflated utricle; apex obtuse. Occasionally, at the base of spores lacking a distinctly inflated utricle, shortly pedicellate structures can be observed, cylindrical, 0.1–0.2 µm wide and 0.5–0.6 µm long. Utricle light golden yellow (2C6), slight at first, becoming distinct at maturity, irregularly adhering to the spore wall, inflated up to 0.5–1.5 µm from the spore wall, asymmetrically inflated. Due to the inflated utricle, spores appear to roll in various orientations in water mounts, so that only a portion of spores are observed in lateral view under the microscope, while the majority appear in other orientations.

Ecology and distribution: Hypogeous, solitary or gregarious, occurring in soil of broad-leaved forest dominated by *Alnus* sp., at elevations of 2500–2600 m, fruiting in autumn. Known only from Yunnan Province, China.

Additional specimen examined: China, Yunnan Province, Dali Bai Autonomous Prefecture, Shibaoshan Mountain, 26°42′ N 99°51′ E, alt. 2601 m, 8 Sep. 2024, Songming Tang, BMDU L24104 (GenBank: ITS = PX560235, nrLSU = PX560237).

Notes: Phylogenetic analyses place this new species, *Hysterangium shibaoshanense*, in Clade II, distinct from a group of species distributed in the Americas and Europe, including *H. aureum, H. coriaceum, H. crassirhachi, H. pterosporum, H. separabile,* and several undescribed *Hysterangium* species, which together form Clade I. Comparison of ITS sequences shows less than 86% similarity between *H. shibaoshanense* and members of Clade I.

Morphologically, *H. shibaoshanense* is characterized by its small, light goldenrod-yellow basidiospores, 2–4 × 0.8–1.5 µm excluding the utricle, which is uncommon in the genus and serves as a key feature distinguishing it from species in

Clade I (Zeller & Dodge, 1929; Castellano, 1988; Castellano & Beever, 1994). Additionally, while basidiospores with an inflated utricle are common in *Hysterangium*, the utricles of *H. shibaoshanense* are more strongly and asymmetrically inflated, in some cases extending from the spore wall beyond the spore width. This causes the ellipsoid spores to assume irregularly globose orientations in mounts. Consequently, although the spores are ellipsoid, they are observed in arbitrary orientations under the microscope, with only a portion appearing in lateral view.

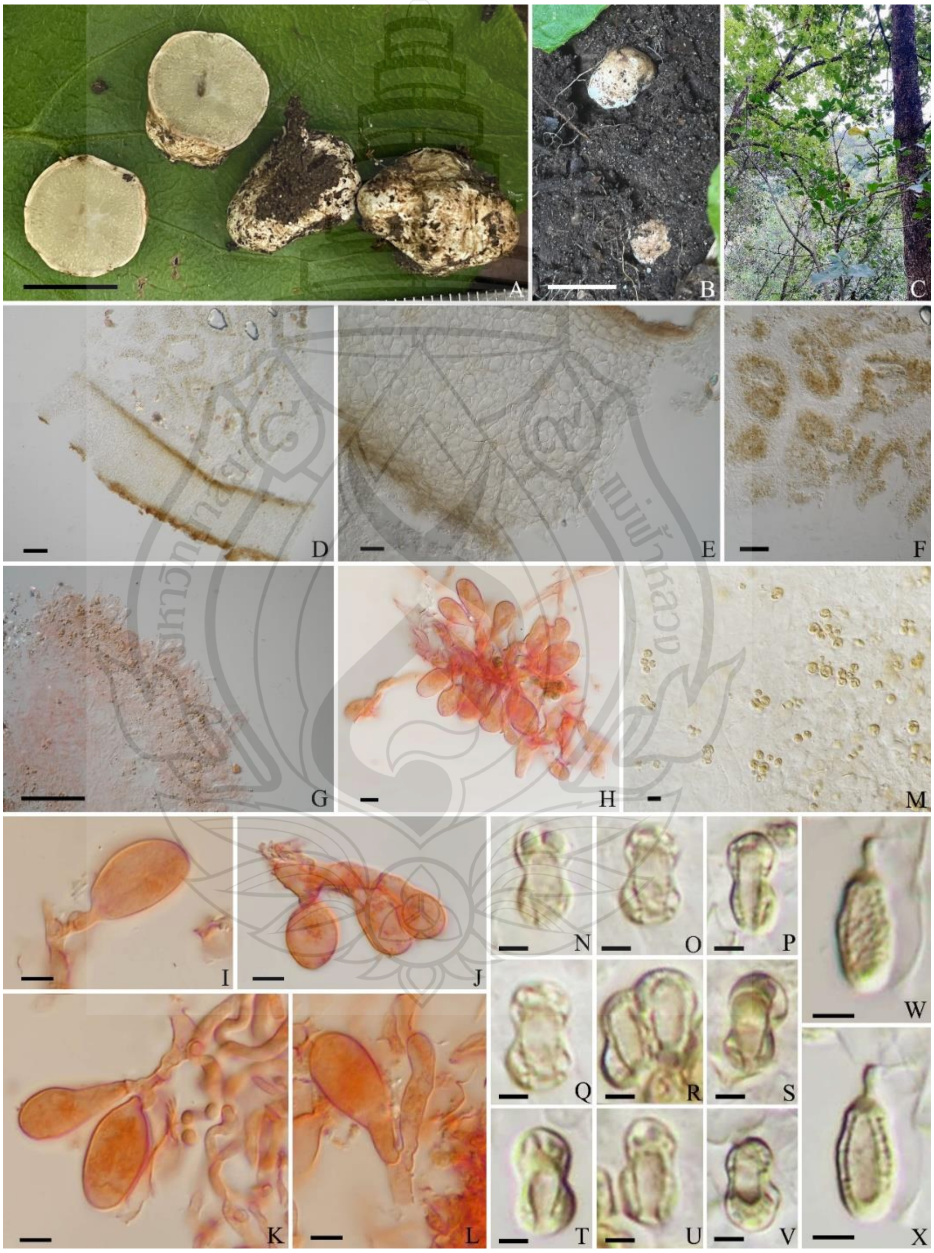


Figure 4.18 *Hysterangium shibaoshanense* morphological characteristics

Figure 4.18 A, B. Fresh basidiomata and gleba; C. Habitat; D. A piece of peridium section in 5% KOH; E. Three-layered structure of the peridium; F. A piece of trama section in 5% KOH; G. Hymenium in Congo red; H–L. Basidia and cystidia in Congo red; M. Spores viewed from different angles in 5% KOH; N–V. Basidiospores with a distinct, asymmetrically inflated utricle in 5% KOH; W, X. Spores without a distinct inflated utricle and shortly pedicellate. Scale bars: A, B = 1 cm; D = 100 μm; E, H = 10 μm; F, G = 50 μm; I–L = 5 μm; M = 2 μm; N–X = 1 μm.

4.2.7 *Melanogaster*

4.2.7.1 Introduction

The genus *Melanogaster* (Paxillaceae, Boletales) was established in the early 19th century, with *M. tuberiformis* Corda designated as the type species (Corda, 1831). Members of this genus are characterized by hypogeous to semi-hypogeous, gasteroid basidiomata, sometimes with hyphal strands at the base or on the surface. The peridium is composed of a prosenchymatous layer; at maturity, the gleba is loculate and gelatinous, with the chambers separated by sterile veins. Basidiospores are yellow to dark brown, and their shapes range from globose and ellipsoid to pyriform or fusiform (Trappe et al., 2009; Moreau et al., 2011; Alvarado et al., 2021).

Melanogaster species typically form ectomycorrhizal associations with both broad-leaved and coniferous trees and are mainly distributed across the Northern Hemisphere, including Europe, North America, and Asia (Wang et al., 2005; Trappe et al., 2009; Tedersoo et al., 2010; Moreau et al., 2011; Türkoğlu & Castellano, 2013). In China, the first specimen of *Melanogaster* was collected in 1915 from the Jinsha River region in Yunnan Province, southwestern China. It was originally identified by Keissler and Lohwag as *M. variegatus* (Vittad.) Tul. & C. Tul. (Lohwag 1937), but was later redescribed by Wang et al. (1995) as a new species, *M. ovoidisporus* Y. Wang. According to current statistics, 16 species of *Melanogaster* have been reported from China, of which 9 are distributed in the southwestern region.

In 2022, Xu et al. described a new species, *Melanogaster panzhihuaensis* L. Fan, X.Y. Yan & Y.Y. Xu, based on a single specimen collected from Panzhihua City in the Jinsha River region. In this study, we report the occurrence of this species in Chuxiong City, central Yunnan Province, where it is also collected and consumed by local people. Furthermore, morphological examination revealed notable differences between the Chuxiong specimens and the original description. We herein provide a detailed morphological account of the Chuxiong collections.

4.2.7.2 Sequence Alignment and Analysis

The internal transcribed spacer (ITS) and nuclear large subunit ribosomal RNA gene (LSU) regions were used in this study to analyze species diversity within the genus *Melanogaster*, as these loci have proven effective for species delimitation in this group. A total of 35 ITS and 10 LSU sequences—comprising both newly generated data and sequences retrieved from NCBI—were analyzed. These sequences represent 19 species of *Melanogaster* (Table 4.10). The analytical methods followed those described in the Materials and Methods section. Phylogenetic clades were considered strongly supported if they exhibited a bootstrap support (BS) value ≥ 70% and/or a posterior probability (PP) ≥ 0.90.

Table 4.10 Taxa information and GenBank accession numbers of the sequences used in *Melanogaster* study

Species Name	**Voucher**	**Origin**	**GenBank No.**	
			ITS	**LSU**
Melanogaster ambiguus	HMAS68237	UK	MW598530	
Melanogaster ambiguus	JC180719NR, 31 (FCO-Fungi)	Spain	MN594286	MN594299
Melanogaster broomeanus	BJTC FAN909	China	MW598531	
Melanogaster broomeanus	BJTC FAN1230-A	China	MW598545	
Melanogaster coccolobae	375 ITCV (holotype)	Mexico	MZ098622	MZ098618
Melanogaster coccolobae	378 ITCV	Mexico	MZ098623	MZ098619
Melanogaster euryspermus	OSC JMT35744	USA	MN984316	
Melanogaster euryspermus	OSC158351 JLF1456	USA	MN984313	
Melanogaster intermedius	K(M)130202	UK	EU784372	
Melanogaster cf. intermedius	K(M)122480	UK	EU784371	
Melanogaster luteus	PAM09082801	France	HQ714780	
Melanogaster luteus	Mon06	Montenegro	HQ714794	
Melanogaster macrosporus	ZB489	Hungary	AJ555526	
Melanogaster minobovatus	BJTC FAN911 (holotype)	China	MW598541	

Table 4.10 (continued)

Species Name	Voucher	Origin	GenBank No.	
			ITS	LSU
Melanogaster quercicola	BJTC FAN1102-A	China	MW598533	
Melanogaster quercicola	BJTC FAN1091 (holotype)	China	MW598532	
Melanogaster panzhihuaensis	HMAS81915 (holotype)	China	MW598551	
Melanogaster panzhihuaensis	**L21235**	**China**	**This study**	**This study**
Melanogaster panzhihuaensis	**L21236**	**China**	**This study**	**This study**
Melanogaster panzhihuaensis	**L20048**	**China**	**This study**	**This study**
Melanogaster panzhihuaensis	**L20049**	**China**	**This study**	**This study**
Melanogaster panzhihuaensis	**Wap1716**	**China**	**This study**	**This study**
Melanogaster obovatus	BJTC FAN808 (epitype)	China	MW598539	
Melanogaster obovatus	BJTC FAN809	China	MW598540	
Melanogaster rivularis	PAM08090514-S190	France	HQ714731	
Melanogaster rivularis	PAM08090514-S285	France	HQ714767	
Melanogaster shanxiensis	HMAS81910 (holotype)	China	MW598550	
Melanogaster spinisporus	BJTC FAN941-A (epitype)	China	MW598548	
Melanogaster spinisporus	BJTC FAN938	China	MW598546	
Melanogaster subglobisporus	HMAS83329 (holotype)	China	MW598534	
Melanogaster tomentellus	BJTC FAN539 (holotype)	China	MW598538	
Melanogaster tuberiformis	JC110130BT, 34 (FCO-Fungi)	Spain	MN594289	MN594302
Melanogaster variegatus	JC180617BT, 35 (FCO-Fungi)	Spain	MN594290	MN594303
Paxillus involutus	Can12.1	France	KF261368	
Paxillus rubicundulus	Orton2905, M-190474 (K)	UK	KU163502	

Note The newly generated sequences are in bold.

4.2.7.3 Results

Phylogenetic Analysis

The ML and Bayesian analyses of the 35 ITS and 10 LSU sequences are shown in Figure 11 with associated bootstrap supports for branches.

In the phylogenetic tree, the 30 ITS sequences from *Melanogaster* revealed the phylogenetic relationship of 19 species, and used representatives of *Paxillus* as outgroups. The phylogenetic analysis showed that the new species is clearly distinct from other species of the genus *Melanogaster*.

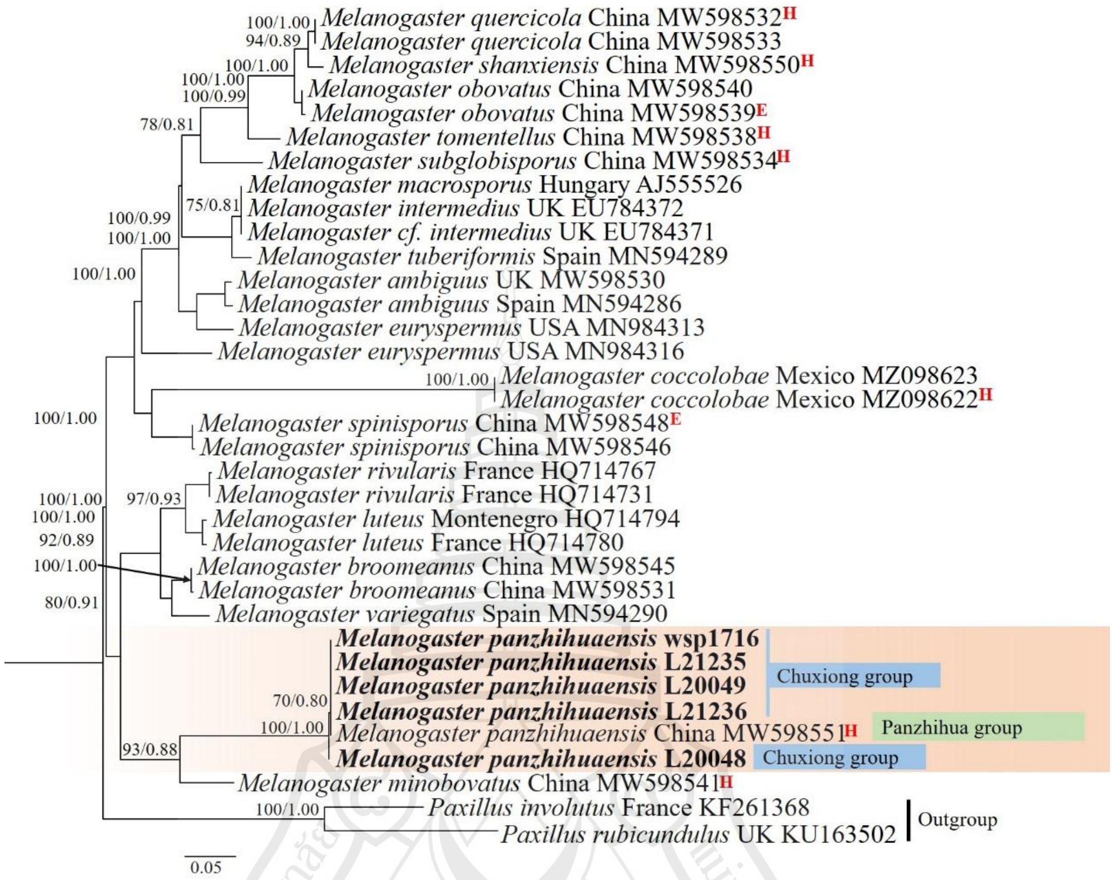


Figure 4.19 Phylogeny derived from a maximum likelihood (ML) analysis of the nrDNA-ITS sequences from *Melanogaster* species

Figure 4.19 Values next to nodes reflect maximum likelihood bootstrap support values (BS), left, and Bayesian posterior probabilities (PP), right. Names of novel species and samples with newly generated sequences are in bold.

Melanogaster panzhihuaensis L. Fan, X.Y. Yan & Y.Y. Xu 2022 Figure 4.20

Description (Figure 4.20): Basidiomata globose to subglobose, with irregular depressions, 1.8-4.6 cm diam., the surface smooth to short, finely fluffy, bright yellowish brown (5D8), staining reddish brown (8D8) where bruised when fresh, and dark brown (8F8) after drying, with rhizomorphs obviously matching the color of the basidiomata surface. KOH reddish brown(8D8) on fresh peridium and black on dried specimens. $FeSO_4$ black on dried specimens. The odor strong when fresh, and diminishes after drying.

Gleba solid, gelatinous-rubbery, pale brown in youth, at maturity black with white to yellow sterile veins.

Peridium thin, 60-120 μm thick, consisting of yellowish-brown interwoven hyphae, with deep yellowish-brown cells on the outer edge, short thick rod-shaped, front shield round, the hyphal color gradually becomes translucent inward.

Basidia 8–10 × 2–3 μm, hyaline narrow-clavate when young, commonly 3-5-spored, sterigmata short, collapsed and disappeared at maturity.

Basidiospore obovate or slender obovate, at first hyaline to light brown(6C8), becoming brown (6E7) individually, and dark brown (6F8) to black in mass at maturity, smooth, blunt top, with truncate-cupped base and short distinct remnants of sterigmata, measured (6.5–) 8.5–10.0 × 4.0–5.0 μm, Q (L/I) = 1.70–2.15 Qm = 1.92 ± 0.21 (n = 50), wall 0.3–0.5 μm thick.

Ecology & Distribution: Hypogeous, in soil under *Pinus yunnanensis* forest, and under the mixed forest of *Keteleeria evelyniana* and *Quercus acutissima* Carruth. Forest. Known only from northwestern Yunnan Province, China.

Additional specimen examined: Yunnan Province: Chuxiong Yi Autonomous Prefecture, Mouding County, Xinqiao Town, Haojiahe village, alt. 1783.3 m, under the mixed forest of *Keteleeria evelyniana* and *Quercus acutissima* Carruth., 7 Aug. 2020, Lin Li，L20048 and L22049；Chuxiong City，Donggua Town，alt. 1667 m，under the mixed forest of *Keteleeria evelyniana* and *Quercus* sp. 16 Oct. 2021, Lin Li, L21235 and L21236 (BMDU L20048: ITS = PX096553, LSU = PX096470; BMDU L20049: ITS = PX096554; BMDU L21235: ITS = PX096555, LSU = PX096471; BMDU L21236: ITS = PX096556, LSU = PX096472).

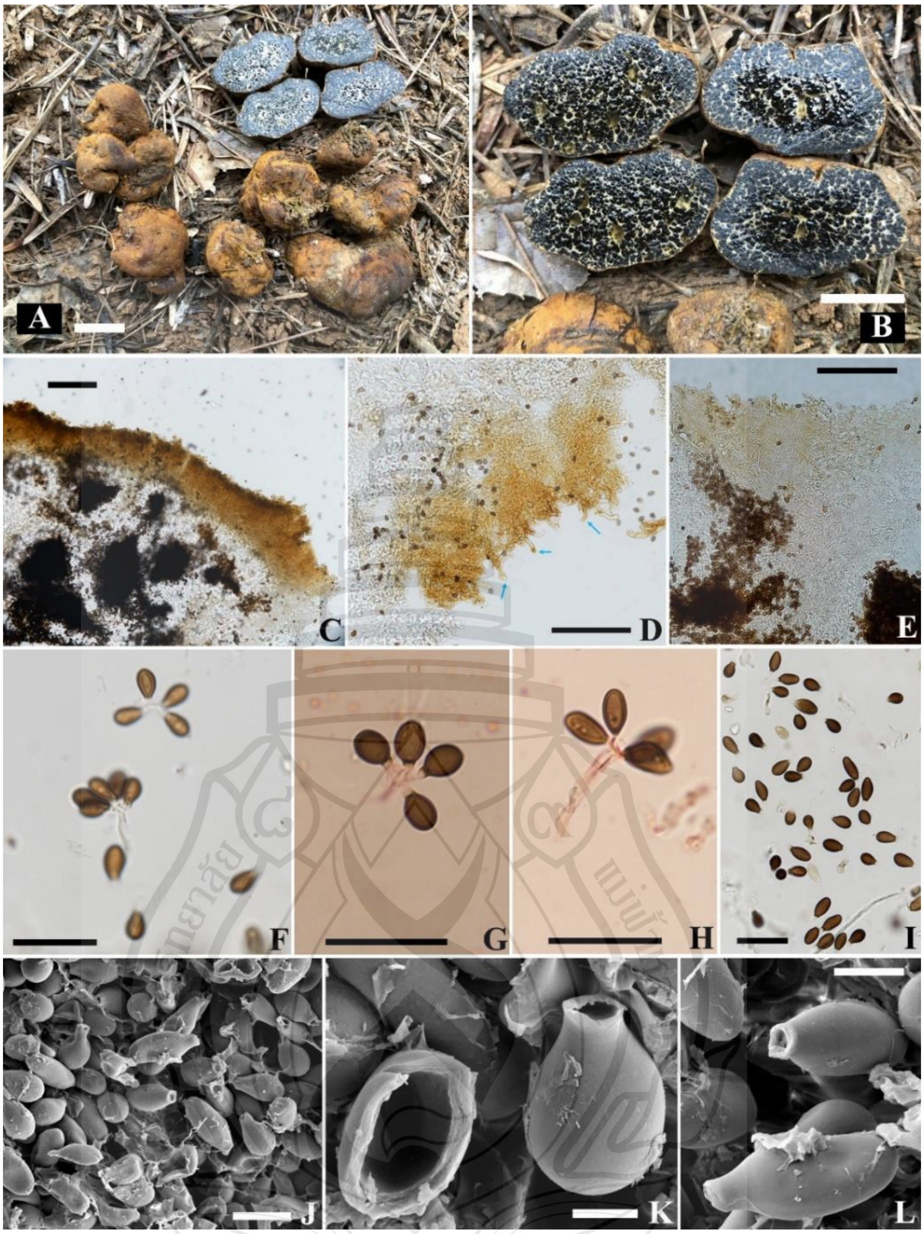


Figure 4.20 *Melanogaster panzhihuaensis* morphological characteristics

Figure 4.20 A.B. Basidiomata; C. A piece of the section of the basidiomata in 5% KOH; D.E. Hyphae composing the peridium; F.G.H. Basidia in 5% KOH and Congo red stain; I. Basidiospores in 5% KOH; J.K.L. SEM ascospores (dry sample). Scale bars: A-B = 1cm; C = 100μm; D-E = 50μm; F-I. = 10μm; J = 5μm; K=1μm; L=2μm.

4.2.7.4 Discussion

In one of the species' habitats, Chuxiong City, the species has been collected and consumed by mushroom hunters. They typically dry the basidiomata for storage and brew them in water to drink when coughing. However, it is not sold in the market. This paper presents a study of the specimens of this species collected from Chuxiong.

For the species *Melanogaster panzhihuaensis*, phylogenetic analysis based on ITS and LSU sequences shows that specimens from the Chuxiong group together with the type specimen from Panzhihua in the same clade with high support, and the ITS sequence similarity is greater than 99.85%. Although the molecular phylogenetic results are consistent, when comparing the morphological characteristics of multiple specimens collected from Chuxiong with the description of the type specimen (HMAS 81915), we noticed distinct morphological differences. The basidiomata from Chuxiong specimens are larger, with a diameter reaching 4.6 cm. Both when fresh and dried, they exhibit a strong odor. The surface of the basidiomata shows distinct rhizomorphs. The color of the gleba is darker when fresh, turning black at maturity. The peridium is thin (60–120 µm thick), and no clamp connections were observed. The basidiospores are obovate, with a rounder apex that does not taper, and are smaller in size, measuring 8.5–10.0 × 4.0–5.0 µm. In contrast, the description based on the type specimen (HMAS 81915) reports the basidiomata as 1.5 × 1.7 cm in size, with no recorded odor, rhizomorphs not distinct, the gleba pale yellow-brown when fresh, the peridium 150–220 µm thick with clamp connections, and basidiospores oval, with a blunt and narrow apex, measuring 8.7–12.4 × 5.2–7.6 µm (Xu et al., 2022). The morphological differences listed above may be related to the different habitats and symbiotic tree species of the two populations.

4.2.8 *Protubera*

4.2.8.1 Introduction

Based on specimens collected from coastal rainforests in southern Brazil, Möller (1895) established the remarkable genus *Protubera*, with *P. maracuja* designated as the type species. This genus is characterized by basidiomata that, at maturity, are enclosed within a thin white to brown epigeous peridium; the peridium is often disrupted by the primordial tissue connecting the peridium and the gleba. The gleba is gelatinous to cartilaginous, olive to brown in color. Basidiospores are small, smooth, subcylindrical, and olive to brown. Species in this genus exhibit a saprotrophic lifestyle (Zeller, 1939; Malloch, 1989; Trierveiler-Pereira et al., 2014). Currently, *Protubera* comprises 15 recognized species, four of which have been reported from China (Li et al., 2018; Liu, 1994; Liu et al., 1996). Based on combined morphological and molecular phylogenetic analyses, we describe one new species in this study.

4.2.8.2 Sequence Alignment and Analysis

The nuclear ribosomal large subunit (nrLSU), RNA polymerase II second largest subunit (RPB2), and ATP synthase subunit 6 (ATP6) regions were used in this study to analyze species diversity within the genus *Protubera*, as these loci have proven effective for species delimitation in this group. A total of 18 nrLSU, 20 RPB2, and 20 ATP6 sequences—including both newly generated data and sequences retrieved from NCBI—were analyzed. These sequences represent 11 species of *Protubera* (Table 4.11). The analytical methods followed those described in the Materials and Methods section. Phylogenetic clades were considered strongly supported if they exhibited a bootstrap support (BS) value ≥ 70% and/or a posterior probability (PP) ≥ 0.90.

Table 4.11 Taxa information and GenBank accession numbers of the sequences used in *Protubera* study

Species Name	Voucher	Origin	GenBank No.		
			LSU	**RPB2**	**ATP6**
Protubera hauluensis	OSC59673	USA	DQ218517	DQ219039	DQ218801
Protubera nothofagi	OSC59699	USA	AY574644	DQ219040	AY574786
Phallogaster pinyonensis	AHF530	Western north American	NG064311	DQ219043	DQ218884
Phallogaster saccatus	T13202	Eastern north American	DQ218595	DQ219038	DQ218882
Protubera beijingensis	HMAS279558	China	MG430511	MG430517	MG430514
Protubera borealis	OKM21898	USA	DQ218516	DQ219106	DQ218800
Protubera jamaicensis	T28248	Colombia	DQ218647	DQ219110	DQ218933
Protubera maracuja	ICN-LTP220	Brazil	KC808513	KF783231	KC808515
Protubera maracuja	ICN-LTP284	Brazil	KC808514	KF783232	KC808516
Protubera maracuja	Garido2550-A	Chile	DQ218518	DQ219111	DQ218802
Protubera nipponica	KH-JPN11-511	Japan	KF783251	KF783234	KF783268
Protubera nipponica	KH-JPN10-509	Japan		KF783233	KF783267
Protubera sabulonensis	T12737	USA	DQ218649	DQ219113	DQ218935

Table 4.11 (continued)

Species Name	Voucher	Origin	GenBank No.		
			LSU	**RPB2**	**ATP6**
Protubera parvispora	OSC59689	New Zealand		DQ219112	DQ218934
Protubera yunnanensis	**HKAS131260**	**China**	This study	This study	This study
Protubera yunnanensis	**HKAS131261**	**China**	This study	This study	This study
Protubera yunnanensis	**HKAS131262**	**China**	This study	This study	This study
Protubera yunnanensis	**HKAS131263**	**China**	This study	This study	This study
Protubera yunnanensis	**HKAS131267**	**China**	This study	This study	This study
Protubera yunnanensis	**HKAS131269**	**China**	This study	This study	This study

Note The newly generated sequences are in bold.

4.2.8.3 Results

Phylogenetic Analysis

The ML and Bayesian analyses of the 18 nrLSU, 20 RPB2, and 20 ATP6 sequences are shown in Figure 21 with associated bootstrap supports for branches.

In the phylogenetic tree, the 16 nrLSU, 18 RPB2, and 18 ATP6 sequences from *Protubera* revealed the phylogenetic relationship of 11 species, and used representatives of *Phallogaster* as outgroups. The phylogenetic analysis showed that the new species is clearly distinct from other species of the genus *Protubera*.

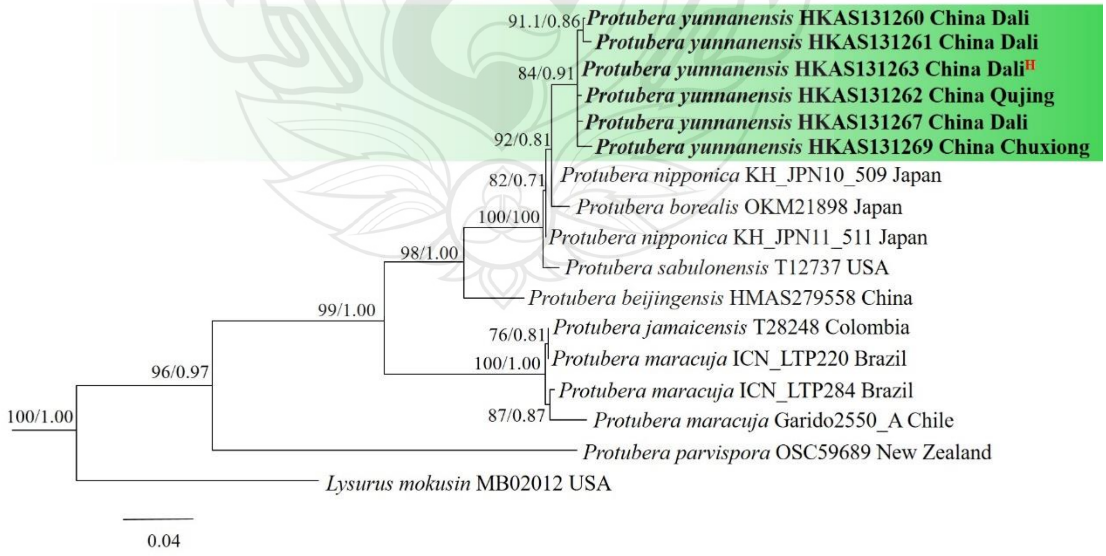


Figure 4.21 Phylogeny derived from a maximum likelihood (ML) analysis of the nrLSU, 20 RPB2, and 20 ATP6 sequences from *Protubera* species.

Figure 4.21 Values next to nodes reflect maximum likelihood bootstrap support values (BS), left, and Bayesian posterior probabilities (PP), right. Names of novel species and samples with newly generated sequences are in bold.

Protubera yunnanensis L. Li, K.D. Hyde & S.H. Li, sp. nov. Figure 22

Mycobank number: MB 860408

Diagnosis: Differs from other *Protubera* spp. by its larger basidiomata, up to 14 cm in diam., and surface remains white throughout its entire development. The peridium is two-layered, 180-340 μm thick, composed of hyaline intertwined hyphae. The hyphae of the outer layer are thicker in diameter than those of the inner layer. The hyphae in the inner layer are filled with a gelatinous substance. Basidiospores are smaller, measuring 2.5–3.0 × 1.0–2.0 μm, individual hyaline, pale brown in mass.

Etymology: yunnanensis, referring to the type specimens were collected in Yunnan, and the species is found in several regions of the province.

Holotype: China, Yunnan province, Mt. Cangshan, Dali University campus, 25º40′ N 100º09′ E, alt. 2105.7 m, gregarious on grassland. 23 Oct. 2023, Lin Li, HKAS131263 (GenBank: nrLSU= PX112446, *rpb2*=XXXX, *atp6*=XXXX).

The basidiomata are spherical or irregularly spherical, with a diameter of 1.0–14.0 cm. The surface is smooth, white to dirty white (3B1), turning dirty yellow (3C3) when dry, and does not change color when injured. The base has abundant white rhizomorphs, often extending from the base, with some thick ones reaching up to 2 mm in diam.

The glebal mass is divided into elongated plates, which are neatly arranged, extending from the inner peridium toward the center of the gleba. Sometimes, they completely fill the gleba and are compressed and deformed at the center; other times, they do not extend to the center, creating a hollow space. The larger basidiomata plates are arranged irregularly, always pressing against each other to fill the gleba. Between the plates is a transparent, gelatinous, viscous to agarlike substance. When immature, the plates are white, but as they mature, they change to gray-green (28E8) or brown-green (29E5), eventually turning dark green (29E8) and nearly dissolving at maturity. When freshly cut, the basidiomata emits a foul odor.

The peridium is two-layered and easily separable from the gleba. The peridium is 180–340 μm thick. The outer layer 150–250 μm thick, composed of hyaline intertwined hyphae, 5–7 μm in diam., with yellow-brown (5D7) pigments. The inner layer 50-100 μm thick, composed of hyaline dense intertwined hyphae, 1–2 μm in diam., filled with gelatinous, viscous substance.

Hymenium is located on the inner walls of plates. Basidia clavate or cylindrical, thin-walled, colorless, commonly 6–8-spored, 14.0–15.5 × 3.5–5.0 μm, collapsing and disappearing at maturity.

Basidiospores ellipsoid, rounded and blunt top, with a truncated base from sterigma, individual spores hyaline, pale brown (5D4) in mass, measuring 2.5–3.0(–3.5) × 1.0–2.0 μm, Q (L/I) =2.00–2.58, Q_m = 2.26 ± 0.22 (n = 50), smooth, thin-walled.

Ecology & Distribution: gregarious or solitary on soil or grassland, alt. 1700–2600m, fruiting in summer to autumn. Known only from northwestern Yunnan Province, China.

Additional specimen examined: China, Yunnan province, Dali Mt. Cangshan, Wuwei temple, alt. 2242m, on grassland under *Pinus armandii,* 6 Sep. 2014, Shuhong Li, HKAS131260; alt. 2600m, on grassland under *Pinus armandii*, 20 Sep. 2015, Songming Tang, HKAS131261; Dali University campus, alt. 2010m, on grassland, 21 Oct. 2024, Lin Li, HKAS131267; Qujing city, Zhanyi county, Gaozhai village, 29 Jul. 2021, Songming Tang, HKAS131262; Chuxiong city, Wuding county, Maojie town, Yangcaidi village, 26 Oct. 2023, Yongchang Zhao, HKAS131269.

Notes: The phylogenetic tree of the genus *Protubera* based on combined nrLSU, *rpb2*, and *atp6* analyses (Figure 4.21) shows that *Protubera yunnanensis* is supported as an independent species with high confidence. When comparing *P. yunnanensis* with other species of *Protubera* in terms of morphology and distribution, several notable differences are observed: *P. parvispora* is distributed in New Zealand, with a glebal mass that is central and single, not divided into elongated plates (Castellano & Beever, 1994); *P. maracuja* is distributed in tropical and subtropical regions, with an outer peridium that is pseudoparenchymatous and an inner peridium containing large crystals that form rosette patterns (Malloch, 1989; Trierveiler-Pereira et al., 2013); *P. jamaicensis* is found in the Neotropics, with a basal mycelial strand extending into the center of the basidiome and a viscid surface (Imai & Kawamura, 1958); *P. borealis* is distributed in Japan and Korea, and although the basidiomata of *P. borealis* can reach 14-15 cm in diameter, they are brownish or grayish with brownish patches (Imai & Kawamura, 1958), whereas the basidiomata of *P. yunnanensis* always remain white throughout their development. *P. sabulonensis* has been reported from Canada, with a peridium less than 70 μm thick (Malloch, 1989); *P. nipponica* is found in Japan, characterized by a very thick peridium, up to 1.0–1.5 mm thick, with a gelatinous layer that is not agar-like (Imai & Kawamura, 1958). *P. kunmingica*, described in Yunnan Province, China, has smaller basidiomata, less than 1 cm in diameter, and large basidiospores measuring 7–10 × 3–5 μm (Liu et al., 1996); *Protubera beijingensis* has gasteroid basidiocarps, 4–5 cm in diameter, with a pale brown color intermixed with light green, and basidiospores measuring 3.4–5.2 × 1.5–2.4 μm (Li et al., 2018a). In addition to the differences listed above, *P. yunnanensis* has the smallest basidiospore size in the genus, measuring 2.5–3.0 × 1.0–2.0 μm. Furthermore, based on both morphological and multigene phylogenetic analysis, specimens collected from Mt. Cangshan, Dali (BMDU 2014081309, 2014090620, 2015092015, 2015092020) were identified as *P. yunnanensis*. These specimens were previously misidentified as a new distribution record of *P. sabulonensis* in China based solely on morphological comparisons (Li et al. 2016b), and this is hereby corrected.

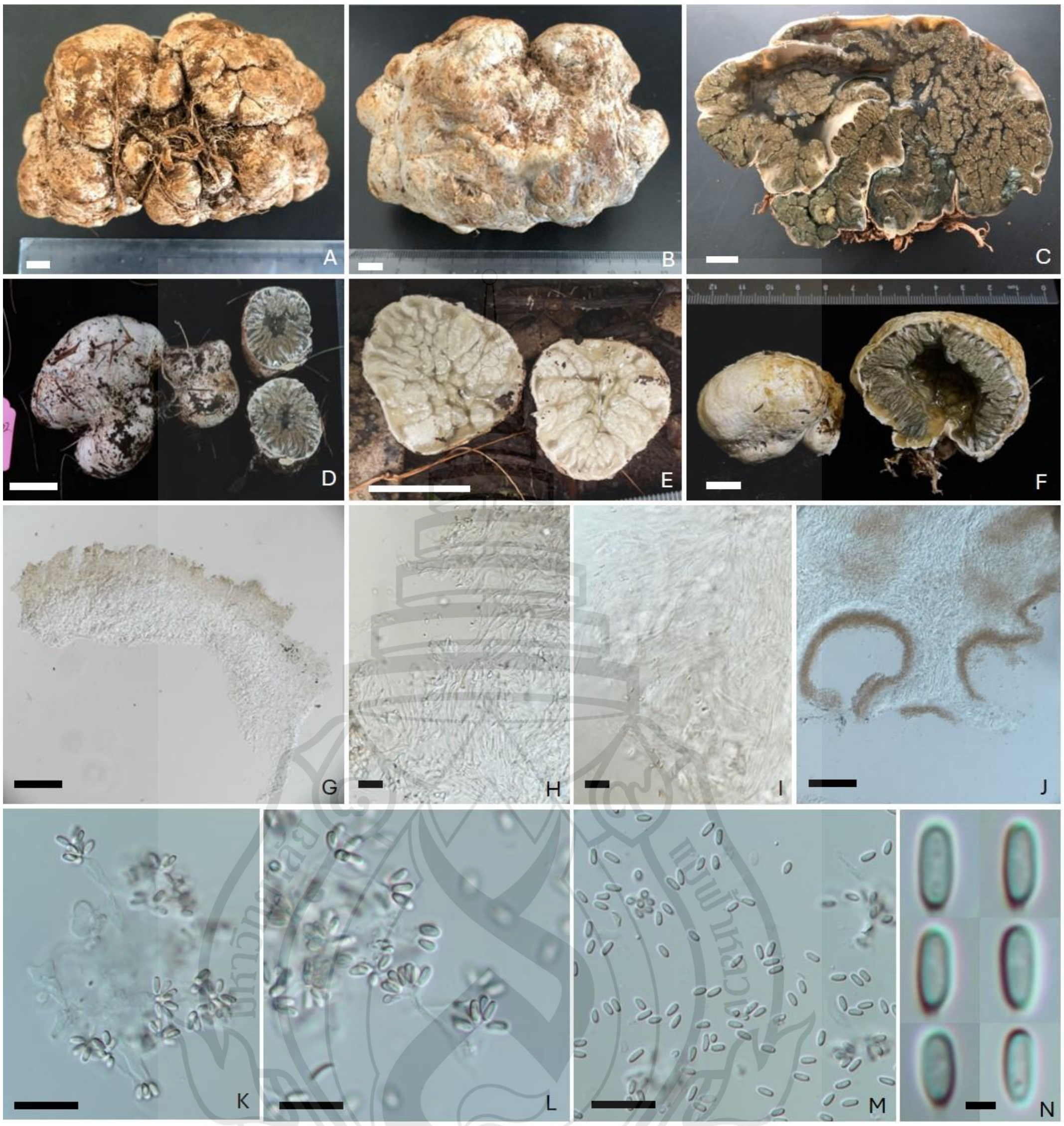


Figure 4.22 *Protubera yunnanensis* morphological characteristics

Figure 4.22 A-F. Bbasidiomata, gleba, and white basal rhizomorph; G. A piece of the section of the peridium in 5% KOH; H. Hyphae composing the outer layer of peridium in 5% KOH; I. Hyphae composing the inner layer of peridium in 5% KOH; J. A piece of the section of the gleba in 5% KOH; K.L. Basidia and basidiospores; M.N. Basidiospores in 5% KOH. Scale bars: A-F = 1cm; G = 100μm; H.I.K-M = 10μm; J = 50μm; N = 1μm.

4.2.9 *Rhizopogon*

4.2.9.1 Introduction

The genus *Rhizopogon* is the largest genus of hypogeous Basidiomycota, with worldwide distribution (Zeller & Dodge, 1918; Smith & Zeller, 1966; Martin, 1998; Molina et al., 1999; Koizumi & Nara, 2016; Li et al., 2016; Wang et al., 2021). Some species are popular edible fungi (Wang et al., 2013). *Rhizopogon* is characterized by globose to subglobose basidiomata, attached by rhizomorphs over the surface, gleba white when young, becoming dark in maturity, finally gelatinized, chambers small, numerous, labyrinthine (Smith & Zeller, 1966; Koukol et al., 2022). All *Rhizopogon* species form obligate ectomycorrhizal associations with Pinacese (Molina & Trappe, 1994; Massicotte et al., 1994; Massicotte et al., 1999; Grubisha et al., 2002).

According to statistics, prior to this study, 11 species of the genus *Rhizopogon* were recorded in China (Liu, 1988; Dai et al., 2010; Li et al., 2016; Wang et al., 2021). Among them, only 4 species reported after 2016 have molecular data (*R. sinoalbidus*, *R. flavidus*, *R. jiyaozi*, and *R. songmaodan*). In this study, a total of 106 *Rhizopogon* specimens were collected from the southwestern region, and after identification, these specimens were assigned to *R. sinoalbidus*, *R. flavidus*, *R. jiyaozi*, and *R. songmaodan*, as well as four additional new species described in this paper. The distribution of *Rhizopogon* 13 species in southwestern China is as follows: *Rhizopogon sinoalbidus* in Yunnan and Xizang province, *R. nigrescens*, *R. flavidus,* and *R. songmaodan* in Yunnan and Sichuan province, *R. jiyaozi* in Yunnan, Sichuan, and Xizang province, *R. fabri*, *R. luteolus*, *R. roseolus*, *R. laojunshanensis*, *R. weixiensis,* and *R. melanocuticularis* in Yunnan province, *R. luteoloides* and *R. zayuensis* in Xizang province.

4.2.9.2 Sequence Alignment and Analysis

The internal transcribed spacer (ITS) region was used in this study to analyze species diversity within the genus *Rhizopogon*, as it has proven to be a useful locus for delimiting species in this group. A total of 140 ITS sequences, including those generated in this study and those retrieved from NCBI, were analyzed. These sequences represent 73 species of *Rhizopogon* (Table 13). The analytical methods followed those described in the Materials and Methods section. Phylogenetic clades were considered strongly supported if they exhibited a bootstrap support (BS) value ≥ 70% and/or a posterior probability (PP) ≥ 0.90.

Table 4.12 Taxa information and GenBank accession numbers of the sequences used in *Rhizopogon* study

Species Name	Voucher	Origin	GenBank No.
Rhizopogon abietis	OSC41455	USA	KC346843
Rhizopogon abietis	Trappe 7603	USA	EU837243
Rhizopogon albidus	AHS 69642	USA	AM085519
Rhizopogon alkalivirens	AHS 69037(holotype)	USA	AF377154
Rhizopogon arctostaphyli	JPT5705(holotype)	USA	AF377167
Rhizopogon arctostaphyli	-	USA	EU726304
Rhizopogon atroviolaceus	AHS68263	USA	AF377131
Rhizopogon bacillisporus	OSC63507	USA	EU837230
Rhizopogon bacillisporus	SOC1307	USA	JN022517
Rhizopogon brunsii	LG1296	USA	AY971814
Rhizopogon brunsii	LG701(holotype)	USA	AY971823
Rhizopogon burlinghamii	JMT17882	USA	AF058303
Rhizopogon colossus	AHS49480	USA	AF071441
Rhizopogon colossus	AHS 49480	USA	AH011348
Rhizopogon corsicus	V. Demoulin s.n17IX1989	Belgium	AM085521
Rhizopogon diabolicus	AHS68424	USA	AF071444
Rhizopogon diabolicus	AHS68404	USA	AF366386
Rhizopogon diabolicus	AHS 68424	USA	AH011349
Rhizopogon diabolicus	AHS68489	USA	AF366387
Rhizopogon ellenae	AHS66137	USA	AF071445
Rhizopogon ellenae	JMT17476	USA	AF058311
Rhizopogon ellenae	-	USA	JF695019
Rhizopogon ellipsosporus	OSC138981	USA	JX310372
Rhizopogon ellipsosporus	OSC138996	USA	JX310373
Rhizopogon evadens	AHS65484	USA	AF062927
Rhizopogon evadens	JMT16402	USA	AF058312
Rhizopogon evadens	JMT12321	USA	AF062932
Rhizopogon exiguus	OSC140477	USA	JX310374
Rhizopogon fallax	AHS66116	USA	AF377143
Rhizopogon fallax	AHS65762	USA	AF377144
Rhizopogon fragrans	AHS60155	USA	AM085523
Rhizopogon fuscorubens	JMT17446	USA	AF058313

Table 4.12 (continued)

Species Name	Voucher	Origin	GenBank No.
Rhizopogon fuscorubens	Ash58b	-	AY880943
Rhizopogon graveolens	PRM619028	Czech	AJ810037
Rhizopogon hawkerae	AHS68417	USA	AF071447
Rhizopogon hawkerae	AHS 68417	USA	AH011351
Rhizopogon idahoensis	-	-	AF224472
Rhizopogon idahoensis	AHS70715	USA	AF377123
Rhizopogonluteolus	JMT22516	Sweden	AF062936
Rhizopogonluteolus	isolate373	Spain	EU423919
Rhizopogonluteolus	DG05-22	Scotland	JQ888192
Rhizopogon milleri	AHS70789(holotype)	-	AF377135
Rhizopogon occidentalis	JMT17564	USA	AF058305
Rhizopogon occidentalis	LCG211	USA	AF062939
Rhizopogon occidentalis	KGP85	USA	DQ822821
Rhizopogon ochraceisporus	AHS65963	USA	AF071439
Rhizopogon ochraceisporus	JMT17944	USA	AF058306
Rhizopogon ochraceisporus	JMT17916	USA	AF062935
Rhizopogon ochraceisporus	AHS58928	USA	AF366389
Rhizopogon ochraceorubens	AHS59643	USA	AF062928
Rhizopogon ochraceorubens	JMT19192	USA	AF071440
Rhizopogon odoratus	AHS 71319	USA	AM085526
Rhizopogon olivaceotinctus	HDT 51086	USA	AJ515509
Rhizopogon pachydermus	K.A.Harrison7290	USA	AM085527
Rhizopogon parksii	JMT17679	USA	AF062930
Rhizopogon parksii	JMT19446	USA	AF058314
Rhizopogon parksii	OSC130792	USA	JX310375
Rhizopogon parvulus	AHS68364	USA	AF071449
Rhizopogon parvulus	MICH AHS 68364	USA	AH011352
Rhizopogon pedicellus	AHS66176	USA	AF377126
Rhizopogon pseudoroseolus	MICH66604	USA	AJ810041
Rhizopogon pseudoroseolus	K98S35	New Zealand	GQ267484
Rhizopogon rocabrunae	17067	Spain	JF908761
Rhizopogon rogersii	JMT17228	USA	AF071437
Rhizopogon rogersii	OSC63539	USA	EU697274
Rhizopogon roseolus	JMT8227	USA	AF058315
Rhizopogon roseolus	RrUP175	Sweden	DQ179127
Rhizopogon roseolus	MA-Fungi 47716	Spain	AJ810064
Rhizopogon roseolus	AT630	Japan	GQ179955
Rhizopogon roseolus	AT632	Japan	GQ179956
Rhizopogon roseolus	Tottori-10	Japan	AB274244
Rhizopogon rudus	AHS71352(holotype)	-	AF377107

Table 4.12 (continued)

Species Name	Voucher	Origin	GenBank No.
Rhizopogon salebrosus	R141SCCO	USA	HQ914327
Rhizopogon salebrosus	AHS69292	USA	AF377156
Rhizopogon sardous	AQUI27-XI-1981	Italy	AM085529
Rhizopogon semireticulatus	JMT7899	USA	AF058307
Rhizopogon semireticulatus	JMT17562	USA	AF062940
Rhizopogon semireticulatus	AHS71326	USA	AF377118
Rhizopogon semireticulatus	AHS71330(holotype)	-	AF377119
Rhizopogon subbadius	AHS58998(holotype)	USA	AF377152
Rhizopogon subbadius	AHS68485	USA	AF377151
Rhizopogon subcaerulescens	-	-	M91613
Rhizopogon subgelatinosus	JMT7624	USA	AF062937
Rhizopogon subgelatinosus	AHS68558	USA	AF377147
Rhizopogon subpurpurascens	AHS65669	USA	AF062929
Rhizopogon subpurpurascens	JMT19168	USA	AF058308
Rhizopogon subsalmonius	JMT17218	USA	AF062938
Rhizopogon subsalmonius	Martin 1653	Spain	AM085530
Rhizopogon succosus	JMT19321	USA	AF062933
Rhizopogon variabilisporus	OSC63496	USA	KC346855
Rhizopogon variabilisporus	OSC134688	USA	KC346856
Rhizopogon verii	G.P.XII-1982	Tunisia	AM085531
Rhizopogon vesiculosus	OSC129175	Canada	HQ385854
Rhizopogon vesiculosus	OSC129170	Canada	HQ385849
Rhizopogon villescens	JMT17681	USA	AF058309
Rhizopogon villosulus	AHS59143	USA	AF071451
Rhizopogon villosulus	JMT19466	USA	AF058310
Rhizopogon vinicolor	JMT17899	USA	AF058316
Rhizopogon vinicolor	JMT19383	USA	AF058304
Rhizopogon vinicolor	JMT20787	USA	AF062941
Rhizopogon vinicolor	OSC129176	Canada	HQ385847
Rhizopogon vulgaris	JMT19154	USA	AF062934
Rhizopogon vulgaris	JMT17998	USA	AF062931
Rhizopogon zelleri	JMT12974	USA	AF062942
Rhizopogon himalayensis	Akatoch89	India	OR187308
Rhizopogon cashmerianus	KASH2922(H)	India	MK692540
Rhizopogon alpinus	TNS-F-61928(H)	Japan	LC128302
Rhizopogon alpinus	TNS-F-65451	Japan	LC128303
Rhizopogon nitidus	TNS-F-65452(H)	Japan	LC128305
Rhizopogon nitidus	TNS-F-65453	Japan	LC128306
Rhizopogon confusus	PRM945153(H)	Czech	HG999784
Rhizopogon confusus	PRM879700	Czech	HG999780

Table 4.12 (continued)

Species Name	Voucher	Origin	GenBank No.
Rhizopogon yakushimensis	TNS:F-54013(H)	Japan	LC216340
Rhizopogon yakushimensis	TNS:F-54014	Japan	LC216339
Rhizopogon songmaodan	HKAS 106767(H)	China	NR174900
Rhizopogon songmaodan	HKAS-106770	China	MN655985
Rhizopogon songmaodan	**L19003**	**China**	**This study**
Rhizopogon jiyaozi	YAAS-L2929(H)	China	NR158906
Rhizopogon jiyaozi	HKAS71302	China	MH827597
Rhizopogon jiyaozi	**L23110**	China	**This study**
Rhizopogon flavidus	YAAS-L2957(H)	China	NR158904
Rhizopogon flavidus	YAAS-L2959	China	KP893815
Rhizopogon sinoalbidus	YAAS-L2949(H)	China	NR158905
Rhizopogon sinoalbidus	HKAS84655	China	MH827596
Rhizopogon sinoalbidus	**L24098**	**China**	**This study**
Rhizopogon sinoalbidus	**L23014**	**China**	**This study**
Rhizopogon sinoalbidus	**L23006**	**China**	**This study**
Suillus kwangtungensis	HKAS71979	China	KU721539
Suillus aurihymenius	SHI-597	China	JN201972
Suillus alpinus	SHI-697A	China	JN201974
Truncocolumella citrina	JLF 2149	USA	MH217566
Truncocolumella citrina	isolate(TDB-2001)	USA	L54097
Truncocolumella pseudocolumella	HKAS131259	China	KP090063
Truncocolumella pseudocolumella	HKAS131259	China	KP090064
Rhizopogon laojunshanensis	**L20074**	**China**	**This study**
Rhizopogon melanocuticularis	**L22409**	**China**	**This study**
Rhizopogon melanocuticularis	**L22410**	**China**	**This study**
Rhizopogon zayuensis	**L22031**	**China**	**This study**
Rhizopogon zayuensis	**L22032**	**China**	**This study**
Rhizopogon weixiensis	**L22401**	**China**	**This study**
Rhizopogon weixiensis	**L22402**	**China**	**This study**
Rhizopogon weixiensis	**L22403**	**China**	**This study**
Rhizopogon weixiensis	**L22404**	**China**	**This study**
Rhizopogon weixiensis	**L22405**	**China**	**This study**
Rhizopogon weixiensis	**L22406**	**China**	**This study**
Rhizopogon weixiensis	**L22407**	**China**	**This study**
Rhizopogon weixiensis	**L22408**	**China**	**This study**

Note The newly generated sequences are in bold.

4.2.9.3 Results

Phylogenetic Analysis

The ML and Bayesian analyses of the 140 ITS sequences are shown in Figure 23 with associated bootstrap supports for branches.

In the phylogenetic tree, the 133 ITS sequences from *Rhizopogon* revealed the phylogenetic relationship of 73 species, and used representatives of *Suillus* and *Truncocolumella* as outgroups. The phylogenetic analysis showed that the new species is clearly distinct from other species of the genus *Rhizopogon*. In addition to the ITS sequences used in this phylogenetic analysis, the LSU sequences were amplified from the newly supplemented specimens in this study and uploaded to NCBI for future study.

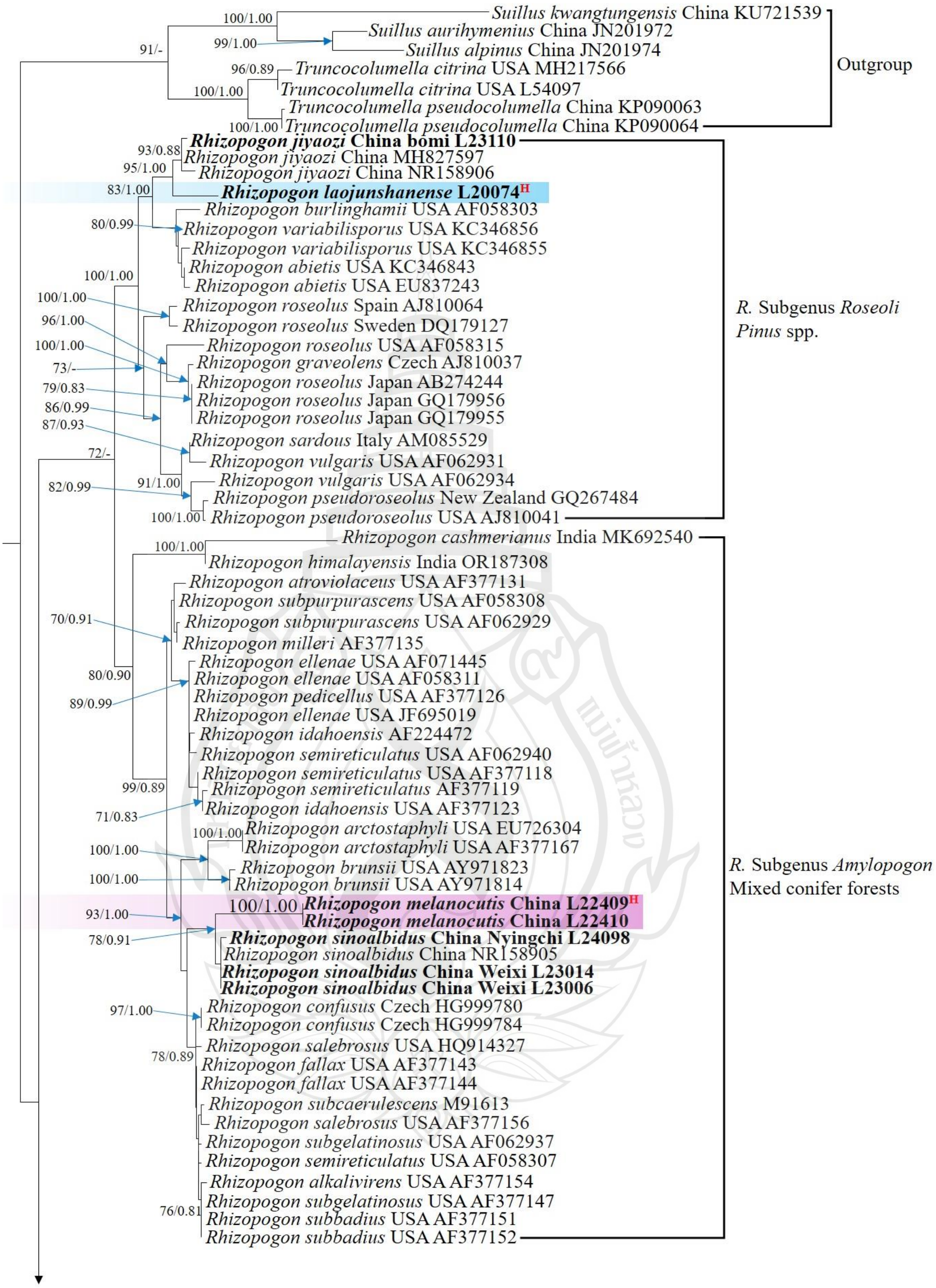


Figure 4.23 Phylogeny derived from a maximum likelihood (ML) analysis of the nrDNA-ITS sequences from *Rhizopogon* species

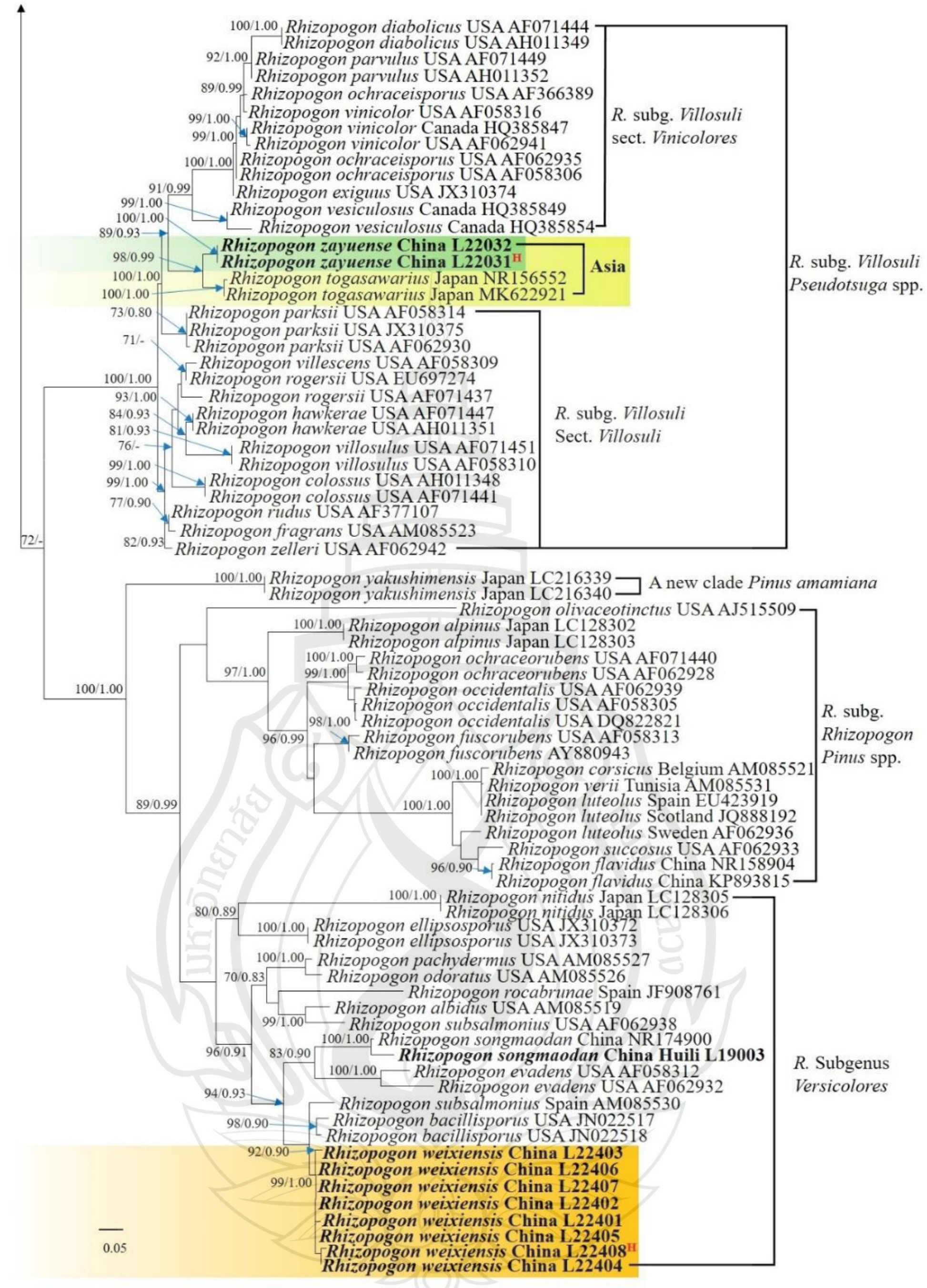


Figure 4.23 (continued)

Figure 4.23 Values next to nodes reflect maximum likelihood bootstrap support values (BS), left, and Bayesian posterior probabilities (PP), right. Names of novel species and samples with newly generated sequences are in bold.

Rhizopogon laojunshanensis L. Li, K.D. Hyde & S.H. Li, sp. nov. Figure 4.24

Mycobank number: MB 860403

Diagnosis: Differs from other *Rhizopogon* spp. by its peridium not changing color when injured, composed of hyaline to pale brown hyphae interwoven, with some hyphal walls attached to dark-colored granules. It has smaller basidiomata, up to 1.5 cm in diam., and hyaline to light brown basidiospores with dimensions of 6.0–8.5 × 2.0–3.0 μm.

Etymology: *laojunshanensis*, refers to the type locality of the Laijunshan Mountain.

Holotype: China, Yunnan province, Lijiang city, Jiuhe town, Laojunshan Mountain, 26°38′ N 99°49′ E, alt. 2734.2 m, in soil under forest of *Pinus yunnanensis* Franch., 17 August 2020, Lin Li, BMDU L20074 (GenBank: ITS = PX096563, LSU = 096593).

Basidiomata globose to subglobose, 1.0–1.5 cm in diam., slightly soft and elastic, khaki (4C6) in color, not changing color when cut or bruised, with rhizomorphs of the same color over the surface. Dried basidiomata are dark yellow-brown (4E8) and show no color change upon contact with KOH. Odor not distinctive.

Gleba spongy, composed of densely arranged irregular hollow chambers, numerous, labyrinthine, white when immature, turning Olive gray to brown-gray (29E5) at maturity, no color change when cut. Trama composed of interwoven hyaline gelatinized hyphae, 80-120 μm wide.

Peridium 170–300 μm thick, not easily detached, composed of hyaline to pale brown (4C4) hyphae interwoven, 5–7 μm in diam., some hyphal walls are attached with dark-colored granules, and no color change when exposed to KOH. Clamp connections absent.

Basidia clavate or short cylindrical, thin-walled, colorless, commonly 4–6-spored, 15.5–18.5 × 4.5–6.0 μm, collapsing and disappearing at maturity. Basidioles measure 12.0–14.5 × 5.5–7.5 μm.

Basidiospores long-ellipsoid, narrowly rounded and blunt top, with a truncated base showing a circular depression, initially hyaline, becoming hyaline to light brown (5C4) at maturity, yellow-brown (5D8) in mass, dimension ranges (5.0–) 6.0–8.5 (–10.0) × 2.0–3.0 μm, Q (L/I) = 2.42–2.94, Q_m = 2.75 ± 0.18 (n = 50), smooth, thin-walled, sometimes with 1–3 guttulate, non-amyloid in Melzer's reagent.

Ecology & Distribution: Hypogeous, or semi-embedded in the soil, under *Pinus yunnanensis* forest, alt. 2700m, fruiting in autumn. Known only from northwestern Yunnan Province, China.

Notes: The phylogenetic tree of the genus *Rhizopogon* based on combined ITS and nrLSU analysis (Figure 4.23) shows that *Rhizopogon laojunshanensis* is supported as an independent species with high confidence. This species belongs to the *Rhizopogon* subgenus *Roseoli* and forms a sister clade with *R. jiyaozi*, with a similarity of 96.25%. Morphologically, *R. laojunshanensis*, apart from its peridium not changing color when injured, shares similar macrocharacteristics with other species in the subgenus *Roseoli*. Only two species from China belong to the subgenus *Roseoli*: *R. jiyaozi* and the new species described in this study, *R. laojunshanensis*. These two species are closely related, both distributed in southwestern China and forming a symbiotic relationship with *Pinus yunnanensis*. Among them, *R. jiyaozi* is the widely distributed and primarily consumed species of *Rhizopogon* in the region. However, the two species show clear morphological differences. The peridium of *R. jiyaozi* discolorates to rose-pink when cut or bruised and is composed of interwoven hyaline hyphae with red-brown pigment, while the peridium of *R. laojunshanensis* shows no color change when bruised, composed of hyaline to pale brown (4C4) hyphae interwoven, with some hyphal walls attached to dark-colored granules. Additionally, the basidiomata of *R. laojunshanensis* are noticeably smaller than those of *R. jiyaozi*.

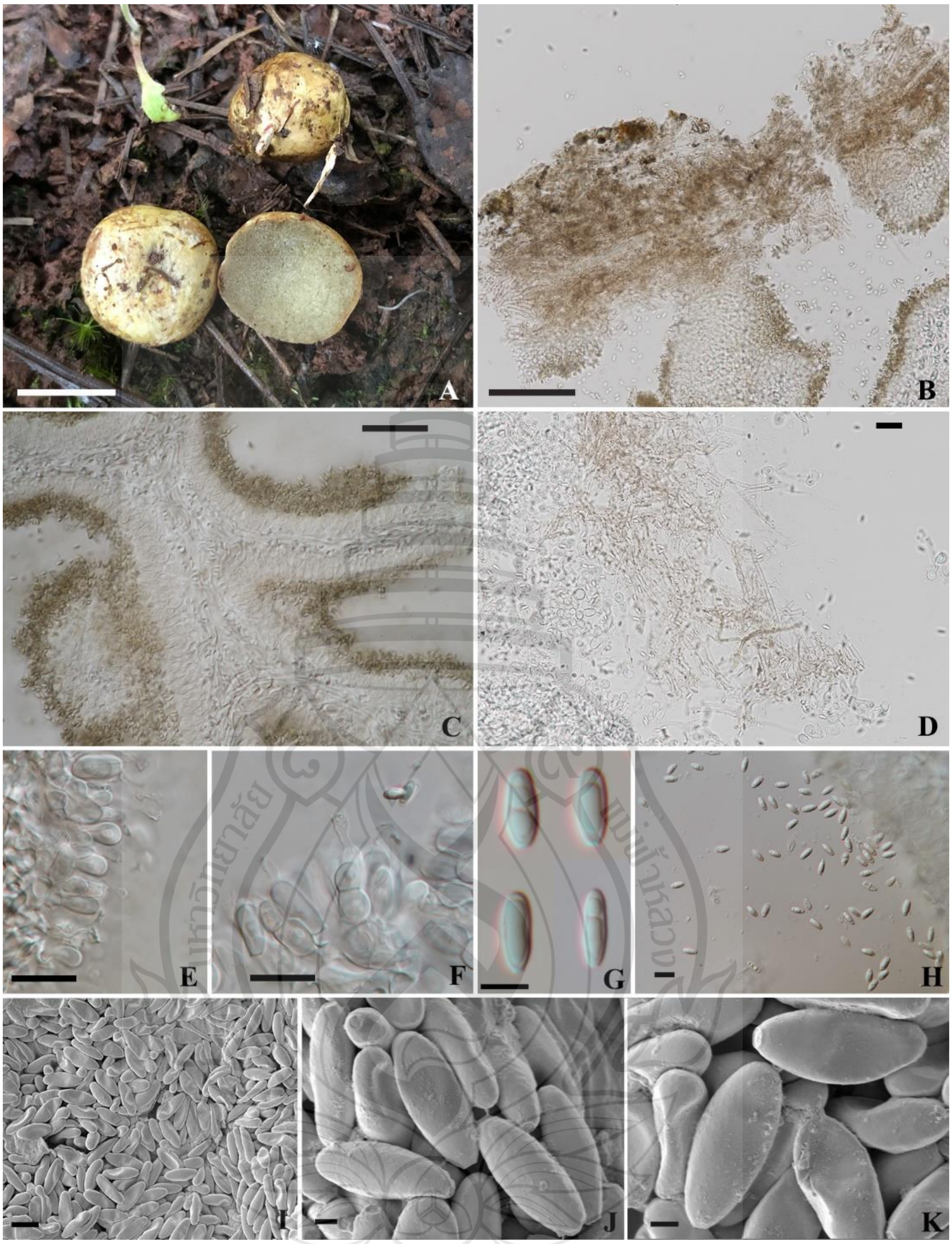


Figure 4.24 *Rhizopogon laojunshanensis* morphological characteristics

Figure 4.24 A. Basidiomata and gleba; B. A piece of the section of the basidiomata in 5% KOH; C. Trama in 5% KOH; D. Hyphae composing the peridium; E.F. Basidia and basidioles in 5% KOH; G.H. Basidiospores; I.J.K. SEM ascospores (dry sample). Scale bars: A = 1cm; B-C = 100μm; D = 10μm; E-F = 20μm; J.I. = 5μm; H=1μm; J.K.=1μm.

Rhizopogon melanocuticularis L. Li, K.D. Hyde & S.H. Li, sp. nov. Figure 4.25

Mycobank number: MB 860404

Diagnosis: Differs from other *Rhizopogon* spp. by its basidiomata gray-brown (4E8) to gray-black (2F4), and its surface covered with a fine, dense, gray-black (2F4) rhizomorph resembling a spider's web. The odor is distinct. The peridium is composed of interwoven hyaline to yellow-brown (5E5) hyphal strands and hyphae, with no color change upon exposure to KOH. Basidiospores are hyaline to light brown, measuring 7.0–9.5 × 2.5–3.5 μm.

Etymology: *melanocuticularis*, refers to the basidiomata surface being gray-black in color.

Holotype: China, Yunnan province, Weixi county, 27°13′ N 99°17′ E, alt. 2484 m, in soil under forest of *Pinus yunnanensis* Franch., 19 May 2022, Shuhong Li, BMDU L22409 (GenBank: ITS = PX096564, LSU = PX096594).

Basidiomata are globose to subglobose, 1.0–3.5 cm in diameter, slightly soft and elastic, gray-brown (4E8) to gray-black (2F4), darkening to black after prolonged exposure or drying. The surface is covered with fine, dense, gray-black (2F4) rhizomorphs resembling a spider's web, and there is no color change upon contact with KOH, not easily fall off. The odor is distinctive.

Gleba spongy, composed of densely arranged irregular hollow chambers, numerous and labyrinthine, white when immature, turning pale yellow-brown (1D5) at maturity, with no color change upon cutting. Trama composed of interwoven hyaline gelatinized hyphae, 50–110 μm wide.

Peridium 180–330 μm thick, easily detached, composed of interwoven hyaline to yellow-brown (5E5) hyphal strands and hyphae, 6–10 μm in diam., with abundant brown extracellular pigment deposits, giving the peridium an overall dark brown (5F8) appearance, no color change upon exposure to KOH. Clamp connections are absent.

Basidia clavate or cylindrical, thin-walled, colorless, commonly 6–8-spored, 15.5–19.5 × 4.0–6.5 μm, collapsing and disappearing at maturity.

Basidiospores long-ellipsoid or subcylindrical, rounded and blunt top, with a truncated base from sterigma, individual spores hyaline, pale brown (5C4) in mass, measuring 7.0–9.5 (–10.0) × 2.5–3.5 μm, Q (L/I) = 2.38–3.64, Q_m = 3.13 ± 0.34 (n = 50), smooth, thin-walled, and non-amyloid in Melzer's reagent.

Ecology & Distribution: Hypogeous, under *Pinus yunnanensis* forest, alt. 2400–3000m, fruiting in summer. Known only from northwestern Yunnan Province, China.

Additional specimen examined: China, Yunnan province, Weixi county, 27°13′ N 99°17′ E, alt. 2484 m, 19 May 2022, Shuhong Li, BMDU L22410 (GenBank: ITS = PX096565, LSU = PX096595); 27°09′ N 99°15′ E, alt. 2994 m, 8 June 2023, Lin Li, BMDU L23201 (GenBank: ITS = PX096566), BMDU L23203 (GenBank: ITS = PX096567).

Notes: The phylogenetic tree of the genus *Rhizopogon* based on combined ITS and nrLSU analysis (Figure 4.23) shows that *Rhizopogon melanocuticularis* is supported as an independent species with high confidence. Additionally, *Rhizopogon melanocuticularis* belongs to the *Rhizopogon* subgenus *Amylopogon* and forms a sister clade with *R. sinoalbidus*, sharing a similarity of 97.8%. *R. melanocuticularis* appears in small quantities in the wild mushroom markets of Weixi County, typically mixed with other *Rhizopogon* species to sell, such as *R. jiyaozi*, *R. sinoalbidus*, and *R. weixiensis*.

Compared to its closely related species *R. sinoalbidus*, *R. melanocuticularis* has basidiomata that are gray-brown to gray-black in color, becoming almost black when dried. The surface is covered with fine, dense, gray-black rhizomorphs. The peridium is thinner, measuring 180–330 μm thick, composed of interwoven yellow-brown hyphae, which appear dark brown in mass, with no color change when exposed to KOH. The odor is distinctive. Basidiospores are longer, measuring 7.0–9.5 (–10.0) × 2.5–3.5 μm, with a Q_m of 3.13. In contrast, *R. sinoalbidus* has basidiomata that are dirty white, pale gray to pale dingy, turning brown when dried. The surface is loosely covered with white to dingy white rhizomorphs. When KOH is applied to the dried brown basidiocarp surface, it turns pink, soon followed by black. The peridium is thicker, ranging from 500–700 μm, with abundant pink to gray or deep blue extracellular pigment deposits when exposed to KOH. The odor is not distinctive. Basidiospores measuring 6.5–7.5 × 2.5–3.0 μm, with a Q_m of 2.78. While *R. sinoalbidus* is widely distributed in the southwestern region, *R. melanocuticularis* has only been found in Weixi County to date.

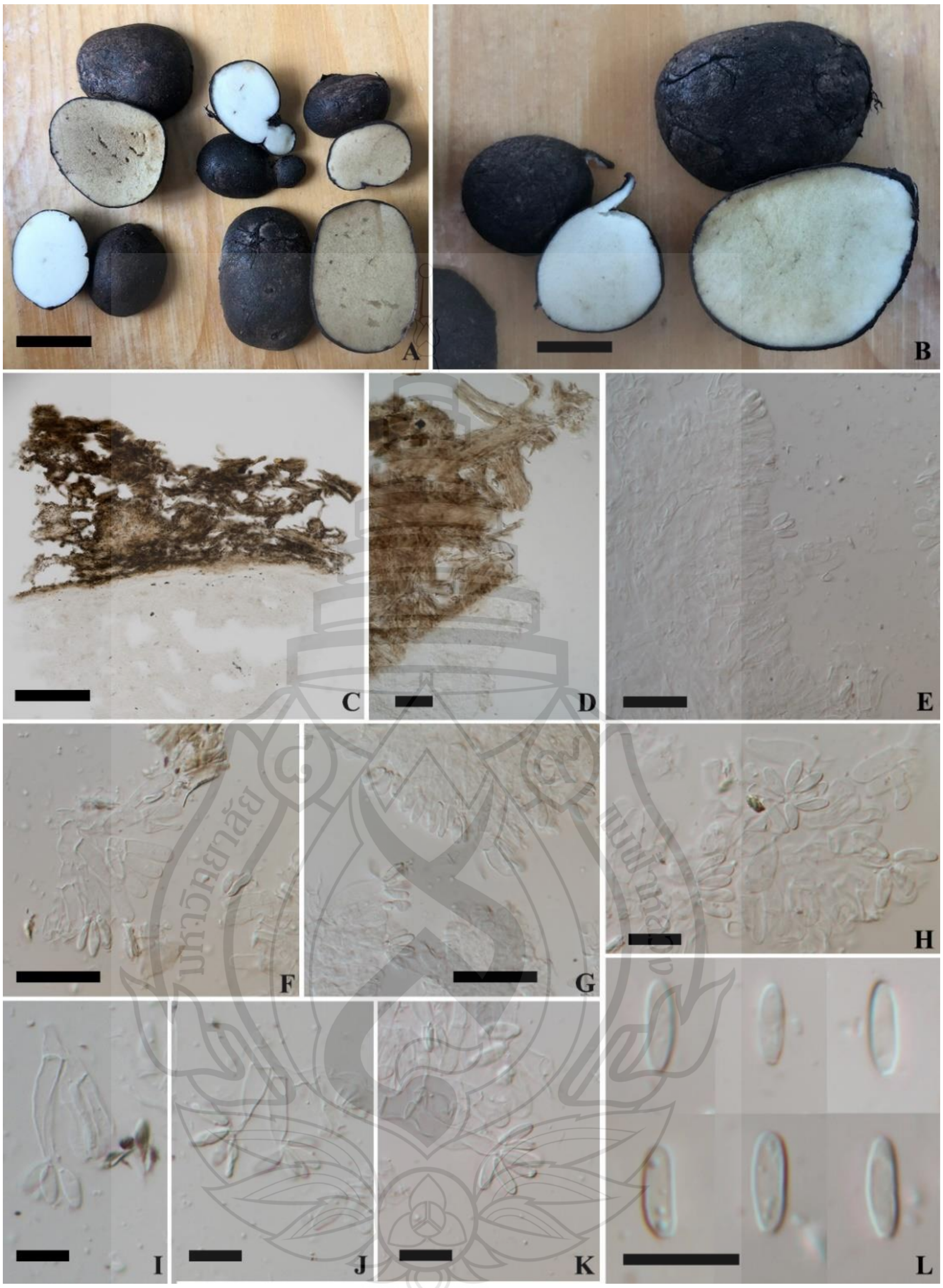


Figure 4.25 *Rhizopogon melanocuticularis* morphological characteristics

Figure 4.25 A.B. Basidiomata and gleba; C. A piece of the section of the basidiomata in 5% KOH; D. Hyphal strands and hyphae composing the peridium in 5% KOH; E.F.G.H. Hymenium in 5% KOH; I.J.K. Basidia and basidiospores; L.Basidiospores. Scale bars: A-B = 1cm; C = 50μm; D-G = 20μm; H-L = 10μm.

Rhizopogon zayuensis L. Li, K.D. Hyde & S.H. Li, sp. nov. Figure 26

Mycobank number: MB 860405

Differs from other *Rhizopogon* spp. by its basidiomata dirty white (2B1), turning gray-black (8F4) to brown-black (8F8) when damaged, surface of the dried basidiomata turns deep green to grayish-black (27F7) upon contact with KOH. The basidiomata surface is covered with sparse, fine white (2B1) rhizomorphs. The peridium is composed of interwoven hyaline to yellow-brown (5E5) hyphae, which produce a blue-green (25E7) pigment upon contact with KOH. Basidiospores are hyaline to tea-brown (5E8), measuring 5.5–6.5 × 2.0–3.5 μm.

Etymology: *zayuense*, refers to the type locality of the Zayu County, Xizang Province, China.

Holotype: China, Xizang province, Zayu county, 28°41′ N 97°27′ E, alt. 2420 m, in soil under forest of *Pseudotsuga forrestii* Craib, 9 August 2022, Lin Li, BMDU L22031 (GenBank: ITS = PX096568, LSU = PX096596).

Basidiomata are globose to subglobose, 1.0–1.5 cm in diam., rubbery, dirty white (2B1), turning gray-black (8F4) to brown-black (8F7) when damaged, surface of the dried basidiomata turns deep green to grayish-black (27F7) upon contact with KOH. The basidiomata surface is covered with sparse, fine white (2B1) rhizomorphs. The odor is not distinctive.

Gleba spongy, composed of densely arranged irregular hollow chambers, numerous and labyrinthine, white when immature, turning olive brown (28E6) at maturity, with no color change upon cutting. Trama composed of interwoven hyaline gelatinized hyphae, 50–80 μm wide.

Peridium 80–150 μm thick, composed of interwoven hyaline to yellow-brown (5E5) hyphae, 2–3 μm in diam. Brown-black (8F7) pigment patches are distributed between the hyphae, which partially dissolve into blue-green (25E7) pigment upon contact with KOH, typically on the side near the gleba.

Basidia clavate or cylindrical, thin-walled, colorless, commonly 4–6-spored, 16.0–18.5 × 3.0–5.5 μm, collapsing and disappearing at maturity.

Basidiospores ellipsoid, rounded and blunt top, with a truncated base showing a circular depression, individual spores hyaline, pale brown (5C4) in mass, measuring 5.5–6.5 (–7.5) × 2.0–3.5 μm, Q (L/I) = 1.70–2.16, Q_m = 1.90 ± 0.11 (n = 50), smooth, thin-walled, sometimes with 1–2 guttulate, and non-amyloid in Melzer's reagent.

Ecology & Distribution: Hypogeous, under *Pseudotsuga forrestii* forest, alt. 2420m, fruiting in summer. Known only from southeastern Xizang Province, China.

Additional specimen examined: China, Xizang province, Zayu county, 28°41′ N 97°27′ E, alt. 2420 m, in soil under forest of *Pseudotsuga forrestii*, 9 August 2022, Lin Li, BMDU L22032 (GenBank: ITS = PX096569, LSU = PX096597).

Notes: The phylogenetic tree of the genus *Rhizopogon* based on combined ITS and nrLSU analysis (Figure 4.23) shows that *Rhizopogon zayuensis* is supported as an independent species with high confidence. Additionally, *Rhizopogon zayuensis* belongs to the *Rhizopogon* subgenus *Villosuli* and forms a unique evolutionary clade with *Rhizopogon togasawarius* Mujic, K. Hosaka and Spatafora (2014) together with similarity to other species of less than 98.1%. *R. zayuensis* is the only species of this subgenus found in China and is the second species of *R.* subg. *Villosuli* was discovered in Asia, after *R. togasawarius* (Mujic et al., 2014). The phylogenetic tree also indicates a close relationship between the two, and this clade, which contains only Asian species, is independent of the two established sections of *R.* subg. *Villosuli*, namely *R.* sect. *Villosuli* and *R.* sect. *Vinicolores* (Grubisha et al., 2002).

Rhizopogon zayuensis is not only classified within *R.* subg. *Villosuli* is based on molecular phylogenetic analysis, but it also aligns morphologically with the key characteristics of members of *R.* subg. *Villosuli* (Grubisha et al., 2002). Of course, *R. zayuensis* also possesses its unique features. The basidiomata of *R. zayuensis* are dirty white (2B1), turning gray-black (8F4) to brown-black (8F7) when damaged, and the surface of the dried basidiomata turns deep green to grayish-black (27F7) when exposed to KOH. The peridium is composed of interwoven hyaline to yellow-brown (5E5) hyphae, with brown-black (8F7) pigment masses distributed between the hyphae. Upon contact with KOH, part of the pigment dissolves into a blue-green (25E7) pigment. The basidiospores measure 5.5–6.5 (–7.5) × 2.0–3.5 μm. Compared to *R. togasawarius,* the basidiomata of *R. togasawarius* are white, quickly staining brown to dull vinaceous red upon disturbance, stains fading to greenish brown with patches of red persisting. Drying to a vinaceous red. Peridium with a subcutis of hyaline yellowish hyphae 2.2–5.7 mm diam. And oriented parallel to the surface of the peridium. Patches of vinaceous pigment are abundant between subcutis hyphae in dried specimens, often obscuring detail. Subcutis with sparse, embedded black granules that are visible in H_2O and dissolve into a green pigment in KOH. The basidiospores measure 6.5–8.6 × 2.5–3.5 μm.

It is worth noting that *Pseudotsuga menziesii* (Douglas-fir) has long been considered the only known plant with which members of *R.* subg. *Villosuli* form ectomycorrhizal associations. This host specificity is unique within the *Rhizopogon* genus, as *Rhizopogon* forms symbiotic relationships with several tree genera within the Pinaceae, and the relationships with host genera are usually distributed across different subgenera of *Rhizopogon* (Massicotte et al., 1994; Molina et al., 1997; Grubisha et al., 2002). Of course, the reason might be that natural specimens described from *R.* subg. *Villosuli* have only been collected from North America, where *Pseudotsuga menziesii* is also one of the dominant groups. Prior to this, the unique symbiotic relationship between *R.* subg. *Villosuli* and *Pseudotsuga menziesii* were questioned due to the report of *R. togasawariana*, a member of *R.* subg. *Villosuli* from Japan that form ectomycorrhizal association with *Pseudotsuga japonica* (Shiras:) Beissn. (Mujic et al., 2014). The new species described in this paper, *R. zayuensis*, from *Pseudotsuga forrestii* Craib forest in Zayu County, Xizang Province, China, also supports the idea that members of *R.* subg. *Villosuli* have other ectomycorrhizal host trees.

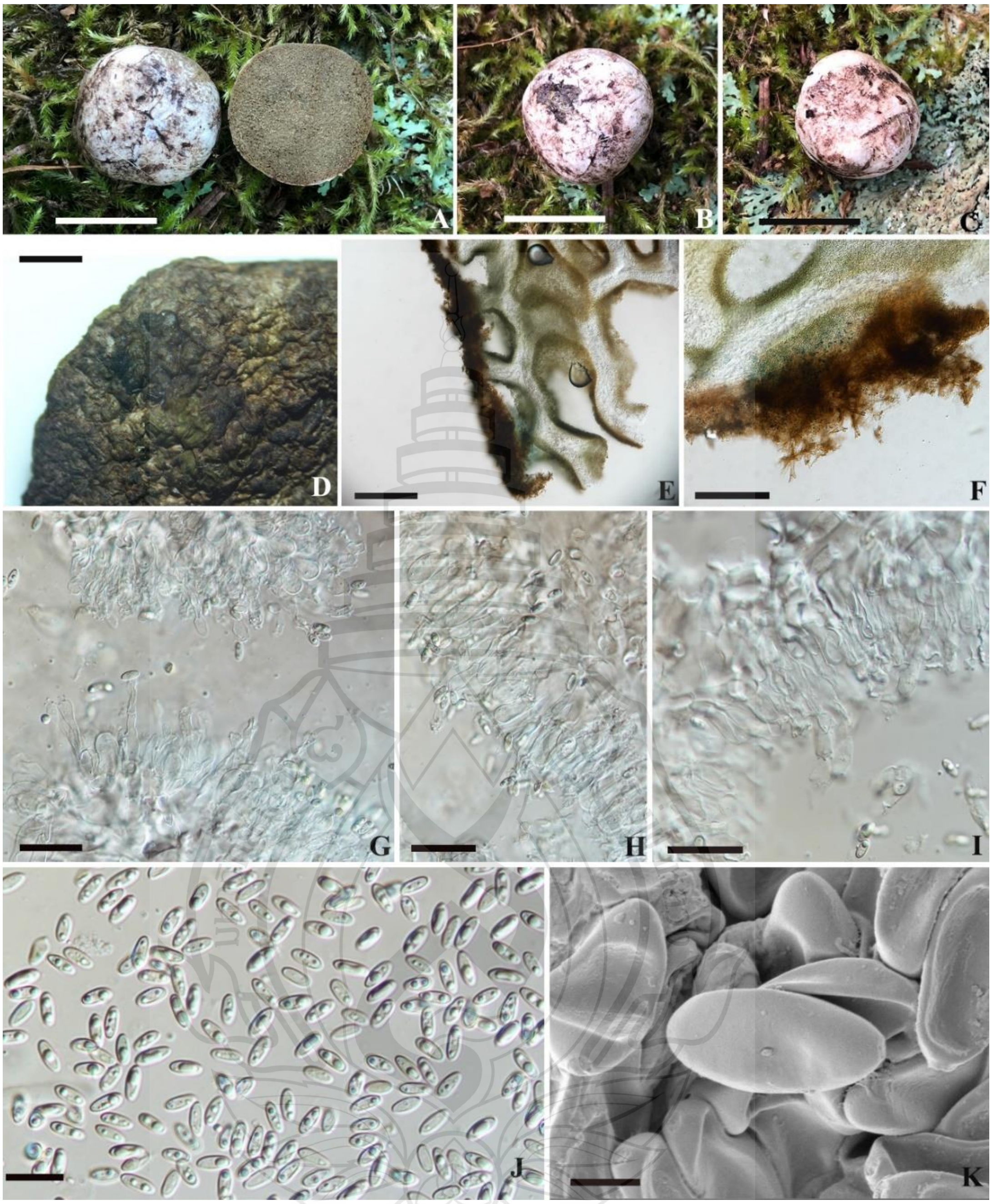


Figure 4.26 *Rhizopogon zayuensis* morphological characteristics

Figure 4.26 A.B.C. Basidiomata; D. Surface of the dried basidiomata turns deep green to grayish-black upon contact with 5% KOH; E.F. A piece of the section of the basidiomata and hyphae composing the peridium in 5% KOH; G.H.I. Hymenium and basidia; J. Basidiospores in 5% KOH; K. SEM ascospores (dry sample). Scale bars: A-C = 1cm; D = 1mm; E = 200μm; F = 100μm; G-I. = 20μm; J = 10μm; K = 2μm.

Rhizopogon weixiensis L. Li, K.D. Hyde & S.H. Li, sp. nov. Figure 4.27

Mycobank number: MB 860406

Differs from other *Rhizopogon* spp. by its basidiomata khaki or pale yellow-brown (5D6), turning red to blood-red (10B8) when damaged or cut, and darkening to reddish-brown (10E8) after some time, fresh basidiomata surface turns rose-pink to red (11B8) upon contact with KOH. Peridium 300–380 μm thick, composed of nearly parallel-arranged and interwoven hyaline to pale brown hyphae, with a large amount of yellowish-ocher (6E8) pigment concentrated on the outer side of the peridium, gradually decreasing towards the inner side, and remaining unchanged in color in KOH solution.

Etymology: weixiensis, refers to the type locality of the Weixi County, Yunnan Province, China.

Holotype: China, Yunnan province, Weixi county, 27°13′ N 99°17′ E, alt. 2493 m, in soil under forest of *Pinus yunnanensis*, 8 May 2022, Lin Li, BMDU L22408 (GenBank: ITS = PX096577, LSU = PX096605).

Basidiomata are globose to subglobose, 1.0–4.5 cm in diam., rubbery, sometimes with a distinct depression resembling the base, though not always present. The basidiomata khaki or pale yellow-brown (5D4), turning red to blood-red (10B8) when damaged or cut, and darkening to reddish-brown (10E8) after some time; the color change is more pronounced in immature basidiomata. The surface of fresh basidiomata turns rose-pink to red (11B8) upon contact with KOH. The rhizomorphs are pale yellow-brown (5D6) and not abundant. Odor not distinctive.

Gleba spongy, composed of densely arranged irregular chambers, numerous and labyrinthine, white when immature, becoming yellowish brown (4D5) at maturity, with no color change upon cutting. Trama composed of interwoven hyaline gelatinized hyphae, 50–100 μm wide.

Peridium 300–380 μm thick, composed of nearly parallel-arranged and interwoven hyaline to pale brown (6B2) hyphae, 3–5 μm in diam., with numerous yellowish-ocher (6E8) pigments on the outer side of the peridium, gradually decrease in size and become less frequent towards the inner side, eventually disappearing, and remaining unchanged in color in 5% KOH solution.

Basidia clavate or cylindrical, thin-walled, colorless, commonly 4–6-spored, 21.0–24.0 × 4.5–5.5 µm, collapsing and disappearing at maturity.

Basidiospores ellipsoid, rounded and blunt top, with a truncated base showing a circular depression 0.5 µm in diam., individual spores hyaline, pale brown (5D4) in mass, measuring 5.5–7.5 × 2.0–3.5 µm, Q (L/I) =2.14–2.87, Q_m = 2.51 ± 0.20 (n = 50), smooth, thin-walled, sometimes with 1–2 guttulate, and non-amyloid in Melzer's reagent.

Ecology & Distribution: Hypogeous, under *Pinus yunnanensis* forest, alt. 2200–2900m, fruiting in early summer. Known only from northwestern Yunnan Province, China.

Additional specimen examined: China, Yunnan province, Weixi county, 27°12′ N 99°17′ E, alt. 2392 m, on soil under forest of *Pinus yunnanensis*, 9 May 2022, Lin Li, BMDU L22401 (GenBank: ITS = PX096570, LSU = PX096598), BMDU L22402 (GenBank: ITS = PX096571, LSU = PX096599), BMDU L22403 (GenBank: ITS = PX096572, LSU = PX096600); 27°10′ N 99°17′ E, alt. 2272 m, on soil under forest of *Pinus yunnanensis*, 9 May 2022, Lin Li, BMDU L22404 (GenBank: ITS = PX096573, LSU = PX096601), BMDU L22405 (GenBank: ITS = PX096574, LSU = PX096602), BMDU L22406 (GenBank: ITS = PX096575, LSU = PX096603); 27°11′ N 99°21′ E, alt. 2820 m, on soil under forest of *Pinus yunnanensis*, 9 May 2022, Lin Li, BMDU L22407 (GenBank: ITS = PX096576, LSU = PX096604).

Notes: The phylogenetic tree of the genus *Rhizopogon* based on combined ITS and nrLSU analysis (Figure 4.23) shows that *Rhizopogon weixiensis* is supported as an independent species with high confidence. Additionally, *Rhizopogon weixiensis* belongs to the *Rhizopogon* subgenus *Versicolores* (Grubisha et al., 2002), with similarity to other species less than 97.89%. *R. weixiensis* is the second species of *R.* subg. *Versicolores* in China with molecular data support, following *R. songmaodan* R. Wang & Fu Q. Yu 2021 (Wang et al., 2021). *Rhizopogon weixiensis* is one of the commonly found edible mushrooms in the wild mushroom market of Weixi County, with the highest yield occurring from late April to June each year, gradually decreasing thereafter and continuing until early July. It has only been reported in the areas surrounding Weixi County. Due to the similar appearance of the basidiomata and their comparable habitats, *R. weixiensis* is often mixed with *R. jiyaozi*, *R. melanocuticularis*, and *R. sinoalbidus* in the market for sale.

Rhizopogon weixiensis exhibits typical characteristics of *R.* subg. *Versicolores*, such as basidiomata surfaces staining pink to red when bruised or cut, and the peridium composed of interwoven hyphae rather than hyphal strands (Grubisha et al., 2002). However, there are exceptions. One typical feature of *R.* subg. *Versicolores* is that the peridium lacks yellow coloration at all stages of development (Smith & Zeller, 1966). Similarly, another species of *R.* subg. *Versicolores* found in Yunnan, *R. songmaodan*, has a white peridium (Wang et al., 2021). The peridium of *R. subsalmonius* and *R. bacillisporus* (Smith & Zeller, 1966), which are phylogenetically close to *R. weixiensis* on the phylogenetic tree, is also white. However, the peridium of *R. weixiensis* is khaki or pale yellow-brown (5D6).

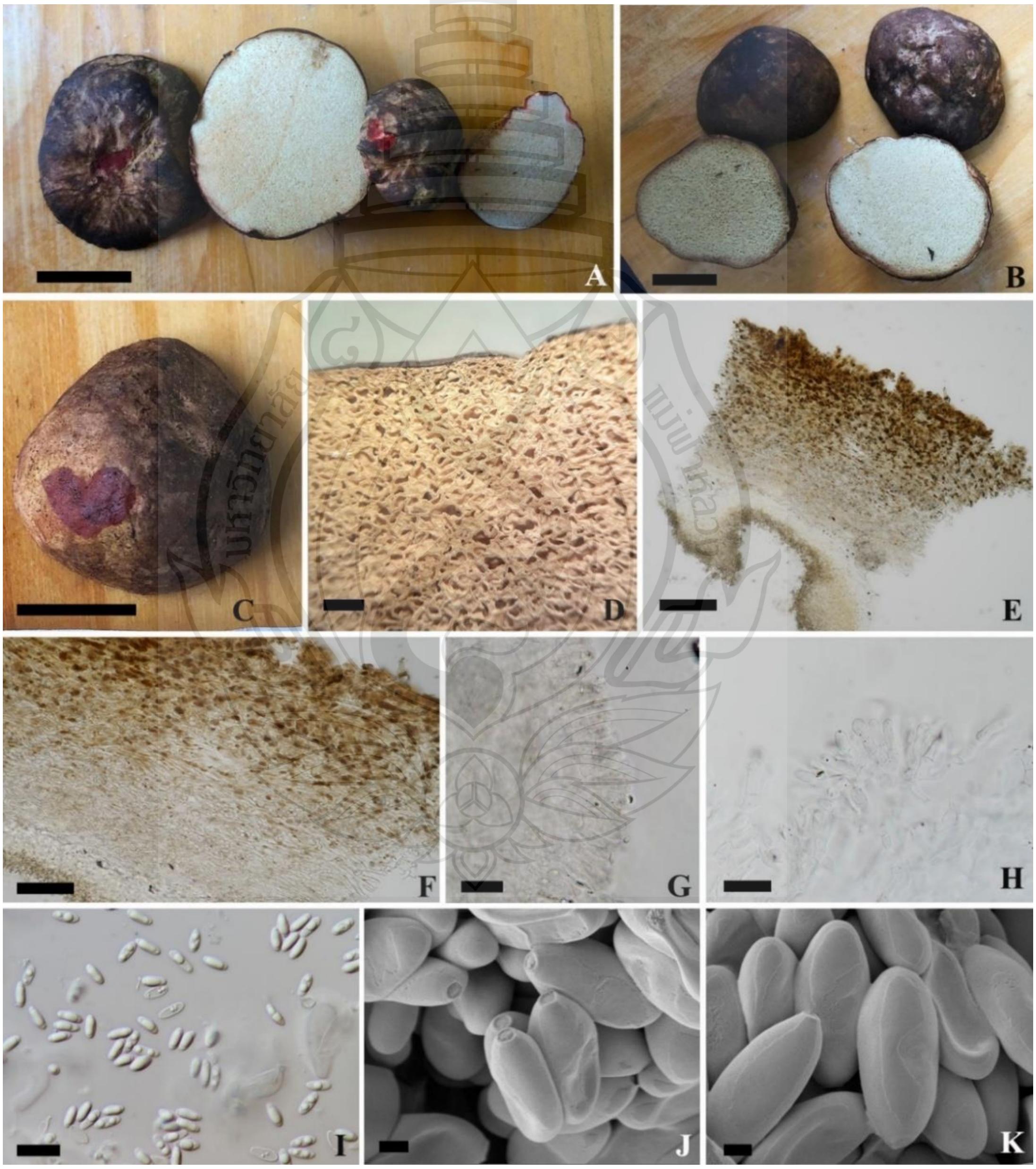


Figure 4.27 *Rhizopogon weixiensis* morphological characteristics

Figure 4.27 A.B. Basidiomata; C. Surface of the dried basidiomata turns rose-pink to red upon contact with 5% KOH; D. Gleba; E. A piece of the section of the basidiomata; F. Hyphae composing the peridium in 5% KOH; G.H. Hymenium and basidia; I. Basidiospores in 5% KOH; J. K. SEM ascospores (dry sample). Scale bars: A-C = 1cm; D = 0.2mm; E = 100μm; F = 50μm; G.H. = 20μm; I = 10μm; J.K. = 1μm.

4.2.10 *Russula*

4.2.10.1 Introduction

The species within the *Russula* genus that form hypogeous basidiomata are characterized by basidiomata that are subglobose to irregularly globose, with a depressed base, and either lack a stipe or possess a degenerate, short stipe. The gleba is ochraceous, labyrinthic, or loculate, with loculae being crowded and sometimes compacted. Basidiospores are globose to subglobose, with ornamentation consisting of isolated spines or spines connected at the base (Elliott & Trappe, 2018). Genera that were once recognized as separate to accommodate hypogeous species, such as *Bucholtzia*, *Cystangium*, *Elasmomyces*, *Gymnomyces*, *Macowanites*, and *Martellia*, have all been incorporated into the genus *Russula* based on molecular phylogenetic studies (Elliott & Trappe, 2018).

According to statistics, there are currently 8+3 species are now known in China (Zhang & Yu, 1990; Tao et al., 1993; Liu, 1998; Sang et al., 2016), among which 6+3 species are distributed in the southwest region: *Russula zangii* in Yunnan, *R. chlorineolens* and *R. gilkeyae* in Xizang, *R. brevipileocystidiata* and *R. megapseudocystidiata* in Sichuan, *R. absphaerocellaris* in Yunnan and Sichuan province. (Zhang & Yu, 1990; Sang et al., 2016; Xu et al., 2019)

4.2.10.2 Sequence Alignment and Analysis

The internal transcribed spacer (ITS) and nuclear large subunit ribosomal RNA gene (LSU) regions were used in this study to analyze species diversity within the genus *Russula*, as these loci have proven effective for species delimitation in this group. A total of 45 ITS and 27 LSU sequences—comprising both newly generated data and sequences retrieved from NCBI—were analyzed. These sequences represent 44 species of *Russula* (Table 4.13). The analytical methods followed those described in the Materials and Methods section. Phylogenetic clades were considered strongly supported if they exhibited a bootstrap support (BS) value ≥ 70% and/or a posterior probability (PP) ≥ 0.90.

Table 4.13 Taxa information and GenBank accession numbers of the sequences used in *Russula* study

Species Name	**Voucher**	**GenBank No. ITS**	**GenBank No. LSU**
Cystangium domingueziae	Trappe26311	KF819811	
Cystangium gamundiae	Trappe26316	KF819810	
Cystangium longisterigmatum	Trappe26350	KF819808	
Cystangium seminudum	H5346	EU019947	
Cystangium sessile	H5038	EU019948	EU019948
Cystangium theodoroui	SLM43I84	DQ403804	
Gloeocystidiellum aculeatum	Wu890714-52	AF506433	AF506433
Gymnomyces abietis	Trappe 16022	AY239347	
Gymnomyces californicus	Trappe 16027	AY239308	
Gymnomyces compactus	Trappe 13565	AY239342	
Gymnomyces monosporus	OSC 61382	KP859259	KP859279
Gymnomyces redolens	SLM42I62	DQ403803	
Gymnomyces setigerus	OSC 29622	AY239317	
Gymnomyces subalpinus	OSC 56196	AY239309	
Gymnomyces xerophilus	SRC648	DQ028476	
Lactarius spinosporus	BJTC FAN445 (holotype)	KY270490	
Russula adulterina	489RUS25	AY061651	
Russula absphaerocellaris	BJTC FAN492 (holotype)	KY270486	KY270493
Russula absphaerocellaris	BJTC FAN448 (paratype)	KY270489	
Russula densiobtusispora	**L21195**	**This study**	**This study**
Russula densiobtusispora	**L21196**	**This study**	**This study**
Russula brevipileocystidiata	BJTC FAN455 (holotype)	KY270487	KY270492
Russula cessans	525/07.219	JN944011	JN940595
Russula cuprea	FH12250	KT934010	KT933871
Russula curtipes	GENT:FH-12-206	KT933995	KT933856
Russula decolorans	1-502IC57(TUB), hue39(TUB)	AY061670	AF325302

Table 4.13 (continued)

Species Name	**Voucher**	**GenBank No. ITS**	**GenBank No. LSU**
Russula emetic	517IS76(TUB), lw81(TUB)	AY061673	AF325305
Russula exalbicans	nl79/93 (TUB)	AF418622	AF325306
Russula fellea	316RUS25(TUB), hue114(TUB)	AY061676	AF325307
Russula kalimna	MEL2238306	EU019927	EU019927
Russula laojunshanensis	**L21200**	**This study**	**This study**
Russula laricina	575/08.681	JN944008	JN940593
Russula lilacea	435/07.213	JN944005	JN940592
Russula mairei	lw113 (TUB), hue54 (TUB)	AF418620	U11926
Russula megapseudocystidiata	BJTC FAN454 (holotype)	KY270488	KY270491
Russula olivacea	523IC55(TUB), hue85(TUB)	AY061699	AF325314
Russula persanguinea	MEL2101880	EU019916	EU019916
Russula purpureoflava	MEL2101898	EU019917	JX266641
Russula queletii	510RUF26(TUB),fo46861(TUB)	AY061711	AF325316
Russula rubrolutea	Trappe 12610	EU019940	EU019940
Russula sichuanensis	53792	JX391969	
Russula tapawera	Trappe 12607	EU019935	EU019935
Russula violacea	322IS55, 93009EL	AY061725	AF218559
Zelleromyces hispanicus	MA-Fungi 37497	AF231911	
Zelleromyces hispanicus	MA-Fungi 37498	AF231912	

Note The newly generated sequences are in bold.

4.2.10.3 Results

Phylogenetic Analysis

The ML and Bayesian analyses of the 45 ITS and 27 LSU sequences are shown in Figure 28 with associated bootstrap supports for branches.

In the phylogenetic tree, the 45 ITS and 27 LSU sequences from *Russula* revealed the phylogenetic relationship of 44 species, and used representatives of *Zelleromyces hispanicus*, *Zelleromyces hispanicus* and *Lactarius spinsporus* as outgroups. The phylogenetic analysis showed that the new species is clearly distinct from other species of the genus *Russula.*

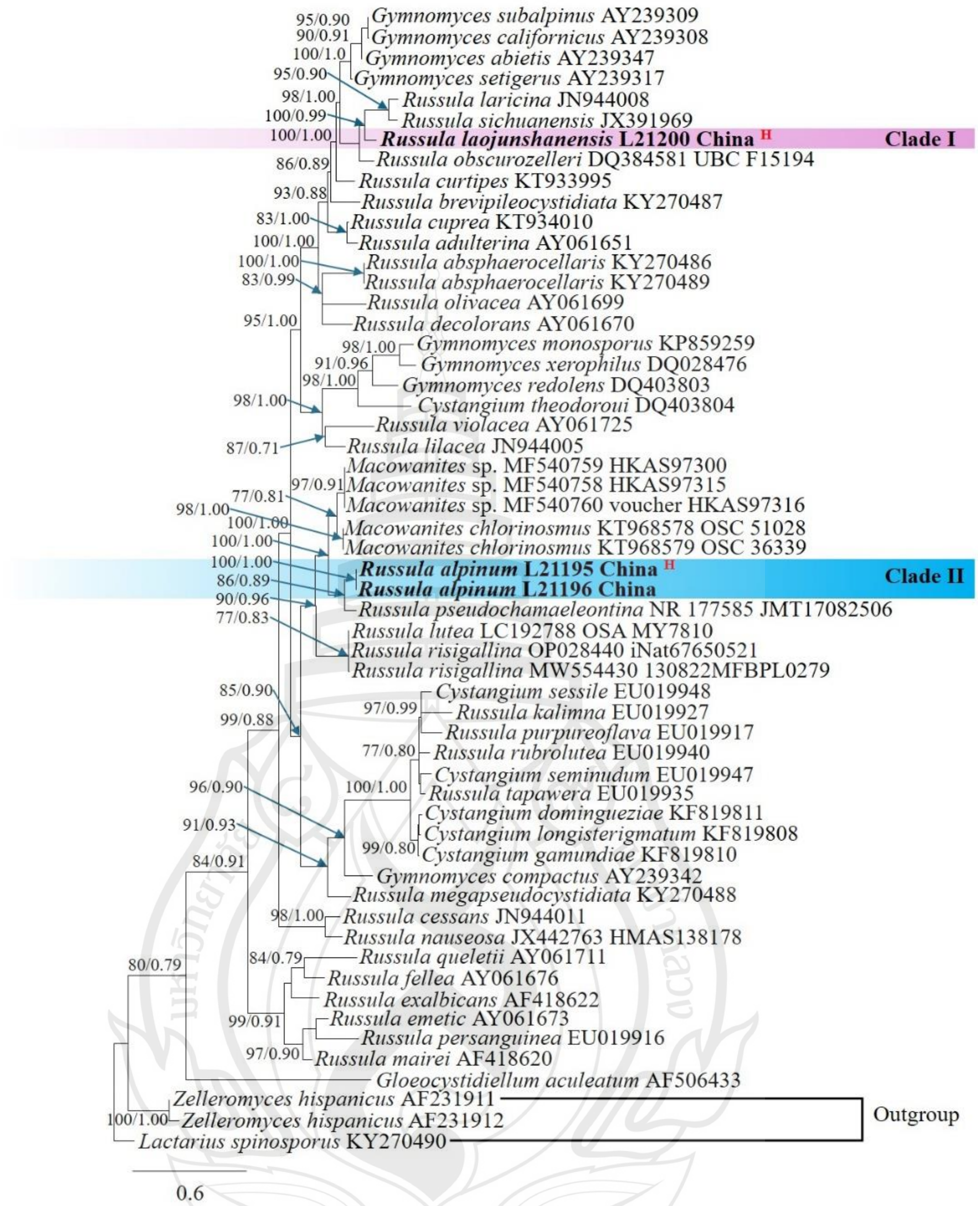


Figure 4.28 Phylogeny derived from a maximum likelihood (ML) analysis of the nrDNA-ITS and LSU sequences from *Russula* species

Figure 4.28 Values next to nodes reflect maximum likelihood bootstrap support values (BS), left, and Bayesian posterior probabilities (PP), right. Names of novel species and samples with newly generated sequences are in bold.

Russula densiobtusispora L. Li, K.D. Hyde & S.H. Li, sp. nov.Figure 29

Mycobank number: MB 860409

Diagnosis: Differs from other *Russula* species by its hypogeous, gasteroid basidiomata, 0.8– 1.0 × 1.2 cm in size, with a short stipe, slightly bulbous at the base, finely hairy, measuring 1.0– 2.0 × 2.0– 3.0 mm; basidiospores globose, hyaline, up to 8–10(–12) μm in diameter, ornamented with isolated, curved, clavate, blunt spines, densely distributed, 2–3 μm long.

Etymology: densiobtusispora, referring to spores with echinulate ornamentation, composed of isolated, curved, clavate, blunt spines, densely distributed, 2–3 μm long.

Holotype: CHINA, Yunnan, Mt. Laojunshan (26 °63N 99 °72E), alt. 3875.7 m, in the forest of *Abies forrestii* var. *smithii* and *Rhododendron* sp. mixed forest, 19 Sep. 2021, Lin Li, BMDU L21195 (GenBank: ITS=PX106474, LSU=PX105442).

Basidiomata 0.8–1.0 × 1.2 cm, subglobose to irregularly globose, depressed at the base, with a short stipe protruding from the base. Peridial surface light cinnamon (6B3), glabrous when mature, slightly hairy in immature specimens. Gleba white to pale khaki (5B6), labyrinthine or loculate; loculae compact and crowded. Odour indistinct.

Stipe short, slightly bulbous at the base, finely hairy, light cinnamon (6B3), 1.0–2.0 × 2.0–3.0 mm, extending into the gleba to form a white, columnar columella that continues upward through the gleba to the apex. The stipe is composed of interwoven, hyaline hyphae; the outermost cells contain light brown (4B6) pigment and abundant cystidia-like outer hyphae, hyaline, 2.0–3.0 μm in diameter, irregularly arranged, either interwoven or prostrate, straight or curved, clavate, blunt-tipped, aseptate.

Peridium 80–140 μm thick, composed of two layers. The outer layer is pseudoparenchymatous, 40–80 μm thick, consisting of subglobose to subangular, hyaline, thin-walled cells measuring 4.0–10.0(–13.0) μm wide; cells of the outermost layer contain light brown (4B6) pigment. Some of these outermost cells expand into cystidia-like outer hyphae, 1.5–3.0 μm in diameter, irregularly arranged, interwoven or prostrate, stubby, blunt-tipped, septate, and commonly found in immature basidiomata, but only occasionally observed in mature specimens. The inner layer is composed of interwoven, hyaline hyphae, 40–60 μm thick; the transition between the two layers is gradual, with the outer layer cells becoming smaller toward the inner layer. The peridium thickens at the junction of the stipe and the base of the basidioma, reaching up to 300 μm.

Basidia 20–25 × 5–10 μm, clavate to broadly clavate, slightly enlarged at the apex, hyaline, 1-spored; sterigmata up to 5–7 μm long. Cystidia 22–40 × 6–12 μm, clavate, slightly enlarged at the apex, with a blunt or occasionally pointed tip, more abundant in immature basidiomata. Hymenophoral trama 20.5–40.0 μm wide, composed of interwoven, hyaline hyphae 1.0–2.5 μm in diameter.

Basidiospores globose, 8–10(–12) μm in diameter (n = 40), excluding ornamentation, hyaline, slightly thick-walled (0.5–1.0 μm thick); ornamentation echinulate, composed of isolated, curved, clavate, blunt spines, densely distributed, 2–3 μm long. Hilar appendix central, conical, straight, 2–4 μm long.

Ecology & Distribution: Hypogeous, solitary, or in groups in the soils under the forest of *Abies forrestii* var. *smithii* and *Rhododendron* sp. mixed forest, alt. 3800–3900m fruiting in autumn. Known only from northwestern Yunnan Province, China.

Additional specimen examined: China, Yunnan Province, Mt. Laojunshan, 26°63N, 99°72E, alt. 3912.3 m, 16 Sep. 2021, Lin Li, BMDU L21196 (GenBank: ITS = PX106475, LSU = PX105443).

Notes: Molecular phylogenetic analysis based on ITS and LSU regions places *Russula densiobtusispora* within a clade closely related to former *Macowanites* Kalchbr. species, a genus previously used to accommodate one gasteroid taxa of Russulales (Elliott & Trappe, 2018). These sequestrate species were characterized by subterranean, gasteroid basidiomata and often possessed a well-developed columella derived from a short basal stipe (Zeller & Dodge, 1919). Similarly, *Russula densiobtusispora* exhibits a short, finely hairy stipe that extends into the gleba to form a central, whitish columella — a feature shared with several former *Macowanites* species.

Interestingly, *Russula densiobtusispora* also forms a well-supported sister relationship with the epigeous *Russula pseudochamaeleontina*, implying that hypogeous sequestration and columella formation have evolved independently and possibly multiple times within the genus. In fact, related studies have already proposed and supported this pattern (Lebel & Tonkin, 2007; Elliott & Trappe, 2018), and supports the hypothesis that transitions between epigeous and hypogeous fruiting strategies in *Russula* may represent ecological adaptations, potentially linked to animal-mediated spore dispersal or high-altitude environmental pressures, rather than ancestral lineage divisions., these conclusions.

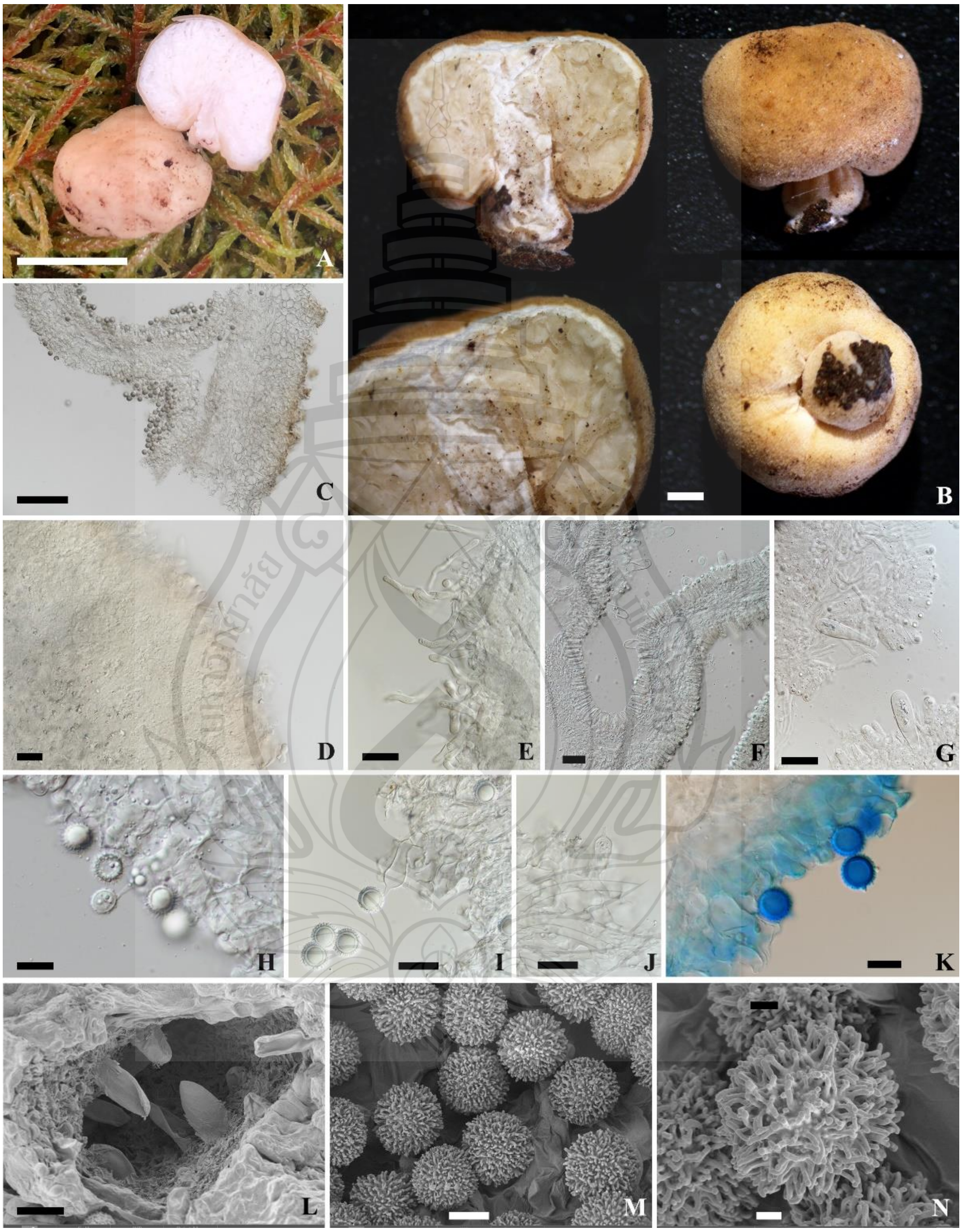


Figure 4.29 *Russula densiobtusispora* morphological characteristics

Figure 4.29 A.B. Basidiomata and gleba; C. A piece of the section of the peridium in 5% KOH; D. Peridium outermost cells expand into cystidia-like outer hyphae; E. Stipe outermost cells expand into cystidia-like outer hyphae; F. Hymenium and hymenophoral trama; G. Cystidia and hyphae of trama; H-J. Basidia and basidiospores in 5% KOH; K. Basidiospores in Lactic phenol cotton blue staining solution; L-N. SEM Cystidia and basidiospore. Scale bars: A = 1cm; B = 1mm; C = 50μm; D-E.M = 5μm; F. H-J.L = 20μm; G.K = 10μm; N = 2μm.

Russula laojunshanensis L. Li, K.D. Hyde & S.H. Li, sp. nov. Figure 30

Mycobank number: MB 860411

Diagnosis: Differs from other *Russula* species by its hypogeous, gasteroid basidiomata that are light pale khaki (4B4) in color, measuring 1.4 × 1.8 cm, with a short, finely hairy stipe 5.0 × 3.0 mm in size. The gleba is light chrome yellow (4B7), labyrinthine to loculate. Basidiospores are globose, hyaline, measuring (4–)6–8 μm in diameter, and ornamented with densely distributed, straight, conical, blunt spines that are either isolated or connected at the base, 0.8–1.2 μm long. The hilar appendix is central, conical, and straight, measuring 2–4 × 1–2 μm.

Etymology: laojunshanensis refers to Mt. Laojunshan, the type locality.

Holotype: CHINA, Yunnan Province, Mt. Laojunshan (26°29N 99°64E), alt. 3705.2 m, in a mixed forest of *Abies forrestii* var. *smithii* and *Rhododendron* spp., 19 Sep. 2021, Lin Li, BMDU L21200 (GenBank: ITS=PX106476).

Basidiomata 1.4 × 1.8 cm, subglobose, slightly depressed at the base, with a short stipe emerging from the base. Peridium surface light pale khaki (4B4), smooth. Gleba light chrome yellow (4B7), strongly labyrinthine or loculate; loculae compact and crowded. Odour indistinct.

Stipe short, finely hairy, pale khaki (4B4), measuring 5.0 × 3.0 mm, extending internally into the gleba to form a white, columnar columella that continues upward through the gleba to the apex. Stipe tissue composed of interwoven, hyaline hyphae; the outermost cells contain abundant cystidia-like outer hyphae that are hyaline, 2.0–4.0 μm wide, irregularly arranged, interwoven or prostrate, straight or curved, clavate, blunt-tipped, and aseptate.

Peridium 100–150 µm thick, pseudoparenchymatous, composed of subglobose to subangular, hyaline, thin-walled cells, 2.0–8.0(–16.0) µm wide. Cells in the outer and inner layers are much smaller and more compact than those in the middle layer, gradually decreasing in size from the center toward the periphery. The outermost cells contain brown (7E5) pigments.

Basidia 14.5–18.0 × 5.5–7.0 µm, clavate to broadly clavate, slightly swollen at the apex, hyaline, 2-spored; sterigmata up to 2–3 µm long. Cystidia are broadly clavate, slightly swollen in the middle, tapering toward the apex or slightly narrowed to a subcapitate shape. Hymenophoral trama 20.0–38.0 µm wide, composed of interwoven, hyaline hyphae 1.0–2.5 µm wide, with irregular, thin-walled, embedded globose cells 4.0–6.0 µm wide.

Basidiospores globose, (4–)6–8 µm in diameter (n = 60), excluding ornamentation; hyaline, with slightly thickened walls (0.5–1.0 µm thick); ornamentation echinulate, consisting of densely distributed, straight, conical, blunt spines, either isolated or fused at the base, 0.8–1.2 µm high. Hilar appendix central, conical, straight, 2–4 × 1–2 µm.

Notes: Molecular phylogenetic analysis based on ITS and LSU regions places *Russula laojunshanensis* in a clade closely related to the previously described *Russula sichuanensis*. However, the two species differ markedly in morphology. *Russula sichuanensis* produces semi-hypogeous to epigeous basidiomata that are russuloid to secotioid, resembling medium-sized, distorted, or aborted agaricoid *Russula*. It features a curly pileus, a white percurrent stipe often concealed by the pileus, and tightly contorted, sinuate lamellae that range in color from cream to yellow (Li et al., 2013). In contrast, *Russula laojunshanensis* forms completely hypogeous basidiomata. Although a short stipe is present, the basidioma is strongly gasteroid, with a nearly enclosed spore-producing tissue (gleba). The original lamellar structure is entirely lost and replaced by compact, crowded labyrinthine chambers. *Russula sichuanensis* occurs at elevations of 3300–3900 m (Li et al., 2013), while *R. laojunshanensis* was found at 3700 m. Both species inhabit high-elevation coniferous forests on the eastern and southeastern edges of the Qinghai–Tibetan Plateau. The evolution toward gasteroid basidiomata in both taxa may represent a specialized ecological adaptation to the cold and harsh montane forest environment.

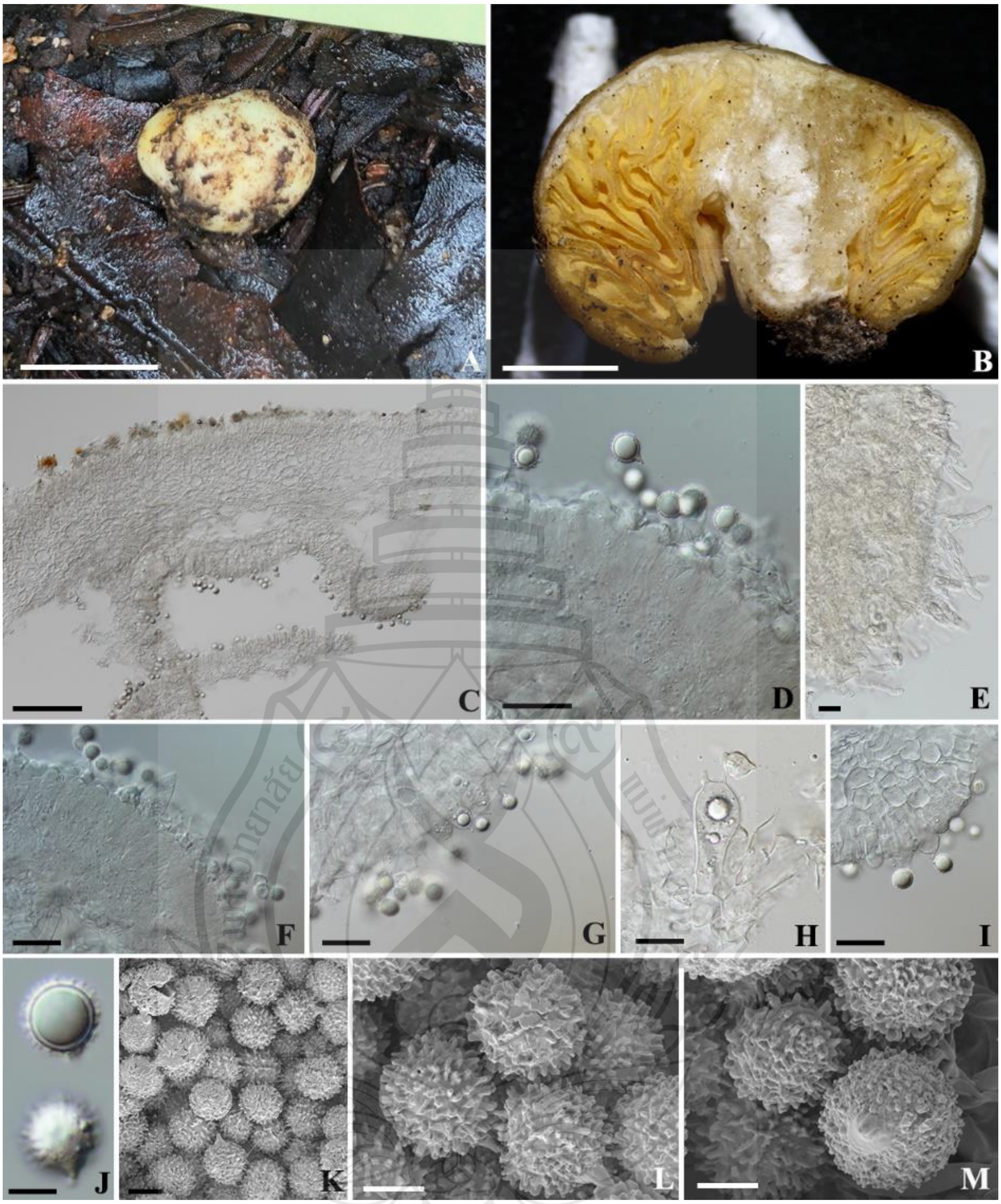


Figure 4.30 *Russula laojunshanensis* morphological characteristics

Figure 4.30 A.B. Basidiomata and gleba; C. A piece of the section of the peridium in 5% KOH; D. Hymenium and basidiospore; E. Stipe outermost cells expand into cystidia-like outer hyphae; F. Cystidia; G-I. Basidia and basidiospores in 5% KOH; J. Basidiospores in 5% KOH; K-M. SEM basidiospore. Scale bars: A = 1cm; B = 5mm; C = 50μm; D.F.G.I = 10μm; E.H.J.K.L = 5μm; M = 2μm.

4.2.11 *Truncocolumella*

4.2.11.1 Introduction

Truncocolumella Zeller (Agaricomycetes, Basidiomycota) is a small genus of false truffles, established by Zeller in 1939 with the type species *T. citrina* Zeller (Zeller, 1939). The main characteristics of this genus are: basidiomata depressed-spheroid to reniform, from a rhizomorphic base and with a central point of attachment; columella stump-like to dendroid, prominent; peridium persistent, not separating from the gleba; gleba pale white when immature, brown when mature; spores smooth, ellipsoid, as seen individually, almost hyaline. Another species, *Truncocolumella rubra*, was named in the same article and later recombined as *Gastroboletus ruber* (Zeller) Cázares & Trappe (Cázares & Trappe, 1991). The third species in this genus, *T. occidentalis* (Malençon) Malençon & Zeller, was reclassified from *Dodgea occidentalis* Malençon (Malençon & Zeller, 1940). Subsequently, *T. citrina* var. *citrina* Zeller and *T. citrina* var. *separabilis* A.H. Sm. were synonymised with *T. citrina* (Smith & Singer, 1959). To date, only *T. citrina* and *T. occidentalis* are recognised within the genus *Truncocolumella*.

Truncocolumella citrina is a common ectomycorrhizal species, frequently found in coniferous forests in North America (Zeller, 1939; Smith & Singer, 1959; Goodman et al., 1998; Wood et al., 1998; Massicotte et al., 2000; Binder & Hibbett, 2007; Twieg et al., 2007; Sato & Toju, 2019). *Truncocolumella occidentalis*, however, is rare and has only been found in Canada (Malençon & Zeller, 1940). Until 2012, *Truncocolumella* species had not been reported outside of North America. That year, a specimen was discovered at Mt. Yala in western Sichuan Province, China. Two additional collections were later

found in the same region. Western Sichuan Province, part of the Hengduan Mountains, is one of the world's most biodiverse areas. Morphological and molecular analyses revealed that these three samples represent a new species, *T. pseudocolumella*, described in this paper.

4.2.11.2 Sequence Alignment and Analysis

The internal transcribed spacer (ITS) region was used in this study to analyze species diversity within the genus *Truncocolumella*, as it has proven to be a useful locus for delimiting species in this group. Thirty ITS sequences from NCBI and this study, representing two species of *Truncocolumella* and selected accessions from the closely-related genera *Chroogomphus*, *Gomphidius*, *Rhizopogon*, *Suillus* (Table 15), including *Gastroboletus vividus* Trappe & Castellano, *Gastroboletus subalpinus* Trappe & Thiers, and *Gastroboletus ruber* (Zeller) Cázares & Trappe as outgroup taxa, were used. The analytical methods followed those described in the Materials and Methods section. Phylogenetic clades were considered strongly supported if they exhibited a bootstrap support (BS) value ≥ 70% and/or a posterior probability (PP) ≥ 0.90.

Table 4.14 Taxa information and GenBank accession numbers of the sequences used in *Truncocolumella* study

Species name	Voucher	Origin	GenBank No.
Gastroboletus vividus	JLF4456	USA	MH213058
Gastroboletus subalpinus	Trappe607-holotype	USA	EU231989
Gastroboletus ruber	OSC 79741	USA	EU697272
Gastroboletus ruber	OSC 69644	USA	EU837224
Gastroboletus ruber	OSC 74672	USA	EU837223
Truncocolumella sp1	OSC 67369	USA	KT968570
Truncocolumella pseudocolumella	**YAAS L2327**	**China**	**KP090063**
Truncocolumella pseudocolumella	**YAAS L2327**	**China**	**KP090064**
Truncocolumella pseudocolumella	**HKAS95533**	**China**	**OR631922**
Truncocolumella pseudocolumella	**HKAS95534**	**China**	**OR631923**
Truncocolumella citrina	JLF 2149	USA	MH217566
Truncocolumella citrina	isolate(TDB-2001)	USA	L54097
Truncocolumella sp2	MR3D88-RHITRU1	USA	OM459721
Truncocolumella sp3	OSC 111948	USA	KF206337

Table 4.14 (continued)

Species name	Voucher	Origin	GenBank No.
Suillus kwangtungensis	HKAS 71979-holotype	China	KU721539
Suillus aurihymenius	HKAS 63129-holotype	China	JN201972
Suillus alpinus	HKAS 63128-holotype	China	JN201974
Gomphidius borealis	IB:NR19990532-holotype	USA	NR_132800
Gomphidius nigricans	OKM 27830	USA	AY077474
Chroogomphus roseolus	HKAS 50552	China	EF423620
Chroogomphus ochraceus	OKM 25472	USA	AH009858
Rhizopogon colossus	MICH AHS49480-holotype	USA	AH011348
Rhizopogon diabolicus	MICH AHS68424-paratype	USA	AH011349
Rhizopogon ellenae	MICH AHS66137-holotype	USA	AH011350
Rhizopogon evadens	MICH AHS65484-holotype	USA	AF062927
Rhizopogon ochraceorubens	MICH AHS59643-holotype	USA	AF062928
Rhizopogon flavidus	YAAS L2957	China	NR_158904
Rhizopogon jiyaozi	YAAS L2929	China	NR_158906
Rhizopogon sinoalbidus	YAAS L2949	China	NR_158905
Rhizopogon songmaodan	HKAS 106767	China	NR_174900

Note The newly generated sequences are in bold.

4.2.11.3 Results

Phylogenetic Analysis

The ML and Bayesian analyses of the 30 ITS sequences are shown in Figure 4.31 with associated bootstrap supports for branches.

In the phylogenetic tree, the 25 ITS sequences from Suillineae revealed the phylogenetic relationship of two species of *Truncocolumella,* and five *Gastroboletus* sequences are used as outgroups. Since there are few *Truncocolumella* sequences in GenBank and these sequences belong to only one species *T. citrina*, we selected sequences of Suillineae for phylogenetic analysis. The analytics include three sequences of the genus *Suillus*; nine sequences of the genus *Rhizopogon*; two sequences of the genus *Chroogmophus*; two sequences of the genus *Gomphidus*; and nine sequences of the genus *Truncocolumella*. Two clades were revealed in the genus *Truncocolumella*; Clade I includes five sequences of *T. citrina* from the USA; Clade II includes four sequences of a new species, *T. pseudocolumella,* from China. The phylogenetic analysis shows that the new species is distinct from *T. citrina*, the type species of *Truncocolumella*. In addition to the ITS sequences used in this phylogenetic analysis, the LSU sequences were amplified from the newly recorded specimens in this study and uploaded to NCBI for future study.

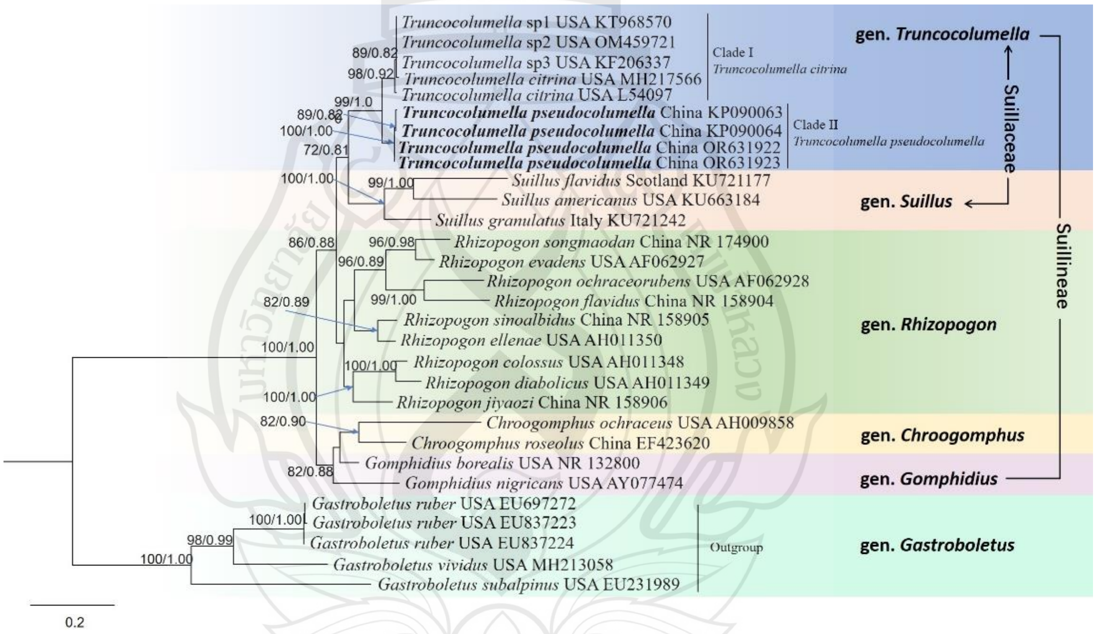


Figure 4.31 Phylogeny derived from a Maximum Likelihood (ML) analysis of the nrDNA-ITS sequences from Suillineae, including *Truncocolumella* species, using *Gastroboletus* as outgroup

Figure 4.31 Values next to nodes present Maximum Likelihood bootstrap support values (BS), left and Bayesian posterior probabilities (PP), right. The names of novel species and samples with newly generated sequences are in bold.

Truncocolumella pseudocolumella Lin Li, S.H. Li & Y. Wang 2024 Figure 4.32

Mycobank number: MB 851721

Diagnosis: Differs from other species in the genus *Truncocolumella* in the basidiomata devoid of any columnar.

Etymology: Pseudocolumella, referring to the absence of the columella.

Holotype: China, Sichuan, Mt. Yala, 30°31′N 101°37′E, alt. 3772.4 m, in the forest of *Quercus guyavifolia* H. Lév. 19 August 2012, Lin Li, (Holotype, HKAS 131259[YAAS L2327]; GenBank ITS=KP090063 and KP090064, LSU=PP112109).

Basidiomata irregularly depressed-globose to pyriform, with a yellowish-tawny (4C8) rhizomorphic base, 1.5–3.0 cm in diameter, the surface typically smooth and dry in appearance, light yellowish-tawny (4C5) with yellowish-brown (4C8) rhizomorphs, colour unchanged on bruising or exposure, elastic, very mature basidiomata deliquesce like *Rhizopogon* (Figure 4.32A). Odour light and pleasant.

Peridium 102–160 μm thick, not separating or evanescent from the gleba at maturity, composed of two layers: outer layer 52–73 μm thick, reddish-tawny (7D7) interwoven hyphae of 1–1.5 μm diameter. The inner layer 40–86 μm thick, consisting of brownish-hyaline nearly parallel interwoven hyphae of 0.5–1.5 μm diameter (Fig. 32D). The boundary between the inner and outer layers gradually transitioning, with changes in hyphae arrangement direction and hyphae colour. The clamp connection clearly visible, dry peridium becoming black (4F4) when encountering 5% KOH (Fig. 32B and C). Gleba pale white when immature, light brown to tawny at maturity, unchanging on bruising or exposure, cavities relatively small, spongy, lacking obvious columnar or dendroid radiating columella (Fig. 32A). Trama (56.0–) 62.5–85.0 μm, composed of hyaline, almost parallel hyphae. Hymenium present on the cavity surface. Basidia narrowly clavate, (15.0–) 17.2–19.5 (–22.4) × 3.5–6.5 (–7.0) μm, 2-4-spored. Sterigmata 2–3 μm (Fig. 32E, G, H and I). Basidiospore ellipsoid, smooth, 7.5– 10.0 (–11.0) × 4.0–5.0 (–5.6) μm, grey to brownish (7D5) in mass, as seen individually, almost hyaline, typically 1–2 guttulate, infrequently 3-guttulate (Figure 4.32F and J), changing to blue in lactophenol cotton blue, not obvious discolouration in Melzer's reagent.

Notes: The genus *Truncocolumella* currently includes three species: *T. citrina*, *T. occidentalis,* and the newly described *T. pseudocolumella* in this study. Based on the original literature descriptions of the species *T. citrina* (Zeller, 1939; Smith & Singer, 1959) and the re-examination of the type specimens of *T. citrina* and *T. occidentalis* by Malençon and Zeller (Malençon & Zeller, 1940), we compared the key distinguishing features of these three species. In terms of macroscopic characteristics, *T. pseudocolumella* differs from other species in the genus *Truncocolumella* in the basidiomata devoid of any columnar. Furthermore, the basidiomata of *T. citrina* have a diameter of 2–4 cm, which is comparable in size to those of *T. pseudocolumella* (1.5–3 cm), but they are two to three times larger than *T. occidentalis*. The basidiomata surfaces of *T. citrina* are distinctly citrine yellow, those of *T. pseudocolumella* are yellowish-tawny, while *T. occidentalis* has white basidiomata, although the colour of *T. occidentalis* remains uncertain due to preservation in alcohol. Microscopically, *T. citrina* has ellipsoid spores measuring 6–10 × 3.5–5.0 µm, with individual spores appearing nearly hyaline. Similarly, *T. pseudocolumella* has ellipsoid spores measuring 7.5–10.0 × 4.0–5.0 µm, also nearly hyaline, showing close resemblance between the two. According to Malençon's description (Malençon & Zeller, 1940), the spores of *T. citrina* are clearly shorter, more oval, and of more irregular shape than those of *T. occidentalis*, in which these bodies are long-elliptic or sometimes subcylindrical. Molecular analysis further demonstrates that *T. pseudocolumella* is distinct from *T. citrin*, with high support for their separation into different species.

4.2.11.4 Discussion

Truncocolumella is an ancient, small genus with only two known species, *T. citrina* and *T. occidentalis*, which were found exclusively on the west Pacific coast of North America until the discovery of the third member, *T. pseudocolumella*, almost a century later in Sichuan, China. *Truncocolumella* is closely related to the epigeous fungi *Suillus*, and both genera are currently classified within the family Suillaceae. The genus *Truncocolumella* is characterised by its prominent, stump-dendroid-like columella, a key morphological feature. However, the new species *T. pseudocolumella* has almost no columella, making it similar to species of *Rhizopogon*, although molecular analysis clearly places it within the genus *Truncocolumella*. In addition to the absence of a columella in the basidiomata of *T. pseudocolumella*, which is a notable distinguishing feature, *T. citrina* and *T. pseudocolumella* also differ in several morphological characteristics: the basidiomata surface of *T. citrina* is distinctly citrine yellow (Zeller, 1939; Smith & Singer, 1959), while that of *T. pseudocolumella* is yellowish-tawny. The peridium of *T. citrina* is 70–100 μm thick, whereas that of *T. pseudocolumella* is thicker, reaching 102–160 μm and is differentiated into two layers based on the orientation of hyphae and changes in hyphal colouration. Additionally, the sterigmata of *T. citrina* are 3–5 μm in length, while those of *T. pseudocolumella* are shorter, measuring 1–2 μm in length. Similarly, based on the reexamination of the morphological structures of the type specimens of *T. citrina* and *T. occidentalis* by Malençon and Zeller (Malençon & Zeller, 1940) and their descriptions, it is evident that *T. pseudocolumella* has larger basidiomata with a yellowish-tawny surface compared to *T. occidentalis*. Additionally, the spores of *T. occidentalis* are long-elliptic, sometimes almost subcylindrical, and are longer than those of both *T. citrina* and *T. pseudocolumella*. *Truncocolumella occidentalis* was reclassified from *Dodgea occidentalis*, and its description is based on a holotype specimen that was preserved in alcohol for 17 years. Since then, no additional reports of this species have been recorded. Unfortunately, molecular data are not available. It is hoped that future collections of this species will help clarify its identity.

Truncocolumella pseudocolumella differs significantly from the North American species, which can be attributed to bio-evolutionary geographical isolation and the unique ecological environment of the Hengduan Mountains in China. *Truncocolumella citrina* is found in coniferous forests of the Pacific Northwest mountains at altitudes around 1200 m (Zeller, 1939; Smith & Singer, 1959), whereas *T. pseudocolumella* is distributed in alpine *Quercus guyavifolia* and *Pinus* forests at 3700 m in the Hengduan Mountains region of China.

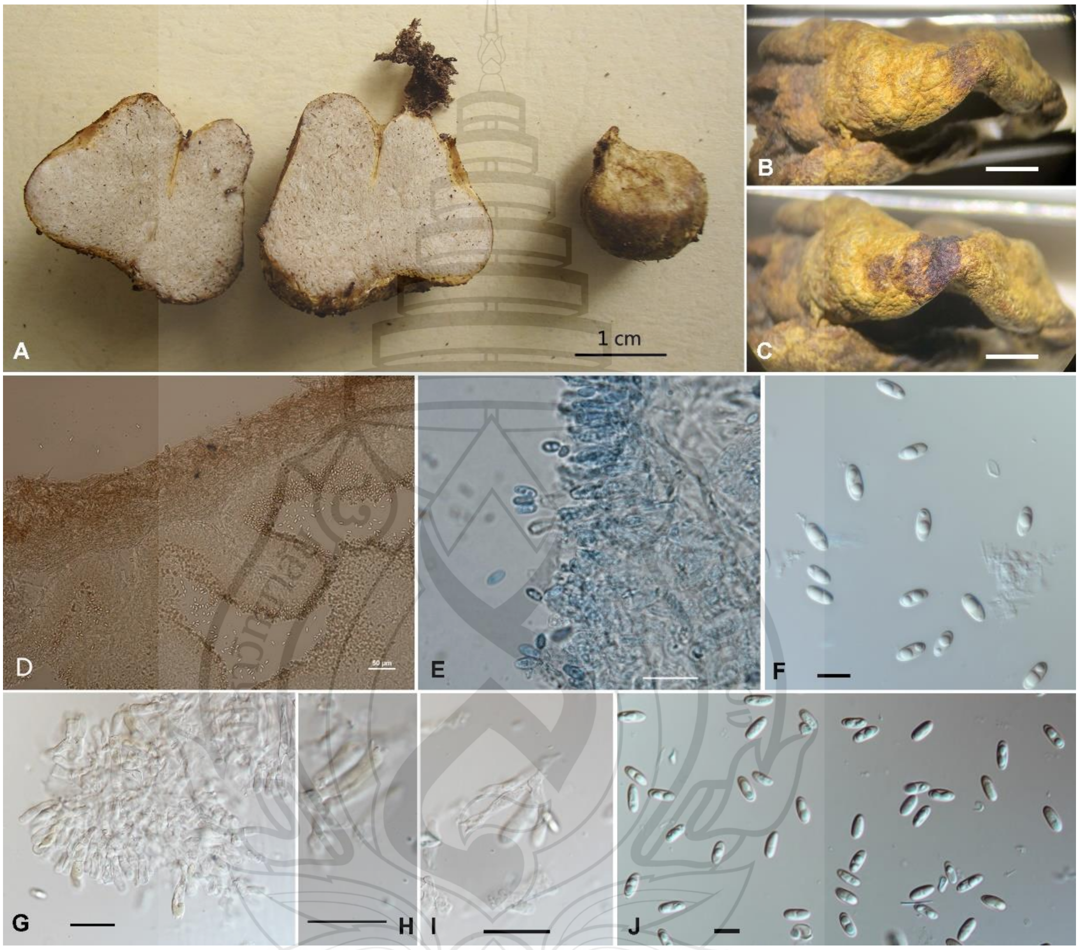


Figure 4.32 *Truncocolumella pseudocolumella* morphological characteristics

Figure 4.32 A Basidiomata; B, C Dry peridium turning black when stained with 5% KOH; D A section of basidiomata in 5% KOH; E A section of the hymenium in lactophenol cotton blue; G-I Basidia in 5% KOH (H. 4-spored basidia, I. 2-spored basidia); F, J Basidiospores in 5% KOH. Scale bars: A = 1 cm; B, C = 0.5 cm; D = 50 μm; E, G, H, I = 20 μm; F, J = 10 μm.

Mucoromycota

4.2.12 *Jimgerdemannia*

4.2.12.1 Introduction

Jimgerdemannia is a young genus within the family Endogonaceae, established in 2017 by Desirò et al., who separated it from the genus *Endogone* (Desirò et al., 2017). Some species within the family Endogonaceae can form ectomycorrhizal associations with various trees, and the truffle-like sporocarps they produce are considered hypogeous fungi. These hypogeous fungi are commonly referred to as “pea truffles.” (Desirò et al., 2017; Yamamoto et al., 2020; Fan, 2023). Currently, the only known pea truffles of the genus *Jimgerdemannia* reported in China were discovered in Taiwan and identified as *Jimgerdemannia flammicorona* [Basionym: *Endogone flammicorona*] (Wong et al., 2017). The new species reported in this study is the only one found in the southwestern region. However, sequence data from GenBank indicates a richer diversity of species in mycorrhizal studies.

4.2.12.2 Sequence Alignment and Analysis

The LSU, TEF1, and *rpb1* region was used in this study to analyze species diversity within the family *Endogonaceae*, as it has proven to be a useful locus for delimiting species in this group. A total of 31 LSU, 34 TEF1, and 14 *rpb1* sequences, including those generated in this study and those retrieved from NCBI, were analyzed. These sequences represent 13 species of *Endogonaceae* (Table 4.15). The analytical methods followed those described in the Materials and Methods section. Phylogenetic clades were considered strongly supported if they exhibited a bootstrap support (BS) value ≥ 70% and/or a posterior probability (PP) ≥ 0.90.

Table 4.15 Taxa information and GenBank accession numbers of the sequences used in *Jimgerdemannia* study

Species Name	Voucher and Origin	GenBank No. LSU	GenBank No. TEF1	GenBank No. rpb1
Endogone pisiformis	OSC 80931 USA		MH449557	MH449558
Endogone pisiformis	KPM NC0024233 JPN	LC002630	LC107391	LC431135
Endogone pisiformis	KPM NC0024230 JPN	LC002629	LC107389	LC431134
Endogone pisiformis	KPM NC0024229 JPN	LC107365	LC107388	LC431133
Endogone corticioides	KPM NC0024742 JPN	LC107370	LC107395	LC431127
Endogone corticioides	KPM NC0024741 JPN	LC107368	LC107393	
Endogone corticioides	KPM NC0024744 JPN	LC107372	LC107396	
Endogone corticioides	KPM NC0024740 JPN	LC107367	LC107392	
Endogone incrassata	OSC T32492 MEX		JF414137	
Endogone incrassata	KPM NC0024209 JPN	LC107362	LC107377	
Endogone incrassata	KPM NC0024214 JPN	LC002621	LC107380	LC431131
Endogone oregonensis	AD153 USA	MF479015	MF479073	
Endogone oregonensis	T36235 USA	MF479016	MF479072	
Endogone botryocarpus	KPM NC0026731 JPN	LC431095	LC431111	
Endogone tuberculosa	OSC 146000 AUS	MF479026		
Vinositunica ingens	KPM NC0024500 JPN	LC431108	LC431124	LC431147
Vinositunica ingens	KPM NC0026747 JPN	LC431107	LC431123	
Vinositunica ingens	KPM NC0024748 JPN	LC431109	LC431125	LC431148
Vinositunica radiata	KPM NC0026742 JPN	LC431106	LC431122	LC431146
Vinositunica radiata	KPM NC0026741 JPN	LC431105	LC431121	
Vinositunica radiata	KPM NC0026740 JPN	LC431104	LC431120	LC431145
Jimgerdemannia lactiflua	KPM NC0024221 JPN	LC002625	LC107383	LC431141
Jimgerdemannia lactiflua	KPM NC0024218 JPN	LC002622	LC107381	
Jimgerdemannia lactiflua	KPM NC0024739 JPN	LC107364	LC107385	
Jimgerdemannia flammicorona	KPM NC0024738 JPN	LC107360	LC107375	
Jimgerdemannia flammicorona	KPM NC0024202 JPN	LC002615	LC107373	
Jimgerdemannia flammicorona	OSC T33849 MEX		JF414140	
Jimgerdemannia flammicorona	OSC T33851 MEX		JF414139	
Jimgerdemannia ambigua	KPM NC0026732 JPN	LC431096	LC431112	
Jimgerdemannia ambigua	KPM NC0026733 JPN	LC431097	LC431113	LC431136
Jimgerdemannia zixishanensis	**L24101 China**	This study	This study	

Table 4.15 (continued)

Species Name	Voucher and Origin	GenBank No. LSU	GenBank No. TEF1	GenBank No. rpb1
Jimgerdemannia laojunshanensis	**L21214 China**	This study	This study	
Jimgerdemannia cangshanensis	**L24100 China**	This study	This study	
Sphaerocreas pubescens	KPM NC0022970 JPN	LC431110	LC431126	LC431151
Sphaerocreas pubescens	NBRC 109377 JPN	LC107618	LC107619	LC431150

Note The newly generated sequences are in bold.

4.2.12.3 Results

Phylogenetic Analysis

The ML and Bayesian analyses of the 31 LSU, 34 *tef1*, and 14 *rpb1* sequences are shown in Figure 4.33 with associated bootstrap supports for branches.

In the phylogenetic tree, the 31 LSU, 34 *tef1*, and 14 *rpb1* sequences from *Endogonaceae* revealed the phylogenetic relationship of 13 species, and used representatives of *Sphaerocreas pubescens* as outgroups. The phylogenetic analysis showed that the new species is clearly distinct from other species of the genus *Endogonaceae*. The sequences from the newly supplemented specimens in this study were uploaded to NCBI for future study.

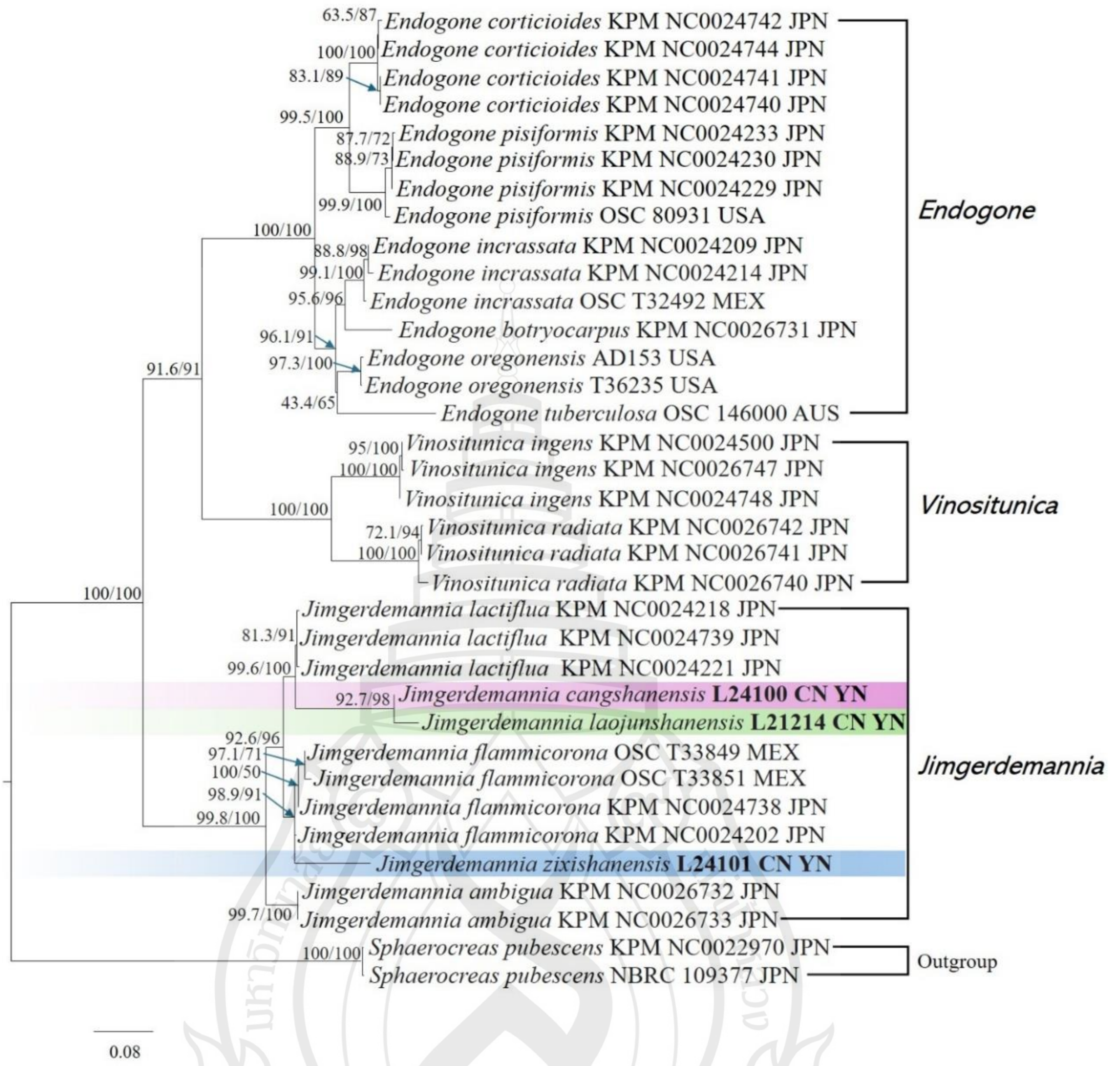


Figure 4.33 Phylogeny derived from a maximum likelihood (ML) analysis of the nrDNA-ITS sequences from *Endogonaceae* species

Figure 4.33 Values next to nodes reflect maximum likelihood bootstrap support values (BS), left, and Bayesian posterior probabilities (PP), right. Names of novel species and samples with newly generated sequences are in bold.

Jimgerdemannia cangshanensis L. Li, K.D. Hyde & S.H. Li, sp. nov. Figure 4.34 I-L

Mycobank number: MB 860412

Diagnosis: Differs from other *Jimgerdemannia* species by its hypogeous sporocarps that are light pale khaki (4B4) in color, measuring 0.5 × 0.8 cm. The gleba is light, pale khaki and lacks a distinct basal region. Zygosporangia are globose to subglobose, 50–80 μm in diameter, hyaline to pale brown.

Etymology: cangshanensis, refers to Mt. Cangshan, the type locality.

Holotype: CHINA, Yunnan Province, Mt. Cangshan (25°34′ N 100°55′ E), alt. 2600 m, in forest of *Pinus yunnanensis* and *Pinus armandii*, 27 Oct. 2024, Lin Li, BMDU L24100 (GenBank: ITS = PX108632, LSU = PX105444, SSU = PX097640).

Sporocarps hypogeous, irregularly subglobose, 0.5 × 0.8 cm in diameter, solitary, light pale khaki (4B4) when mature; surface smooth, becoming wrinkled when dry. The surface is covered with sparse, fine white rhizomorphs. Gleba light pale khaki, without a distinct basal region. Glebal tissue composed of irregular locules filled with densely packed zygospores.

Peridium 100–160 μm thick, composed of interwoven, hyaline, thin-walled hyphae 1.0–2.0 μm wide; the outermost layer contains yellowish brown pigment and is 20–30 μm thick. Zygosporangia globose to subglobose, 50–80 μm in diameter, hyaline to pale brown, thick-walled (2–3 μm), lacking subtending hyphae. The zygosporangial wall is persistent and tightly adherent to the spore. Gametangia not observed. Hyphae hyaline, aseptate, 1–2 μm wide, thin-walled, sparsely branched.

Jimgerdemannia zixishanensis L. Li, K.D. Hyde & S.H. Li, sp. nov. Figure 34 E-H

Mycobank number: MB 860413

Diagnosis: Differs from other *Endogone* species by its hypogeous sporocarps that are light pale khaki (4B4) in color, measuring 1.0–1.5 × 1.8–2.0 cm. The gleba is khaki to yellowish brown and contains a distinct sterile base composed of white tissue. Numerous rhizomorphic strands extend from the base. Zygosporangia are globose, 100–120 μm in diameter, hyaline to light brown.

Etymology: zixishanensis, refers to Mt. Zixishan, the type locality.

Holotype: CHINA, Yunnan Province, Mt. Zixishan (25°00′ N 101°23′ E), alt. 2106.2m, in forest of *Pinus yunnanensis*, 9 Nov. 2024, Yinong Li, BMDU L24101 (GenBank: LSU=PX105445, SSU = PX097641).

Sporocarps hypogeous, globose to subglobose, 1.0–1.5 × 1.8–2.0 cm in diameter, solitary, light pale khaki (4B4) when mature; surface smooth, becoming wrinkled when dry. Gleba khaki to yellowish brown, with a distinct sterile base consisting of white tissue. Numerous rhizomorphic strands arise from the base. Glebal tissue composed of irregular locules filled with zygospores embedded in sparse, interwoven hyphae.

Peridium 150–240 µm thick, composed of interwoven, hyaline to light brown, thin-walled hyphae, 1–2 µm wide; scattered with pigmented dark brown deposits. Zygosporangia globose, 100–120 µm in diameter, hyaline to light brown, thick-walled (2–3 µm), lacking subtending hyphae. The zygosporangial wall is persistent and tightly adherent to the spore. Gametangia not observed. Hyphae hyaline, aseptate, 3–8 µm wide, thin-walled, sparsely branched.

Jimgerdemannia laojunshanensis L. Li, K.D. Hyde & S.H. Li, sp. nov. Figure 4.34 A-D

Mycobank number: MB 860414

Diagnosis: Differs from other *Endogone* species by its hypogeous sporocarps that are light pale khaki (4B4) in color, measuring 0.5 × 1.0 cm. The gleba is golden brown and contains a distinct basal region composed of white sterile tissue. A few rhizomorphic strands extend from the base. Zygosporangia are globose, 90–115 µm in diameter, pale golden brown.

Etymology: laojunshanensis, refers to Mt. Laojunshan, the type locality.

Holotype: CHINA, Yunnan Province, Mt. Laojunshan (26 °29N 99 °64E), alt. 3612.4m, in forest of *Abies forrestii* var. *smithii* and *Rhododendron* spp., 19 Sep. 2021, Yinong Li, BMDU L21214 (GenBank: LSU=PX105446, SSU = PX097642).

Sporocarps hypogeous, globose to subglobose, 0.5 × 0.8 cm in diameter, solitary, light pale khaki (4B4) when mature; surface smooth, becoming wrinkled when dry. Gleba golden to golden brown, with a distinct basal portion consisting of white sterile tissue. A few rhizomorphic strands arise from the base. Glebal tissue composed of irregular locules filled with densely packed zygospores.

Peridium 50–80 µm thick, composed of densely interwoven, hyaline, thin-walled hyphae, 0.5–1.0 µm wide. Zygosporangia globose, 80–100 µm in diameter, pale golden brown, thick-walled (1–2 µm), lacking subtending hyphae. The zygosporangial wall is persistent and tightly adherent to the spore. Gametangia not observed. Hyphae hyaline, aseptate, 1–2 µm wide, thin-walled, sparsely branched.

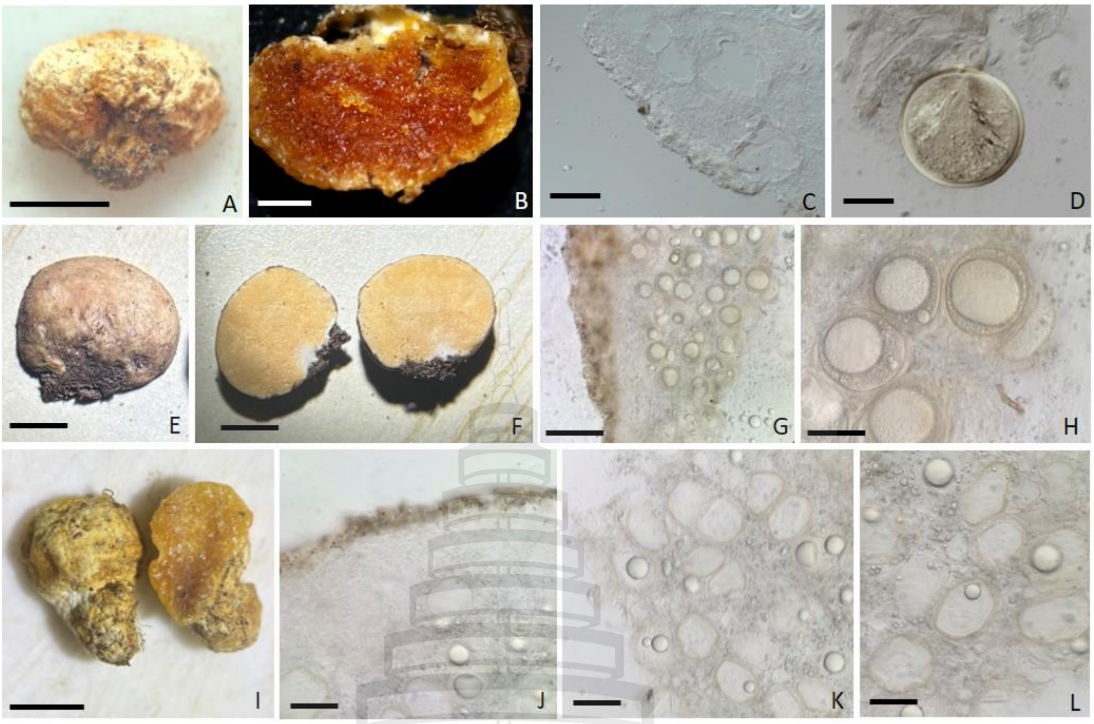


Figure 4.34 A-D *Jimgerdemannia laojunshanensis*

Figure 4.34 A. Sporocarps; B.gleba; C. A piece of the section of the peridium in 5% KOH; D. Zygosporangia in 5% KOH. E-H *Jimgerdemannia zixishanensis* E. Sporocarps; F.gleba; G. A piece of the section of the peridium in 5% KOH; H. Zygosporangia in 5% KOH. I-L *Jimgerdemannia cangshanensis* I. Sporocarps and gleba; J. A piece of the section of the peridium in 5% KOH; K.L. Zygosporangia in 5% KOH. Scale bars: A.E.F.I = 0.5cm; B = 0.2cm; G.K.L. = 100µm; C.D.H.J = 50µm.

4.3 Species Diversity and Distribution of Hypogeous Fungi in Southwestern China

This study compiled the distribution data of hypogeous fungi in southwestern China based on extensive field investigations in major forested regions and wild mushroom markets, combined with literature records, specimen collections, and taxonomic identification. A total of 166 species of hypogeous fungi were documented in the region, belonging to three fungal phyla: Ascomycota (2 orders, 5 families, 8 genera, 91 species), Basidiomycota (5 orders, 14 families, 20 genera, 72 species), and Glomeromycota (1 order, 1 family, 1 genus, 3 species).

4.3.1 Distribution and Habitat

This section provides annotations for all 166 species, including information on known distribution localities, habitat characteristics, and available DNA sequence data. For each genus, species-specific distribution maps are presented. In cases where species are commonly encountered in wild mushroom markets, only the initial collection sites (i.e., type or first-reported localities) are shown on the maps to avoid redundancy

Ascomycota

Eurotiales G.W. Martin ex Benny & Kimbr. 1980

Elaphomycetaceae Tul. ex Paol. 1889

Elaphomyces T. Nees 1820

The genus of *Elaphomyces* is cosmopolitan, but mainly distributed in the Northern Hemisphere. All species within it produce hypogeous ascocarps. Ascomata globose to subglobose, pale brown, brown to black, surface smooth or warted, often encrusted with mycelia, plant rootlets, and soil particles. Gleba solid, cottony and pale at first, becoming a powdery spore mass at maturity, purplish brown, dark brown to black, sometimes blue or olive green (Castellano & Stephens, 2017). In forest ecosystems, *Elaphomyces* typically forms ectomycorrhizal associations with plants and can also be parasitized by some fungi, such as *Tolypocladium* spp. and *Cordyceps* spp. (Mains, 1957; Yamamoto et al., 2022).

According to statistics, there are currently 6 species of the *Elaphomyces* genus reported in China. Among them, 3 are distributed in the southwestern region: *E. granulatus* in Yunnan, *E. tuberculatus*, and *E. muricatus* in Xizang province (Zhang, 1991a; Xu, 1999). The *Elaphomyces* species in the southwestern region are quite diverse. Although obtaining ascocarps is challenging, studies related to the microbial community associated with ectomycorrhizal and research on *Tolypocladium* have detected more *Elaphomyces* species sequences. (Yu et al., 2021b; Yu et al., 2021a)

Elaphomyces granulatus Fr. 1829

Distribution & Specimen information: under *Quercus* spp., ascocarp from summer to early autumn. Yunnan Province: Lijiang, Mt. Yulongshan, alt. 3100m, 8

Sept. 1986, HKAS 17867 (Zang Mu 10816); Kunming Huangtupo, B. C. Zhang 596, 5 May 1990, HMAS 60246. Xizang Province: Nyingchi, Bayi, alt. 3100, 12 Aug. 1996, HXZE 881(Xu A.S. 96104); Milin, alt. 3150m, 1 Sep. 1996, HXZE 982 (Xu A.S. 96205), alt.3100m, 12 Sep. 1996, HXZE 1011(Xu A.S. 96234); Nyingchi, Mt. Sejila(near Lulang Forest Sea), alt. 3500m, 30 Jul. 1997, HXZE 1231(Xu A.S.97117)

Notes: No sequence data available from southwestern China. No edible or medicinal use.

Elaphomyces muricatus Fr. 1829

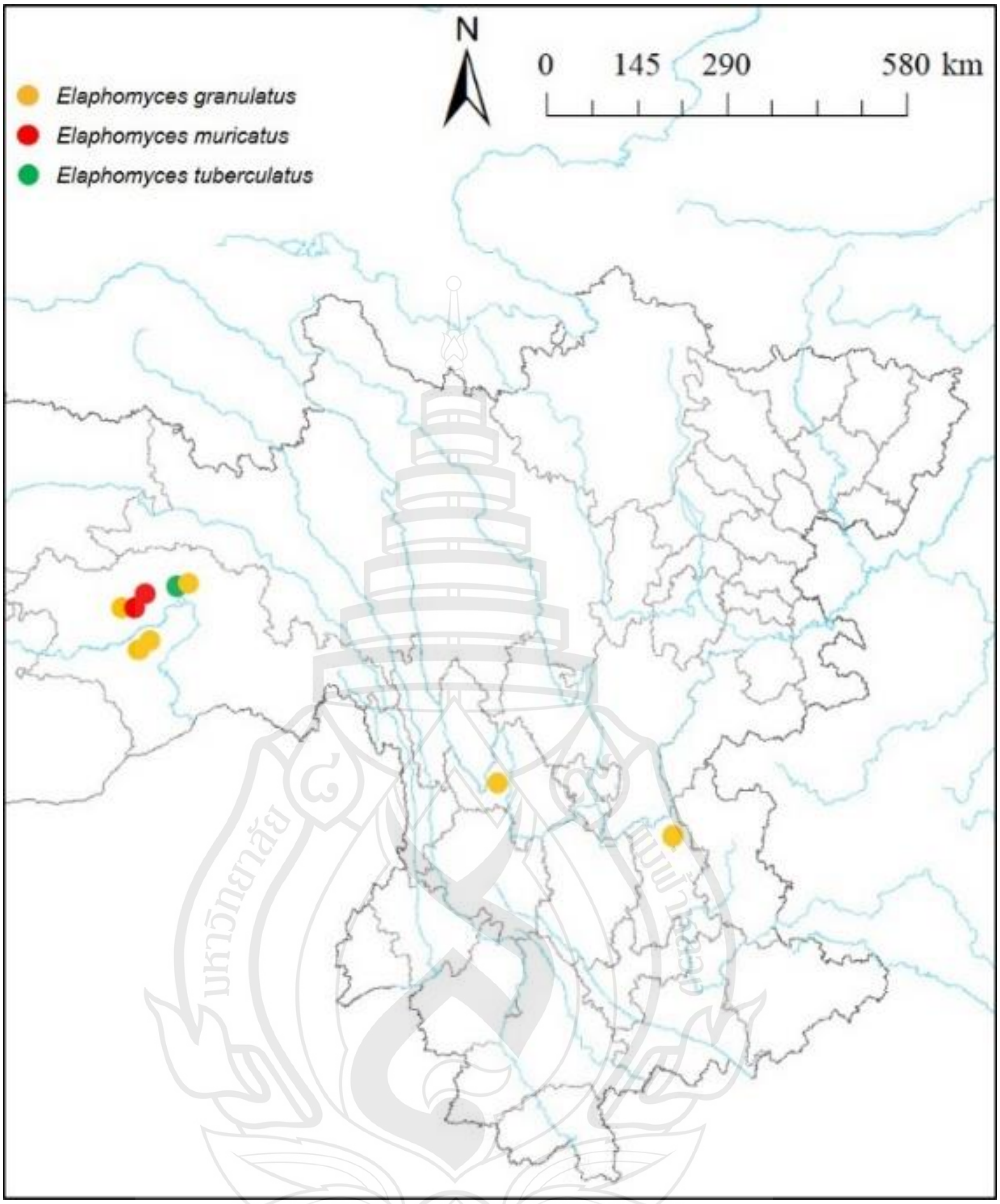
Distribution & Specimen information: under *Quercus* spp., ascocarp from summer. Xizang Province: Nyingchi, Bayi, alt. 3050, 2 Aug. 1997, HXZE 1253 (Xu A.S. 97139), alt. 3000, 5 Aug. 1996, HXZE 816 (Xu A.S. 96040), Juemushan, alt. 3160, parasitized by *Cordyceps ophioglossoides*, 20 Aug. 1997, HXZE 1240 (Xu A.S. 97126)

Notes: No sequence data available from southwestern China. No edible or medicinal use.

Elaphomyces tuberculatus A-S. Xu 1999

Distribution & Specimen information: under *Abies georgei* var. *smithii*, ascocarp from summer. Xizang Province: Nyingchi, Mt. Sejila, alt. 3600, 28 Jul. 1997, HXZE 1230 (Xu A.S. 97116)

Notes: Holotype HXZE1230. No sequence data available from southwestern China. No edible or medicinal use.



Note Dots on the map indicate specimen collection localities. Each species is represented by a unique color, as shown in the legend in the upper-left corner of the figure. The number of dots in the same color reflects the number of specimens recorded for that species. The size of the dots is not meaningful and is adjusted solely for spatial layout clarity on the map. This applies to all species distribution maps provided for each genus or family in this section.

Figure 4.35 Distribution map of *Elaphomyces* species in southwestern China

Pezizales J. Schröt. 1897

Discinaceae Benedix 1962

Hydnotrya Berk. & Broome 1846

Hydnotrya is a genus within the Discinaceae family, where all species produce hypogeous ascocarps. Their ascomata are hollow to convoluted with simple or folded chambers, even nearly solid, lined with recognizable hymenium. *Hydnotrya* species usually form a symbiotic relationship with both conifer and broadleaf trees and are distributed throughout the northern hemisphere (Trappe, 1975; Spooner, 1992; Trappe & Castellano, 2000; Stielow et al., 2010; Xu et al., 2018; Slavova et al., 2021).

According to statistics, there are currently 11 species are now known in China, among which 8 species are distributed in the southwest region: *Hydnotrya cubispora* and *H. cerebriformis* in Xizang, *H. laojunshanensis*, *H. badia*, *H. oblongispora*, *H. zayuensis*, and *H. puberula* in Yunnan, *H. nigricans* in Sichuan province (Xu, 2000; Li L et al., 2013; Xu et al., 2018; Li L. et al., 2023).

Hydnotrya badia L. Fan, Y.W. Wang & Y.Y. Xu 2018

Distribution & Specimen information: under conifers (*Pinus* sp.), ascocarp from autumn. Yunnan Province: Huize, 9 Oct. 2013, BJTC FAN270 (CAO800)

Notes: Holotype BJTC FAN270, ITS = NR161070, LSU = NG0664145, EF1a = OP846120. No edible or medicinal use.

Hydnotrya cerebriformis Harkn (1899)

Distribution & Specimen information: under *Abies georgei*, ascocarp from spring. Xizang Province: Milin, Zhagonggou, alt. 3450m, 16 Apr. 1996, HXZE1059 (Xu A. S. 96282). This species has been reported to be mainly distributed in northern China, with only a single specimen record from the southwestern region.

Notes: No sequence data available from southwestern China. No edible or medicinal use.

Hydnotrya cubispora (E.A. Bessey & B.E. Thomps.) Gilkey (1939)

Distribution & Specimen information: under *Abies* sp., ascocarp from summer. Xizang Province: Nyingchi, Mt. Sejila, alt. 3650m, 30 Jul. 1997, HXZE1214(Xu A. S. 97100); alt. 3600, 28 Jul. 1997, HXZE1228 (Xu A. S. 97114); alt. 4000, 30 Jul. 1997, HXZE1227 (Xu A. S. 97113).

Notes: No sequence data available from southwestern China. No edible or medicinal use.

Hydnotrya laojunshanensis Lin Li et al. (2013) Figure 36

Distribution & Specimen information: under *Abies* spp., ascocarp from late summer and early autumn. Yunnan Province: Lijiang, Mt. Laojunshan, alt. 3786m, 30 Aug. 2012, YAAS L2425; alt. 3540m, 12 Sep. 2019, HKAS131239, alt. 3805m, 21 Sep. 2021, HKAS131240, 131241, 131242. Shangri-La, alt. 3978m, 30 Aug. 2012, HKAS95802.

Notes: Holotype YAAS L2425: ITS = NR132886. Other specimen HKAS131239(BMDU L21197): ITS = ON982592, LSU = ON982620; HKAS131240(BMDU L21211): ITS = ON982580, LSU = ON982621, HKAS131241(BMDU L21212): ITS = ON982593, LSU = ON982622, HKAS131242(BMDU L21215): ITS = ON982594, LSU = ON982623; HKAS95802: ITS = OP908303. No edible or medicinal use.

Figure 4.36 *Hydnotrya laojunshanensis* morphological characteristics

Figure 4.36 A.B.C. Ascomata; D. Section of hymenium in 5% KOH; E. A peridium section in 5% KOH; F. Asci in 5% KOH; G. Ascospores released from asci in 5% KOH; H.I. Ascospores under SEM; J.K. Single ascospore in 5% KOH. Scale bars: A.B.C = 1 cm; D = 100 μm; E.F = 50 μm; G = 30 μm; H. I. J. K = 10 μm.

Hydnotrya nigricans L. Fan, Y.W. Wang & Y.Y. Xu 2018

Distribution & Specimen information: under conifers (*Pinus* sp.), ascocarp from winter. Sichuan Province: Panzhihua, 16 Jan. 2014, BJTC FAN349 (CAO1424).

Notes: Holotype BJTC FAN349, ITS = NR161071, LSU = NG066415. No edible or medicinal use.

Hydnotrya oblongispora Lin Li & S.H. Li 2023 Figure 4.37

Distribution & Specimen information: under *Abies forrestii* Coltm.-Rog, ascocarp from late summer and early autumn. Yunnan Province: Lijiang, Jiuhe Township, alt. 3737m, 12 Aug. 2020, HKAS131236 (BMDU L20069); alt. 3946m, 12 Aug. 2020, HKAS131237 (BMDU L20067), alt. 3811m, 19 Sep. 2021, HKAS131238 (BMDU L21217).

Notes: Holotype HKAS131236 (BMDU L20069): ITS = OM232079, LSU = ON982627. Other specimen HKAS131237 (BMDU L20067): ITS = OM232075, LSU = ON982626; HKAS131238 (BMDU L21217): ITS = OM232084, LSU = ON982625. No edible or medicinal use.

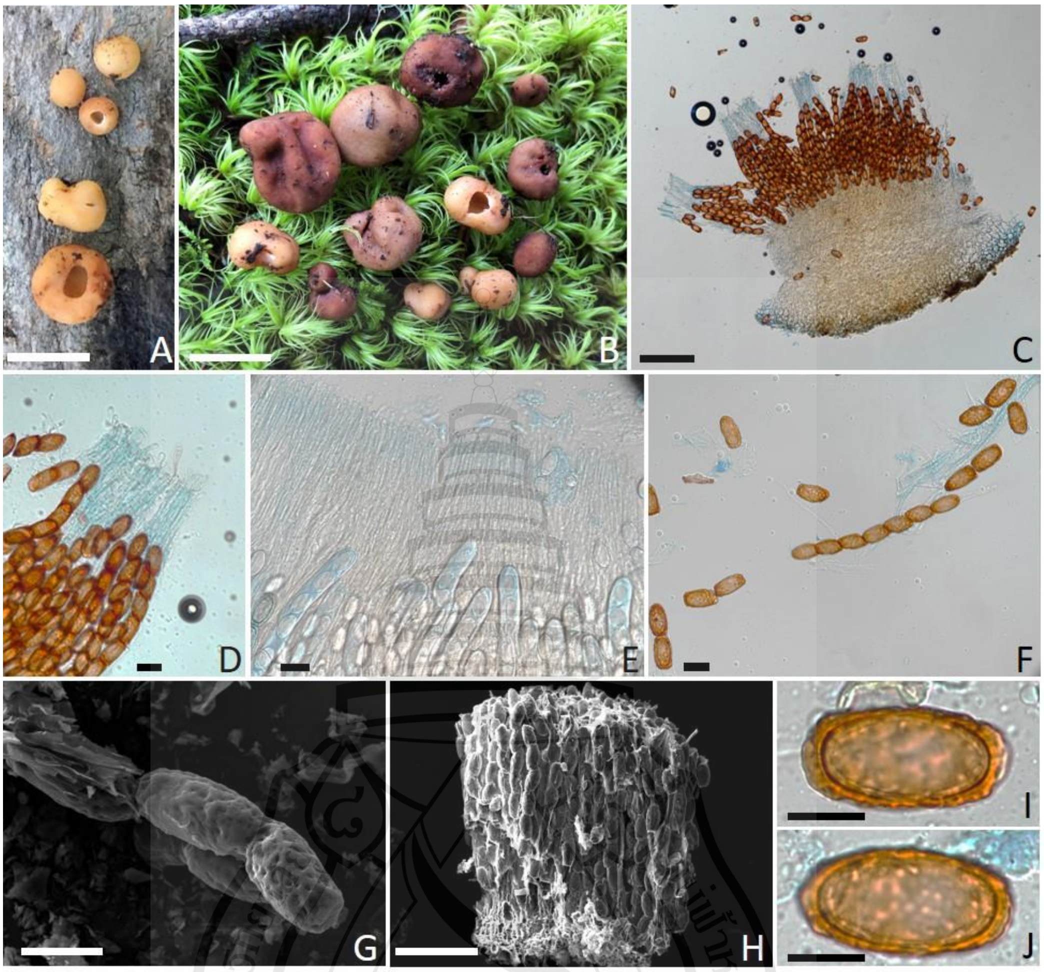


Figure 4.37 *Hydnotrya oblongispora* morphological characteristics

Figure 4.37 A.B. Ascomata with different openings; C. A piece of section of the ascomata in lactophenol cotton blue; D.E. Paraphyses in lactophenol cotton blue; F. Asci in lactophenol cotton blue; G. Ascospores under SEM; H. Asci under SEM; I.J. Single ascospore in 5% KOH and lactophenol cotton blue. Scale bars: A. B = 1 cm; C = 100 μm; D–G = 10 μm; H = 50 μm; I.J = 5 μm.

Hydnotrya puberula L. Fan, Y.W. Wang & Y.Y. Xu 2018

Distribution & Specimen information: under conifers (*Pinus* sp.), ascocarp from winter. Yunnan Province: Baoshan, 28 Dec. 2011, BJTC FAN721(CAO630).

Notes: Holotype BJTC FAN721, ITS = NR 161072, LSU = NG066416. No edible or medicinal use.

Hydnotrya zayuensis Lin Li & S.H. Li 2023 Figure 4.38

Distribution & Specimen information: under *Abies* spp., ascocarp from late summer and early autumn. Xizang Province: Zayu, alt. 3770m, 11 Aug. 2022, BMDU L22027; alt. 3840m, 15 Jul. 2022, BMDU L22024.

Notes: Holotype BMDU L22027: ITS = OP908305, LSU = OP908302. Other specimen BMDU L22024: ITS = OP908304, LSU = OP908301. No edible or medicinal use.

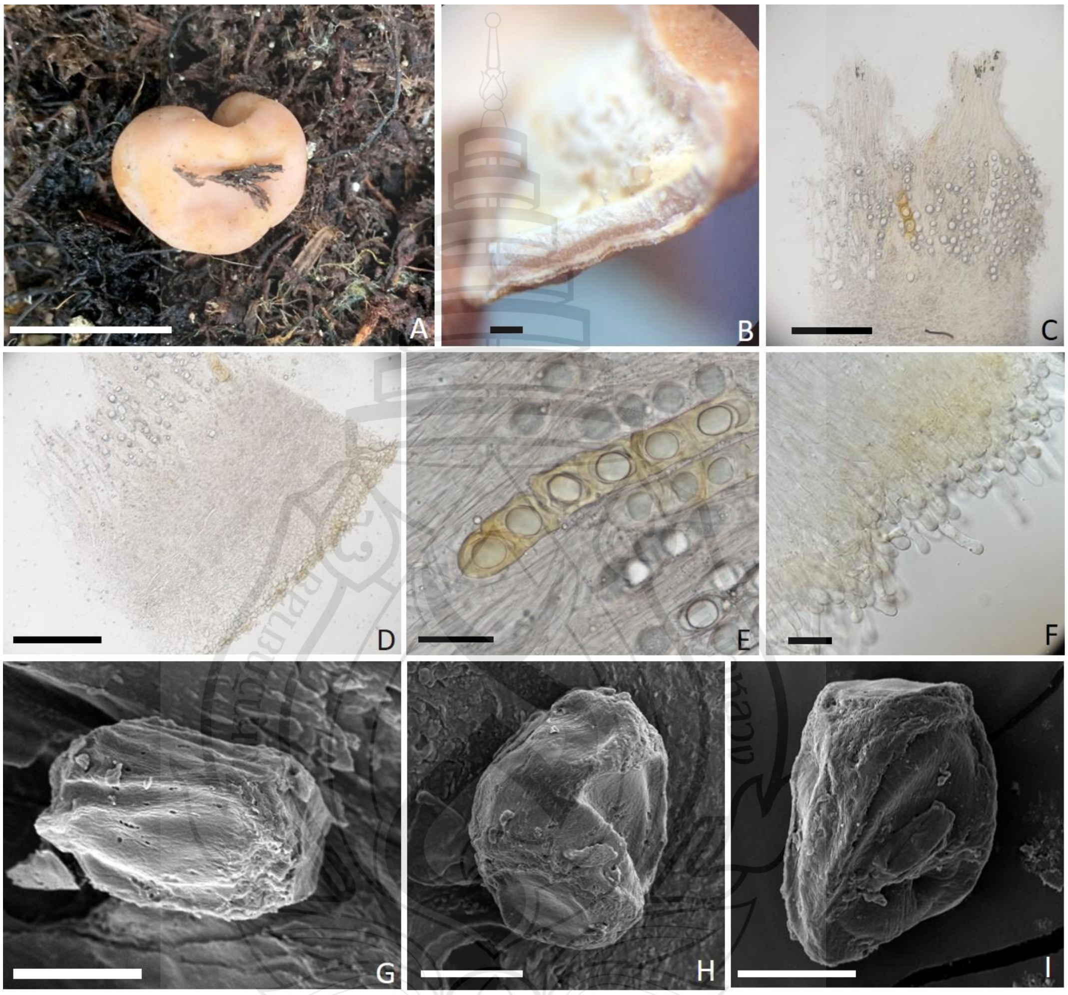


Figure 4.38 *Hydnotrya zayuensis* morphological characteristics

Figure 4.38 A. Ascomata. B. Section of ascomata, with hymenium-lined chambers. C. Section of hymenium in 5% KOH; D. Peridium in 5% KOH; E. Asci and ascospores in 5% KOH; F. Paraphyses in 5% KOH; G.H.I. Ascospores under SEM. Scale bars: A =1cm; B = 1 mm; C.D = 100 μm; E = 20 μm; F-I = 10 μm.

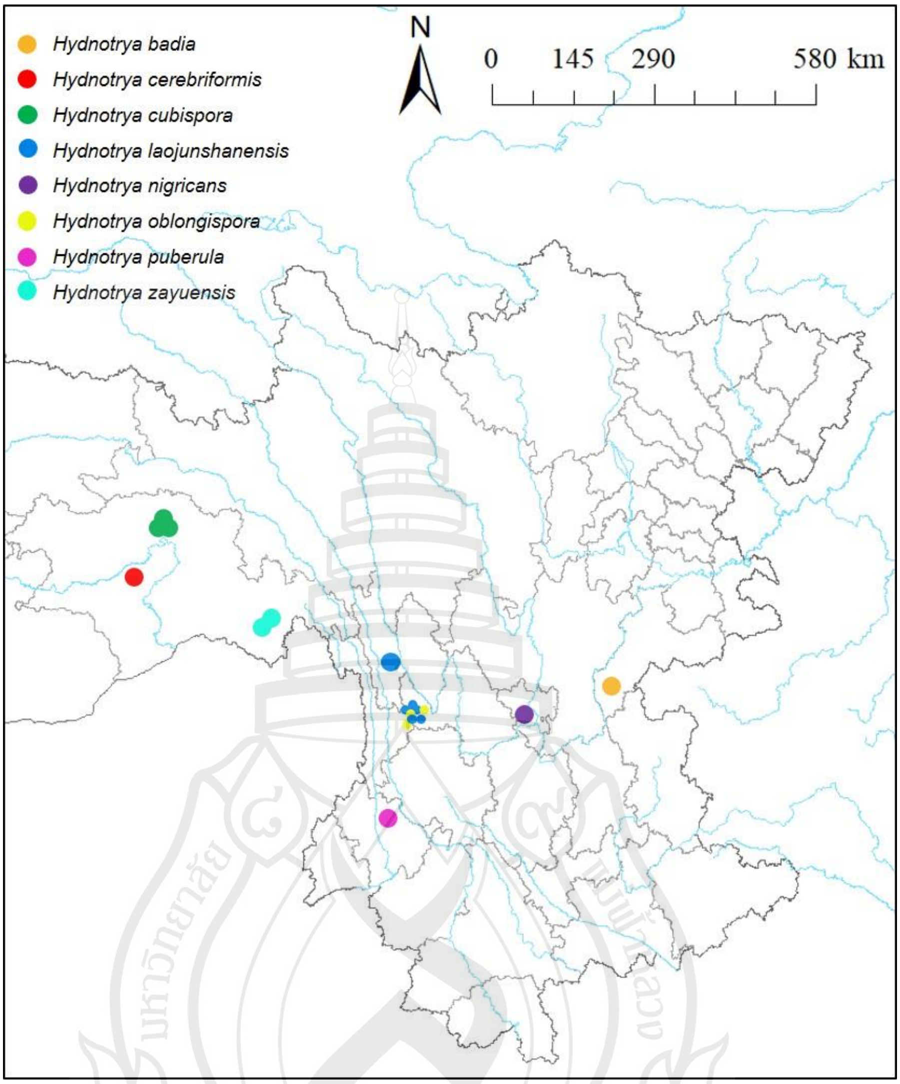


Figure 4.39 Distribution map of *Hydnotrya* species in southwestern China

Pezizaceae Dumort. 1829

Hydnobolites Tul. & C. Tul. 1843

The genus *Hydnobolites* is a truffle-like genus found exclusively in northern temperate forests. All known species in this genus have small ascomata and form ectomycorrhizal associations with a variety of tree species. (Trappe et al., 2009; Healy et al., 2013; Kovács et al., 2011; Li et al., 2019a; Wan et al., 2022). It was characterized as having generally lobed or folded ascomata with a pseudoparenchymatous cortex that could be changed to a loose hyphal area toward the hymenium. The hymenium was poorly arranged around veins that led to the surface with openings generally between folds of the ascomata. Ellipsoid to pyriform, eight-spored asci were irregularly arranged between canals in the medullary area and whereas globose spores with strongly projecting spines were scattered within the asci (Tulasne & Tulasne, 1843; Wan et al., 2022).

According to statistics, there are currently 10 species are now known in China, among which 8 species are distributed in southwest region: *Hydnobolite translucidus*, *H. subrufus*, *H. lini*, *H. tenuiperidius*, *H. yunnanensis* in Yunnan, *H. sichuanensis* in Sichuan, *H. cerebriformis* in Xizang province, *H. canaliculatus* in Yunnan and Sichuan province (Li et al., 2019a; Wan et al., 2022; Fan, 2022).

Hydnobolites canaliculatus L. Fan, Meng Chen & Ting Li 2019

Distribution & Specimen information: in humic soil under mixed forest, ascocarp from winter. Sichuan Province: Panzhihua, 23 Nov. 2014, BJTC FAN444 (CAO1534); Yunnan Province: Xundian, 10 Jan. 2015, BJTC FAN495 (CAO1564).

Notes: Holotype BJTC FAN444 (CAO1534): ITS = MK192803, LSU =MK208505. Other specimen BJTC FAN495 (CAO1564): ITS = MK192802, LSU = MK208506. No edible or medicinal use.

Hydnobolites cerebriformis Tul. & C. Tul. 1843

Distribution & Specimen information: in humic soil under trees of *Pinus wallichiana* and associated with *Rosa* sp., ascocarp from autumn. Xizang Province: Yadong county, alt. 3187m, 4 Aug. 2021, YNAU0318 (wsp1171); alt. 3185m, 10 Oct. 2021, YNAU0972 (wsp1171-1).

Notes: YNAU0318 (wsp1171): ITS = OM758123, LSU = OP642328; YNAU0972 (wsp1171-1): ITS = OM758124, LSU = OP642329. No edible or medicinal use.

Hydnobolites lini S.P. Wan & F.Q. Yu 2022

Distribution & Specimen information: in humic soil under a pure *Pinus armandii* forest, ascocarp from autumn. Yunnan Province: Ludian county, alt. 2270m, 29 Oct. 2021, YNAU0860 (wsp1723); alt. 2245m, 22 Aug. 2021, YNAU0362 (wsp1246); alt. 1868m, 29 Oct. 2021, YNAU0829 (wsp1694).

Notes: Holotype YNAU0860 (wsp1723): ITS = OM758149, LSU = OP642357. Other specimen YNAU0362 (wsp1246): ITS = OM758125, LSU = OP642330; YNAU0829 (wsp1694): ITS = OM758148, LSU = OP642356. No edible or medicinal use.

Hydnobolites sichuanensis S.P. Wan & F.Q. Yu 2022

Distribution & Specimen information: in humic soil under *Abies chensiensis* Tiegh. and *Picea wilsonii* Mast. forest or trees dominated by *Quercus guyavifolia* and associated with *Picea wilsonii*, ascocarp from autumn. Sichuan Province: Litang county, alt. 3911m, 10 Oct. 2021, YNAU0705 (wsp1575); alt. 3932m, 10 Oct. 2021, YNAU0712 (wsp1582).

Notes: Holotype YNAU0705 (wsp1575): ITS = OM758132, LSU = OP642337. Other specimen YNAU0712 (wsp1582): ITS = OM758133, LSU = OP642338. No edible or medicinal use.

Hydnobolites subrufus S.P. Wan & F.Q. Yu 2022

Distribution & Specimen information: in humic soil under a pure *Quercus guyavifolia* forest, ascocarp from later summer. Yunnan Province: Huize county, alt. 2354m, 14 Aug. 2016, HKAS95869 (wsp769).

Notes: Holotype HKAS95869 (wsp769): ITS = MT174248, LSU = OP642325. No edible or medicinal use.

Hydnobolites tenuiperidius S.P. Wan & F.Q. Yu 2022

Distribution & Specimen information: in humic soil under *Pinus armandii* forest, ascocarp from winter. Yunnan Province: Kunming city, alt. 2238m, 14 Nov. 2021, YNAU0900 (wsp1752); alt. 2256m, 14 Nov. 2021, YNAU0901 (wsp1753); Nanhua county, Wujie, alt. 2222m, 14 Nov. 2021, YNAU0899 (wsp1751); alt. 2256m, 14 Nov. 2021, YNAU0986 (wsp1829); alt. 2250m, 14 Nov. 2021, YNAU0987 (wsp1830).

Notes: Holotype YNAU0899 (wsp1751): ITS = OM758150, LSU = OP642358. Other specimen YNAU0900 (wsp1752): ITS = OM758151, LSU = OP642359; YNAU0901 (wsp1753): ITS = OM758152, LSU = OP642360; YNAU0986 (wsp1829): ITS = OP740748, LSU = OP799842; YNAU0987 (wsp1830): ITS = OP740749, LSU = OP799843. No edible or medicinal use.

Hydnobolites translucidus S.P. Wan & F.Q. Yu 2022

Distribution & Specimen information: in humic soil under a pure *Quercus guyavifolia* Levl. forest or *Pinus armandii* forest, ascocarp from autumn to winter. Yunnan Province: Huize county, alt. 2433m, 14 Aug. 2016, HKAS95861 (wsp761); Kunming City, alt. 2462m, 14 Aug. 2016, HKAS95859 (wsp758); alt. 1920m, 24 Sep.

2021, YNAU0568(wsp1435); 27 Jan. 2022, YNAU0988(wsp1919).

Notes: Holotype HKAS95861 (wsp761): ITS = MT174247, LSU = OP642324. Other specimen HKAS95859 (wsp758): ITS = OM758120, LSU = OP642323; YNAU0568(wsp1435): ITS = OM758130, LSU = OP642335; YNAU0988(wsp1919): ITS = OP740750, LSU = OP799844. No edible or medicinal use.

Hydnobolites yunnanensis L. Fan, Meng Chen & Ting Li 2019

Distribution & Specimen information: in humic soil under mixed forest, ascocarp from winter. Yunnan Province: Xundian, 10 Jan. 2015, BJTC FAN732 (FL1501).

Notes: Holotype BJTC FAN732 (FL1501): ITS = MK192821, LSU =MK208509. No edible or medicinal use.

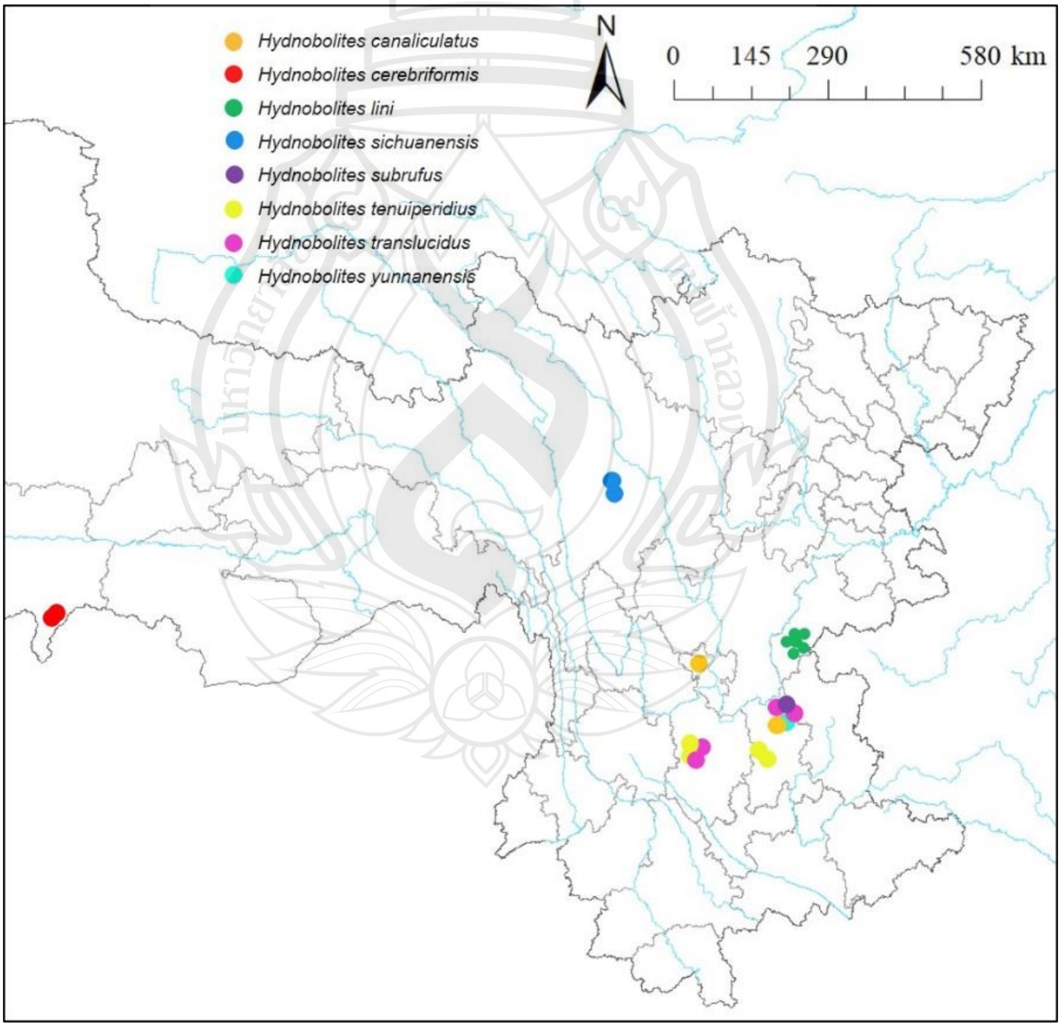


Figure 4.40 Distribution map of *Hydnobolites* species in southwestern China

Pachyphlodes Zobel 1854

The genus *Pachyphlodes* is a Northern Hemisphere-distributed group, occurs in temperate and subtropical regions. Species form hypogeous, truffle-like ascomata. *Pachyphlodesis* is characterized by ascomata with a thick, fleshy, usually warted excipulum. Ascomata colors include black, reddish-brown to yellowish brown, and orange. The ascospores are globose and ornamented with acute to capitate spines(Tulasne & Tulasne, 1844). Many species also produce mitospore mats on the soil surface (Healy et al., 2013, 2015). They form ectomycorrhizal associations with hosts in the Betulaceae Gray, Fagaceae Dumort., Juglandaceae A. Rich. ex Kunth, Pinaceae Spreng. ex F. Rudolphi, and Salicaceae Mirb. (Smith et al., 2007; Lindner & Banik, 2009; Stefani et al., 2009; Tedersoo et al., 2009, 2010; Bonito et al., 2011, Li et al., 2019b, Liu et al., 2020, Fan & Cai, 2015).

According to statistics, there are currently 5 species are now known in China, among which 3 species are distributed in the southwest region: *Pachyphlodes excavata P. atropurpurea* in Yunnan, *P. depressus* in Yunnan and Sichuan province (Li et al., 2019b, Fan & Cai 2015).

Pachyphlodes atropurpurea J.W. Liu, S.P. Wan & F.Q. Yu 2020

Distribution & Specimen information: specimens were bought from the mushroom market, then collected and mixed with the black truffle for sale by mushroom hunters, ascocarp from winter. Yunnan Province: Kunming mushroom market, 12 Dec. 2019, HKAS107304 (LJW-M27).

Notes: Holotype HKAS107304 (LJW-M27): ITS = MT939294, LSU = MT939258). Edible, occasionally seen at the black truffle market.

Pachyphloeus depressus L. Fan 2015

Distribution & Specimen information: in soil under forest dominated by *Pinus armandii*, ascocarp from autumn. Yunnan Province: Qiaojia, 10 Oct. 2013, BJTC FAN302 (Cao807); Sichuan Province: Huili, 10 Oct. 2013, BJTC FAN324 (Cao826).

Notes: Holotype BJTC FAN302 (Cao807): ITS = NR154314, LSU = NG060155. Other specimen BJTC FAN324 (Cao826): ITS = KP027406, LSU = KT220751. No edible or medicinal use.

Pachyphlodes excavata J.W. Liu, S.P. Wan & F.Q. Yu 2020

Distribution & Specimen information: specimens were bought from the mushroom market, then collected and mixed with the black truffle for sale by mushroom hunters, ascocarp from winter. Yunnan Province: Kunming mushroom market, 12 Dec. 2019, HKAS107305 (LJW-M32).

Notes: Holotype HKAS107305 (LJW-M32): ITS = MT939295, LSU = MT939259. Edible, occasionally seen at the black truffle market.

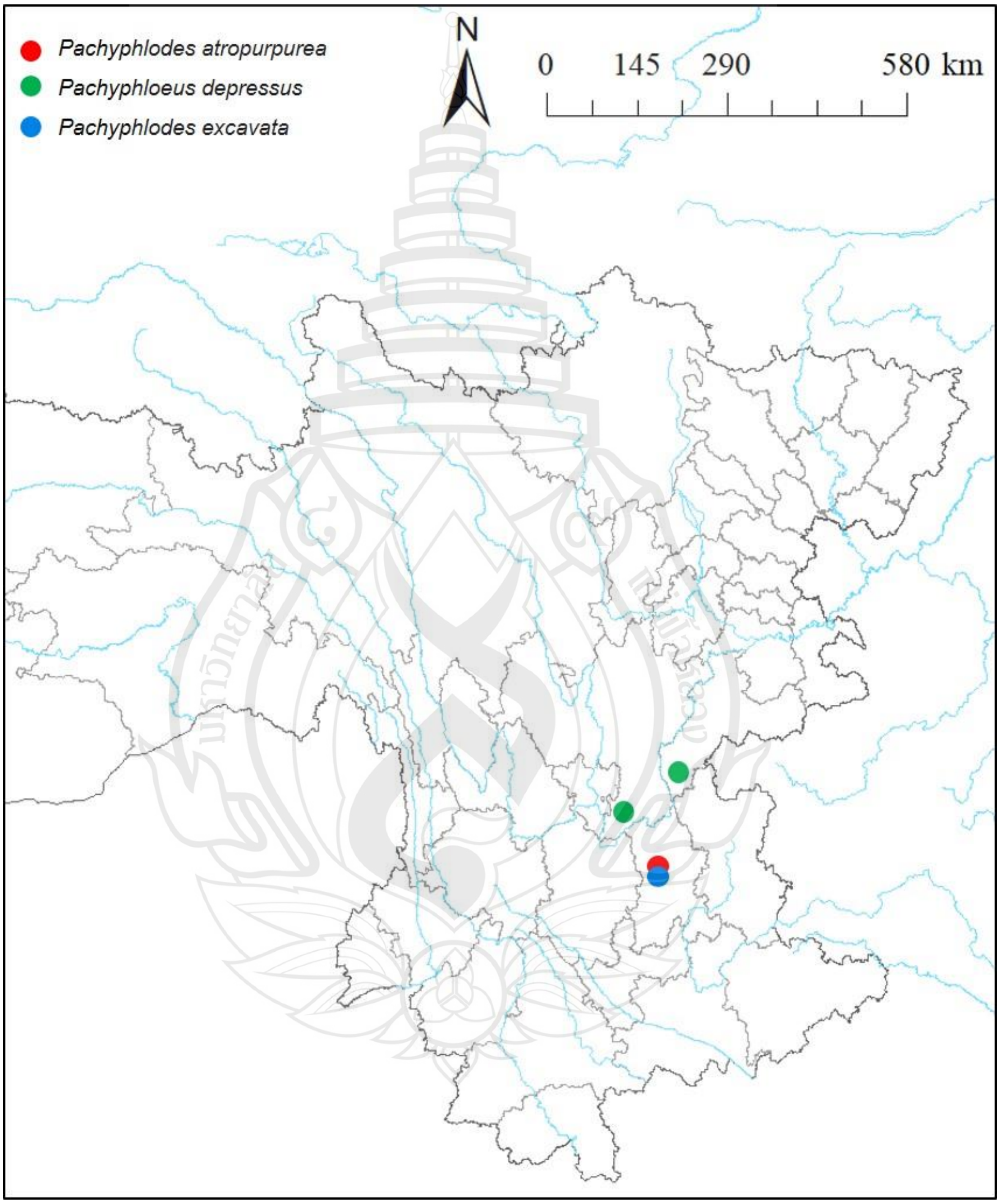


Figure 4.41 Distribution map of *Pachyphlodes* species in southwestern China

Pyronemataceae Corda 1842

Genea Vittad. 1831 [Synonymy: *Genabea* Tul. & C. Tul. 1844]

The genus *Genea* is a Northern Hemisphere distributed group, species with odoriferous hypogeous ascomata. They form ectomycorrhizal associations with hosts in the *Quercus* spp. or *Larix mastersiana* Hedh. & Wils. (Zhang, 1991b; Montecchi & Sarasini, 2000; Gori, 2005; Smith et al., 2006; Trappe, 2009). *Genea* are characterized by ascomata with more or less lobed and have a basal tuft of hyphae that attaches the ascomata to the substrate. Asci inamyloid, uniseriate, cylindrical, typically contain eight verrucose spores (Trappe, 1979; Smith, 2007; Guevara-Guerrero, 2012; Alvarado, 2016).

According to statistics, there are currently 5 species are now known in China, among which 2 species are distributed in southwest region: *Genea fragilis* and *Genea variabilis* in Sichuan province (Tao, 1988; Zhang, 1991b).

Genea fragilis (Tul. & C. Tul.) B.C. Zhang 1991

Distribution & Specimen information: in *Larix mastersiana* woods, ascocarp from autumn. Sichuan Province: Wenchuan, Wolong Natural Reserve, alt. 2250m, 22 Sep. 1989, HMAS60282 (Zhang645).

Notes: No sequence data available from southwestern China. No edible or medicinal use.

Genea variabilis B.C. Zhang 1991

Distribution & Specimen information: in *Larix mastersiana* woods, ascocarp from autumn. Sichuan Province: Wenchuan, Wolong Natural Reserve, alt. 2200m, 22 Sep. 1989, HMAS60280 (Zhang621); Heroes' Valley, alt. 2300m, 23 Sep. 1989, HMAS60281 (Zhang626)

Notes: Holotype HMAS60280 (Zhang621). No sequence data available. No edible or medicinal use.

Stephensia Tul. & C. Tul. 1845

The genus *Stephensia* is a Northern Hemisphere distributed group, species form hypogeous ascomata (De-Vries, 1985; Gyosheva et al., 2012; Kumar et al., 2017). *Stephensia* species have been considered ECM (Molina et al., 1992; Rinaldi et al., 2008), mycorrhizal with orchids (Bidartondo et al., 2004; Wang et al., 2016), or non-mycorrhizal (Hutchison, 1989; Tedersoo et al., 2010). *Stephensia* is characterized by an ascomata with brownish tomentose, truffle interior with meandering canals, globose smooth spores (except for *S. peyronelii* with ellipsoid spores), and non-amyloid asci (Gyosheva et al., 2012; Kumar et al., 2017)

According to statistics, there is currently one species are now known in China, *Stephensia bombycina*, collected from Yunnan province (Wang et al., 2016).

Stephensia bombycina (Vittad.) Tul. & C. Tul. 1851

Distribution & Specimen information: in mixed forest contact *Pinus yunnanensis* and *Corylus* sp., ascomata were found at 10-15 cm depth below the ground, around 30 cm away from the stem of a plant of Tianma (inoculated with mycelium of *Armillariella* sp.), which was planted one year ago. Ascocarp from summer. Yunnan Province: Kunming, Chenggong, Shuihaizi village, Xiya villa, alt. 1972m, 3 Jun. 2015, HMAS86978.

Notes: HMAS86978: ITS = KU556814, LSU = KU556815, rpb2 = KU932574, EF1a = KU932439. No edible or medicinal use.

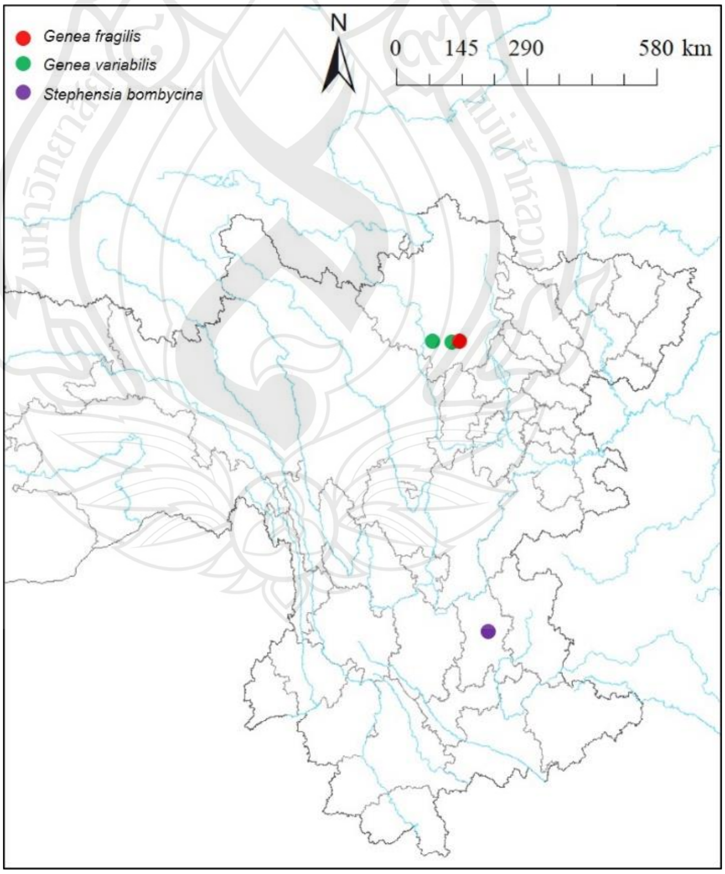


Figure 4.42 Distribution map of Pyronemataceae (*Genea, Stephensia*) species in southwestern China

Tuberaceae F. Berchtold & J. Presl 1820

Choiromyces Vittad. 1831

The genus *Choiromyces* is a Northern Hemisphere-distributed group. Species form hypogeous, truffle-like ascomata, some species are edible. *Choiromyces* is characterized by a thin peridium, forming a pseudoparenchymatic structure. They present a solid gleba conspicuously marbled with sterile veins surrounding the irregular but persistent hymenium, which is called ptycothecium (Læssøe & Hansen, 2007). Asci are indehiscent, clavate to saccate in shape, shortly stipitate, non-amyloid in Melzer’s reagent. They usually contain eight globose, hyaline or pale yellowish-brown spores ornamented at maturity (Zhang & Minter, 1989; Christina et al., 2009; Moreno et al., 2012; Wang et al., 2022). *Choiromyces* species form ectomycorrhizae with hosts in the Pinaceae conifers (Maia et al., 1996; Wang et al., 2022).

According to statistics, there are currently 5 species are now known in China, among which 2 species are distributed in the southwest region: *Choiromyces cerebriformis* in Yunnan, *C. sichuanensis* in Sichuan province (Tao, 1988; Chen et al., 2016; Yuan et al., 2021; Wang et al., 2022).

Choiromyces cerebriformis T.J. Yuan, S.H. Li & Y. Wang 2021

Distribution & Specimen information: under *Abies* sp. and *Rhododendron* sp. mixed forest, or under *Pinus yunnanensis* mixed forest, ascocarp from autumn to winter. Yunnan Province: Shangri-La, alt. 4005m, 24 Sep. 2020, HKAS107566 (YAAS8890); Chuxiong, alt. 1834m, 1 Nov. 2019, YAAS TJ16-1 and YAAS TJ16-2.

Notes: Holotype HKAS107566 (YAAS8890): ITS = MW209701. Other specimen YAAS TJ16-1: ITS = MT672013, YAAS TJ16-2: ITS = MT672014. Edible.

Choiromyces sichuanensis S.P. Wan, Ran Wang & F.Q. Yu 2022

Distribution & Specimen information: in humic soil under *Picea* spp. forest, ascocarp from autumn. Occasionally, *C. sichuanensis* is found mixed with Chinese white truffles (*Tuber panzhihuanense* Deng X.J. &Wang Y.) and sold together at the mushroom market. Sichuan Province: Songpan county, mushroom market, 29 Oct. 2020, YNAU003 (wsp971), YNAU002 (wsp971-1), YNAU004 (wsp971-2).

Notes: Holotype YNAU003 (wsp971): ITS = MW380902. Other specimen YNAU002 (wsp971-1): ITS = OM417587, YNAU004 (wsp971-2): ITS = OK585070. Edible, occasionally seen at the white truffle market.

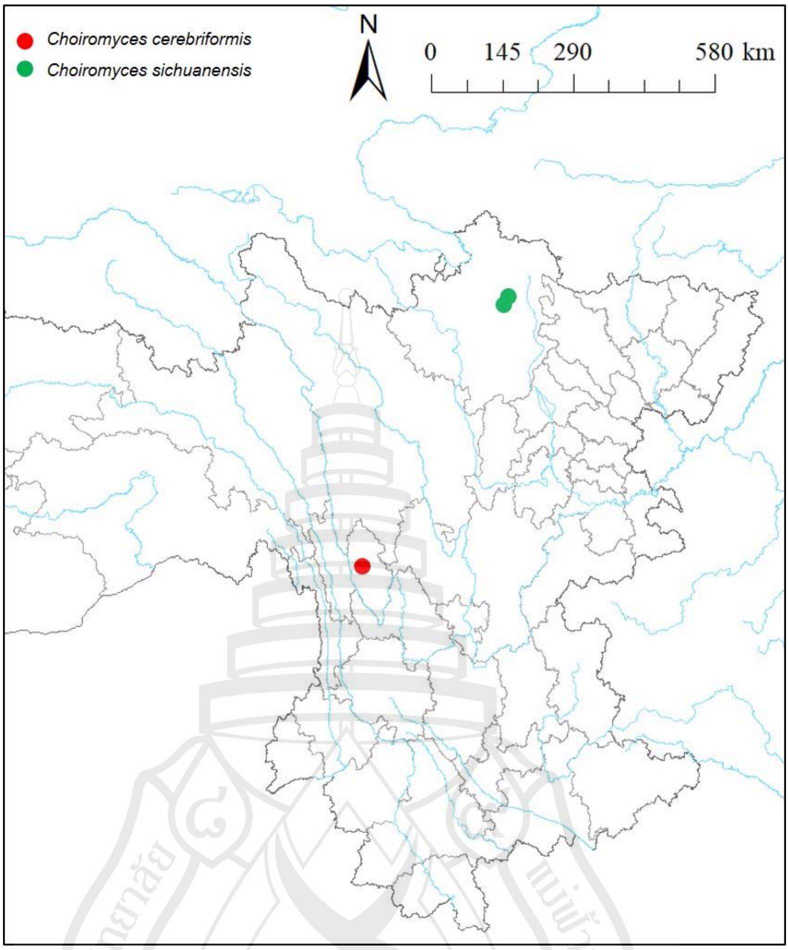


Figure 4.43 Distribution map of *Choiromyces* species in southwestern China

Tuber P. Micheli ex F.H. Wigg. 1780

Notes: The genus *Tuber* is a Northern Hemisphere-distributed group. *Tuber* characterized by ascomata subglobose or tuberiform, usually without rhizomorph, some species with a basal excavation; gleba is solid with a marbled pattern; ascospores are irregularly arranged within the asci, generally 1-5 spored, with some species having up to 8 spores. The ascospores are ellipsoid or subglobose, ranging in color from golden yellow to yellowish-brown, or dark brown to black, ornamented with spines, reticulations, or spiny-reticulations. Due to their pleasant aroma, many species have been appreciated since the 15th century as fragrant edible truffles.

According to statistics, there are currently 74 species are now known in China, among which 59 species are distributed in the southwest region (Fan et al., 2022; Guo & Bau, 2023; Fu & Fan, 2023; Li L. et al., 2024a).

Tuber albicavum Y. Wang, S.H. Li & Lin Li. 2024 Figure 44

Distribution & Specimen information: under *Abies forrestii* var. *smithii* forest, ascoma from autumn. Yunnan Province: Lijiang city, Jiuhe town, alt. 3753.4 m, 19 Sep. 2021, HKAS 131256 (BMDU L21210); alt. 3846 m, 12 Sep. 2020, HKAS 131255 (BMDU L20077).

Notes: Holotype HKAS 131256 (BMDU L21210): ITS = PP151577, LSU = PP151587. Other specimen HKAS 131255 (BMDU L20077): ITS = PP151578, LSU = PP151588. Edible.

Figure 4.44 *Tuber albicavum* morphological characteristics

Figure 4.44 A.B. Ascomata; C.D. A piece of section of the peridium and bristle-like hyphae in 5%KOH; E. A cavity section and hyphae extending beyond the chamber surface in 5%KOH; F.G. Asci in 5%KOH; H.I. Ascospores in 5%KOH. Scale bars: A.B = 1 cm; C.D = 50 µm; E = 10 µm; F.G = 30µm; H.I = 10 µm.

Tuber alboumbilicum Y. Wang & Shu H. Li. 2014

Distribution & Specimen information: in the soil of a forest dominated by *Tsuga chinensis* (Franch.) Pritz, ascoma from autumn. Sichuan Province: Danba county, alt. 2710 m, 17 Aug. 2012, YAAS L2324.

Notes: Holotype YAAS L2324: ITS = KJ742702. Unknown edible.

Tuber badium S.P. Wan 2016

Distribution & Specimen information: in the soil of a pine forest dominated by *Pinus armandii*, ascoma from winter. Sichuan Province: Huidong county, Dec. 2012, HKAS88789 (wsp 090); no specific location BJTC FAN381; Yunnan Province: Chuxiong city, Mt. Zixi, alt. 2201m, 12 Dec. 2023, BMDU L3152; no specific location on HKAS126383.

Notes: Holotype HKAS88789: ITS = KX904889, LSU = KY013652. Other specimen BMDU L3152: ITS = PP151584, BJTC FAN371: ITS = OM256747, LSU = OM366192, *tef1* = OM649597, *rpb2* = OM584255, BJTC FAN381: ITS = OM256748, LSU = OM366193, *tef1* = OM649598, *rpb2* = OM584256, HKAS126383: ITS = OQ025132. Edible occasionally appears in wild mushroom markets.

Tuber baoshanense S.P. Wan. 2017

Distribution & Specimen information: in humus soil under a pure *Pinus armandii* forest, alt. 2200 m, provided by the seller in Baoshan city, Yunnan province, Oct. BJTC FAN400 2012, HKAS88788 (wsp002).

Notes: Holotype HKAS88788: ITS = NR178114. Other specimen BJTC FAN400: LSU = OM366197, *tef1* = OM649602, *rpb2* = OM584260. Edible.

Tuber bomiense K.M. Su & W.P. Xiong. 2014

Distribution & Specimen information: in the soil of *Pinus densata* Mast. forest, ascoma from autumn. Xizang Province: Bomi county, Yigong town, 16 Oct. 2011, YAAS SKM101 and YAAS SKM106. Yunnan Province: no location information recorded, HKAS88805, BJTC FAN467 (Fan et al., 2022).

Notes: Holotype YAAS SKM101: ITS = KC517480. Other specimen YAAS SKM106: ITS = KC517481; HKAS88805: LSU = KY174959; BJTC FAN467: ITS = OM265247, LSU = OM366206, *tef1* = OM649611. Unknown edible.

Tuber caoi L. Fan. 2016

Distribution & Specimen information: in the soil of *Pinus armandii* forest, ascoma from winter. Yunnan Province: Huize county, 1 Dec. 2012, BJTC FAN271 (Fan 032); 4 Sept 2013, BJTC FAN293 (Cao 726); Qiaojia county, 17 Oct 2013, BJTC FAN309 (Cao 815); 17 Oct 2013, BJTC FAN321 (Cao 816).

Notes: Holotype BJTC FAN271: ITS = KP276183, LSU = KP276197, *tef1* = KP276216, *rpb2* = OM584237. Paratype BJTC FAN309: ITS = KP276181, LSU = KP276199, *tef1* = KP276218. Other specimens BJTC FAN293: ITS = OM256788, LSU = KP276198, *rpb2* = OM584240; *tef1* = KP276217; BJTC FAN321: ITS = OM256748, LSU = KP276200, *tef1* = KP276219. Unknown edible.

Tuber calosporum S.P. Wan 2016

Distribution & Specimen information: in the soil of a mixed forest dominated by *Pinus yunnanensis*, ascoma from autumn. Yunnan Province: Huize, provided by the sellers, Oct. 2014, HKAS88790 (wsp143), HKAS88751 (wsp145), HKAS88791 (wsp186), HKAS88794 (wsp382).

Notes: Holotype HKAS88790: ITS = KT444598. Paratype HKAS88751: ITS = KT444600, HKAS88791: ITS = KT444597, HKAS88794: ITS = KT444599. Other specimen BJTC FAN301: ITS = OM246568, LSU = OM366182, *tef1* = OM649586, *rpb2* = OM584242; BJTC FAN418: ITS = OM246573, LSU = OM366202, *tef1* = OM649607, *rpb2* = OM584265. *Tuber calosporum* mitochondrion complete genome NC053885 (Li H. et al., 2018). Edible, this species is commonly found in the truffle markets of Southwest China.

Tuber conditum T. J. Yuan, S. H. Li & X. H. Wang 2025

Distribution & Specimen information: in soil under mixed forest, with *Pinus yunnanensis* dominant, ascoma from autumn. Yunnan Province: Changning county, 20 Sep. 2020, YAAS L3385, YAAS L3682.

Notes: Holotype YAAS L3385: ITS = ON454665, LSU = ON428901. Paratype YAAS L3682: ITS = ON454667. Unknown edible.

Tuber crassitunicatum L. Fan & X.Y. Yan. 2018

Distribution & Specimen information: in soil of mixed forest dominated by *Pinus yunnanensis* in Yunnan, and in soil under *Vitex negundo* var. *heterophylla* in Shanxi, ascoma from autumn to winter. Yunnan Province: Xundian county, 16 Jan. 2015, BJTC FAN465 (Cao, 1560). Also distribution in Shanxi Province: Yangcheng county, Manghe, date unknown, HMAS 88575 (as *Tuber lyonii*); Yuanqu county, Houwentang Village in Lishan Mountain, 1 Aug. 1990, HMAS 88577 (as *Tuber lyonii*). This species is uncommon and has only been reported from the above-mentioned specimens from Yunnan and Shanxi.

Notes: Holotype BJTC FAN465: ITS = NR184354, LSU = NG088329, *tef1* = OM649610, *rpb2* = OM584268. Other specimen HMAS 88575: ITS = MH115296; HMAS 88577: ITS = MH115297. Unknown edible.

Tuber depressum S.P. Wan 2016

Distribution & Specimen information: in the soil of a pine forest dominated by *Pinus armandii*, ascoma from winter. Sichuan Province: Huidong county, Dec. 2012, HKAS 95396 (wsp098), HKAS 88895 (wsp093), HKAS 88896 (wsp094); 3 Nov. 2006, HKAS 52006 (CJ411), This specimen was first collected by Chen J. in Sichuan Province and identified as *T. excavatum*. Subsequently, in 2011, Fan et al., reidentified it as *T. neoexcavatum* using ITS single-gene phylogenetic analysis. However, with the collection of additional specimens, Wan et al., in 2016 redefined this specimen as *T. depressum* based on a combined analysis of ITS and LSU multi-gene phylogenetics and morphological characteristics.

Notes: Holotype HKAS 95396: ITS = KX904892, LSU = KY013655. Other specimen HKAS 88895: ITS = KX904890, LSU=KY013653, HKAS 88896: ITS = KX904891, LSU= KY013654, HKAS 52006: ITS = GQ217540, LSU= GU797140. Unknown edible.

Tuber glabrum L. Fan & S. Feng. 2014

Distribution & Specimen information: in the soil of a mixed forest dominated by *Pinus yunnanensis*, ascoma from autumn. Yunnan Province: Huize, 8 Sep. 2012, BJTC FAN228 (J. Z. Cao711), BJTC FAN232 (J. Z. Cao715), and BJTC FAN233 (J. Z. Cao716).

Notes: Holotype BJTC FAN228: ITS = NR153217, LSU = NG088326, *tef1* = OM649581, *rpb2* = OM584234. Other specimen BJTC FAN232: ITS = KF002727, LSU = OM366179, *tef1* = OM649583, *rpb2* = OM584236; BJTC FAN379: ITS = OM246572; BJTC FAN378: ITS = OM246571; BJTC FAN365: ITS = OM246570; BJTC FAN303: ITS = OM246569; BJTC FAN287: ITS = OM246567. Unknown edible.

Tuber griseolivaceum L. Fan & K.B. Huang. 2017

Distribution & Specimen information: in soil of forest dominated by *Pinus* *yunnanensis* or *Pinus armandii*, ascoma from winter. Sichuan Province: Panzhihua city, 29 Nov. 2014, BJTC FAN434 (Cao 1525). Yunnan Province: Xundian county, 14 Jan. 2015, BJTC FAN469 (Cao 1567).

Notes: Holotype BJTC FAN469: ITS = KY428921, LSU = NG088330, *tef1* = OM649612. Other specimen BJTC FAN470: ITS = KY428923; BJTC FAN434: ITS = KY428922. Unknown edible.

Tuber huiliense L. Fan 2022

Distribution & Specimen information: in soil of forest dominated by *Pinus armandii*, ascocarp occurring in autumn. Sichuan Province: Huili county, 4 Sep. 2013, BJTC FAN288.

Notes: Holotype BJTC FAN288: ITS = OM256781, LSU = OM366181, *tef1* = OM649585, *rpb2* = OM584238. Unknown Edible.

Tuber huidongense Y. Wang. 2002

Distribution & Specimen information: in soil under *P. armandii*-dominated forests mixed with *Betula alnoides* Buch.-Ham. ex D. Don, *Myrsine africana* L., and *Cotoneaster franchetii* Bois in Yunnan and Sichuan, or under *Cyclobalanopsis glauca* Oerst. in Taiwan, ascoma from winter. Sichuan Province: Huidong county, Jiangzhou, Minde village, alt. 2070 m, 226 Nov. 1989, Y. Wang 89923 (IFS); Huidong county, wild edible mushroom market, 3 Nov. 2006, HKAS 52008 (J. Chen 410), HKAS 52015 (J. Chen 419); Huili county, wild edible mushroom market, 6 Nov. 2006, HKAS 52016 (J. Chen 420). Yunnan Province: Kunming city, Shuanglong, Jiulongwan, Erdanshishan, alt. 2260 m, 10 Nov. 2007, HKAS 55304 (JD-03); Dali city, Xiangyun county, 320 national highway, toll station, alt. 1950 m, 2 Dec. 2007, HKAS 55305 (XY-03). Taiwan: Nan-tou county, alt. 1200 m, Dec. 2002, H.T. Hu 0201 (as Holotype of *T. furfuraceum*, in NTUF). This species is widely distributed in China, and there have also been reports of specimens from Korea (Gwon et al., 2022).

Notes: Holotype Y. Wang 89923 (IFS): ITS = DQ478668 and DQ478632. Other specimen HKAS 52008: ITS = FJ797881 and FJ797881, HKAS 52015: ITS = FJ797882 and FJ797882; HKAS 52016: ITS = FJ797883 and FJ797883; HKAS 55304: ITS = FJ797878 and FJ797878; HKAS 55305: ITS = FJ797877 and FJ797877; BJTC FAN101: LSU = OM366156, *tef1* = OM649562, *rpb2* = OM584208; BJTC FAN103: ITS = MH115294, LSU = MH115301; BJTC FAN104: LSU = OM366158, *tef1* =

OM649563, *rpb2* = OM584209. Edible.

Tuber huizeanum L. Fan & C.L. Hou. 2013

Distribution & Specimen information: in the soil of a pine forest, ascoma from winter. Yunnan Province: Huize county, 28 Dec. 2011, BJTC FAN186 (J.Z. Cao 513); Kunming city, from the Kunming mushroom market, 20 Jan. 2011, BJTC FAN144 (J.Z. Cao 107). This species is a common white truffle in the Jinshajiang Valley.

Notes: Holotype BJTC FAN186: ITS = JQ910651, LSU = NG059991, *tef1* = OM649575. Other specimen BJTC FAN144: ITS = JN870100, LSU = KT067697, *tef1* = KT032182; BJTC FAN314: ITS = KT067685, LSU = KT067692, *tef1* = OM649588, *rpb2* = OM584246; BJTC FAN313: ITS = KT067684, LSU = KT067691, *tef1* = KT067715, *rpb2* = OM584245. Edible, commonly found in the truffle markets of Southwest China.

Tuber humilireticulatum L. Fan 2022

Distribution & Specimen information: under *Pinus* sp. mixed forest, ascocarp from winter. Yunnan Province: Kunming city, 28 Nov. 2011, BJTC FAN174, BJTC FAN175, BJTC FAN189; Huize county, 12 Nov. 2015, BJTC FAN485, BJTC FAN486.

Notes: Holotype BJTC FAN174: ITS = KT067677, LSU = OM366168, *tef1* = KT067724, *rpb2* = OM584224. Other specimen BJTC FAN175: ITS = KT067689, SSU= KT067700, *tef1* = KT067723; BJTC FAN189: ITS = KT067689, LSU = OM366171, SSU= KT067705, *tef1* = KT067718, *rpb2* = OM584226; BJTC FAN485: ITS = OM286866; BJTC FAN486: ITS = OM286867. Unknown edible.

Tuber jinshajiangense L. Fan. 2016

Distribution & Specimen information: in soil of pine forest or under *Populus* sp., ascoma from winter. Sichuan Province: Huili county, 20 Dec. 2002, BJTC FAN124 (L. Fan 024), as *T. californicum*; Qingchuan county, Tangjiahe, alt. 1500 m, under *Populus* sp., 17 Sep. 1989, HMAS 60217 (B.C. Zhang 606), as *T. californicum*. Yunnan Province: Baoshan city, 27 Nov. 2014, BJTC FAN406 (Cao 1502), BJTC FAN407 (Cao 1503), BJTC FAN410 (Cao 1506), BJTC FAN412 (Cao 1507), and BJTC FAN451 (Cao 1692).

Notes: Holotype BJTC FAN124: ITS = KP276177, LSU = NG059985. Other specimen BJTC FAN406: ITS = KX575841, LSU = OM366199, *tef1* = OM649604, *rpb2* = OM584262; BJTC FAN407: ITS = KX575842, LSU = OM366200, *tef1* =

OM649605, *rpb2* = OM584263; BJTC FAN410: ITS = KX575843; BJTC FAN412: ITS = KX575844, LSU = KX575848; BJTC FAN451: ITS = KX575845, LSU = KX575849. Edible. This species is common in the Jinsha River Basin. Although it was initially identified as *T. californicum* by Cao (2010), it was later reclassified as *T. jinshajiangense* after Fan (2016) reexamined the specimens. It is currently only found in the Yunnan and Sichuan provinces of China.

Tuber laojunshanense Y. Wang, S.H. Li & Lin Li. 2024 Figure 45

Distribution & Specimen information: under *Abies forrestii* var. *smithii* forest, ascoma from autumn. Yunnan Province: Lijiang city, Jiuhe town, alt. 3856 m, 16 Sep. 2022, HKAS 131253 (BMDU L22070); alt. 3645 m, 11 Aug. 2020, BMDU L20065, BMDU L20066; alt. 3875 m, 19 Sep. 2021, HKAS131251 (BMDU L21213), HKAS131252 (BMDU L21216), HKAS131254 (BMDU L21218), BMDU-L21218a.

Notes: Holotype HKAS 131253 (BMDU L22070): ITS = PP151583, LSU = PP151593. Other specimen BMDU L20065: ITS = PP151573; BMDU L20066: ITS = PP151574; HKAS131251 (BMDU L21213): ITS = PP151579, LSU = PP151589; HKAS131252 (BMDU L21216): ITS = PP151580, LSU = PP151590; HKAS131254 (BMDU L21218): ITS = PP151581, LSU = PP151591; BMDU-L21218a: ITS = PP151582, LSU = PP151592. Edible.

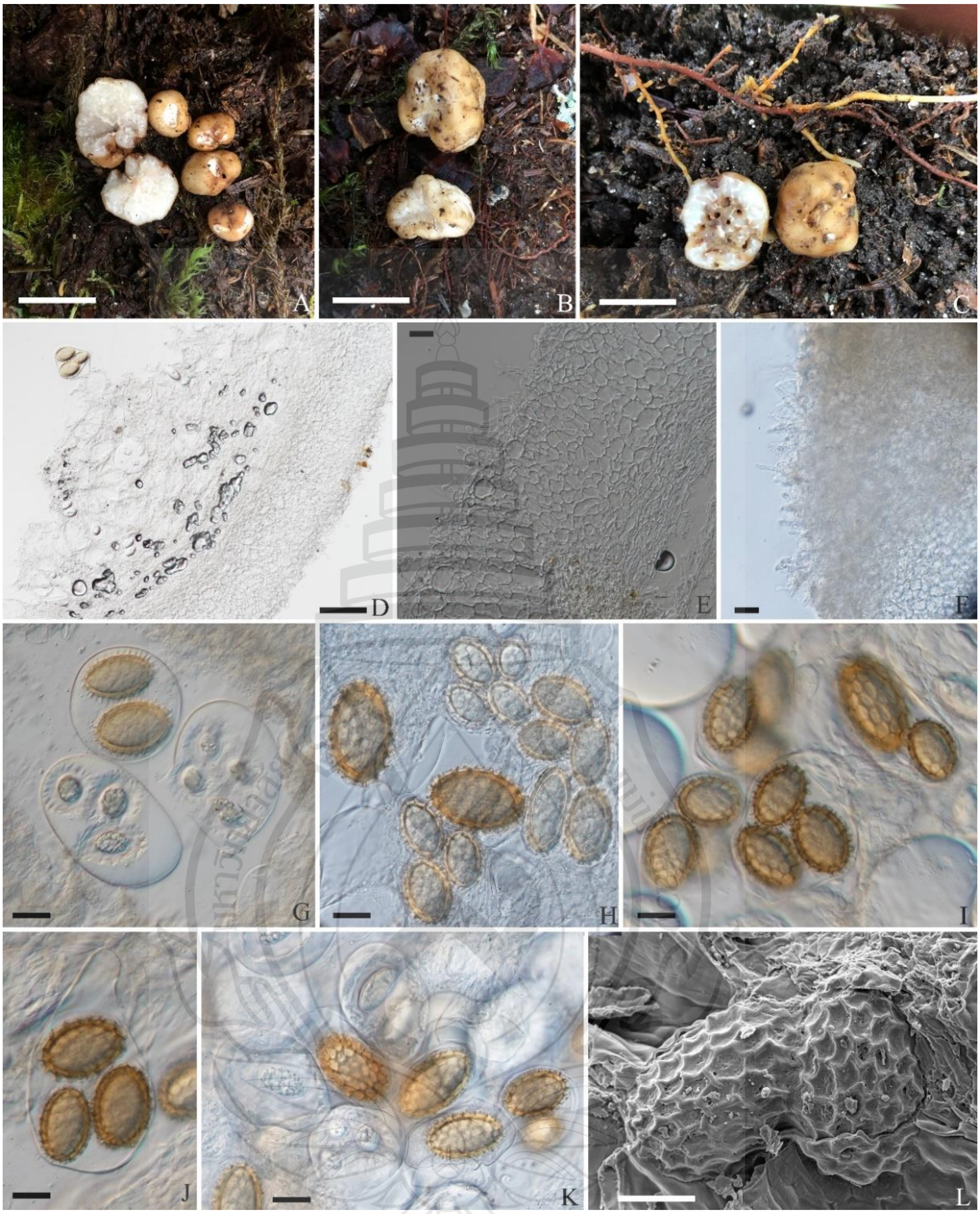


Figure 4.45 *Tuber laojunshanense* morphological characteristics

Figure 4.45 A.B.C. ascomata and gleba; C.D.E. a piece of the section of peridium in 5% KOH; F. peridium outer layer and cystidia in 5% KOH; G-K. Asci and ascospores; L. SEM ascospores (dry sample). Scale bars: A, B, C = 1 cm; D = 100 µm; E-L = 10 µm.

Tuber latisporum Juan Chen & P.G. Liu. 2007

Distribution & Specimen information: in the soil of pine or *Quercus* spp. forest, ascoma from autumn to winter. Yunnan Province: Kunming city, Kunming wild edible mushroom market, 31 Oct. 2003, HKAS 44315 (J. Chen 144); Kunming city, Haikou county, alt. 2100m, 30 Nov. 2003, HKAS 42380 (Z.L. Yang 3608), HKAS 41317 (Z.L. Yang 3607); Kunming city, Chenggong county, alt. 2000m, 26 Nov. 1997, HKAS 30838B (M. Zang 12891).

Notes: Holotype HKAS 44315: ITS = NR119620. Other specimen HKAS 30838B: ITS = DQ898185; HKAS 42380: ITS = DQ898184; BJTC FAN125: ITS = KT067676, LSU = KT067695, *tef1* = KT067725, *rpb2* = OM584214; BJTC FAN126: ITS = KP276189, LSU = KP276204, *tef1* = KP276205, *rpb2* = OM584215. Edible. This species is one of the most common species in the truffle markets of Southwest China.

Tuber lijiangense L. Fan & J.Z. Cao. 2011

Distribution & Specimen information: in the soil of a pine forest, ascoma from autumn to winter. Yunnan Province: Lijiang city, Yongsheng county, 29 Oct. 2006, HKAS 52005 (J. Chen 404).

Notes: Holotype HKAS 52005: ITS = KF805727. Other specimen BJTC FAN307: ITS = KP276188, LSU = KP276203, *tef1* = KP276206, rpb2 = OM584244. Edible. Although the holotype specimen HKAS 52005 was initially identified as *T. borchii* var. *sphaerosperma* Malençon by Chen (2007), it was later reclassified as *T. lijiangense* after Fan et al. (2011) reexamined the specimen. This species is common in Southwest China.

Tuber liui A-S. Xu. 1999

Distribution & Specimen information: in the soil of the *Quercus aquifolioides* forest, ascoma from autumn. Xizang Province: Milin county, Zaxiraodeng town, Tianbunong, alt. 3100 m, 1 Sep. 1996, HXZE 984 (A.S. Xu 96207).

Notes: Holotype HXZE 984: ITS = DQ478660 and DQ478636. Unknown edible.

Tuber liyuanum L. Fan & J.Z. Cao. 2013

Distribution & Specimen information: in soil of pine forest, ascoma from autumn to winter. Yunnan Province: Huize county, 22 Oct. 2011, BJTC FAN162 (S.P.

Li 001); 28 Dec. 2011, BJTC FAN187 (J.Z. Cao 514).

Notes: Holotype BJTC FAN162: ITS = JQ771191, LSU = KT067698, *tef1* = KT067710, *rpb2* = OM584218. Other specimen BJTC FAN187: ITS = JQ771193, LSU = KT067704, *tef1* = KT067719. Edible. This species is a common truffle found under pine trees in the Jinshajiang Valley of Southwest China.

Tuber magnireticulatum L. Fan 2022

Distribution & Specimen information: under *Pinus armandii* forest, ascocarp from winter. Sichuan Province: Huidong county, 4 Jan. 2016, BJTC FAN537.

Notes: Holotype BJTC FAN537: ITS = OM256767, LSU = OM366212, *tef1* = OM649617. Unknown edible.

Tuber marroninum T. J. Yuan, S. H. Li & X. H. Wang 2025

Distribution & Specimen information: in soil under mixed forest, with *Pinus yunnanensis* dominant, ascocarp from autumn. Yunnan Province: Xiangyun county, 20 Sep. 2020, YAAS L3694, YAAS L3695.

Notes: Holotype YAAS L3694: ITS = ON454668, LSU = ON428904, *tef1* = OQ305199, *rpb2* = OQ305202. Paratype YAAS L3695: ITS = OQ297680. Unknown edible.

Tuber melanorufum L. Fan. 2023

Distribution & Specimen information: under *Pinus* forest, ascoma from autumn. Yunnan Province: Huize county, 31 Oct. 2020, BJTC FAN1273; 21 Oct. 2020, BJTC FAN1274, BJTC FAN1276, BJTC FAN1287.

Notes: Holotype BJTC FAN1273: ITS = NR191213. Other specimen BJTC FAN1274: ITS = OP852123, BJTC FAN1276: ITS = OP852124. Unknown edible.

Tuber melanoumbilicatum S.P. Wan, Rui Wang & F.Q. Yu 2024

Distribution & Specimen information: under a *Pinus* sp. forest, ascoma from winter. Yunnan Province: Baoshan City, 5 Dec. 2020, YNAU017 (wsp1006), YNAU018 (wsp1006-1).

Notes: Holotype YNAU017 (wsp1006): ITS = OK625304, LSU = OR661815, *tef1* = OR832379, *rpb2* = OR832411. Other specimen YNAU018 (wsp1006-1): ITS = OK625305, LSU = OR661816; *tef1* = OR832380; *rpb2* = OR832412. Unknown edible.

Tuber microexcavatum S.P. Wan, Rui Wang & F.Q. Yu 2024

Distribution & Specimen information: collected from *Platycarya strobilacea* Maxim., occasionally on *Pinus armandii* Franch., ascoma from summer. Yunnan Province: Luquan County, 11 Aug. 2022, 5 Dec. 2020, YNAU1263 (wsp2087), YNAU1264 (wsp2087-1).

Notes: Holotype YNAU1263 (wsp2087): ITS = OR250184; LSU = OR661838; *tef1* = OR832381; *rpb2* = OR832413. Other specimen YNAU1264 (wsp2087-1): ITS = OR250185; LSU = OR661839; *tef1* = OR832382; *rpb2* = OR832414. Unknown edible.

Tuber neoexcavatum L. Fan & Yu Li. 2013

Distribution & Specimen information: in the soil of *Pinus yunnanensis* forest, and *P. armandii* forest, ascoma from winter. Yunnan Province: Baoshan city, 28 Dec. 2011, BJTC FAN184.

Notes: Holotype BJTC FAN184: ITS = JX458715. Other specimen BJTC FAN330: ITS = OM287841. Edible. This species is commonly found in the Jinsha River basin and is also frequently seen in markets. Since its formal naming, numerous specimens have been used in various studies, resulting in a substantial amount of available sequence data.

Tuber panzhihuanense X.J. Deng & Y. Wang. 2013

Distribution & Specimen information: in calcareous soils with pH 6.8–7.6 under trees of *P. yunnanensis* at alt. 1719–2538 m, ascoma from autumn to winter. Sichuan Province: Panzhihua city, Yanbian county, alt. 2000m, 24 Nov. 2010, HKAS72015 (X.J. Deng 267) and HKAS72016 (X.J. Deng 268); 23 Nov. 2010, HKAS72024 (X.J. Deng 276), HKAS72025 (X.J. Deng 277), HKAS72030 (X.J. Deng 282), and HKAS72013 (X.J. Deng 265). Yunnan Province: Kunming city, 29 Nov. 2010, HKAS72008 (X.J. Deng 260); Zhangtong village, 10 Dec. 2010, HKAS72011 (X.J. Deng 263); Qujin, 13 Dec. 2010, HKAS72014 (X.J. Deng 266); Huize, 18 Dec. 2010, HKAS72069 (X.J. Deng 325) and HKAS72077 (X.J. Deng 333); Yongsheng, 21 Dec. 2010, HKAS72026 (X.J. Deng 278).

Notes: Holotype HKAS72015: ITS = NR120126. Other specimen HKAS72077: ITS = JQ978655; HKAS72069: ITS = JQ978654; HKAS72030: ITS = JQ978653; HKAS72026: ITS = JQ978652; HKAS72025: ITS = JQ978651; HKAS72024: ITS =

JQ978650; HKAS72016: ITS = JQ978649; HKAS72014: ITS = JQ978647; HKAS72013: ITS = JQ978646; HKAS72011: ITS = JQ978645; HKAS72008: ITS = JQ978644; HKAS95328: LSU = KY174962; HKAS95329: LSU = KY174963. Edible. This species is the most common white truffle in the Jinshajiang Valley and is also the primary white truffle traded in the truffle markets of Southwest China.

Tuber parvomurphium L. Fan. 2016

Distribution & Specimen information: in soil under conifers dominated by *Pinus armandii*, ascoma from autumn to winter. Yunnan Province: Qiaojia county, 10 Dec. 2013, BJTC FAN298 (Cao 803); 16 Nov. 2013, BJTC FAN323 (Cao & Fan 825).

Notes: Holotype BJTC FAN298: ITS = KP276186, LSU = NG059981, *tef1* = KP276214, *rpb2* = OM584241. Paratype BJTC FAN323: ITS = KP276185, LSU = KP276191, *tef1* = KP276215. Other specimen BJTC FAN421: ITS = OM256795. Unknown edible.

Tuber polymorphosporum S.P. Wan. 2017.

Distribution & Specimen information, in humus soil under a pure *Pinus armandii* forest, ascoma from winter. Yunnan Province: Baoshan city, Changning county, alt. 2499m, 5 Nov. 2014, HKAS88793 (wsp263), HKAS88799 (wsp387), HKAS88800 (wsp388).

Notes: Holotype HKAS88793: ITS = : KX262072, LSU = KY013652. Paratype HKAS88799: ITS = KX262073, HKAS88800: ITS = KX262074. Other specimen BJTC FAN411: ITS = OM256793; BJTC FAN413: ITS = OM256794; BJTC FAN544: ITS = OM256802. Unknown edible.

Tuber pseudobrumale Y. Wang & Shu H. Li. 2014

Distribution & Specimen information: in the soil of *Pinus yunnanensis* forest, ascoma from winter. Yunnan Province: Huize, alt. 1816m, 7 Dec. 2013, YAAS L3181. An additional 5 specimens are preserved at the BJTC.

Notes: Holotype YAAS L3181: ITS = KJ742703. Other specimen BJTC FAN458: ITS = OM287846; BJTC FAN346: ITS = OM287843; BJTC FAN334: ITS = OM287842; BJTC FAN322 ITS = OM287839, LSU = OM366186, *tef1* = OM649591, *rpb2* = OM584249; BJTC FAN306: ITS = OM287838, LSU = OM366183, *tef1* = OM649587, *rpb2* = OM584243. Edible.

Tuber pseudoexcavatum Y. Wang, G. Moreno, Riousset, Manjón & G. Riousset 1998

Distribution & Specimen information: in soils under *Pinus yunnanensis* or *P. armandii* forests, at alt. 2000–3000 m, ascoma from autumn to winter. Sichuan Province: Huidong County, 26 Nov. 1989, collected by A.P. Li and Y.W. Li, deposited in the Herbarium of the Institute of Forestry and Soil Sciences: IFS 89912, IFS 89913, IFS 89914, IFS 89916, IFS 89919, IFS 89920, IFS 89922, IFS 89911, IFS 89910, IFS 89915, IFS 89917, and IFS 89918. When Dr. Yun Wang first described the species (Wang et al., 1998), he also examined ascomata imported in January 1995 from Yunnan Province, China, to the French and Spanish markets, deposited as AH18384, AH18385, and AH18387 in the Herbarium of the University of Alcalá Additionally, he studied ascomata imported from China and collected from pine forest soils in Yunnan, deposited as L. J. Riousset Herbarium 01 14 03 95 (preserved in the L. J. Riousset Herbarium). The authors designated L. J. Riousset Herbarium 01 14 03 95 as the holotype, and AH 18387 as an isotype. Yunnan Province: Changning County, found in soil under a pure *Pinus armandii* forest, alt. 2500 m, 20 Sep. 2020, collected by S.H. Li, specimens deposited as YAAS L3932 and YAAS L3933 in the Herbarium of the Biotechnology and Germplasm Resources Institute, Yunnan Academy of Agricultural Sciences (YAAS). The presence of this species may not be uncommon in the southwestern region of China. One mycorrhiza sequence in GenBank (accession no. LC202054) indicates that the species also occurs in Taiwan, China.

Notes: Holotype: L. J. Riousset Herbarium 01 14 03 95. Several sequences derived from other specimens or isolates are available in GenBank. Edible.

Tuber pseudofulgens L. Fan & X.Y. Sang 2022

Distribution & Specimen information: in the soil of a mixed forest dominated by *Pinus* spp., ascocarp from autumn to winter. Yunnan Province: Baoshan city, 27 Nov. 2014, BJTC FAN399; Kunming city, Dongchuanqu, 22 Sep. 2014, BJTC FAN368; Sichuan Province: Huidong county, Sep. 2014, BJTC FAN388.

Notes: Holotype BJTC FAN399: ITS = OM256757, LSU = OM366196, *tef1* = OM649601, *rpb2* = OM584259. Other specimen BJTC FAN368: ITS = OM256745, LSU = OM366191, *tef1* = OM649596, *rpb2* = OM584254; BJTC FAN388: ITS = OM256755, LSU= OM366194, *tef1* = OM649599, *rpb2* = OM584257. Unknown edible.

Tuber pseudohimalayense G. Moreno, Manjón, J. Díez & García-Mont. 1997

Distribution & Specimen information: in the soil of pine and oak forest, ascoma from autumn to winter. The holotype specimen AH 18331 consists of a single ascocarp. It was imported from China to Spain and donated by a truffle trading company, with no detailed habitat information available. According to the author’s description, it was probably imported from China in January of 1995 and is preserved at The Herbarium of the University of Alcalá (AH). However, this species is widely distributed in China and is one of the most common varieties in the truffle markets. It is bought in large quantities every year and is referred to as "female truffle" in Southwest China.

Notes: Holotype AH 18331: LSU = FJ233104 and GU979110. Other specimen HKAS126409: ITS = OQ025152; BJTC FAN541: ITS = OM287847; BJTC FAN354: ITS = OM287844; BJTC FAN328: ITS = OM287840; BJTC FAN297: ITS = OM287837; BJTC FAN266: ITS = OM287836; BJTC FAN216: ITS = OM287835; BJTC FAN161: ITS = OM287834; BJTC FAN160: ITS = OM287833; BJTC FAN123: ITS = OM287832; BJTC FAN121: ITS = OM287831; BJTC FAN122: ITS = MF627983, LSU = OM366162, *tef1* = OM649567, *rpb2* = OM584213. Edible.

Tuber pseudomagnatum L. Fan. 2013

Distribution & Specimen information: in the soil of *Pinus yunnanensis* forest, ascoma from autumn. Yunnan Province: Huize, 22 Cot. 2011, BJTC FAN163 (Shao-pin Li 002). An additional 12 specimens from the Jinshajiang Valley are preserved at the BJTC. Below are their specimen numbers and molecular data.

Notes: Holotype BJTC FAN163: ITS = JQ771192, LSU = KP276192, *tef1* = KP276208, *rpb2* = OM584219. Other specimen BJTC FAN391: ITS = OM265244, LSU = OM366195, *tef1* = OM649600, *rpb2* = OM584258; BJTC FAN532: ITS = OM265248; BJTC FAN403: ITS = OM265246; BJTC FAN393: ITS = OM265245; BJTC FAN390: ITS = OM265243; BJTC FAN389: ITS = OM265242; BJTC FAN376: ITS = OM265241; BJTC FAN372: ITS = OM265240; BJTC FAN367: ITS = OM265239; BJTC FAN366: ITS = OM265238; BJTC FAN299: ITS = KP276184, LSU = KP276193, *tef1* = KP276209; BJTC FAN315: ITS = KT067686, LSU = KT067693, *tef1* = KT067711. Edible. This is one of the more common species in the Jinshajiang Valley.

Tuber pseudosphaerosporum L. Fan. 2013.

Distribution & Specimen information: in the soil of *Pinus armandii* forest, ascoma from winter. Yunnan Province: Huize, Daibu town, 11 Nov. 2012, BJTC FAN250 (Shuang Feng 017) and BJTC FAN260 (Xiao-yong Li 008).

Notes: Holotype BJTC FAN250: ITS = NR153229, LSU = NG059982, *tef1* = OM649584. Other specimen BJTC FAN260: ITS = KF744062; BJTC FAN394: ITS = OM256789. Unknown Edible.

Tuber qujingense S.P. Wan 2021

Distribution & Specimen information: in humic soil under a pure *Pinus armandii* forest, ascoma from autumn. Yunnan Province: Huize county, alt. 2415m, 12 Aug. 2016, HKAS 95823 (wsp721).

Notes: Holotype HKAS 95823: ITS = KX904885, LSU = KY013659. Unknown Edible.

Tuber shidianense S.P. Wan. 2016

Distribution & Specimen information: in the soil of a mixed forest dominated by *Pinus yunnanensis*, ascoma from winter. Yunnan Province: Baoshan city, Shidian county, alt. 2335 m, 3 Nov. 2014, HKAS88770 (wsp256), HKAS88771 (wsp 352).

Notes: Holotype HKAS88770: ITS = KT444595, LSU = NG228760. Paratype HKAS88771: ITS = KT444596, LSU = KY174961. Edible.

Tuber shii L. Fan & Y.W. Wang. 2016

Distribution & Specimen information: in soil under forest dominated by *Pinus armandii*, ascoma from winter. Sichuan Province: Huili county, 23 Nov. 2014, BJTC FAN436 (Cao 1527); 28 Nov. 2014, BJTC FAN409 (Cao 1505); Panzhihua city, 23 Nov. 2014, BJTC FAN429 (Cao 1520), BJTC FAN430 (Cao 1521), BJTC FAN431 (Cao 1521), BJTC FAN433 (Cao 1524), BJTC FAN437 (Cao 1528). Yunnan Province: Huize county, 28 Nov. 2014, BJTC FAN404 (Cao 1500), BJTC FAN405 (Cao 1501); Baoshan city, 27 Nov. 2014, BJTC FAN408 (Cao 1504), BJTC FAN414 (Cao 1507-2).

Notes: Holotype BJTC FAN409: ITS = KX555454, LSU = KX555465, *tef1* = OM649606, *rpb2* = OM584264. Other specimen BJTC FAN431: ITS = KX555462, LSU = OM366204, *tef1* = OM649609, *rpb2* = OM584267; BJTC FAN405: ITS = KX555453, LSU = KX555464, *tef1* = OM649603, *rpb2* = OM584261; BJTC FAN436: ITS = KX555458, LSU = KX555469; BJTC FAN429: ITS = KX555461; BJTC

FAN430: ITS = KX555456, LSU = KX555467; BJTC FAN433: ITS = KX555457, LSU = KX555468; BJTC FAN437: ITS = KX555463; BJTC FAN404: ITS = KX555459; BJTC FAN408: ITS = KX555460; BJTC FAN414: ITS = KX555455, LSU = KX555466. Edible. This species is distributed in Yunnan and Sichuan, but it is not common in market.

Tuber sinense K. Tao & B. Liu. 1989   Figure 46

Distribution & Specimen information: in the soil of mixed forest, coniferous trees are primarily *Pinus armandii* and *P. yunnanensis*, broadleaf trees are primarily *Viburnum cylindricum* Buch.-Ham. ex D. Don and *Alnus cremastogyne* Burkill., ascoma from autumn. Sichuan Province: Huidong county, 13 Sep. 1988, MHSU 1633; 23 Sep. 1988, MHSU 1631. The above only includes specimen information from the initial report; this species is more widely distributed in the Jinsha River basin of Yunnan and Sichuan Provinces. Yunnan Province: Chuxiong city, Mt. Zixishan, 22 Oct. 2023, BMDU L23104.

Notes: Holotype MHSU 1633. Isotype specimen BJTC FAN108: ITS = MF627968, LSU = OM366160, *tef1* = OM649565, *rpb2* = OM584211; BJTC FAN110: ITS = MF627970, LSU = OM366161, *tef1* = OM649566, *rpb2* = OM584212; BMDU L23104: ITS = PV871200. Edible. This species is common in Yunnan and Sichuan provinces, China. It is popular in the Southwest truffle markets and is traded in large quantities.

Figure 4.46 *Tuber sinense* morphological characteristics

Figure 4.46 A.B.C. Collected ascomata; D. Gleba; E. Surface of the ascomata; F-J. Asci and ascospores. Scale bars: A, B, C, D = 1 cm; E = 0.5 mm; F, G = 50 μm; H, I, J = 20 μm.

Tuber sinoaestivum J.P. Zhang & P.G. Liu 2013

Distribution & Specimen information: in the soil of a pine forest dominated by *Pinus armandii*, ascoma from winter. Sichuan Province: Huidong County, alt. 2280m, 25 Nov. 2009, HKAS 59105 (Zhang JP 153), Daqiao mountain, alt. 2250 m, 26 Nov.

2009, HKAS 59108 (Zhang JP 158), HKAS 59110 (Zhang JP 156); Gaji market, 23 Oct 2003, HKAS 44347 (Chen J 141). Gaji mountain, alt. 2280 m, 25 Nov. 2009, HKAS 59095 (Zhang JP 143); Haiba mountain, alt. 2300 m, 23 Nov. 2009, HKAS 59100 (Zhang JP 140); Yezu mountain, alt. 2300 m, 27 Oct. 2009, HKAS 59101 (Zhang JP 65).

Notes: Holotype HKAS 59105(Zhang et al., 2013), no sequence data from holotype. Other specimen HKAS 59101: ITS = JN896357, *tub2* = JQ348406, HKAS 59108: ITS = JN896351, HKAS 59095: ITS = JN896354, HKAS 59100: ITS = JN896355, *tub2* = JQ348408. Edible, Common in the market in Yunnan and Sichuan provinces.

Tuber sinoalbidum L. Fan & J.Z. Cao. 2011

Distribution & Specimen information: in the soil of the forest, ascoma from winter. Sichuan Province: Panzhihua city, 31 Dec. 2007, BJTC FAN105. There is only one record of this species, which is the type specimen.

Notes: Holotype BJTC FAN105: ITS = MH115298, LSU = MH115299. Unknown edible.

Tuber sinoborchii Ting Li & L. Fan 2022

Distribution & Specimen information: in the soil of a pine forest dominated by *Pinus armandii*, ascocarp from winter. Yunnan Province: Huize county, 12 Nov. 2011, BJTC FAN171, BJTC FAN169, BJTC FAN173.

Notes: Holotype BJTC FAN171: ITS = OM286802, LSU = OM366167, *tef1* = OM649573, *rpb2* = OM584223. Other specimen BJTC FAN169: ITS = OM286800, LSU= OM366166, *tef1* = OM649572, *rpb2* =OM584222; BJTC FAN173: ITS = OM286804. Unknown edible.

Tuber sinoexcavatum L. Fan & Yu Li. 2011

Distribution & Specimen information: in the soil of *Pinus* sp. forest, ascoma from winter. Sichuan Province: Panzhihua city, 20 Dec. 2007, BJTC FAN130.

Notes: Holotype BJTC FAN130: LSU = OM366163, *tef1* = OM649568, *rpb2* = OM584216. Other specimen BJTC FAN166: ITS = JX458718, LSU = OM366165, *tef1* = OM649571, *rpb2* = OM584221. Uncommon, unknown edible.

Tuber sinomonosporum J.Z. Cao & L. Fan. 2014

≡ ***Paradoxa sinensis*** L. Fan & J.Z. Cao, Mycotaxon 120: 473, 2012

Distribution & Specimen information: The only specimen information for this species is recorded in the article where its synonymy (*Paradoxa sinensis*) was published. Yunnan Province: Kunming, from the local mushroom market, 20 Dec. 2010, BJTC FAN150 (Jin-Zhong Cao 113).

Notes: Holotype BJTC FAN150: ITS = KF002729. Edible but rare.

Tuber sinoniveum S.P. Wan & F.Q. Yu. 2016

Distribution & Specimen information: in the soil of *Pinus armandii* forest, ascoma from winter. Yunnan Province: Baoshan city, Shuizhai village, alt. 2500 m, 1 Nov. 2014, HKAS88792 (wsp 234).

Notes: Holotype HKAS88792: ITS = KX904882, LSU = NG059736. Unknown edible.

Tuber sinosphaerosporum L. Fan, J.Z. Cao & Yu Li. 2013

Distribution & Specimen information: in the soil of a conifers forest, ascoma from winter. Yunnan Province: Baoshan city, 11 Dec. 2010, BJTC FAN135; Kunming city, Chenggong county, 18 Dec. 2010, BJTC FAN136.

Notes: Holotype BJTC FAN135: ITS = JX092086, LSU = NG059983, *tef1* = OM649569, *rpb2* = OM584217. Other specimen BJTC FAN136: ITS = JX092087, LSU = KP276196, *tef1* = KP276211; BJTC FAN137: ITS = OM286797; BJTC FAN204: ITS = OM286810. Unknown edible.

Tuber songlu S.P. Wan 2021

Distribution & Specimen information: in humic soil under a pure *Pinus armandii* forest, ascoma from autumn. Yunnan Province: Huize county, alt. 2415m, 14 Aug. 2016, HKAS 95771 (wsp695); December 2016, HKAS 95777 (wsp701), HKAS 95851 (wsp749).

Notes: Holotype HKAS 95771: ITS = KX904883, LSU = KY013656. Other specimen HKAS 95777: ITS = KX904884, LSU = KY013657; HKAS 95851: ITS = KX904886, LSU = KY013658. Unknown edible.

Tuber taiyuanense B. Liu. 1985

Distribution & Specimen information: This species was originally described from Shanxi Province: Taiyuan city, Zhangjiahe, Eastern Hill, in soil of pine forest, 25 Sep. 1983, HBSU 2319, this type specimen was unfortunately destroyed in a fire. A neotype specimen was later designated by Cao et al. (2011), Beijing: Dongling

Mountains, in soil of a pines forest, 20 Aug. 1998, HMAS 75888A (H.A. Wen, X.Q. Zhang & Z. Wang 294). This species is widely distributed in China and can be found in Hebei, Hubei, Shanxi, Sichuan, and Yunnan provinces, among other regions (Fan et al., 2022).

Notes: Holotype HMAS 75888A. Other specimen BJTC FAN199: ITS = OM311191; BJTC FAN164: ITS = OM311182, LSU = OM366164, *tef1* = OM649570, *rpb2* = OM584220; BJTC FAN220: LSU = OM366174, *tef1* = OM649578, *rpb2* = OM584231; BJTC FAN225: LSU = OM366176, *tef1* = OM649580, *rpb2* = OM584233. Edible.

Tuber tomentellum L. Fan & H.Y. Fu. 2023

Distribution & Specimen information: under a *Pinus* forest, ascoma from autumn. Yunnan Province: Kunming city, Oct. 2020, BJTC FAN1340, BJTC FAN1346; Dali city, Xiangyun county, 21 Oct. 2020, BJTC FAN1330.

Notes: Holotype BJTC FAN1340: ITS = OP852127. Other specimen BJTC FAN1330: ITS = OP852126, BJTC FAN1346: ITS = OP852128. Unknown edible.

Tuber turmericum L. Fan. 2015

Distribution & Specimen information: in the soil of mixed forest, ascoma from winter. Yunnan Province: Kunming city, Xundian county, 12 Jan. 2015, BJTC FAN473 (Cao 1568-2), BJTC FAN472 (Cao 1568-1); 10 Jan. 2015, BJTC FAN459 (Cao 1553); 11 Jan. 2015, BJTC FAN471 (Cao 1568), FAN474 (Cao 1568-3), FAN475 (Cao 1568-4), FAN476 (Cao 1568-5), FAN477 (Cao 1568-6); Qujing city, Huize county, 12 Jan. 2015, FAN482 (S.P. Li 1580). This is a rare species, found only in Sichuan and Yunnan provinces in Southwest China.

Notes: Holotype BJTC FAN473: ITS = NR147444. Paratype BJTC FAN472: ITS = KT758836; Other specimen BJTC FAN471: ITS = KT758835, LSU = OM366208, *tef1* = OM649613, *rpb2* = OM584269; BJTC FAN459: ITS = KT758834; BJTC FAN474: ITS = KT758838; BJTC FAN475: ITS = KT758839; BJTC FAN476: ITS = KT758840; BJTC FAN477: ITS = KT758841; BJTC FAN482: ITS = KT758842. Unknown edible.

Tuber umbilicatum Juan Chen & P.G. Liu. 2006

Distribution & Specimen information: in the soil of *Pinus yunnanensis* forest, ascoma from autumn. Yunnan Province: Chengjiang county, Tigu village, alt. 1900–

2000 m, 31 Oct. 2003, HKAS 44316 (J. Chen 145). This species is only found in Southwest China. Additionally, two specimens are preserved in HKAS, and six specimens are preserved in BJTC.

Notes: Holotype HKAS 44316: ITS = GU979031, LSU = GU979086. Other specimen BJTC FAN344: ITS = OM311220, LSU = OM366188, *tef1* = OM649593, *rpb2* = OM584251; BJTC FAN225: ITS = MH115325, LSU = MH115326; BJTC FAN212: ITS = OM311201, LSU = OM366173, *tef1* = OM649577, *rpb2* = OM584228; *T. umbilicatum* mitochondrion complete genome: MW924655. Edible.

Tuber umbilicicavatum Y. Wang, S.H. Li & Lin Li. 2024 Figure 4.47

Distribution & Specimen information: under *Abies forrestii* var. *smithii* forest, ascoma from autumn. Yunnan Province: Lijiang city, Jiuhe town, alt. 3870.7 m, 19 Sep. 2021, HKAS 131258 (BMDU L21189); alt. 3916 m, 19 Sep. 2021, HKAS 131257 (BMDU L21187).

Notes: Holotype HKAS 131258 (BMDU L21189): ITS = PP151575, LSU = PP151585. Other specimen HKAS 131257 (BMDU L21187): ITS = PP151576, LSU = PP151586. Edible.

Figure 4.47 *Tuber umbilicicavatum* morphological characteristics

Figure 4.47 A.B. ascomata; C. a piece of the section of the ascomata in 5%KOH; D. asci in lactophenol; E. 1-spored, 2-spored, and 4-spored asci; F. SEM ascospores (dry sample); G-O. ascospores and ascus; Scale bars: A, B = 1 cm; C-D = 100 μm; E. G-O = 20 μm; F = 10 μm

Tuber variabilisporum L. Fan & Ting Li 2022

Distribution & Specimen information: in the soil of a pine forest dominated by *Pinus armandii*, ascoma from winter. Sichuan Province: Panzhihua city, 16 Jan. 2014, BJTC FAN362, BJTC FAN330.

Notes: Holotype BJTC FAN362: ITS = OM287845, LSU = OM366190, *tef1* = OM649595, *rpb2* = OM584253. Other specimen BJTC FAN330: ITS = OM287841. Unknown edible.

Tuber verrucosivolvum S.P. Wan. 2016

Distribution & Specimen information: in the soil of a pine forest dominated by *Pinus armandii*, ascoma from winter. Sichuan Province: Huidong county, Dec. 2012, HKAS 88863 (wsp060), HKAS 88864 (wsp061).

Notes: Holotype HKAS 88863: ITS = KX904887, LSU = KY013650. Other specimen HKAS 88864: ITS = KX904888, LSU= KY013651. Unknown edible.

Tuber wanglangense L. Fan. 2018

Distribution & Specimen information: in the soil of *Picea* sp. forest, ascoma from autumn. Sichuan Province: Pingwu county, Wanglang Nature Preservation Area, alt. 2500 m, 18 Sep. 1989, HMAS 60220 (B.C. Zhang 610). This is a rare species.

Notes: Holotype HMAS 60220: ITS2 = DQ478637, In GenBank, it is mistakenly labeled under the specimen number “Y. Wang 610”. Unknown edible.

Tuber wenchuanense L. Fan & J.Z. Cao. 2013

Distribution & Specimen information: in the soil of *Larix mastersiana* Rehder & E.H. Wilson forest, ascoma from autumn. Sichuan Province: Wenchuan county, 22 Sep. 1989, HKAS60239 (B.C. Zhang 618), HKAS60241A (B.C. Zhang 622). Based on the nucleotide sequence data of *Tuber wenchuanense* in public databases (GenBank), Mleczko et al. (2023) reconstructed the distribution of this species, revealing that the distribution of this species can be described as Holarctic and covers all three continents: Europe, Asia, and North America.

Notes: Holotype HKAS60239: ITS = JX267044, LSU = MH115327. Other specimen BJTC FAN833: ITS = OM311256, LSU = OM366222, *tef1* = OM649629, *rpb2* = OM584280. Unknown edible.

Tuber wumengense L. Fan. 2016

Distribution & Specimen information: in soil of mixed forest dominated by *Pinus armandii* or *P. yunnanensis*, ascoma from summer to autumn. Yunnan Province: Huize, 24 Aug. 2012, BJTC FAN218A (J.Z. Cao 701A), BJTC FAN218B (J.Z. Cao 701B); 4 Sep. 2013, BJTC FAN292 (J.Z. Cao 725). Hubei Province: Shennongjia, 19 Sep. 1990, HMAS 60229, as *T. borchii* (B.C. Zhang 668). An additional 15 specimens are preserved at the BJTC.

Notes: Holotype BJTC FAN218A: ITS = NR147441, LSU = NG059992, *tef1* = KT067714, *rpb2* = OM584230. Paratype BJTC FAN218B (J.Z. Cao 701B): ITS =

KT067690, LSU = KT067708, *tef1* = KT067713. Other specimen HMAS 60229: ITS = KT067687; BJTC FAN292: ITS = KT067683, LSU = KT067709, *tef1* = KT067716, *rpb2* = OM584239. Unknown edible.

Tuber xanthomonosporum Qing & Yun Wang. 2015

Distribution & Specimen information: in the soil of *Pinus yunnanensis* forest, ascoma from winter. Sichuan Province: Panzhihua city, 15 Nov. 2012, YAAS L3185 (Y. Qing Pan1201), YAAS L3186 (C.Y. Liu Pan-No-10), YAAS L3187 (C.Y. Liu Pan-No-40). This species is unique and rare, with only three known specimens, all from Panzhihua City, Sichuan Province.

Notes: Holotype YAAS L3185: ITS = NR172748. Other specimen YAAS L3186: ITS = KJ162155; YAAS L3187: ITS = KJ162156. Unknown edible.

Tuber xizangense A-S. Xu. 1999

Distribution & Specimen information: in the soil of a fir forest, ascoma from autumn. Xizang Province: Nyingchi city, Bayi town, Caishichang, alt. 3200 m, 17 Aug. 1997, HXZE 1307 (A.S. Xu 97193).

Notes: Holotype HXZE 1307. No sequence data available. Unkown edible.

Tuber yigongense L. Fan & W.P. Xiong. 2018

Distribution & Specimen information: in the soil of *Pinus* sp. forest, ascoma from summer to winter. Xizang Province: Nyingchi city, Bomi county, Yigong town, Jiangsegang village, alt. 2230 m, 15 Nov. 2016, BJTC FAN731 (W.P. Xiong X00486), BJTC FAN730 (W.P. Xiong X00485); 26 July 2016, BJTC FAN728 (W.P. Xiong X00454-1), BJTC FAN729 (W.P. Xiong X00454-2). Since its publication, this species has only been recorded from specimens collected in Nyingchi, Xizang Province, China.

Notes: Holotype BJTC FAN731: ITS = MF663714, LSU = OM366216. Other specimen BJTC FAN728: ITS = MF663717; BJTC FAN729: ITS = MF663716, LSU = OM366192, *tef1* = OM649623, *rpb2* = OM584277; BJTC FAN730: ITS = MF663715; BJTC FAN1185: ITS = OM287858. Edible.

Tuber yunnanense S.P. Wan, Rui Wang & F.Q. Yu. 2024

Distribution & Specimen information: under *Pinus* sp. forest, ascoma from autumn to winter. Yunnan Province: Nujiang, Gongshan County, 29 Oct. 2020, YNAU019 (wsp973-1), YNAU020 (wsp973-2), YNAU0107 (wsp974-3). Sichuan Province: 12 Sep. 2021, YNAU0491 (wsp1365).

Notes: Holotype YNAU019 (wsp973-1): ITS = OK625306, LSU = OR661811, *tef1* = OR813081, *rpb2* = OR832407. Other specimen YNAU020 (wsp973-2): ITS = OK625307, LSU = OR661812, *tef1* = OR813082, *rpb2* = OR832408; YNAU0107 (wsp974-3): ITS = OR665397, LSU = OR661813, *tef1* = OR813083, *rpb2* = OR832409; YNAU0491 (wsp1365): ITS = OR250186, LSU = OR661814, *tef1* = OR813084, *rpb2* = OR832410. Unknown edible.

Tuber zhongdianense X.Y. He, Hai M. Li & Y. Wang. 2004

Distribution & Specimen information: in the soil of *Quercus pannosa* Hand.-Mazz. forest, ascoma from autumn. Yunnan Province: Shangri-La (Diqing), Zhongdian county, alt. 3000m, 15 Oct. 2002, Y. Wang 0299 (IFS). One of the species is distributed in the high-altitude mountainous regions of Yunnan Province. One additional specimen is preserved in HKAS 45388B, and seventeen specimens are preserved in BJTC.

Notes: Holotype Y. Wang 0299 (IFS): ITS = NR119621. Other specimen HKAS 45388B: ITS = DQ898186, BJTC FAN176: ITS = KP276178, LSU = KP276201, *tef1* = KP276213; BJTC FAN178: ITS = KT067679, LSU = KT067701, *tef1* = KT067722, *rpb2* = OM584225; BJTC FAN189: ITS = KT067689, LSU = KT067705, *tef1* = KT067718. Edible.

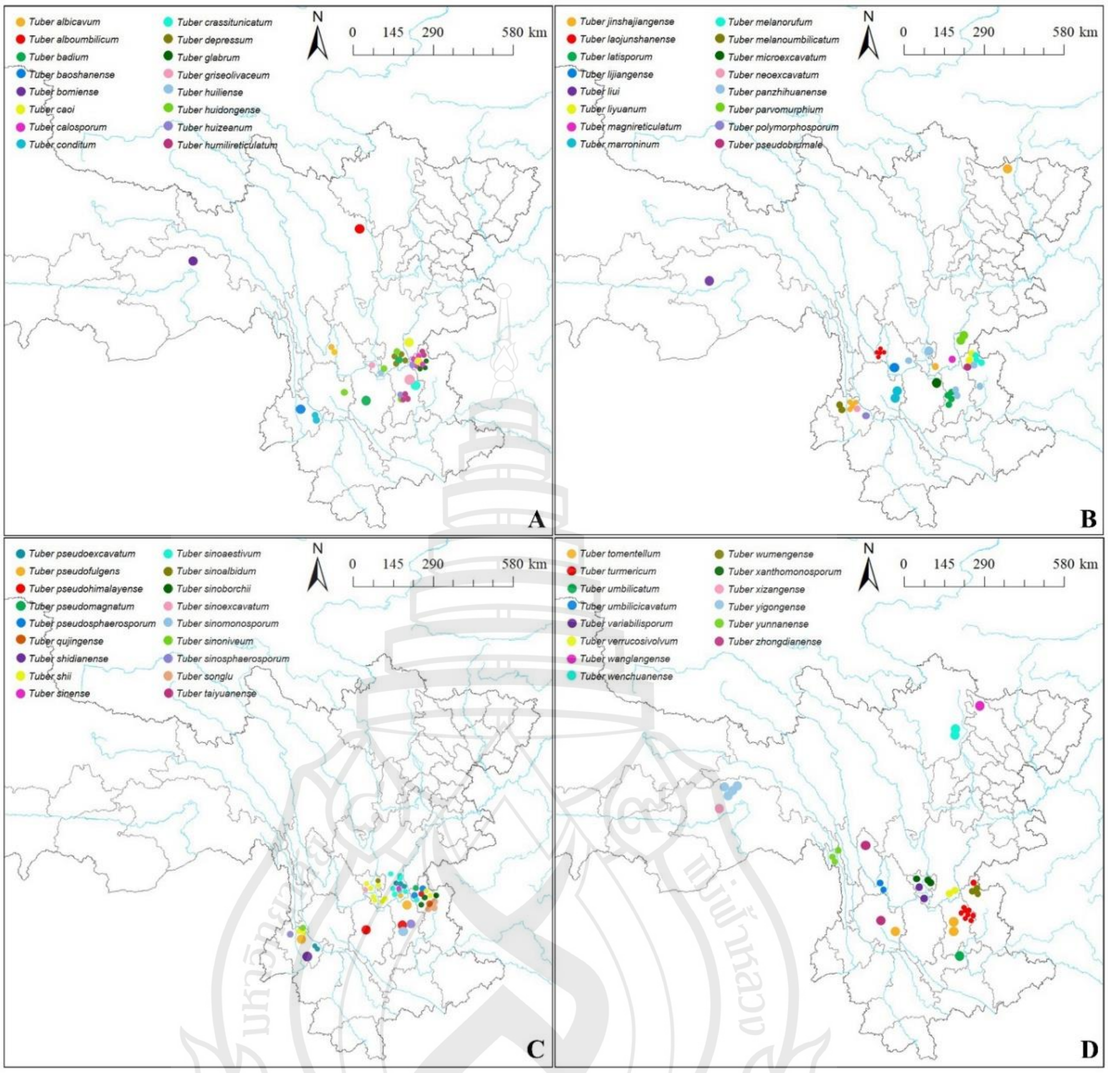


Figure 4.48 Distribution map of *Tuber* species in southwestern China

Basidiomycota

Agaricales Underw. 1899

Hydnangiaceae G äum. & C.W. Dodge 1928

Hydnangium Wallr. 1839

The genus *Hydnangium* is the globally distributed group (Malajczuk et al., 1982; Chu-Chou & Grace, 1983). *Hydnangium* species form hypogeous or semi-hypogeous, red or pink basidiomata, with a rhizomorphic base. gleba is composed of numerous irregulars, labyrinthine arranged chambers, lacking columella, basidia 2-spored, basidiospore spherical with spines. (Wang & Yang, 2003; Campos, 2004; Cooper, 2014). They form ectomycorrhizal associations with hosts in the *Eucalyptus* spp.(Smith

& Read, 1997; Tagu et al., 2002; Martin et al., 2007; da Silva Coelho et al., 2010).

According to statistics, there is currently one species are now known in China, *Hydnangium carneum*, collected from Yunnan province (Wang & Yang, 2003).

Hydnangium carneum Wallr. 1839

Distribution & Specimen information: in humic soil near the rhizosphere of *Eucalyptus* spp. trees, basidiomata from autumn to winter. Yunnan Province: Kunming City, Heilongtan, Kunming Institute of Botany, 16 Oct. 2002, HKAS42321 (YZL3601); 10 Feb. 2003, HKAS42290 (ZLF161).

Notes: No sequence data available from southwestern China. No edible or medicinal use.

Hymenogastraceae Vittad. 1831

Hymenogaster Vittad. 1831

The genus *Hymenogaster* is the globally distributed group, which is a species-rich genus of false truffles. As of now, according to the records from the MycoBank database, about 170 species of this group have been reported globally. *Hymenogaster* is characterized by basidiomata with some degree of columella development from almost none to having a narrow percurrent one but never a stipe-columella, spores mostly under 20 μm long, ellipsoid to ovoid in shape (some species lacking a pronounced beak), ornamentation in the form of a warty-wrinkled mostly adnate perisporium (Soehner, 1922; Smith, 1966, Stielow et al., 2011). They form ectomycorrhizae with the plant.

According to statistics, there are currently 40 species and variants are now known in China, among which 8 species are distributed in the southwest region: *Hymenogaster zunhuaensis*, *H. gilkeyae*，*H. niveus, H. latifusisporus*, and in Yunnan, *H. tener* in Yunnan and Xizang, *H. xizangensis* and *H. zayuensis* in Xizang, *H. subnanus* in Sichuan province (Liu et al., 1996; Liu, 1998; Liu et al., 2002; Fan, 2023; Li et al., 2024a; Li et al., 2024b). Among them, *H. zayuensis* is a new species described in the present study.

Hymenogaster gilkeyae Zeller & C.W. Dodge 1934

Distribution & Specimen information: in soil under *Quercus acutissima* forest, basidiomata from summer. Yunnan Province: Kunming Xishan Park, 11 Sep. 1933, HKAS24536.

Notes: No sequence data available from southwestern China. No edible or medicinal use.

Hymenogaster latifusisporus K. Tao, M.C. Chang & B. Liu 1996

Distribution & Specimen information: in soil 1-2 cm deep under a *Quercus variabilis* Bl. forest, basidiomata from summer. Yunnan Province: Kunming, 11 Aug. 1990, MHSU 2044 (M.C Chang 410) (Liu, 1998; Tao et al., 1996).

Notes: Holotype MHSU 2044. No sequence data available from southwestern China. No edible or medicinal use.

Hymenogaster niveus Vittad. 1831 emend. Stielow et al., 2011

= ***Cortinomyces niveus*** (Vittad.) Bougher & Castellano, Mycologia 85(2): 280 (1993)

= ***Protoglossum niveum*** (Vittad.) T.W. May, Muelleria 8(3): 287 (1995)

Distribution & Specimen information: in the leaf litter layer of a *Quercus acutissima* forest, basidiomata from summer. Yunnan Province: Kunming City, Heilongtan, Kunming Institute of Botany, 11 Aug. 1990, MHSU2072 (Wang & Chang 423). According to the *Fungi of China* (Liu, 1998), this species is also distributed in Shanxi Province, China. Stielow et al. (2011) revised *Hymenogaster niveus* based on ITS phylogenetic analysis, suggesting that it belongs to a cryptically diverse species complex. Unfortunately, molecular data for the Chinese specimens of this species are currently unavailable.

Notes: No sequence data available from southwestern China. No edible or medicinal use.

Hymenogaster subnanus K. Tao, B. Liu & M.C. Chang 1996

Distribution & Specimen information: in the litter layer under a bamboo forest, basidiomata from summer. Sichuan Province: Dujiangyan city, Mt. Qingcheng, 19 Aug. 1990, MHSU2098 (M.C Chang 223), (Liu, 1998; Tao et al., 1996). According to the *Fungi of China* (Liu, 1998), this species is also distributed in Shanxi Province, China, occurring 5-10 cm deep in soil under *Forsythia suspensa* (Thunb.) Vahl.

Notes: Holotype MHSU2098. No sequence data available from southwestern China. No edible or medicinal use.

Hymenogaster tener Berk. 1844

Distribution & Specimen information: in soil under *Quercus acutissima* Carr.

or *Quercus aquifolioides* forest, basidiomata from summer. Xizang Province: Nyingchi, Mt. Sejila, alt. 3000m, 30 Jul. 1995, MHSU2229 (XAS002); Yunnan Province: Kunming, Heilongtan park, 10 Aug. 1990, MHSU2102 (CMC376).

Notes: No sequence data available from southwestern China. No edible or medicinal use.

Hymenogaster xizangensis K. Tao, B. Liu & A-S. Xu 1996

Distribution & Specimen information: in soil under *Quercus aquifolioides* Rehder & E. H. Wils. forest, basidiomata from summer. Xizang Province: Nyingchi, Mt. Sejila, alt. 3000m, 24 Jul. 1995, MHSU2228 (XAS001).

Notes: Holotype MHSU2228 (XAS001). No sequence data is available. No edible or medicinal use..

Hymenogaster zayuensis L. Li, K.D. Hyde & S.H. Li, sp. nov. Figure 15

Distribution & Specimen information: in soil under *Quercus pannosa* Hand.-Mazz., basidiomata from summer to autumn. Xizang Province: Zayu, alt. 3410 m, 11 August 2022, Lin Li, BMDU L22039, BMDU L22041.

Notes: Holotype BMDU L22039: ITS=PX096319, LSU=PX096419. Other specimen BMDU L22041: ITS = PX096320, LSU = PX096420. No edible or medicinal use.

Hymenogaster zunhuaensis L. Fan & T. Li, Diversity 16(5, no. 303): 10 (2024)

Distribution & Specimen information: in soil under *Quercus acutissima* forest, basidiomata from summer. Yunnan Province: Kunming, Heilongtan park, 10 Aug. 1990, HMAS83129 ex MHSU 2034 (M.C. Chang & L. Wang 408). This specimen was initially morphologically identified as *Hymenogaster cerebellum* Cavara 1893 (Liu, 1998). Subsequently, in 2024, Ting et al. (2024b) reidentified this specimen (HMAS83129 ex MHSU 2034), along with specimens collected from Hebei, Shaanxi, and Shanxi Provinces, as a new species, *Hymenogaster zunhuaensis*. However, the molecular data of specimen HMAS83129 ex MHSU 2034 were not explicitly provided in the paper. The holotype of *Hymenogaster zunhuaensis* was collected from Hebei Province: Tangshan City, Zunhua County, alt. 107m, 17 Sep. 2017, in soil under *Castanea mollissima* Bl., BJTC FAN1062 (XYY 062).

Notes: Holotype BJTC FAN1062: ITS = NR197568, LSU = PP622363. No edible or medicinal use.

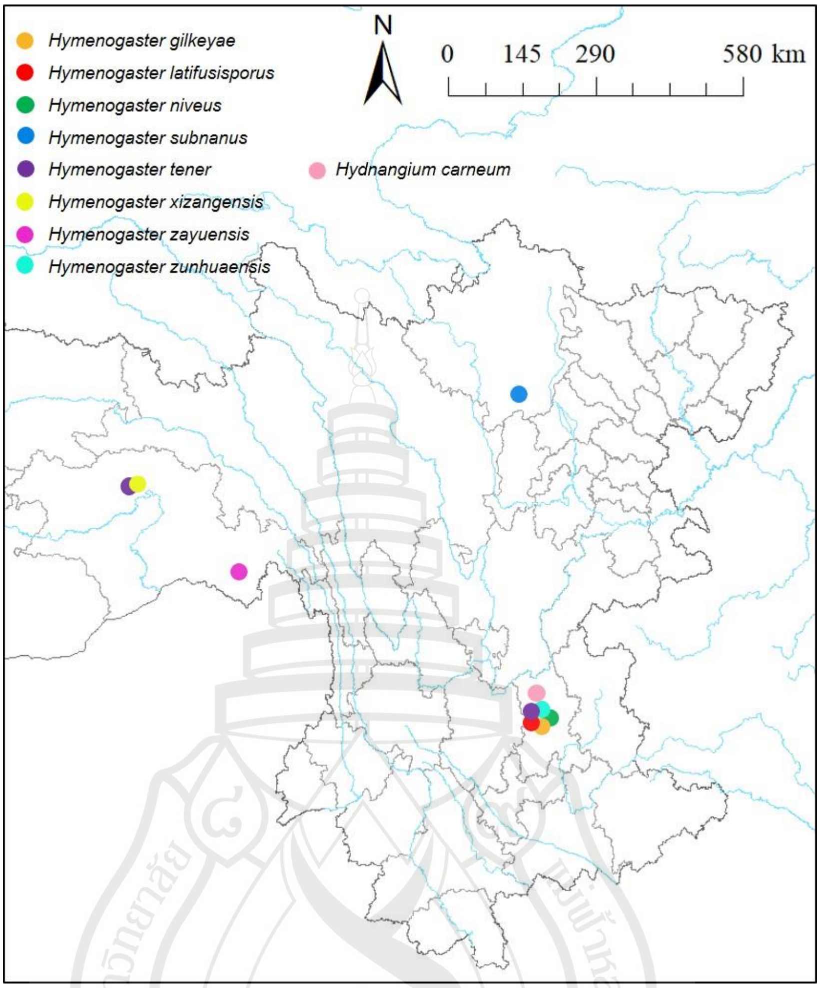


Figure 4.49 Distribution map of *Hymenogaster*, *Hydnangium* species in southwestern China

Boletales E.-J. Gilbert 1931

Boletaceae Chevall. 1826

Chamonixia Rolland 1899

The genus *Chamonixia* is a Northern Hemisphere distributed group (Rolland, 1899; Montecchi & Sarasini, 2000; Mleczko et al., 2009; Smith & Singer, 1959; Eckblad & Ellingsen, 1984; Orihara et al., 2016a). Species form hypogeous basidiomata. *Chamonixia* is characterized by the gasteroid, spongy basidiomata often with bluish discoloration and basidiospores with 6-12 large, blunt, longitudinal ridges.

According to statistics, prior to this study, only one species of this genus was recorded in China, *Chamonixia caespitosa*, based on a collection by Dr. Harry Smith in 1922 from Sichuan Province, China. (Eckblad & Ellingsen, 1984; Liu, 1998). Eight specimens were collected from Yunnan Province in this study. Based on morphological characteristics and molecular phylogenetic analyses, they are supported as representing a new species, *Chamonixia laojunshanensis*.

Chamonixia caespitosa Rolland 1899

Distribution & Specimen information: under *Picea* sp. forest, basidiomata from autumn. Sichuan Province: Maerkang city, Matang village, alt. 3500m, 12 Sep. 1922, this specimen is preserved at UPS (the Department of Systematic Botany, Uppsala University, Sweden), without information on collection number (Eckblad & Ellingsen, 1984).

Notes: No sequence data available from China. No edible or medicinal use.

Chamonixia laojunshanensis L. Li, K.D. Hyde & S.H. Li, sp. nov. Figure 4.11

Distribution & Specimen information: in soil under *Abies forrestii*, basidiomata from autumn. Yunnan Province: Lijiang, Jiuhe, Mt. Laojunshan, alt. 3946 m, 15 Sep. 2022, Lin Li, BMDU L22053, BMDU L22054, BMDU L22055, BMDU L22056, BMDU L22057, BMDU L22058, BMDU L22059, BMDU L22060; alt. 3715 m, 28 Sep. 2021, Lin Li, BMDU L21208, BMDU L21209.

Notes: Holotype BMDU L22053: ITS=PX105425, LSU=PX096422, SSU=PX097606. Other specimens BMDU L21208: ITS = PX105426; BMDU L21209: ITS = PX105427; BMDU L22054: ITS = PX105428, LSU = PX096423, SSU=PX097607; BMDU L22055: ITS = PX105429, LSU = PX096424, SSU= PX097608; BMDU L22057: ITS = PX105431, LSU = PX096426, SSU= PX097610; BMDU L22059: ITS = PX105433, LSU = PX096428, SSU= PX097612; BMDU L22060: ITS = PX105434, LSU = PX096429, SSU= PX097613. No edible or medicinal use.

Gastroboletus Lohwag 1926

The genus *Gastroboletus* has been reported from North and South America, Asia, South Africa, Western Europe, and eastern Australia (Cázares & Trappe, 1991; Horak, 1977; Lohwag, 1926; Nouhra & Castellano, 1995; Nouhra et al., 2002; Singer & Smith, 1964; Smith & Singer 1959; Trappe & Castellano, 2000; Thiers & Trappe, 1969; Wang et al., 2014). *Gastroboletus* species are characterized by forming hypogeous, boletus-like basidiomata, that have a poorly developed or absent stipe with irregularly arranged tubes. The tubes are often not vertically oriented geotropically and may be covered by a well-developed, persistent peridial membrane or blocked by outgrowths of wall tissue. Spores are elliptical to spindle-shaped, smooth, brown to golden brown, spores not forcibly discharged from the basidia, forming ectomycorrhizal (Lohwag, 1926; Nouhra et al., 2002).

According to statistics, there are currently only two known species of the genus *Gastroboletus* in China. One is *Gastroboletus boedijnii*, the type species of the genus, which was described by Lohwag based on a specimen collected by Handel-Mazzetti in 1916 from Yunnan Province, China (Lohwag, 1926; Lohwag, 1937). The other related species reported in China is *G. thibetanus*, collected from Xizang Province (Wang et al., 2014). Then, in 2016, Wu et al., proposed a new combination, *Neoboletus thibetanus*, based on the specimen characteristics collected from Yunnan and Xizang Province, studied through comparisons with *G. thibetanus* (Wu et al., 2016a). In the same year, Wu et al., once again suggested a new combination, *Sutorius thibetanus*, based on their research on the family Boletaceae (Wu et al., 2016b).

Gastroboletus boedijnii Lohwag 1937

Distribution & Specimen information: The specimen information in the original literature is brief. The holotype was collected from Yunnan Province: Lijiang City, southern slope of Yulong Snow Mountain, Yuhu Village (referred to as “Ngulukö” in the original literature, which is likely a transliteration from the Naxi language), alt. 2900m, collected by Handel-Mazzetti from late September to early October 1916, housed in the Natural History Museum in Vienna, E. 12951.

Notes: No sequence data available. No edible or medicinal use.

Sutorius thibetanus (Shu R. Wang & Yu Li) G. Wu & Zhu L. Yang 2016

= ***Gastroboletus thibetanus*** Shu R. Wang & Yu Li, in Wang, Wang, Wang & Li, Mycotaxon 129(1): 80 (2015) [2014]

= ***Neoboletus thibetanus*** (Shu R. Wang & Yu Li) Zhu L. Yang, B. Feng & G. Wu, in Wu, Zhao, Li, Zeng, Feng, Halling & Yang, Fungal Diversity: [17] (2015)

Distribution & Specimen information: on soil under *Abies forrestii* var. *smithii* Viguié & Gaussen, basidiomata from summer to autumn. Xizang Province: Nyingchi, Mt. Sejila, 26 Jul. 2012, HMJAU30001 (WSR T10020); 11 Aug. 2013, HMJAU30002 (WSR T50457); 11 Aug. 2013, HMJAU30005 (WSR T50467); 4 Oct. 2013, HMJAU30006 (LS T18269); Bomi, alt. 3400-3500m, 22 Jun. 2009, HKAS57093

(Feng364). Yunnan Province: Shangri-La, alt. 3700m, 3 Sep. 2013, HKAS82600 (Feng1494).

Notes: Holotype HMJAU30001 (WSR T10020). Other specimen HKAS57093: LSU = KF112326, RPB1= KF112496, RPB2 = KF112655. No edible or medicinal use.

Rossbeevera T. Lebel & Orihara [as '*Rosbeeva*'] 2012

The basidiomata of *Rossbeevera* are not commonly encountered. Species of this genus have been reported from Australia, New Zealand, China, and Japan. *Rossbeevera* is characterized by ellipsoid to fusiform basidiospores with 3–5 longitudinal ridges, bluish-green to cyanescent discoloration of basidiomata, and a thin cutis-like whitish peridium (Lebel et al., 2012a, 2012b; Orihara et al., 2016b; Orihara, 2018). In a 2020 study, the authors used molecular systematics to transfer the type species of *Rossbeevera*, *Rossbeevera pachydermis*, to the genus *Leccinum*, supporting the inclusion of *Rossbeevera* species within the broadly circumscribed *Leccinum* genus (Kuo & Ortiz-Santana, 2020), although the morphological differences between the two genera were not discussed in the study. However, it is noteworthy that *Rossbeevera* species are characterized by fusiform basidiospores with 3–5 longitudinal ridges, which is a distinct feature, whereas species of *Leccinum* typically possess smooth, fusiform basidiospores.

According to statistics, there are currently 3 species are now known in China, among which 1 species is distributed in the southwest region: *Rossbeevera yunnanensis* in Yunnan (Orihara et al., 2012a, Hosen et al.,2019).

Rossbeevera yunnanensis Orihara & M.E. Sm. 2012

Distribution & Specimen information: in mixed forest dominated by *Pinus yunnanensis* Franch., *Lithocarpus mairei* (Schottky) Rehder, *L. dealbatus* (Hook. f. & Thomson ex Miq.) Rehder, and unidentified *Quercus* spp, basidiomata from autumn. Yunnan Province: Chuxiong Yi Autonomous Prefecture, Mt. Zixi, 19 Sep. 2010, HKAS70689 (MES420), isotype KPM-NC0017850.

Notes: Holotype HKAS70689 (MES420): LSU = JN979437. No edible or medicinal use.

Octaviania Vittad. 1831

The genus *Octaviania* is a Northern Hemisphere-distributed group. Species form hypogeous to emergent basidiomata. *Octaviania* is characterized by basidiomata sessile or with a rudimentary stipe at the base, surface glabrous, floccose, or occasionally scaly to warty, often discolouring when rubbed or bruised, gleba more or less rubbery, marbled， with dextrinoid or non-amyloid basidiospores with coarse, conical to pyramidal ornamentation. *Octaviania* species form ectomycorrhizas with woody plants (Chu-Chou & Grace, 1983; Frank et al., 2006; Orihara et al., 2012b).

According to statistics, there are currently 4 species are now known in China, among which 1 species is distributed in the southwest region: *Octaviania potteri* in Xizang province (Liu et al., 1996; Orihara et al., 2021).

Octaviania potteri (Singer & A.H. Sm.) Orihara, Healy & M.E. Sm. 2021

= ***Octaviania asterosperma*** var. *potteri* Singer & A.H. Sm. 1961

Distribution & Specimen information: under *Quercus aquifolioides* forest, ascocarp from summer. Xizang Province: Nyingchi, Mt. Juemushan, alt. 3150m, 19 Aug. 1995, MHSU2231 (XAS004).

Notes: No sequence data available from southwestern China. No edible or medicinal use.

Turmalinea Orihara & N. Maek. 2015

The genus *Turmalinea* is currently distributed only in Asia (Orihara et al., 2016). Species form hypogeous basidiomata. *Turmalinea* is characterized by subglobose to depressed-globose to reniform, rubbery, sessile or rarely with a short stipe at the base, surface smooth to slightly felty, pale pink to pink, or white to brownish white, often turning blue to indigo-blue when rubbed or bruised (Orihara et al., 2016).

According to statistics, there is currently 1 species are now known in China, *Turmalinea chrysocarpa*, collected from Yunnan province (Orihara et al., 2016).

Turmalinea chrysocarpa Orihara & Z.W. Ge 2015

Distribution & Specimen information: under a broad-leaved forest, ascocarp from autumn. Yunnan Province: Gejiu City, Manhao Town, alt. 860m, 24 Sep. 2011, HKAS70601 (Ge-ZW3098), isotype KPM-NC18068.

Notes: Holotype HKAS70601 (Ge-ZW3098): ITS = KC552003, LSU = KC552051, SSU = KC552216, ATP6 = KP222888. No edible or medicinal use.

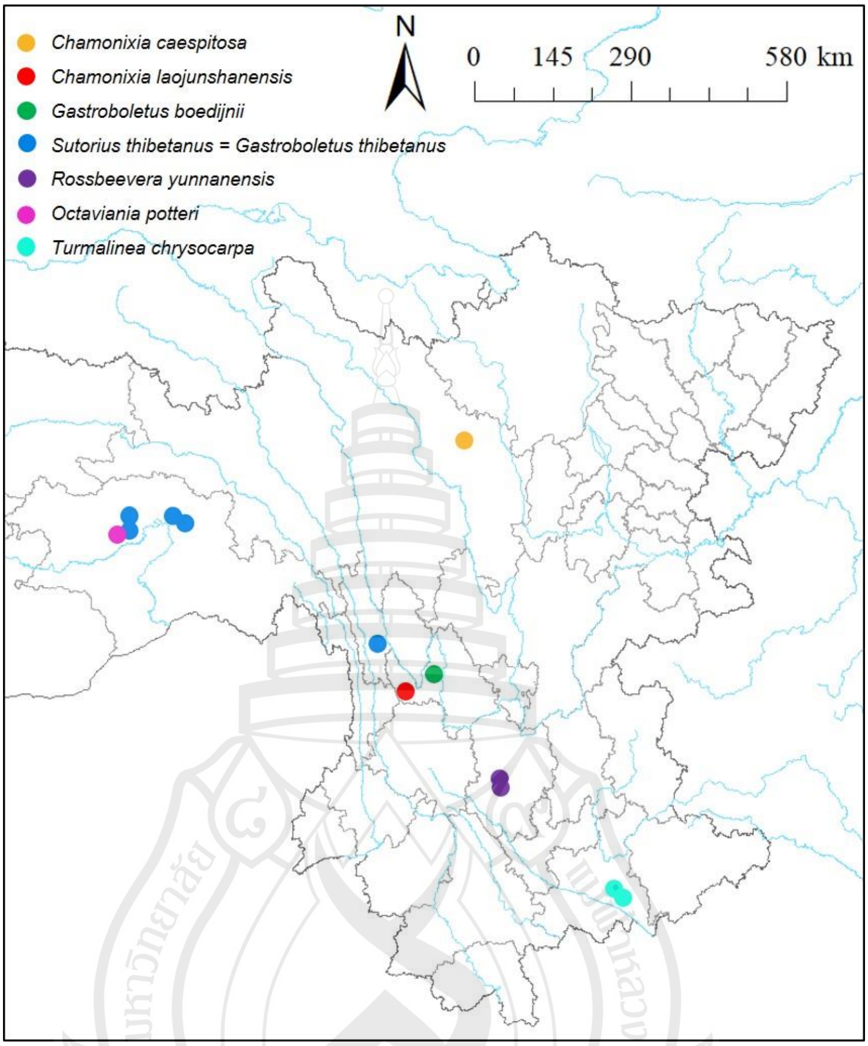


Figure 4.50 Distribution map of hypogeous Boletaceae (*Chamonixia, Gastroboletus, Sutorius, Rossbeevera*, *Octaviania, Turmalinea*) species in southwestern China

Paxillaceae Lotsy 1907

Melanogaster Corda 1831

The genus *Melanogaster* is a Northern Hemisphere-distributed group. Species form hypogeous or semi-hypogeous basidiomata, some species are edible or medicinal. *Melanogaster* is characterized by sometimes with mycelial strands at the base or surface, gleba with gelatinous small chambers separated by sterile veins scattered throughout when mature, yellow to dark brown, globose, ellipsoid, pyriform or fusiform basidiospores (Trappe et al., 2009; Moreau et al., 2011; Alvarado et al., 2021). *Melanogaster* species usually form ectomycorrhizae with both broadleaved and conifer trees (Wang et al., 2005; Trappe et al., 2009; Tedersoo et al., 2010; Moreau et al., 2011; Türkoğlu & Castellano, 2013)

According to statistics, there are currently 16 species are now known in China, among which 9 species are distributed in the southwest region: *Melanogaster fusisporus*, *M. ovoidisporus*, *M. obovatus***,** *M. tomentellus*, *M. diqingensis*, *M. truncatisporus* in Yunnan. *M. cyaneus* and *M. broomeanus* in Sichuan province. *M. panzhihuaensis* in Yunnan and Sichuan provinces. (Liu et al., 1989; Wang et al., 1995; Liu, 1988; Xu et al., 2022; Yuan et al., 2024)

Melanogaster broomeanus Berk. 1843

Distribution & Specimen information: Sichuan Province: Songpan County, 30 Aug. 1991, HKAS 24811 (Mingsheng Yan 1729). There is no clear habitat information for this specimen in the literature (Liu, 1998).

Notes: The specimen (HKAS 24811) lacks available molecular data, but Xu et al., provided three ITS sequences of *M. broomeanus* in their study on the diversity of *Melanogaster* in China (Xu et al., 2022). However, the origin of the specimens that produced these sequences cannot be determined from the article. They are BJTC FAN909: ITS = MW598531; BJTC FAN1230-A: ITS = MW598545; BJTC FAN1230-B: ITS = MW598547. No edible or medicinal use.

Melanogaster cyaneus T.J. Yuan, Shu H. Li & Raspé, MycoKeys 107: 149 (2024)

Distribution & Specimen information: in mainly reddish brown soils under *Castanea mollissima* Bl., basidiomata from autumn. Sichuan Province: Panzhihua City, Yanbia County, Shuanglong village, alt. 1970m, 16 Aug. 2020, HKAS129200 ex YAAS-TJ75-1; alt. 2000m, 16 Aug. 2020, YAAS-TJ75-2.

Notes: Holotype HKAS129200 ex YAAS-TJ75-1: ITS = ON427476, LSU = ON427489, rpb2 = ON533869. Other Specimen YAAS-TJ75-2: ITS = ON427477, LSU = ON427490, rpb2 = ON533870. No edible or medicinal use.

Melanogaster diqingensis T.J. Yuan, Shu H. Li & Raspé, MycoKeys 107: 149 (2024)

Distribution & Specimen information: in brown soil under *Quercus aquifolioides* Rehd. et Wils., basidiomata from autumn. Yunnan Province: Diqing Autonomous Prefecture, Shangri-La County, Baishuitai Village, alt. 2380m, 25 Sep.

2020, HKAS 121212 ex YAAS-WXH_9068.

Notes: Holotype HKAS 121212 ex YAAS-WXH_9068: ITS = ON427482, LSU = ON427495, rpb2 = ON533874. No edible or medicinal use.

Melanogaster fusisporus Y. Wang 1995

Distribution & Specimen information: under *Quercus* sp. or *Abies* sp. forest, basidiomata from summer to autumn. Yunnan Province: Kunming, 10 Aug. 1990, MHSU2005(CMC-WL-WDS239). The holotype of this species (OSC4416) was collected on 27 Jul. 1975 from Hokkaido, Japan, and is currently housed in the Herbarium of Oregon State University (Wang et al., 1995). According to the *Fungi of China*, this species is also distributed in Heilongjiang Province, China (MHSU 2004), and Taiwan (Trappe OSC 8396) (Liu, 1998). Xu et al. (2022) re-examined the Chinese specimens and confirmed that *M. fusisporus* is morphologically distinct based on its large, fusiform, brown basidiospores. However, the DNA data of the holotype specimen could not be successfully obtained at this time.

Notes: No sequence data available. No edible or medicinal use.

Melanogaster obovatus (K. Tao, Ming C. Chang & B. Liu) L. Fan & X.Y. Yan 2022

≡***Melanogaster fusisporus* var. *obovatus*** K. Tao, Ming C. Chang & B. Liu, 1995.

Distribution & Specimen information: in soil under *Quercus* sp., basidiomata from autumn. Yunnan Province: Kunming, 10 Aug. 1990, MHSU2008 (WL224). According to the *Fungi of China*, this species is also distributed in Shanxi and Jilin Province, China (Liu, 1998).

Notes: Epitype BJTC FAN808: ITS = MW598539, LSU = MW598516, from Shanxi province. No edible or medicinal use.

Melanogaster ovoidisporus Y. Wang 1995

Distribution & Specimen information: The holotype was collected from Yunnan Province: Lijiang City, on the west bank of the Jinshajiang River, Shajia Village, alt. 1975m, 10 Aug. 1915, collection number Handel-Mazzetti 7583, currently housed in the Handel-Mazzetti Herbarium (HM1372). This specimen was first identified in 1937 by Keissler and Lohwag as *Melanogaster variegatus* (Vittad.) Tul. & C. Tul. 1851 (Lohwag, 1937). Later, in 1995, Wang Yun re-examined this specimen, and based on morphological features, described it as a new species, *Melanogaster ovoidisporus*, designating this specimen as the holotype (Wang et al., 1995). According to the *Fungi of China* (Liu, 1998), this species is also distributed in Shanxi Province, China.

Notes: Holotype HM1372. No sequence available. No edible or medicinal use.

Melanogaster panzhihuaensis L. Fan, X.Y. Yan & Y.Y. Xu 2022 Figuer 20

Distribution & Specimen information: Sichuan Province: Panzhihua City, in soil under *Pinus yunnanensis* forest, 1995, HMAS 81915 ex MHSU2266 (Z.J. Gu 6). Yunnan Province: Chuxiong Yi Autonomous Prefecture, Mouding County, Xinqiao Town, Haojiahe village, alt. 1783.3 m, under the mixed forest of *Keteleeria evelyniana* and *Quercus acutissima* Carruth., 7 Aug. 2020, Lin Li，L20048 and L22049；Chuxiong City，Donggua Town，alt. 1667 m, under the mixed forest of *Keteleeria evelyniana* and *Quercus* sp. 16 Oct. 2021, Lin Li, L21235 and L21236.

Notes: Holotype HMAS 81915: ITS = MW598551, LSU = MW589529. Other Specimen BMDU L20048: ITS = PX096553, LSU = PX096470; BMDU L20049: ITS = PX096554; BMDU L21235: ITS = PX096555, LSU = PX096471; BMDU L21236: ITS = PX096556, LSU = PX096472. Edible.

In one of the species’ habitats, Chuxiong City, the species has been collected and consumed by mushroom hunters. They typically dry the basidiomata for storage and brew them in water to drink when coughing. However, it is rarely sold in the market.

Melanogaster tomentellus L. Fan, X.Y. Yan & Y.Y. Xu 2022

Distribution & Specimen information: in soil under *Pinus* sp. forest, basidiomata from winter. Yunnan Province: Kunming, Haikou Town, alt. 1900m, 8 Jan. 2016, BJTC FAN539 (Cao1707).

Notes: Holotype BJTC FAN539 (Cao1707): ITS = MW598538, LSU = MW589524. No edible or medicinal use.

Melanogaster truncatisporus T.J. Yuan, Shu H. Li & Raspé, MycoKeys 107: 149 (2024)

Distribution & Specimen information: in mainly brown soils under *Castanea mollissima* and *Pinus yunnanensis*, basidiomata from autumn. Yunnan Province: Nujiang Autonomous Prefecture, Lanping County, alt. 2030m, 26 Oct. 2020, YAAS TJ83 and YAAS TJ109; Lanping County, Zhongpai township, Xinchangping village, alt. 1990m, 26 Oct. 2020, KUN-HKAS129199 ex YAAS-TJ87; Gongshan County, alt.

1800m, 25 Sep. 2020, YAAS L5346.

Notes: Holotype KUN-HKAS129199 ex YAAS-TJ87: ITS = ON427479, LSU = ON427492, rpb2 = ON533872. Other specimens YAAS TJ83: ITS = ON427478, LSU = ON427491, rpb2 = ON533871; YAAS TJ109 ITS = ON427480, LSU = ON427493, rpb2 = ON533873; YAAS L5346 ITS = ON427481, LSU = ON427494. No edible or medicinal use.

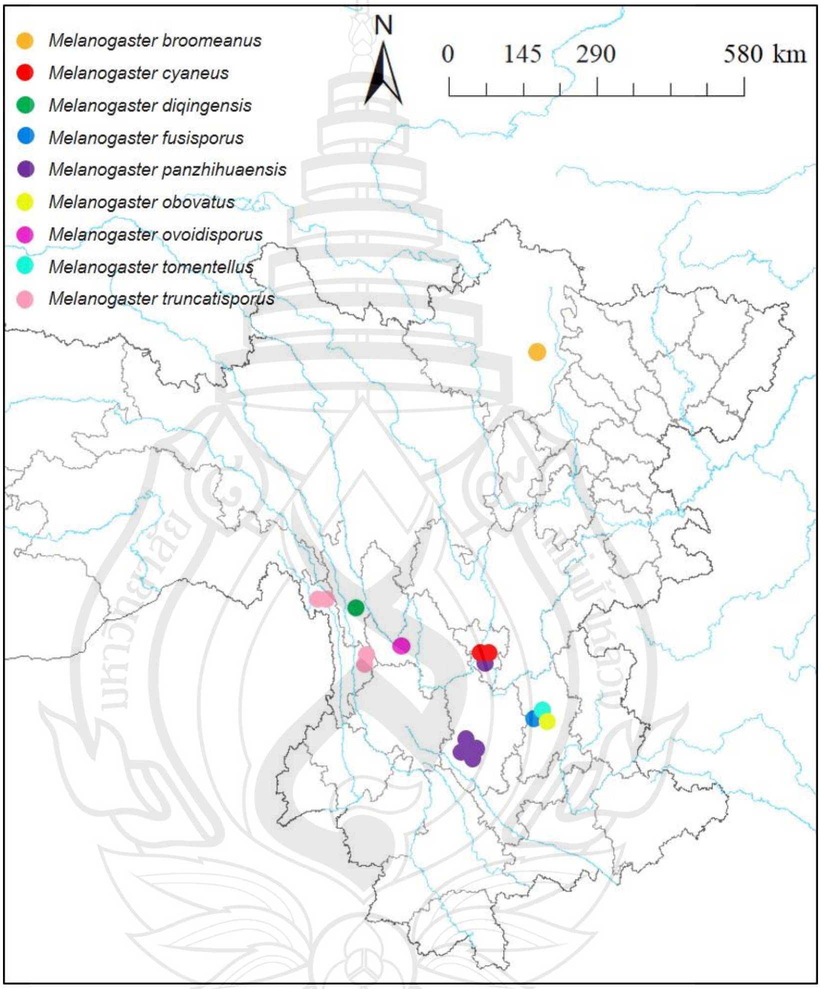


Figure 4.51 Distribution map of *Melanogaster* species in southwestern China

Rhizopogonaceae Gäum. & C.W. Dodge 1928

Rhizopogon Fr. 1817

The genus *Rhizopogon* is the largest genus of hypogeous Basidiomycota, with worldwide distribution (Zeller & Dodge, 1918; Smith & Zeller, 1966; Martin, 1998; Molina et al., 1999; Koizumi & Nara, 2016; Li et al., 2016; Wang et al., 2021). Some species are popular edible fungi (Wang et al., 2013). *Rhizopogon* is characterized by globose to subglobose basidiomata, attached by rhizomorphs over the surface, gleba white when young, becoming darkens in maturity, finally gelatinized, with chambers small, numerous, and labyrinthine (Smith & Zeller, 1966; Koukol et al., 2022). All *Rhizopogon* species form obligate ectomycorrhizal associations with Pinaceae (Molina & Trappe, 1994; Massicotte et al., 1994; Massicotte et al., 1999; Grubisha et al., 2002).

According to statistics, prior to this study, 11 species of the genus *Rhizopogon* were recorded in China (Liu, 1988; Dai et al., 2010; Li et al., 2016; Wang et al., 2021). Among them, only 4 species reported after 2016 have molecular data (*R. sinoalbidus*, *R. flavidus*, *R. jiyaozi*, and *R. songmaodan*). In this study, a total of 106 *Rhizopogon* specimens were collected from the southwestern region, and after identification, these specimens were assigned to *R. sinoalbidus*, *R. flavidus*, *R. jiyaozi*, and *R. songmaodan*, as well as four additional new species described in this paper. The distribution of *Rhizopogon* 13 species in southwestern China is as follows: *Rhizopogon sinoalbidus* in Yunnan and Xizang province, *R. nigrescens*, *R. flavidus*, and *R. songmaodan* in Yunnan and Sichuan province, *R. jiyaozi* in Yunnan, Sichuan, and Xizang province, *R. fabri*, *R. luteolus*, *R. roseolus*, *R. laojunshanensis*, *R. weixiensis*, and *R. melanocuticularis* in Yunnan province, *R. luteoloides* and *R. zayuensis* in Xizang province.

Rhizopogon fabri Trappe 1975

Distribution & Specimen information: under *Pinus* spp. forests, basidiomata collected in summer. Yunnan Province: Lijiang City, Judian County, Jinsha River Valley, alt. 1950 m, 10 Jun. 1981, HKAS7901 (Xingjiang Li 971); Shangri-La, Nixi Township, alt. 3200 m, 4 Jul. 1981, HKAS8019 (Junrong Tao 1); alt. 3100 m, 4 Jul. 1981, HKAS8028 (Junrong Tao 5); Zhongdian County, alt. 3000 m, 29 Jun. 1981, HKAS7837 (Xingjiang Li 909). According to the *Flora of China Fungi VII*, this species is also distributed in Guangdong province (Liu, 1988).

Notes: No sequence data available from southwestern China. No edible or medicinal use.

Rhizopogon flavidus Lin Li & Shu H. Li 2016 Figure 52

Distribution & Specimen information: under *Pinus yunnanensis* forests, basidiomata from spring to summer. Yunnan Province: Dali, Jianchuan, alt. 2339.5m, 3 Aug. 2013, YAAS L2957, L2956, L2959 and L2961; Lijiang, Lashihai, alt. 2480.6m, 31 Jul. 2013, YAAS L2924.

Notes: Holotype YAAS L2957: ITS = NR158904. Other specimen YAAS L2956: ITS = KP893814, YAAS L2959: ITS = KP893815. No edible or medicinal use.

Figure 4.52 *Rhizopogon flavidus* morphological characteristics

Figure 4.52 A-D. Basidiomata and gleba; E. A piece of the section of peridium in 5% KOH; F.G. Trama in 5% KOH (G represents the overmature and autolyzed hymenial tissue.); H-K. Basidiospores in 5% KOH. Scale bars: A-D = 1cm; E.F.G = 50µm; H = 10µm; I-K = 1µm.

Rhizopogon jiyaozi Lin Li & Shu H. Li 2016 Figure 53

Distribution & Specimen information: under *Pinus yunnanensis* , or *Pinus densata* Mast. forests, basidiomata from spring to summer. Yunnan Province: Lijiang, Lashihai, alt. 2480.6m, 31 Jul. 2013, YAAS L2929, L2911, L2916, L2918, L2925, L2927, L2930, L2931 and L2932; Ninglang, HKAS71302, Dali, Jianchuan, alt.

2339.5m, 3 Aug. 2013, YAAS L2950; Shangri-La, Mt. Baima, alt. 3291.2m, 26 Aug. 2013, YAAS L2395, L2399 and L2409. Xizang Province: Nyingchi, Bomi, alt. 2739.7m, 18 Oct. 2023, BMDU L23110, L23111. These are newly recorded distributions in Xizang province from the current survey.

Notes: Holotype YAAS L2929: ITS = NR158906. Other specimen YAAS L2911: ITS = KP893825, YAAS L2916: ITS = KP893826, YAAS L2918: ITS = KP893827, YAAS L2925: ITS = KP893828, YAAS L2927: ITS = KP893829, YAAS L2930: ITS = KP893831, YAAS L2931: ITS = KP893832, YAAS L2932: ITS = KP893833, YAAS L2950: ITS = KP893834, YAAS L2409: ITS = KP893837, YAAS L2399: ITS = KP893836, YAAS L2395: ITS = KP893835; HKAS71302: ITS = MH827597, LSU = MH827602, rpb2 = MH827609, tef1 = MH827592; BMDU L23110: ITS = PP237772, LSU = PP237774, L23111: ITS = PP237773, LSU = PP237775. Edible, commonly found in mushroom markets.

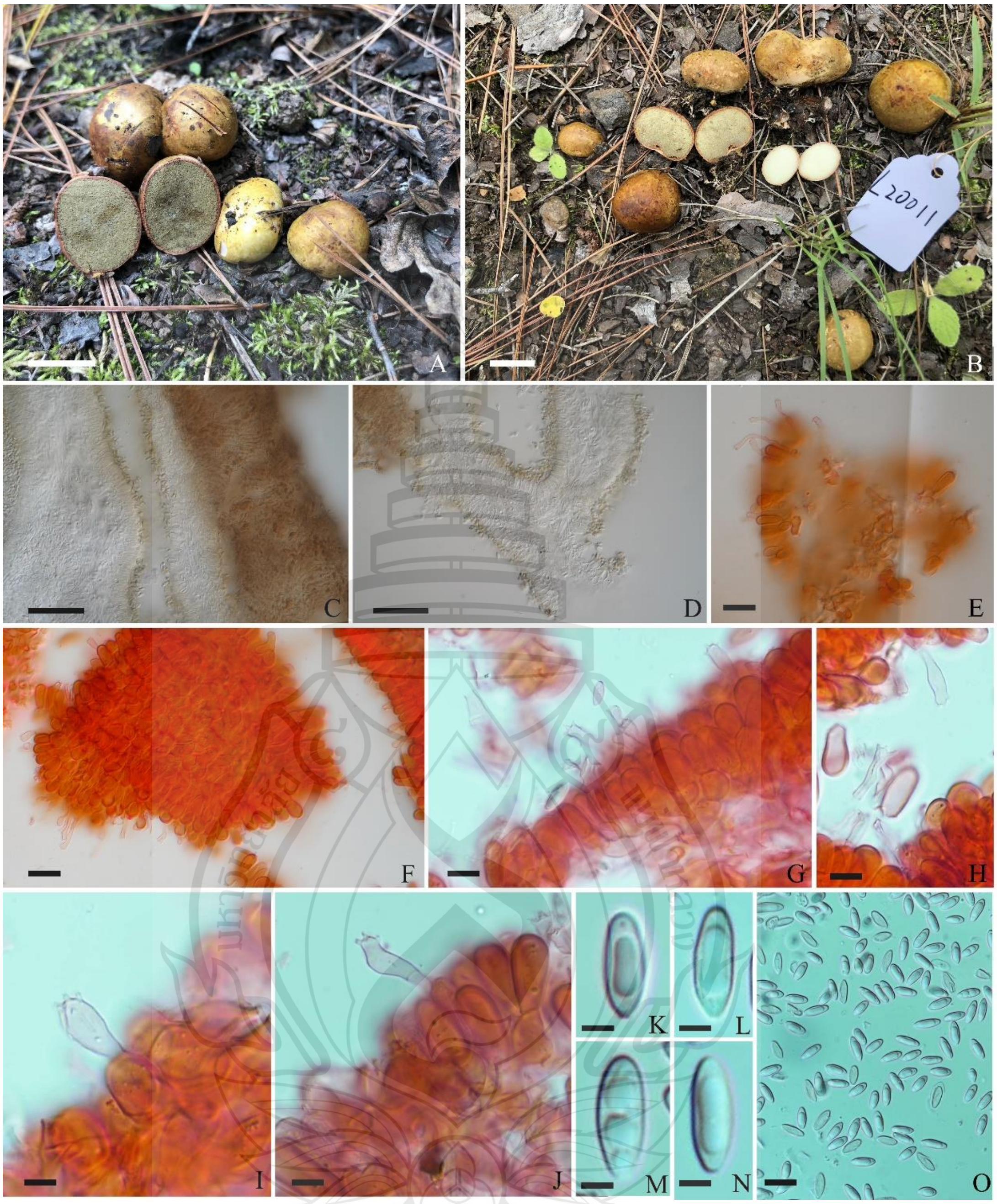


Figure 4.53 *Rhizopogon jiyaozi* morphological characteristics

Figure 4.53 A.B. Basidiomata and gleba; C. A piece of the section of the basidiomata in 5% KOH; D. Trama in 5% KOH; E-J. Basidia and basidioles in congo red staining solution; K-O. Basidiospores in 5% KOH. Scale bars: A.B = 1cm; C.D = 50µm; E.F = 20µm; G.H = 10µm; I. J.O = 5µm; K-N = 2µm.

Rhizopogon luteoloides A.H. Sm. 1966

Distribution & Specimen information: in soil under *Pinus densata* Mast. and *Quercus aquifolioides* Rehder et Wils. mixed forests, basidiomata collected in summer. Xizang Province: Mt. Sejila, alt. 3200m, 6 Aug. 1995, MHSU 2230 (Asheng Xu 003).

Notes: No sequence data available from southwestern China. No edible or medicinal use.

Rhizopogon luteolus Fr. 1817

Distribution & Specimen information: in soil under *Pinus densata* Mast. forests, basidiomata collected in summer to autumn. Yunnan Province: Shangri-La, Zhongdian Town, Dabao Temple, alt. 3450m, 20 Sep. 1993, HKAS26776, Xiaozhongdian Town, Bigu Village, alt. 3300m, 26 Jul. 1986, HKAS17506 (M. Zang 10484), alt. 3800m, 29 Jul. 1986, HKAS17606 (M. Zang 10587). According to the *Flora of China Fungi VII*, this species is also distributed in Nei Mongol province (Liu, 1988).

Notes: No sequence data available from southwestern China. No edible or medicinal use.

Rhizopogon nigrescens Coker & Couch 1928

Distribution & Specimen information: basidiomata collected in summer to autumn. Yunnan Province: Shangri-La, Zhongdian Town, Jisha Village, alt. 3200m, under *Picea* sp. forests, 24 Jul. 1986, HKAS17442 (M. Zang 10418). Sichuan Province: Xichang City, alt. 2000m, 13 Sep. 1983, HKAS11815 (M. S. Yuan). According to the *Flora of China Fungi VII*, this species is also distributed in Guangdong province (Liu, 1988).

Notes: No sequence data available from southwestern China. Edible, but has not been observed in markets in southwestern China.

Rhizopogon sinoalbidus Lin Li & Shu H. Li 2016 Figure 4.54

Distribution & Specimen information: under *Pinus* spp. forests, basidiomata from spring to summer. Yunnan Province: Dali Bai Autonomous Prefecture, Jianchuan County, alt. 2339.5m, 3 Aug. 2013, YAAS L2949, L2944, L2946, L2947, L2948, L2953, and L2954; Lijiang City, HKAS84655; Shangri-La City, Weixi County, L23006, L23014. Xizang Province: Nyingchi City, alt. 3052.5m, 26 Jul. 2024, BMDU L24098. This is a newly recorded distribution in Xizang province from the current survey. Sequences of this species, isolated from mycorrhizal tips of *Pinus tabuliformis* in Shaanxi, are recorded in GenBank, indicating that this species is also distributed in Shaanxi, China.

Notes: Holotype YAAS L2949: ITS = NR158905. Other specimen YAAS L2944: ITS = KP893816, YAAS L2946: ITS = KP893817, YAAS L2947: ITS = KP893818, YAAS L2948: ITS = KP893819, YAAS L2953: ITS = KP893821, YAAS L2954: ITS = KP893822; HKAS84655: ITS = MH827596, LSU = MH827601, *rpb2* = MH827608, *tef1* = MH827591; BMDU L23006: ITS = PX096560, LSU = PX096590; BMDU L23014: ITS = PX096561, LSU = PX096591; BMDU L24098: ITS = PX096559, LSU = PX096589; Edible, commonly found in mushroom markets.

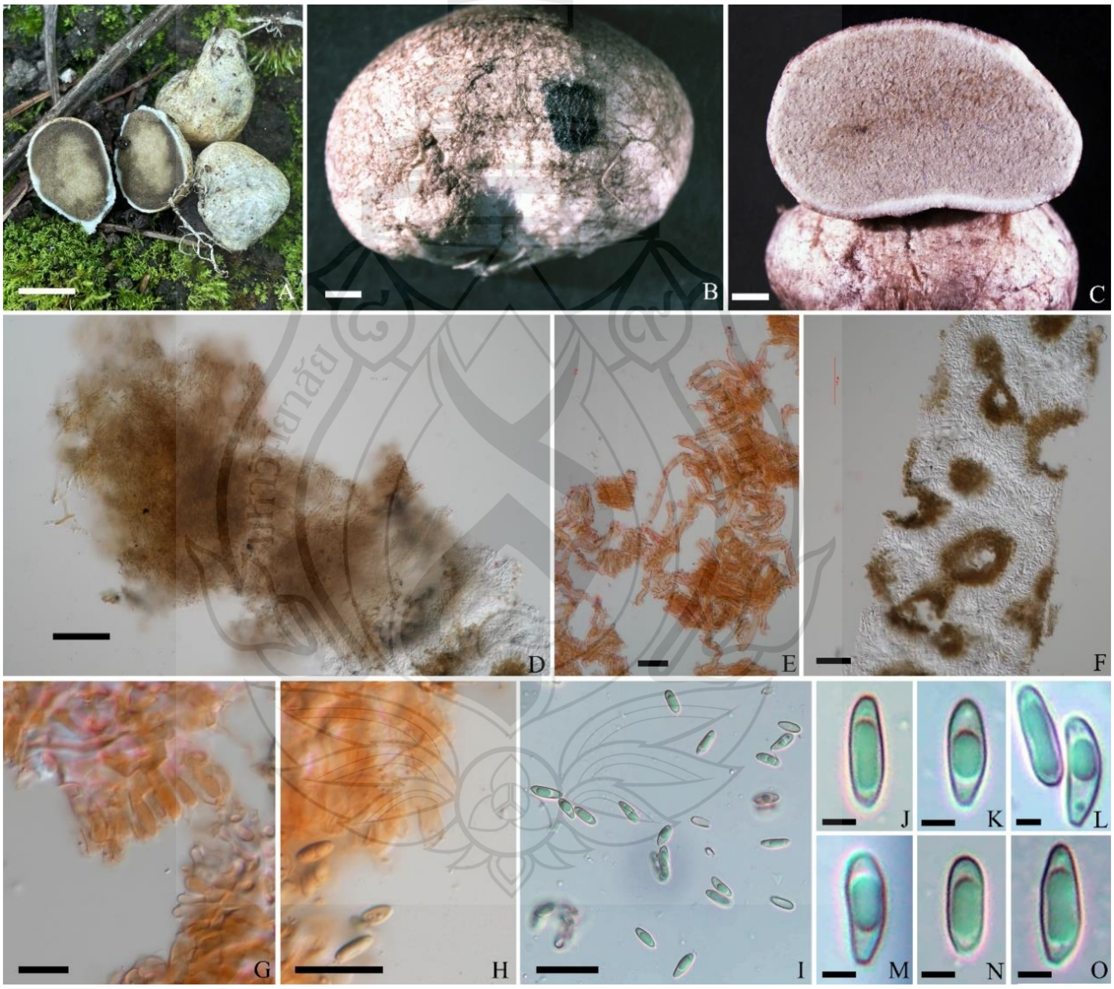


Figure 4.54 *Rhizopogon sinoalbidus* morphological characteristics

Figure 4.54 A.B.C. Basidiomata and gleba (B. Basidiocarp surface exhibiting a blackish blue reaction when treated with 5% KOH); D. A piece of the section of the peridium in 5% KOH; E. Hyphae forming the peridium in congo red staining solution;

F. Trama in 5% KOH; G.H. Basidia and basidioles in congo red staining solution; I-O. Basidiospores in 5% KOH. Scale bars: A = 1cm; B.C = 2 mm; .D = 100μm; E.H.I = 10μm; F.G = 20μm; J-O = 2 μm.

Rhizopogon songmaodan R. Wang & Fu Q. Yu 2021 Figure 4.55

Distribution & Specimen information: under *Pinus* spp. forests, basidiomata from spring to summer. Yunnan Province: Kunming, alt. 2710m, 13 Apr. 2019, HKAS106765, HKAS106766, HKAS106767 (razy-236), HKAS106768 (razy-237); Sichuan Province: Liangshan, alt. 2634m, 6 Jul. 2019, HKAS106770 (razy-252); Huili, wild mushroom market, 8 May 2019, BMDU L19003. This is a newly recorded distribution in Sichuan province from the current survey.

Notes: Holotype HKAS106767 (razy-236): ITS = NR174900. Other specimen HKAS106765: ITS = MN655981, HKAS106766: ITS = MN655982, HKAS106768: ITS = MN655984, HKAS106770: ITS = MN655985, BMDU L19003: ITS = PX096562. Edible, commonly found in mushroom markets.

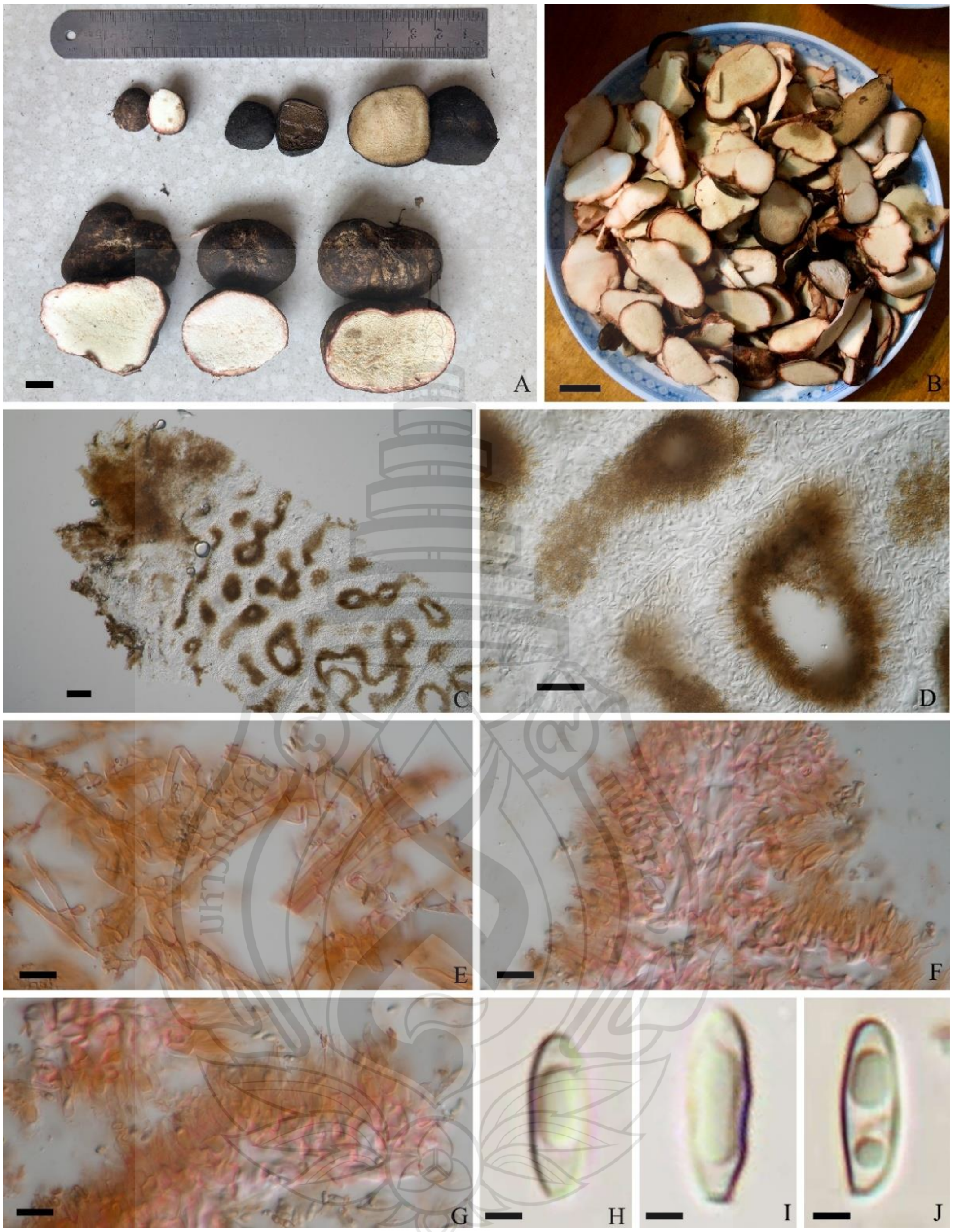


Figure 4.55 *Rhizopogon songmaodan* morphological characteristics

Figure 4.55 A.B. Basidiomata and gleba; C. A piece of the section of the basidiomata in 5% KOH; D. Trama in 5% KOH; E. Hyphae forming the peridium in Congo red staining solution; F.G. Basidia and basidioles in Congo red staining solution; H-J. Basidiospores in 5% KOH. Scale bars: A.B = 1cm; C = 100 μm; D = 50μm; E.G = 10 μm; F = 20 μm; H.I. J = 2μm.

Rhizopogon roseolus (Corda) Th. Fr. 1909

= ***Rhizopogon rubescens*** (Tul. & C. Tul.) Tul. & C. Tul., *G. bot. ital.* 2(1): 58 (1845) [1844]

= ***Rhizopogon rubescens*** **var.** ***ochraceus*** A.H. Sm., *Mem. N. Y. bot. Gdn* 14(2): 99 (1966)

= ***Rhizopogon rubescens*** **var.** ***pallidimaculatus*** A.H. Sm., *Mem. N. Y. bot. Gdn* 14(2): 97 (1966)

= ***Rhizopogon rubescens*** **var.** ***rileyi*** A.H. Sm., Mem. N. Y. bot. Gdn 14(2): 98 (1966)

Distribution & Specimen information: under *Pinus* sp. forests, basidiomata collected in summer. Yunnan Province: Lijiang city, Judian Town, Jinsha River, alt. 1855m, 9 Jun. 1981, HKAS7877 (Xingjiang Li 968); Mt. Yulong Snow, Yufeng Temple, alt. 2700m, 17 Sep. 1993, HKAS26715 (Y. Doi), alt. 2740m, 22 May. 1984, HKAS16949 (Jianxun Xi 504); Shangri-La, Bitahai, alt. 3600m, 24 Jun. 1981, HKAS7952 (Anmin Li 2), Xiaozhongdian Town, Bigu Forest Farm., alt. 3260m, 28 Jul. 1986, HKAS17984 (Keke Chen 71); Nujiang Lisu Autonomous Prefecture, Fugong County, Zhiziluo (formerly Bijiang County), 4 Jul. 1978, HKAS3858 (Daqian Ji); Kunming city, Jindian Forest Farm., 14 Sep. 1991, HKAS23523 (M. Zang 11894), Gangtou Village, 11 Aug. 1990, MHSU2206 (Lei Wang & Mingchang Chang 377), Qiongzhu temple, MHSU2207 (Lei Wang & Mingchang Chang 340). According to the *Flora of China Fungi VII*, this species is also distributed in Shanxi and Guangdong provinces (Liu, 1988).

Notes: No sequence data is available from southwestern China. Edible. Notably, *Rhizopogon roseolus* was not detected among the extensive collection of *Rhizopogon* specimens in this study. However, its closely related species, *Rhizopogon jiyaozi*, is widely distributed in southwestern China. Critically, all specimens previously identified as *Rhizopogon roseolus* in the literature lack available molecular sequence data. Further verification of this situation requires additional evidence.

Rhizopogon laojunshanensis L. Li, K.D. Hyde & S.H. Li, sp. nov. Figure 24

Distribution & Specimen information: in soil under *Pinus yunnanensis* Franch., basidiomata from summer. Yunnan Province: Lijiang city, Jiuhe town, Mt. Laojunshan, alt. 2734.2 m, 17 August 2020, Lin Li, BMDU L20074.

Notes: Holotype BMDU L20074: ITS = PX096563, LSU = 096593. No edible or medicinal use.

Rhizopogon melanocuticularis L. Li, K.D. Hyde & S.H. Li, sp. nov. Figure 4.25

Distribution & Specimen information: in soil under *Pinus yunnanensis* Franch., basidiomata from summer. Yunnan Province: Weixi county, alt. 2484 m, 19 May 2022, Shuhong Li, BMDU L22409, BMDU L22410; alt. 2994 m, 8 June 2023, Lin Li, BMDU L23201, BMDU L23203.

Notes: Holotype BMDU L22409: ITS = PX096564, LSU = PX096594. Other specimens BMDU L22410: ITS = PX096565, LSU = PX096595; BMDU L23201: ITS = PX096566; BMDU L23203: ITS = PX096567. Edible, commonly seen in wild mushroom markets.

Rhizopogon zayuensis L. Li, K.D. Hyde & S.H. Li, sp. nov. Figure 26

Distribution & Specimen information: in soil under *Pseudotsuga forrestii* Craib, basidiomata from summer. Xizang Province: Zayu county, alt. 2420 m, 9 August 2022, Lin Li, BMDU L22031, BMDU L22032.

Notes: Holotype BMDU L22031: ITS = PX096568, LSU = PX096596. Other specimen BMDU L22032: ITS = PX096569, LSU = PX096597. No edible or medicinal use.

Rhizopogon weixiensis L. Li, K.D. Hyde & S.H. Li, sp. nov. Figure 27

Distribution & Specimen information: in soil under *Pinus yunnanensis* Franch., basidiomata from summer. Yunnan Province: Weixi county, alt. 2493 m, 8 May 2022, Lin Li, BMDU L22408; alt. 2392 m, 9 May 2022, Lin Li, BMDU L22401, BMDU L22402, BMDU L22403; alt. 2272 m, 9 May 2022, Lin Li, BMDU L22404, BMDU L22405, BMDU L22406; alt. 2820 m, 9 May 2022, Lin Li, BMDU L22407.

Notes: Holotype BMDU L22408: I ITS = PX096577, LSU = PX096605. Other specimens BMDU L22401: ITS = PX096570, LSU = PX096598, BMDU L22402: ITS = PX096571, LSU = PX096599, BMDU L22403: ITS = PX096572, LSU = PX096600; BMDU L22404: ITS = PX096573, LSU = PX096601, BMDU L22405: ITS = PX096574, LSU = PX096602, BMDU L22406: ITS = PX096575, LSU = PX096603; BMDU L22407: ITS = PX096576, LSU = PX096604. Edible, commonly seen in wild mushroom markets.

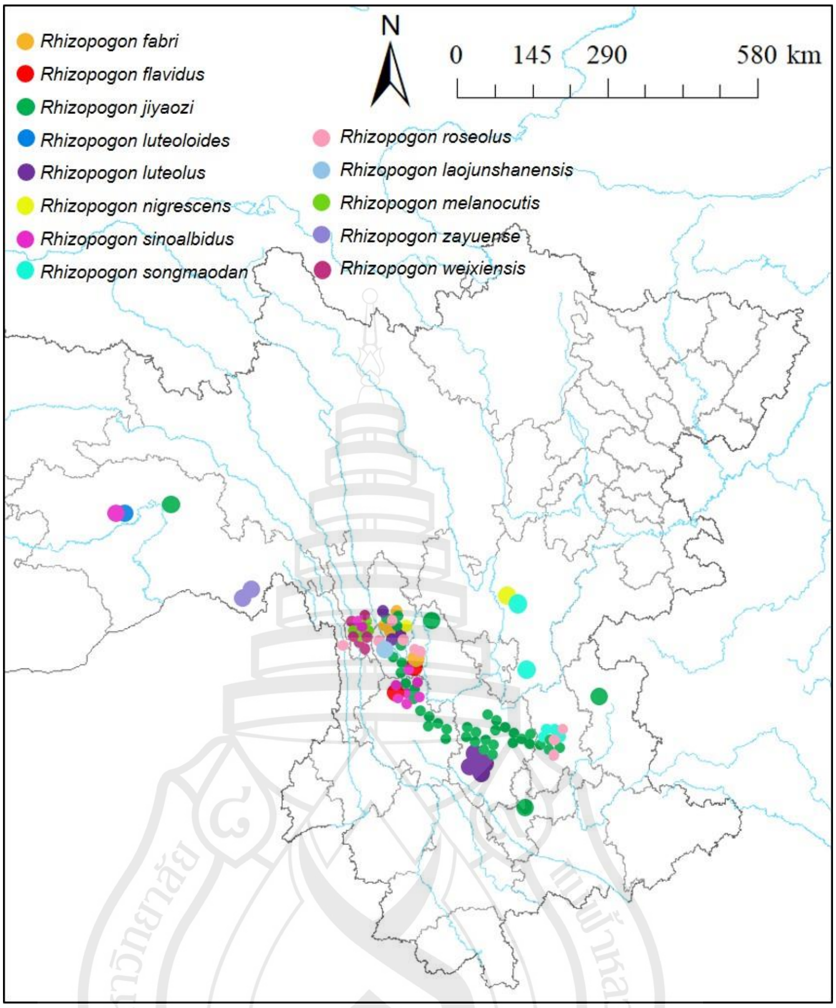


Figure 4.56 Distribution map of *Rhizopogon* species in southwestern China

Sclerodermataceae Corda 1842

Scleroderma Pers. 1801

The genus *Scleroderma* is a species-rich groups throughout the tropics (Corrales et al., 2022); some species are edible or medicinal (Dai & Yang, 2008). *Scleroderma* is characterized by the subglobose, pyriform, or subturbinate basidiomata, a firm peridium, which dehisces at maturity, and globose, colored, and ornamented basidiospores (Guzmán, 1970). *Scleroderma* species form ectomycorrhizal with Cistaceae, Dipterocarpaceae, Fagaceae, Juglandaceae, Myrtaceae, Mimosaceae, and Pinaceae (Corrales et al., 2022).

According to statistics, there are currently 20 species are now known in China, among which 8 species are distributed in the southwest region: *Scleroderma yunnanense*, *S. venenatum var. macrosporum*, *S. suthepense*, *S. erubescens*, *S. separatum*, *S. squamulosum*, *S. vinaceum*, *S. cepa* in Yunnan province (Zhang et al., 2020; Li, 2003; Liu et al., 2005; Zhang et al., 2013; Li et al., 2015; Dai et al., 2010; Dai & Yang, 2008; Wang et al., 2020; Wu et al., 2023).

Scleroderma cepa Fr. 1829

Distribution & Specimen information: on the soil under Fagaceae and *Pinus yunnanensis*, basidiomata from summer. Yunnan Province: NIOHP YN20190904-01(no detailed specimen information), collected by the National Institute of Occupational Health and Poison Control, Chinese Center for Disease Control and Prevention.(Li H. et al., 2020; Li H. et al., 2023; Li H. et al., 2024; Yuan et al., 2024).

Notes: NIOHP YN20190904-01: ITS = MN919351. Poisonous, occasionally collected for consumption, leading to poisoning incidents.

Scleroderma erubescens Z.W. Ge, R. Wu & L.R. Zhou 2023

Distribution & Specimen information: on soil in *Castanea spp.* and *Pinus yunnanensis* mixed forest, basidiomata from summer to autumn. Yunnan Province: Kunming, Luquan, alt. 1680m, 19 Jul. 2019, HKAS126621 (Ge4828); Nujiang, Fugong, alt. 1700m, 3 Aug. 2011, HKAS73740 (Zhu363); Baoshan, Longyang, alt. 1700m, 17 Sep. 2019, HKAS126622 (Ge4356) (Wu et al., 2023).

Notes: Holotype HKAS126621 (Ge 4828): ITS = OQ554978. Paratype HKAS73740 (Zhu363): ITS = OQ554977, HKAS126622 (Ge4356): ITS = OQ554976. No edible or medicinal use.

Scleroderma separatum Z.W. Ge, R. Wu & L.R. Zhou 2023

Distribution & Specimen information: under *Carya illinoinensis* and *Pinus yunnanensis* mixed forest, basidiomata from summer to autumn. Yunnan Province: Yuxi, Xinping, alt. 1520m, 2 Aug. 2018, HKAS126618 (Ge4148), HKAS126619 (Zhou31); Dali, Yangbi, ail. 1500m, 25 Aug. 2020, HKAS126620 (Ge5394) (Wu et al., 2023).

Notes: Holotype HKAS126618 (Ge4148): ITS = OQ554973. Paratype HKAS126619 (Zhou31): ITS = OQ554974, HKAS126620 (Ge5394): ITS = OQ554975. No edible or medicinal use.

Scleroderma squamulosum Z.W. Ge, R. Wu & L.R. Zhou 2023

Distribution & Specimen information: on the soil under Fagaceae and *Pinus yunnanensis*, basidiomata from summer. Yunnan Province: Nujiang, Lushui, alt.

3100m, 6 Aug. 2011, HKAS71482 (Hao373); Xishuangbanna, Jinghong, Dadugang, alt. 2380m, 22 Jul. 2007, HKAS54573(Tang342); Dehong, Yingjiang, Tongbiguan Nature Reserve, alt. 2170m, 17 Jul. 2009, HKAS56778 (Tang821); Baoshan, Longling, alt. 2500m, 30 Jul. 2014, HKAS87110 (Liu464); Yuxi, Xinping, alt. 2935m, 26 Jul. 2011, HKAS70439 (Ge2935) (Wu et al., 2023).

Notes: Holotype HKAS71482 (Hao373): ITS = OQ554979. Paratype HKAS54573 (Tang342): ITS = OQ554982, HKAS56778 (Tang821): ITS = OQ554980, HKAS87110 (Liu464): ITS = OQ554981, HKAS70439 (Ge2935): ITS = OQ554983. No edible or medicinal use.

Scleroderma suthepense Kumla, Suwannar. & Lumyong 2013

Distribution & Specimen information: basidiomata from summer to autumn. Yunnan Province: Dehong, Mangshi, 19 Aug. 2015, NIOHP MS20150819-25; Xishangbanna, Jinghong, alt. 910m, 27 Jul. 2016, NIOHP JH2016-0727-052 (Zhang et al., 2020).

Notes: NIOHP MS20150819-25: ITS = MH513624; NIOHP JH2016-0727-052: ITS = MH513626. No edible or medicinal use.

Scleroderma venenatum* var. *macrosporum Y.Z. Zhang, C.Y. Sun & Hai J. Li 2020

Distribution & Specimen information: on soil in *Delonix regia* forest, basidiomata from autumn. Yunnan Province: Lincang, alt. 1630m, 29 Aug. 2015, NIOHP Li 150829-04; Honghe, Mile, alt. 1790m, 8 Nov. 2016, NIOHP MLMY20160808009 & NIOHP MLMY20160808016 (Zhang et al., 2020).

Notes: Holotype NIOHP Li 150829-04: ITS = MH513634. Paratype NIOHP MLMY20160808009: ITS = MH513632, MLMY20160808016: ITS = MH513630. No edible or medicinal use.

Scleroderma vinaceum Z.W. Ge, R. Wu & L.R. Zhou 2023

Distribution & Specimen information: in forest dominated by *Castanea* spp., basidiomata from summer. Yunnan Province: Nujiang, Gongshan, alt. 1520m, 1 Aug. 2011, HKAS73723 (Zhu346); Nujiang, Lushui, alt. 2825m, 6 Aug. 2010, HKAS69055 (Guo63), alt. 2400m, 5 Aug. 2011, HKAS73764 (Zhu387); Nujiang, Fugong, alt. 2400m, 5 Aug. 2011, HKAS73183 (Qin197); Chuxiong, Mt. Zixi, alt. 2400m, 18 Sep. 2010, HKAS61712 (Ge2789); Chuxiong, Lufeng, alt. 2320m, 12 Aug. 2021, HKAS126617(Ge5651) (Wu et al., 2023).

Notes: Holotype HKAS73723 (Zhu346): ITS = OQ554987. Paratype HKAS69055(Guo63): ITS = OQ554984, HKAS73764(Zhu387): ITS = OQ554988, HKAS73183(Qin197): ITS = OQ554985, HKAS61712(Ge2789): ITS = OQ554986, HKAS126617(Ge5651): ITS = OQ554989. No edible or medicinal use.

Scleroderma yunnanense Y. Wang 2013

Distribution & Specimen information: under *Pinus kesiya* var. *langbianensis* forest, basidiomata from autumn. Yunnan Province: Puer, 15 Sep. 2011, HKAS79633 (Ji S1101), HKAS79664 (Ji S1102), HKAS79665 (Ji S1103); Xishuangbanna, Puwen, 24 Sep. 2011, HKAS79666 (Ji S1104), HKAS79667(Ji S1105), HKAS79668 (Ji S1106) (Zhang et al., 2013).

Notes: Holotype HKAS79633 (Ji S1101): ITS = JQ639040–JQ639043. Other specimen HKAS79664 (Ji S1102): ITS = JQ639044 & JQ639045, HKAS79665 (Ji S1103): ITS = JQ639046. Edible, commonly found in mushroom markets.

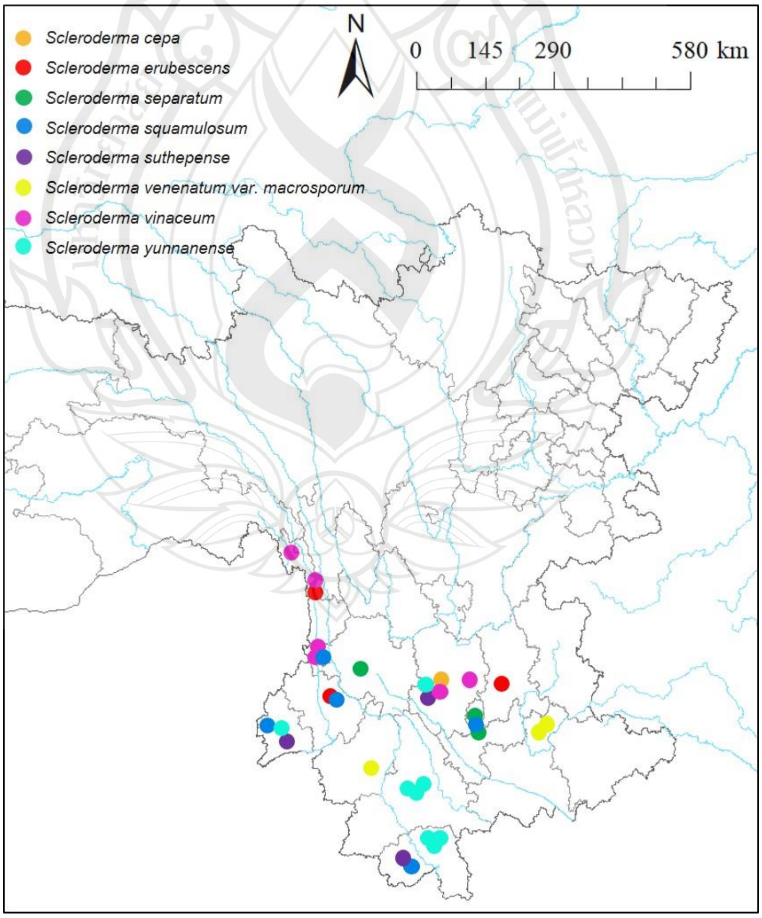


Figure 4.57 Distribution map of *Scleroderma* species in southwestern China

Suillaceae Besl & Bresinsky 1997

Truncocolumella Zeller 1939

Truncocolumella is a rare genus of hypogeous fungi, currently found only three species distributed in the Pacific Northwest and the Chinese Hengduan Mountains Ranges. *Truncocolumella* is characterized by basidiomata irregularly depressed-globose to pyriform, the surface typically smooth and dry in appearance, with some rhizomorphs, elastic, very mature basidiomata deliquesce like *Rhizopogon*. Gleba white when young, becoming darkens in maturity, finally gelatinized, chambers small, numerous, labyrinthine, obvious stump-like or dendroid-radiating columella (Zeller, 1939; Smith & Singer, 1959; Li et al., 2024b).

According to statistics, there is currently 1 species are now known in China, *Truncocolumella pseudocolumella*, collected from Sichuan province (Li et al., 2024b).

Truncocolumella pseudocolumella Lin Li, S.H. Li & Y. Wang 2024 Figure 4.32

Distribution & Specimen information: under *Quercus guyavaefolia* and *Pinus sp.* forest, basidiomata from autumn. Sichuan Province: Yala Snowy Mountains, alt. 3772m, 19 Aug. 2012, HKAS131259 ex YAAS L2327; Ganzi, 22. Aug. 2014, HKAS 95533, HKAS 95534.

Notes: Holotype HKAS131259: ITS = KP090063 & KP090064, LSU = PP112109. Other specimen HKAS 95533: ITS = OR631922, LSU = PP112108, HKAS 95534: ITS = OR631923. No edible or medicinal use.

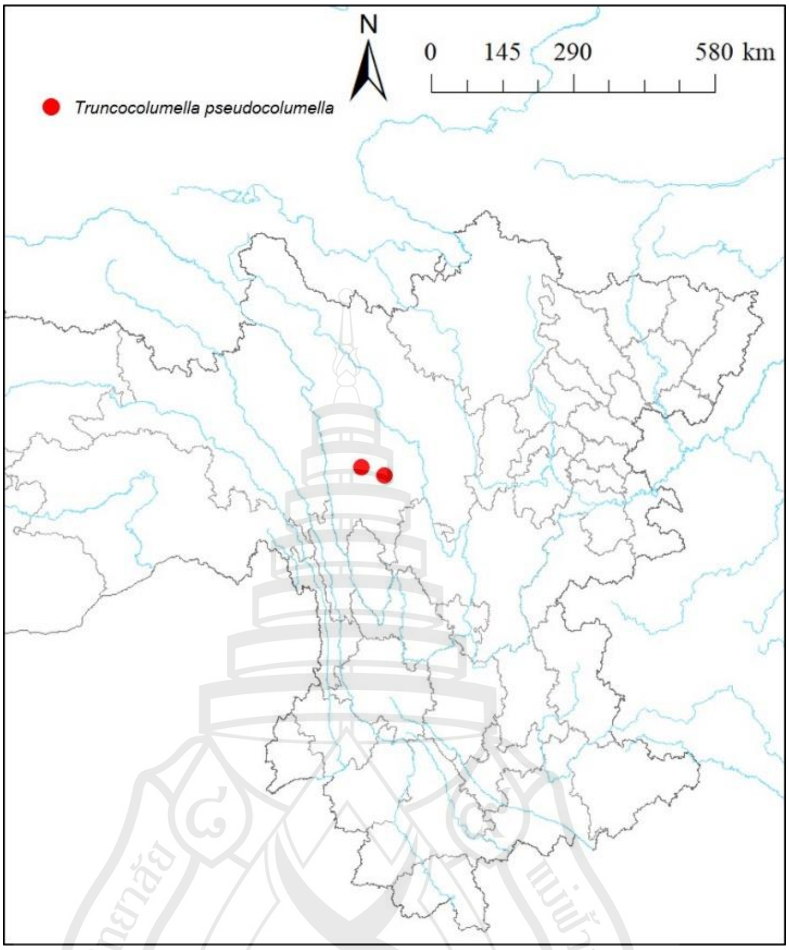


Figure 4.58 Distribution map of *Truncocolumella* species in southwestern China

Gomphales Jülich 1982

Gomphaceae Donk 1961

Gautieria Vittad. 1831

The genus *Gautieria* was described and reported across the Northern Hemisphere, South America, and Oceania, typically forming ectomycorrhizal associations with symbiotic tree partners, such as Pinaceae, Betulaceae, and Fagaceae (Vittadini, 1831; Liu, 1998; Bougher & Lebel, 2001; Vidal et al., 2023). Species form hypogeous, truffle-like basidiomata. *Gautieria* is characterized by a subglobose to reniform basidiomata with a thin and easily evanescent peridium, a cartilaginous gleba with simple or branched columella and a coralloid type of development, often elongated, labyrinthiform cavities, basidiospores with longitudinal ribs (Zeller & Dodge, 1918; Cunningham, 1944; Zeller, 1948; Rauschert, 1975; Beaton et al., 1985; Vidal et al., 2023).

According to statistics, there are currently 12 species are now known in China (Liu, 1998; Ying, 1984, 1995; Bau & Liu, 2013; Yang et al., 2023; Li et al., 2025), among which 3 species are distributed in the southwest region: *G. pallida* in Sichuan Province, *G. shennongjiaensis* in Hubei and Xizang Provinces, and *G. zixishanensis* is distributed in Yunnan Province (Liu, 1998; Bau & Liu, 2013; Yang et al., 2023; Li et al., 2025).

Gautieria pallida Harkn. 1934

Distribution & Specimen information: The record of this species' distribution in China is briefly mentioned in the *Flora of Fungi in China VII* as follows: the specimen was collected from Chengkou County, Sichuan Province, with no habitat or date of collection recorded, and the collector and specimen number are R. P. Farges (FH1) 1566) (Liu, 1998).

Notes: No sequence data available. No edible or medicinal use.

Gautieria shennongjiaensis K. Tao, Ming C. Chang & B. Liu 1996

Distribution & Specimen information: in soil under *Quercus aquifolioides*, *Pinus yunnanensis*, or *Pinus densata* forest, basidiomata from summer to autumn. Xizang Province: Nyingchi, Sejila Mountain, alt. 3000m, 24 Aug. 1995, MHSU 2232 (Xu AS 005); alt. 3050m, 10 Sep. 1995, MHSU 2235 (Xu AS 007); Bomi County, Zhamu forest farm rear mountain, alt. 2700m, 14 Sep. 1995, MHSU 2236 (Xu AS 009)；Zayu County, Xiazayu Town, Xiayue Village， alt. 2200m, 16 Sep. 1995, MHSU 2238 (Xu AS 0011). According to the *Flora of China Fungi VII*, the type specimen of this species was collected from Shennongjia, Hubei Province, MHSU 2259 (Liu, 1988).

Notes: Holotype MHSU 2259. No sequence data available. No edible or medicinal use.

Gautieria zixishanensis L. Li, K.D. Hyde & S.H. Li, 2025 Figure 4.13

Distribution & Specimen information: in soil under *Pinus yunnanensis* and *Quercus* sp. mixed forest, basidiomata from winter. Yunnan Province: Chuxiong city, Zixi Mountain, alt. 2053.7m, 2 Nov. 2023, BMDU L23106, BMDU L23107.

Notes: Holotype BMDU L23106: ITS = PQ672295, nrLSU = PQ672297. Other Specimen BMDU L23107: ITS = PQ672296 nrLSU = PQ672298. No edible or medicinal use.

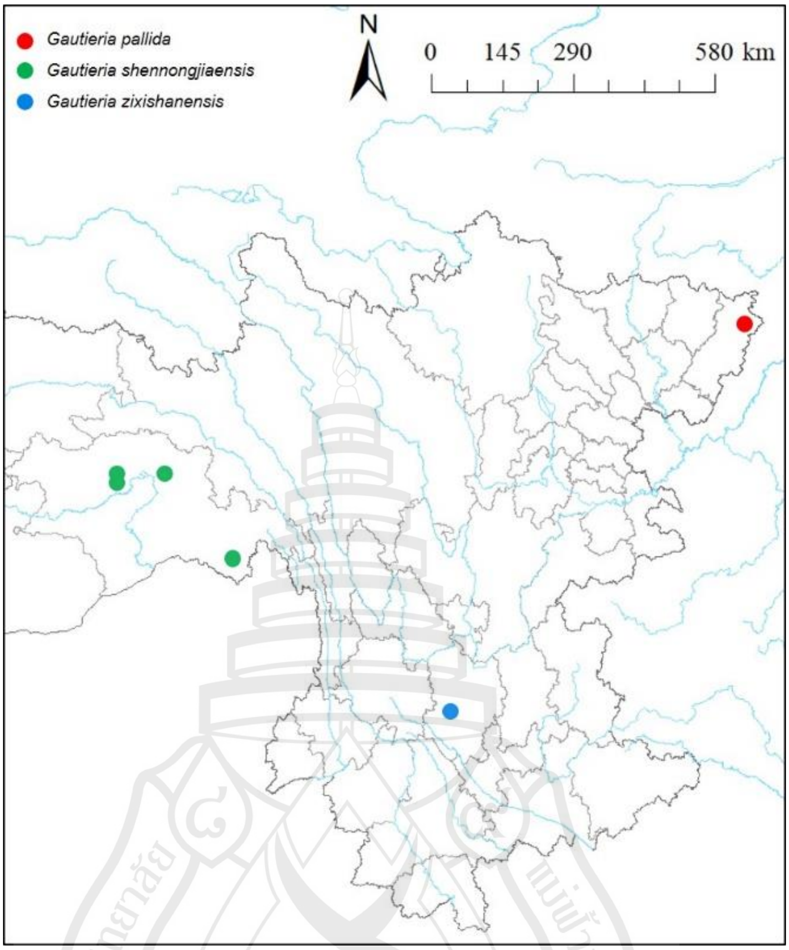


Figure 4.59 Distribution map of *Gautieria* species in southwestern China

Hysterangiales K. Hosaka & Castellano 2007

Hysterangiaceae E. Fisch. 1898

Hysterangium Vittad. 1831

The genus *Hysterangium* is a worldwide distributed group. *Hysterangium* is characterized by with olive–green, gelatinous gleba, and ellipsoid, hyaline spores that usually have a loose or closely appressed, wrinkled utricle (Hosaka et al., 2006; Guevara et al., 2008). Many *Hysterangium* species form ectomycorrhizal associations with plants (Castellano, 1988; Castellano, 1999).

According to statistics, there are currently 16 species are now known in China, among which 5 species are distributed in southwest region: *Hysterangium strobilus* was collected by Dr. Harry Smith in Sichuan Province, China, in 1922, that is the first discovery of the *Hysterangium* genus in China (Eckblad & Ellingsen, 1984); *H. obtusum* and *H. fuscum* in Yunnan province; *H. latiappendiculatum* in Xizang province (Xu & Liu, 2003; Liu et al., 2005). In this study, a new species, *Hysterangium alpinum*, collected from high-elevation fir forests in Yunnan, is described.

Hysterangium fuscum Harkn. 1899

Distribution & Specimen information: under *Quercus* sp. forest, ascocarp from summer to autumn. Yunnan Province: Anning city, Bijia Mountain in Wenquan, 6 August 1990, MHSU2140.

Notes: No sequence data available. No edible or medicinal use.

Hysterangium latiappendiculatum A-S. Xu & B. Liu 2003

Distribution & Specimen information: in soil under *Quercus aquifolioides* Rehder & E. H. Wilson in Sarg. forest, basidiomata from summer. Xizang Province: Nyingchi, Nongyuannanshan, alt. 3150m, 24 Aug. 1996, HXZE913 (Xu AS 96136).

Notes: Holotype HXZE913 (Xu AS 96136). No sequence data available. No edible or medicinal use.

Hysterangium obtusum Rodway 1920

Distribution & Specimen information: under *Quercus acutissima* and *Rubus* sp. mixed forest, basidiomata from summer to autumn. Yunnan Province: Kunming city, Heilongtan, Kunming Institute of Botany, 11 August 1990, MHSU2148 (Chang M. C. & Wamg L. 301), MHSU2149 (Wang L 292), MHSU2150 (Wang L 304).

Notes: No sequence data available. No edible or medicinal use.

Hysterangium strobilus Zeller & C.W. Dodge 1929

Distribution & Specimen information: under *Picea* sp. or *Quercus* sp. forest, basidiomata from autumn. Sichuan Province: northwestern Sichuan (no specific location), under *Picea* sp. forest, alt. 3500m, 12 Sep. 1922, there is no information on the collection number of the specimen in the original literature, the specimen is kept at the Institute of Systematic Botany, Uppsala University, Sweden; western Sichuan, Huidong, under *Quercus* sp. forest, 14 Sep. 1988, MHSU2170 (Tao K 274).

Notes: No sequence data available. No edible or medicinal use.

Hysterangium alpinum L. Li, K.D. Hyde & S.H. Li, sp. nov. Figure 4.17

Distribution & Specimen information: in soil under *Abies forrestii*, basidiomata from autumn. Yunnan Province: Lijiang city, Juhe town, Mt. Laojunshan, alt. 3875.7m, 19 Sep. 2021, Lin Li, BMDU L21199.

Notes: Holotype BMDU L21199: ITS=PX108631 LSU=PX105441. No edible or medicinal use.

Hysterangium shibaoshanense L. Li & K.D. Hyde sp. nov. Figure 4.18.

Distribution & Specimen information: in the soil of a broad-leaved forest dominated by *Alnus* sp., basidiomata from autumn. Yunnan Province: Dali Bai Autonomous Prefecture, Jianchuan county, Shibaoshan Mountain, alt. 2508.8 m, 8 Sep. 2024, Songming Tang, BMDU L24099, alt. 2601 m, 8 Sep. 2024, Songming Tang, BMDU L24104.

Notes: Holotype BMDU L24099: ITS=PX560236, LSU= PX560238. Other specimen BMDU L24104: ITS= PX560235, LSU= PX560237. No edible or medicinal use.

Phallogastraceae Castellano, T. Lebel, Davoodian & K. Hosaka 2021

Protubera Möller 1895

The genus *Protubera* is characterized by enclosed white to brownish epigeous (when mature) basidiomata, gelatinous or cartilaginous gleba of olivaceous or brownish tinges; small, smooth, and subcylindrical basidiospores of olivaceous to brownish tinges (Zeller, 1939; Malloch, 1989; Trierveiler-Pereira et al., 2014).

According to statistics, there are currently 5 species are now known in China, among which 3 species are distributed in the southwest region: *Protubera kunmingica, P. nipponica,* and *P. yunnanensis* in Yunnan province (Li et al., 2018; Liu, 1994; Liu et al., 1996).

Protubera kunmingica (M. Zang, K. Tao & X.X. Liu) G.J. Li & R.L. Zhao 2018

≡ ***Kobayasia kunmingica*** M. Zang, K. Tao & X.X. Liu, Journal of Shanxi University, Natural Science 19(3): 321. 1996.

Distribution & Specimen information: under *Eucalyptus globulis* Lab. forest, basidiomata from autumn. Yunnan Province: Kunming, Jindian, alt. 1900m, 14 Sep. 1994, HKAS23523 (Zang M 11894-1).

Notes: Holotype HKAS23523 (Zang M 11894-1). No sequence data available. No edible or medicinal use.

Protubera nipponica Kobayasi 1938

Distribution & Specimen information: under *Lithocarpus* sp. and *Castanopsis* sp. forest, basidiomata from summer. Yunnan Province: Puer, Jingdong, Mt. Ailao, alt. 2300m, 27 Aug. 1991, HKAS23813 (Song G353); Honghe, Pingbian, Mt. Dawei Natural Reserve, alt. 2220m, 4 Jul. 1992, HKAS26139 (Liu PG1288)

Notes: No sequence data available from southwestern China. No edible or medicinal use.

Protubera yunnanensis L. Li, K.D. Hyde & S.H. Li, sp. nov. Figure 4.22

Distribution & Specimen information: gregarious on grassland, under bamboo and *Pinus*, basidiomata from summer. Yunnan Province: Mt. Cangshan, Dali University campus, alt. 2105.7m, 23 Oct. 2023, Lin Li, HKAS131263, 21 Oct. 2024, Lin Li, HKAS131267; Wuwei temple, alt. 2242m, 6 Sep. 2014, Shuhong Li, HKAS131260; alt. 2600m, 20 Sep. 2015, Songming Tang, HKAS131261; Qujing city, Zhanyi county, Gaozhai village, 29 Jul. 2021, Songming Tang, HKAS131262; Chuxiong city, Wuding county, Maojie town, Yangcaidi village, 26 Oct. 2023, Yongchang Zhao, HKAS131269.

Notes: Holotype HKAS131263: LSU= PX112446. Other specimens HKAS131267: LSU = PX112444; HKAS131260: LSU = PX112447; HKAS131261: LSU = PX112442; HKAS131262: LSU = PX112443; HKAS131269: LSU = PX112445. No edible or medicinal use.

Trappeaceae P.M. Kirk 2008

Trappea Castellano 1990

The genus *Trappea* species form hypogeous to epigeous basidiomata. *Trappea* characterized by basidiomata globose to irregularly lobed, white when fresh and quickly staining pink or brown when exposed, gleba green to olive, with scattered to abundant sterile locules contained in a gelatinized layer located just within the true peridium; locules empty, columella distinct, dendroid to subpercurrent, basidiospores smooth, hyaline to pale green, ellipsoid to oblong (Castellano, 1990; Davoodian et al., 2021).

According to statistics, there is currently 1 species are now known in China, *Trappea cinnamomea*, collected from Xizang province (Xu & Luo, 2003).

Trappea cinnamomea A-S. Xu & D.Q. Luo 2003

Distribution & Specimen information: in the forest of *Quercus aquifolioides* Rehd. & Wils., basidiomata from autumn. Xizang Province: Nyingchi, Lulang, east slope of Mt. Sejila, alt. 3200m, 29 Sep. 2001, HXZE1723 (Xu A. S. 2001-107).

Notes: Holotype HXZE1723 (Xu A. S. 2001-107). No sequence data available. No edible or medicinal use.

Phallogastraceae (Locq. 1974) Castellano, T. Lebel, Davoodian & K. Hosaka 2021

Phallogaster Morgan 1893

The genus *Phallogaster* species is mostly distributed in North America, Central America, Europe, and Asia (Thaxter, 1893; Furia & Bernicchia, 1982; Dogan, 2006; Yang & Ge, 2008; Davoodian et al., 2021). *Phallogaster s*pecies form hypogeous to epigeous basidiomata, characterized by subglobose to irregularly subovoid, with a tapered to irregularly shaped base, single or caespitose, with white rhizomorphs, sometimes discolouring pinkish. Basidiome surface smooth to velvety to finely tomentose, white to clay white, discolouring pinkish to salmon buff to reddish or faint lilac at exposure. Columella branched, whitish to semi-translucent, gelatinized. Gleba varies from green, olivaceous green, greyish olive to pale olive; gelatinized, loculate, locules small. Spores smooth, elongate-ellipsoid to oblong, thin-walled, utricle absent, hyaline singly, olive buff to honey yellow or pale green in mass. Clamp connections present. Odour sometimes fetid. Species apparently saprotrophic, occurring on decaying wood or leaves, or partially buried in litter (Thaxter, 1893; Yang & Ge, 2008; Davoodian et al., 2021).

According to statistics, there is currently one species are now known in China, *Phallogaster saccatus*, collected from Sichuan province (Yang & Ge, 2008).

Phallogaster saccatus Morgan 1893

Distribution & Specimen information: among fallen wood in a forest dominated by *Picea* and *Abies*, basidiomata from summer. Sichuan Province: Xiangcheng county, Reda, alt. 3700m, 15 Jul. 2004, HKAS 45512 (Yang & Ge, 2008).

Notes: No sequence data available from southwestern China. No edible or medicinal use.

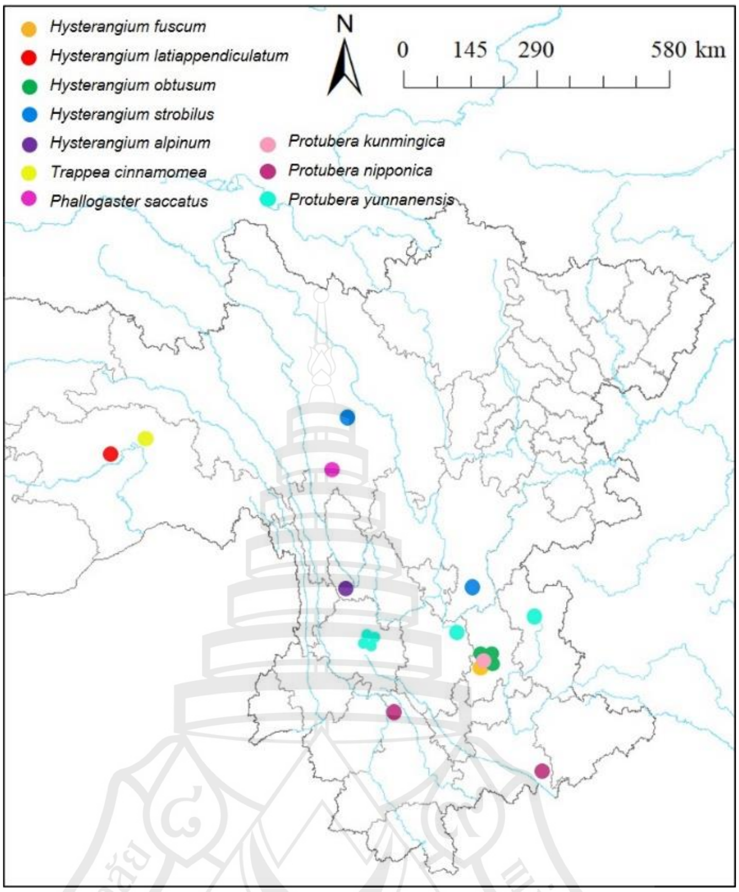


Figure 4.60 Distribution map of *Hysterangium, Protubera, Trappea,* and *Phallogaster* species in southwestern China

Russulales Kreisel ex P.M. Kirk, P.F. Cannon & J.C. David 2001

Albatrellaceae Nuss 1980

Leucogaster R. Hesse 1882

The genus *Leucogaster* species is mostly distributed in the Northern Hemisphere (Zeller & Dodge, 1924; Fogel, 1975), and only one is reported from Australia (Beaton et al., 1985). Species form hypogeous basidiomata. *Leucogaster* is characterized by gleba with a compact, gelatinous mass, which is composed of round to polygonal chambers, basidiospores globose or subglobose, with reticulate ornamentation, including the perisporium, which might be more adherent to the cell wall or more loosely attached (Tofilovska et al., 2023).

According to statistics, there are currently 2 species are now known in China, they are all distributed in southwest region: *Leucogaster solidus* in Yunnan, *L. foveolatus* in Sichuan province(Li et al., 2021; Liu & Tao, 1996).

Leucogaster foveolatus (Harkn.) Zeller & C.W. Dodge 1924

Distribution & Specimen information: in soil under *Quercus* sp. mixed forest, ascocarp from autumn. Sichuan Province: Huidong, Yanba, alt. 2350m, 17 Sep. 1988, MHSU 2146 (Tao352).

Notes: No sequence data is available. No edible or medicinal use.

Leucogaster solidus L. Fan & T. Li 2021

Distribution & Specimen information: in soil under *Pinus armandii* mixed forest, ascocarp from winter. Yunnan Province: Kunming, 28 Dec. 2012, BJTC FAN733.

Notes: Holotype BJTC FAN733: ITS = MW938546. No edible or medicinal use.

Leucophleps Harkn. 1899

The genus *Leucophleps* species is mostly distributed in the Northern Hemisphere (Fogel & Trappe, 1978; Albee-Scott, 2005, 2007). Species form hypogeous basidiomata. *Leucophleps* is characterized by a peridium white to pallid yellow, after application of KOH, changing to pink through vinaceous red or lilac, confluent with mediostratum. Gleba white, locules labyrinthiform, filled with spores embedded in a subgelatinous mass, which often exudes as a white, milky latex when damaged. Columella lacking. Basidiospores are hyaline, nonamyloid, globose to broadly ellipsoid, spinose; the spines are embedded in a gelatinous matrix (Fogel, 1979; Albee-Scott, 2007).

According to statistics, there is currently one species are now known in China. *Leucophleps spinispora* was collected by Dr. Harry Smith in Sichuan Province, China, in 1922, which is the first discovery of the *Leucophleps* genus in China (Eckblad & Ellingsen, 1984).

Leucophleps spinispora Fogel 1979

Distribution & Specimen information: in soil under *Abies sp.* forest, basidiomata from autumn. Sichuan Province: alt. 3300-3400m, 27 Sep. 1922.

Notes: The specimen is stored in the Systematic biology-Department of Organismal Biology, Uppsala University, Sweden, but the specimen number is not listed in the original literature. No sequence data available from southwestern China. No edible or medicinal use (Eckblad & Ellingsen, 1984).

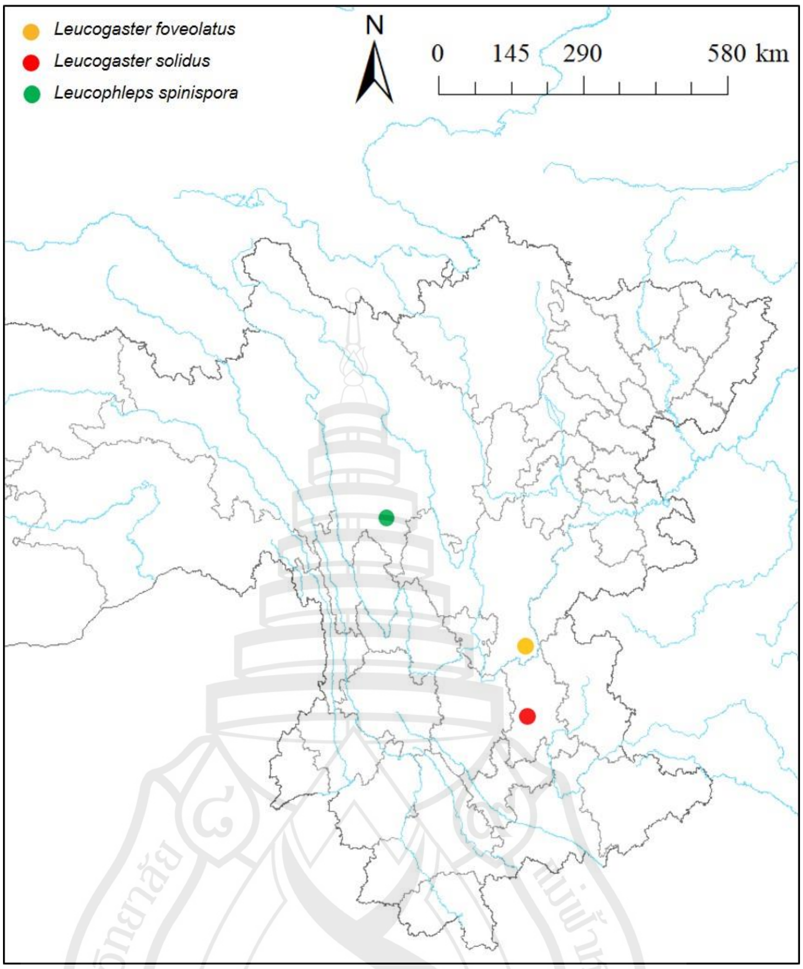


Figure 4.61 Distribution map of hypogeous Albatrellaceae (*Leucogaster, Leucophleps*) species in southwestern China

Russulaceae Lotsy 1907

The Russulaceae family is a species-rich group traditionally composed the species that form epigeous basidiomata. However, recent molecular evidence suggests that species within the Russulaceae family that form hypogeous basidiomata all originate from the epigeous genera *Russula* and *Lactarius*. Therefore, there is substantial molecular evidence supporting that *Cystangium*, *Gymnomyces*, *Macowanites*, *Martellia*, and *Elasmomyces* are synonymous with *Russula*, while *Zelleromyces* and *Arcangeliella* are synonymous with *Lactarius* (Miller et al., 2001; Eberhardt, 2002; Miller & Buyck, 2002; Larsson & Larsson, 2003; Eberhardt & Verbeken, 2004; Nuytinck et al., 2004; Binder et al., 2005; Lebel & Tonkin, 2007; Buyck et al., 2010; Verbeken et al., 2014; Elliott & Trappe, 2018).

Lactarius Pers. 1797

The species within the *Lactarius* genus that form hypogeous basidiomata, which characterized by basidiomata subglobose, somewhat lobed, sessile, gleba labyrinthoid, with locules irregularly arranged, empty or partially filled, Latex rather abundant or absent, columella absent. Basidiospores ellipsoid, hyaline, with a myxosporium of continuous or interrupted ridges forming an incomplete reticulum (Calonge, 2000; Calonge & Martín, 2000). Species form ectomycorrhizal with plants (Molina & Trappe, 1982).

According to statistics, there are currently 5 species are now known in China, among which 1 species is distributed in the southwest region: *Lactarius spinosporus* in Sichuan province.(Zhang & Yu, 1990; Tao et al., 1993; Liu, 1998; Li et al., 2018; Sang et al., 2016)

Lactarius spinosporus X.Y. Sang & L. Fan 2016

Distribution & Specimen information: under *Pinus armandii* mixed forest, basidiospores from winter. Sichuan Province: Panzhihua, 23 Nov. 2014, BJTC FAN445 (CJZ1536).

Notes: Holotype BJTC FAN445(CJZ1536): ITS = KY270490, LSU = KY270494. No edible or medicinal use.

Russula Pers. 1796

The species within the *Russula* genus that form hypogeous basidiomata are characterized by basidiomata that are subglobose to irregularly globose, with a depressed base, and either lack a stipe or possess a degenerate, short stipe. The gleba is ochraceous, labyrinthic, or loculate, with loculae being crowded and sometimes compacted. Basidiospores are globose to subglobose, with ornamentation consisting of isolated spines or spines connected at the base (Elliott & Trappe, 2018). Genera that were once recognized as separate to accommodate hypogeous species, such as *Bucholtzia*, *Cystangium*, *Elasmomyces*, *Gymnomyces*, *Macowanites*, and *Martellia*, have all been incorporated into the genus *Russula* based on molecular phylogenetic studies (Elliott & Trappe, 2018).

According to statistics, there are currently 8 species are now known in China (Zhang & Yu, 1990; Tao et al., 1993; Liu, 1998; Sang et al., 2016), among which 6 species are distributed in the southwest region: *Russula zangii* in Yunnan, *R. chlorineolens* and

R. gilkeyae in Xizang, *R. brevipileocystidiata* and *R. megapseudocystidiata* in Sichuan, *R. absphaerocellaris* in Yunnan and Sichuan province (Zhang & Yu, 1990; Sang et al., 2016; Xu et al., 2019). Two new species were discovered in Yunnan Province in this study and described as *Russula densiobtusispora* and *Russula laojunshanensis*.

Russula absphaerocellaris X.Y. Sang & L. Fan 2016

Distribution & Specimen information: under *Pinus yunnanensis* mixed forest, basidiomata from winter. Yunnan Province: Kunming, 10 Jan. 2015, BJTC FAN492 (Cao1566). Sichuan Province: Panzhihua, 23 Nov. 2014, BJTC FAN448 (CJZ1539).

Notes: Holotype BJTC FAN492 (Cao1566): ITS = KY270486, LSU = KY270493. Paratype BJTC FAN448 (CJZ1539): ITS = KY270489. No edible or medicinal use.

Russula brevipileocystidiata X.Y. Sang & L. Fan 2016

Distribution & Specimen information: under *Pinus yunnanensis* mixed forest, basidiomata from winter. Sichuan Province: Panzhihua, 23 Nov. 2014, BJTC FAN455 (CJZ1539-1).

Notes: Holotype BJTC FAN455(CJZ1539-1): ITS = KY270487, LUS = KY270492. No edible or medicinal use.

Russula chlorineolens Trappe & T.F. Elliot 2018

= Macowanites chlorinosmus A.H. Sm. & Trappe, Mycologia 55(4): 423 (1963)

Distribution & Specimen information: under mixed broadleaf-conifer forest, basidiomata from autumn to winter. Xizang Province: Nyingchi, Lulang, 13 Nov. 2016, HKAS9315 (wsp815), HKAS9316 (wsp816); Medog, 8 Nov. 2016, HKAS9300 (wsp800) (Xu et al., 2019).

Notes: HKAS9315 (wsp815): ITS = MF540758, HKAS9316 (wsp816) : ITS = MF540759; HKAS9300 (wsp800) : ITS = MF540760. No edible or medicinal use.

Russula gilkeyae (Zeller & C.W. Dodge) Trappe & T.F. Elliott 2018

=**Gymnomyces gilkeyae** (Zeller & C.W. Dodge) Trappe, T. Lebel & Castellano, Mycotaxon 81: 199 (2002)

=**Hydnangium gilkeyae** Zeller & C.W. Dodge, Ann. Mo. bot. Gdn 22: 371 (1935)

=**Martellia gilkeyae** (Zeller & C.W. Dodge) Singer & A.H. Sm., Mem. Torrey bot. Club 21(3): 32 (1961) [1960]

=**Octaviania gilkeyae** (Zeller & C.W. Dodge) Svrček, Fl. ČSR, B-1, Gasteromycetes: 192 (1958)

Distribution & Specimen information: in soil under *Pinus densata* forest, basidiomata from autumn. Xizang Province: Nyingchi, Mt. Sejila, alt. 3000m, 10 Sep. 1995, MHSU2234 (Xu AS 008) (Liu et al., 1996).

Notes: No sequence data available from southwestern China. No edible or medicinal use.

Russula megapseudocystidiata X.Y. Sang & L. Fan 2016

Distribution & Specimen information: under *Pinus yunnanensis* mixed forest, basidiomata from winter. Sichuan Province: Panzhihua, 23 Nov. 2014, BJTC FAN454 (CJZ1534-1).

Notes: Holotype BJTC FAN454 (CJZ1534-1): ITS = KY270488, LSU = KY270491. No edible or medicinal use.

Russula zangii Trappe & T.F. Elliott 2018

= Macowanites yunnanensis M. Zang [as 'Mlacowanites'], in Zang & Yuan, Acta bot. Yunn. 21(1): 37 (1999)

Distribution & Specimen information: under *Pinus armandii* Franch. forest, basidiomata from summer. Yunnan Province: Chuxiong, Mt. Zixi, alt. 2200m, 24 Jul. 1997, HKAS30518, HKAS30519 (Zang & Yuan, 1999).

Notes: Holotype HKAS30518. No sequence data available from southwestern China. No edible or medicinal use.

Russula densiobtusispora L. Li, K.D. Hyde & S.H. Li, sp. nov. Figure 4.29

Distribution & Specimen information: solitary, or in groups in the soils under the *Abies forrestii* var. *smithii* and *Rhododendron* sp. mixed forest, basidiomata from autumn. Yunnan Province: Lijiang city, Jiuhe towm, Mt. Laojunshan, alt. 3875.7 m, 19 Sep. 2021, Lin Li, BMDU L21195, alt. 3912.3 m, 16 Sep. 2021, Lin Li, BMDU L21196.

Notes: Holotype BMDU L21195: ITS=PX106474, LSU=PX105442. Other specimens BMDU L21196: ITS = PX106475, LSU = PX105443. No edible or medicinal use.

Russula laojunshanensis L. Li, K.D. Hyde & S.H. Li, sp. nov. Figure 4.30

Distribution & Specimen information: solitary in the soils under the *Abies forrestii* var. *smithii* and *Rhododendron* sp. mixed forest, basidiomata from autumn.

Yunnan Province: Lijiang city, Jiuhe town, Mt. Laojunshan, alt. 3705.2 m, 19 Sep. 2021, Lin Li, BMDU L21200.

Notes: Holotype BMDU L21200: ITS = PX106476. No edible or medicinal use.

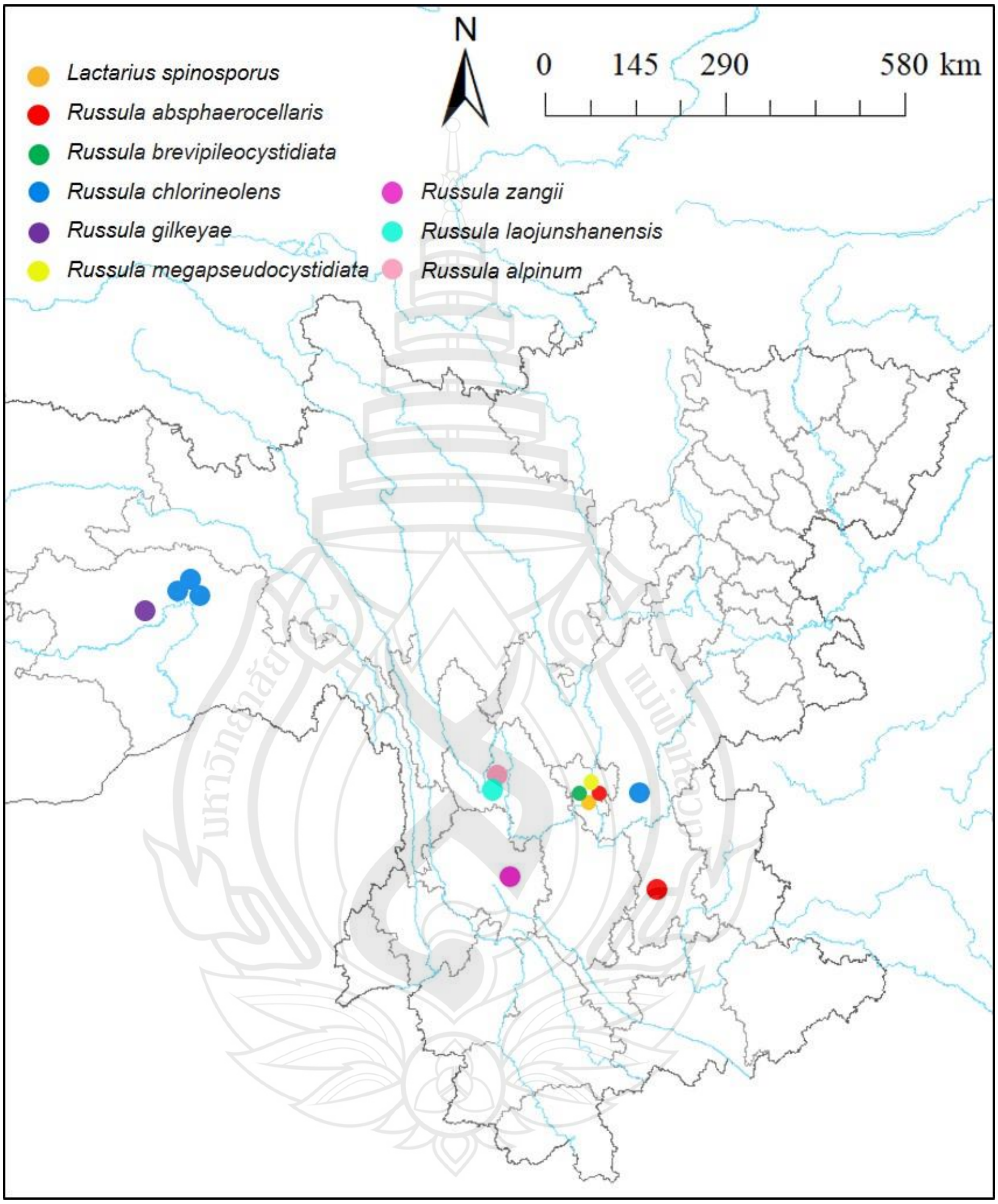


Figure 4.62 Distribution map of hypogeous *Lactarius, Russula* species in southwestern China

Mucoromycota

Endogonales Jacz. & P.A. Jacz. 1931

Endogonaceae Paol. 1889

Jimgerdemannia Trappe, Desirò, M.E. Sm., Bonito & Bidartondo 2017

Jimgerdemannia is a young genus within the family Endogonaceae, established in 2017 by Desirò et al., who separated it from the genus *Endogone* (Desirò et al., 2017). Some species within the family Endogonaceae can form ectomycorrhizal associations with various trees, and the truffle-like sporocarps they produce are considered hypogeous fungi. These hypogeous fungi are commonly referred to as “pea truffles.” (Desirò et al., 2017; Yamamoto et al., 2020; Fan, 2023). Currently, the only known pea truffles of the genus *Jimgerdemannia* reported in China was discovered in Taiwan and identified as *Jimgerdemannia flammicorona* [Basionym: *Endogone flammicorona*] (Wong et al., 2017). The new species reported in this study is the only one found in the southwestern region. However, sequence data from GenBank indicate a richer diversity of species in mycorrhizal studies.

Jimgerdemannia cangshanensis L. Li, K.D. Hyde & S.H. Li, sp. nov. Figure 4.34

Distribution & Specimen information: solitary in the soils under the *Pinus yunnanensis* and *Pinus armandii* mixed forest, basidiomata from autumn to winter. Yunnan Province: Dali city, Mt. Cangshan, alt. 2600 m, 27 Oct. 2024, Lin Li, BMDU L24100.

Notes: Holotype BMDU L24100: ITS = PX108632, LSU = PX105444, SSU = PX097640. No edible or medicinal use.

Jimgerdemannia zixishanensis L. Li, K.D. Hyde & S.H. Li, sp. nov. Figure 4.34

Distribution & Specimen information: solitary in the soils under the *Pinus yunnanensis* forest, basidiomata from winter. Yunnan Province: Chuxiong city, Mt. Zixishan, alt. 2106.2 m, 9 Nov. 2024, Yinong Li, BMDU L24101.

Notes: Holotype BMDU L24101: LSU=PX105445, SSU = PX097641. No edible or medicinal use.

Jimgerdemannia laojunshanensis L. Li, K.D. Hyde & S.H. Li, sp. nov. Figure 4.34

Distribution & Specimen information: solitary in the soils under the *Abies forrestii* var. *smithii* and *Rhododendron* sp. mixed forest, basidiomata from autumn. Yunnan Province: Lijiang city, Jiuhe town, Mt. Laojunshan, alt. 3612.4 m, 19 Sep. 2021, Yinong Li, BMDU L21214.

Notes: Holotype BMDU L21214: LSU=PX105446, SSU = PX097642. No edible or medicinal use.

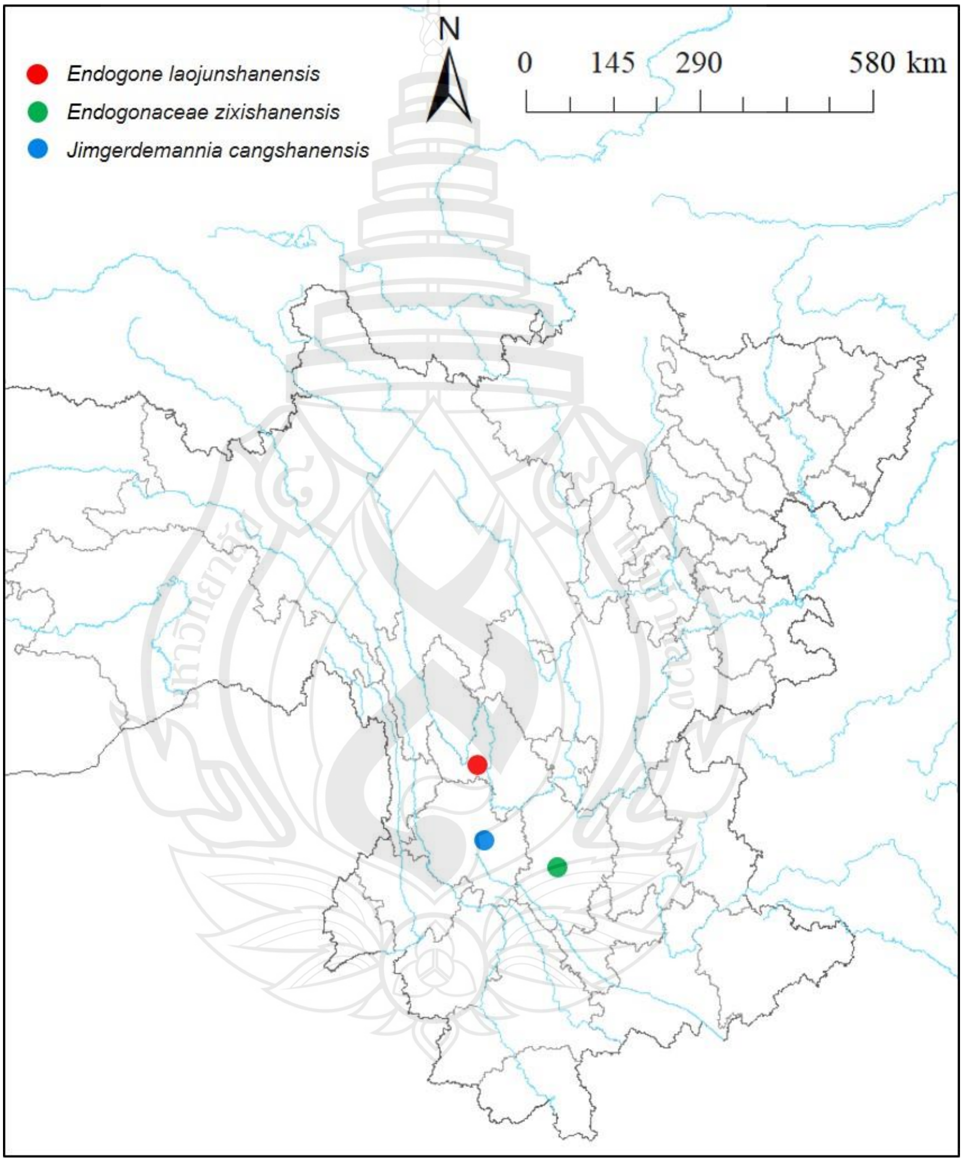


Figure 4.63 Distribution map of hypogeous Endogonaceae (*Jimgerdemannia, Endogone*) species in southwestern China

Glomeromycota

Glomerales J.B. Morton & Benny 1990

Glomeraceae Piroz. & Dalpé 1989

Glomus Tul. & C. Tul. 1844

The genus *Glomus* is common arbuscular mycorrhizal fungi (AMF) in forest soils. Only Fan reported in 2023 (Fan, 2023) that at least one species of the genus *Glomus* was found in the *Pinus armandii* Franch. forest in southwestern China, capable of forming truffle-like fruiting bodies with a diameter of up to 2 cm. These fruiting bodies had yellow-brown spores and were considered subterranean fungi. However, the aforementioned literature did not provide a specific description of the species and lacked available sequences.

4.3.2 Distribution Features

Based on the above statistics, we analyzed the specimen records of the 166 hypogeous fungal species distributed in Southwest China and obtained the following findings.

First, among the 166 hypogeous fungal species recorded in Southwest China, 133 have their type specimens collected from this region. Among them, 116 species are currently known only from Southwest China. Additionally, 56 species are represented by a single specimen record in this region. Notably, 38 of these are known solely from their type specimens, suggesting that these species have not been rediscovered or recollected since their original description. Similarly, 44 species have more than one specimen record, but all were collected from the same locality, with no evidence of broader geographic distribution. These patterns indicate that the distribution ranges of these species in Southwest China are highly restricted and that they are difficult to detect and collect. These two groups of rare species total 100, accounting for 60.2% of the hypogeous fungal species recorded in the region (Figure 4.64).

Second, 22 species are considered relatively widespread and commonly encountered—i.e., those with high occurrence frequency or abundant specimen records—accounting for 13.3% of the regional hypogeous mycobiota (Figure 4.64). These species primarily belong to the genera *Tuber*, *Rhizopogon*, and *Scleroderma*, as well as *Protubera yunnanensis* (Table 4.16).

Third, local people in Southwest China have a long-standing tradition of collecting and consuming hypogeous fungi. According to our investigation, 38 species are known to be edible, representing 22.9% of the total (Figure 4.64). Among them, 17 species are well-liked and heavily traded in local wild mushroom markets, including 12 species of *Tuber*, 4 species of *Rhizopogon*, and *Scleroderma yunnanensis* (Figure 4.64, Table 4.16). Interestingly, four additional edible species—*Pachyphlodes atropurpurea*, *Pachyphlodes excavata*, *Choiromyces cerebriformis*, and *Choiromyces sichuanensis*—are not intentionally collected but are occasionally found as admixtures in truffle markets.

Fourth, market investigations revealed an intriguing pattern: while *Tuber* species are widely traded beyond the local area, most other edible hypogeous fungi are only popular among local communities, with trade occurring almost exclusively within the region. These practices are often shaped by ethnic and regional traditions. For example, although *Rhizopogon jiyaozi* and *Rhizopogon sinoalbidus* have relatively wide distributions in Southwest China, they are mainly consumed and traded in specific areas such as Chuxiong Yi Autonomous Prefecture, Yulong Naxi Autonomous County (Lijiang), Jianchuan County (Dali Bai Autonomous Prefecture), and Weixi Lisu Autonomous County. *Rhizopogon songmaodan* is widely consumed and sold only in the Liangshan Yi Autonomous Prefecture, whereas *Rhizopogon weixiensis* is commonly utilized only in Weixi County. Another case is *Melanogaster panzhihuaensis*, which is collected and sun-dried by a small number of local residents in Chuxiong Prefecture and used as a herbal tea believed to relieve coughing and clear the lungs, though it is not available in local markets.

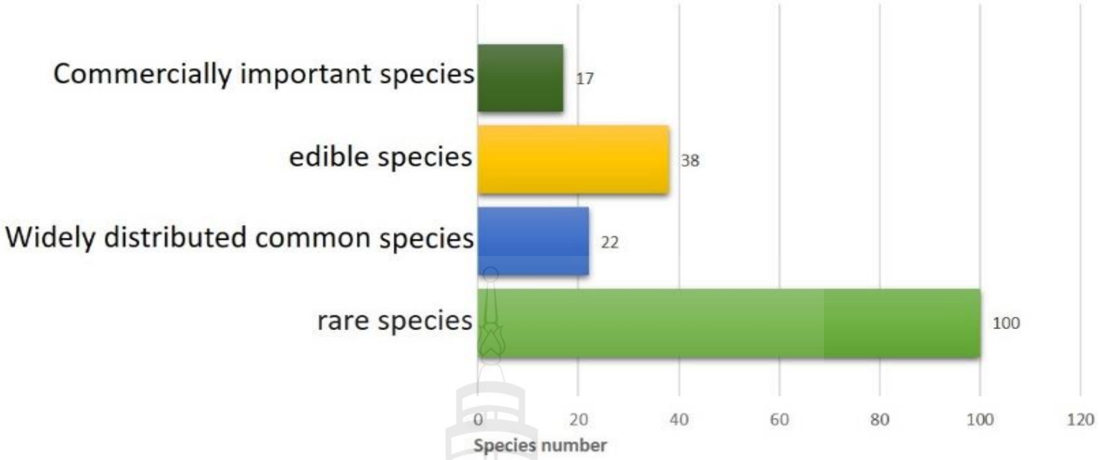


Figure 4.64 Species number of hypogeous fungi in southwestern China

Table 4.16 List of widely distributed common hypogeous fungi in southwest China (22 species)

Species	Distribution	Habitat	Edibility & Trade
Protubera yunnanensis	Dali, Chuxiong, Qujing in Yunnan	Saprophytic	No
Rhizopogon jiyaozi	Nyingchi in Xizang, Central and NW Yunnan	Ectomycorrhizal with Pinus	Yes
Rhizopogon sinoalbidus	Nyingchi in Xizang, NW Yunnan	Ectomycorrhizal with Pinus	Yes
Rhizopogon songmaodan	Huili in Sichuan, Central Yunnan	Ectomycorrhizal with Pinus	Yes
Rhizopogon weixiensis	NW Yunnan	Ectomycorrhizal with Pinus	Yes
Scleroderma erubescens	NW, West, and Central Yunnan	Associated with *Castanea* spp. and *Pinus yunnanensis*	No
Scleroderma separatum	NW and Central Yunnan	Associated with *Carya illinoinensis* and *Pinus yunnanensis*	No
Scleroderma squamulosum	Most regions in Yunnan	Associated with Fagaceae and *Pinus* spp.	No
Scleroderma vinaceum	NW and Central Yunnan	Associated with *Castanea* spp.	No
Scleroderma yunnanense	Most regions in Yunnan	Associated with *Pinus* spp.	Yes
Tuber calosporum	Central and NE Yunnan	Mixed forests	Yes
Tuber huidongense	SW Sichuan, Central Yunnan	Mixed forests	Yes

Table 4.16 (continued)

Species	**Distribution**	**Habitat**	**Edibility & Trade**
Tuber huizeanum	Central and NE Yunnan	*Pinus* forests	Yes
Tuber latisporum	Central Yunnan	Mixed forests	Yes
Tuber lijiangense	NW and Central Yunnan	*Pinus* forests	Yes
Tuber liyuanum	Jinsha River Basin in Sichuan and Yunnan	*Pinus* forests	Yes
Tuber neoexcavatum	West and Central Yunnan	*Pinus* forests	Yes
Tuber panzhihuanense	Jinsha River Basin in Sichuan and Central Yunnan	*Pinus* forests	Yes
Tuber pseudohimalayense	SW Sichuan, Central and NW Yunnan	*Pinus* forests	Yes
Tuber pseudomagnatum	Jinsha River Basin in Central and NE Yunnan	*Pinus* forests	Yes
Tuber sinense	SW Sichuan, Central Yunnan	Mixed forests	Yes
Tuber sinoaestivum	SW Sichuan, Northern Yunnan	*Pinus* forests	Yes

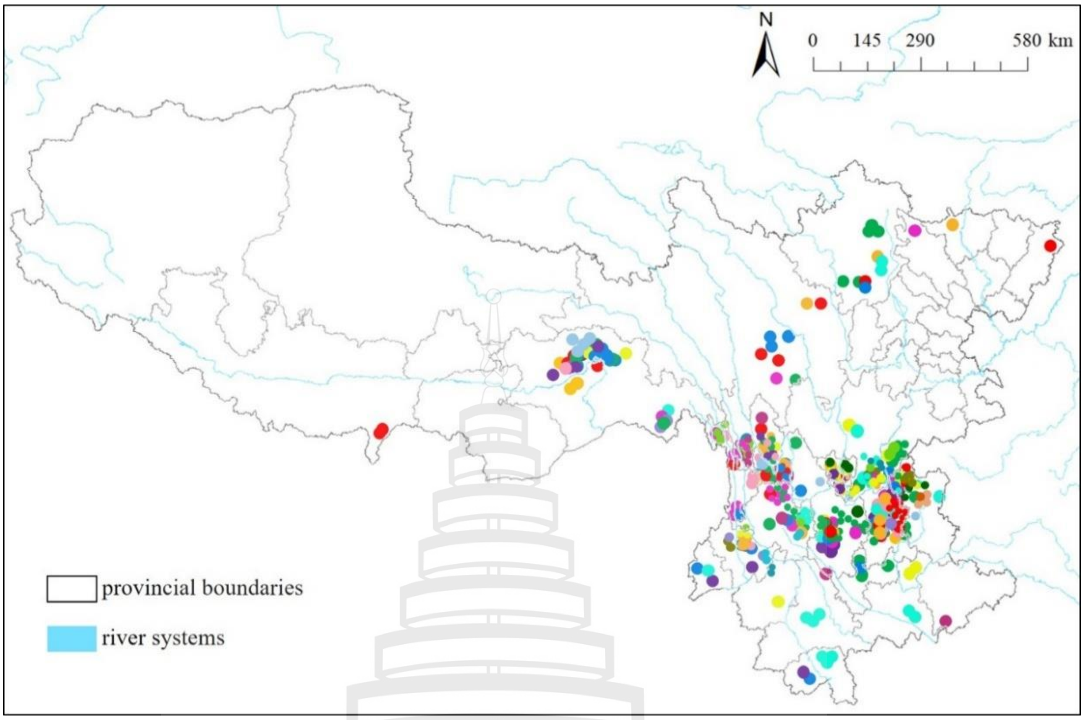
Using Geographic Information System (GIS), we integrated watershed and elevation data with distribution records of hypogeous fungi in southwestern China to analyze their distribution patterns and characteristics (Figures 4.65, 4.68). The results are as follows:

1. Distribution across river basins: A total of 166 species of hypogeous fungi have been recorded in southwestern China. After excluding specimens lacking locality information, 447 specimens representing 161 species were included in the analysis. The *UpSet plot* analysis revealed patterns of species distribution across different river basins (Figure 4.66). The Jinsha River basin harbors the highest diversity, with 110 species, followed by the Nujiang River basin with 26 species, the middle and lower reaches of the Yarlung Zangbo River with 21 species, and the middle reaches of the Lancang River with 11 species. All other basins host fewer than six species.

Regarding endemism at the basin level (Figure 4.66), 92 species were found exclusively in the Jinsha River basin, 13 species were restricted to the Nujiang River basin, and 14 species occurred only in the middle and lower Yarlung Zangbo River basin. Other basins each contained fewer than six unique species. Overall, 84.5% of the species were confined to a single basin, indicating a high degree of local endemism.

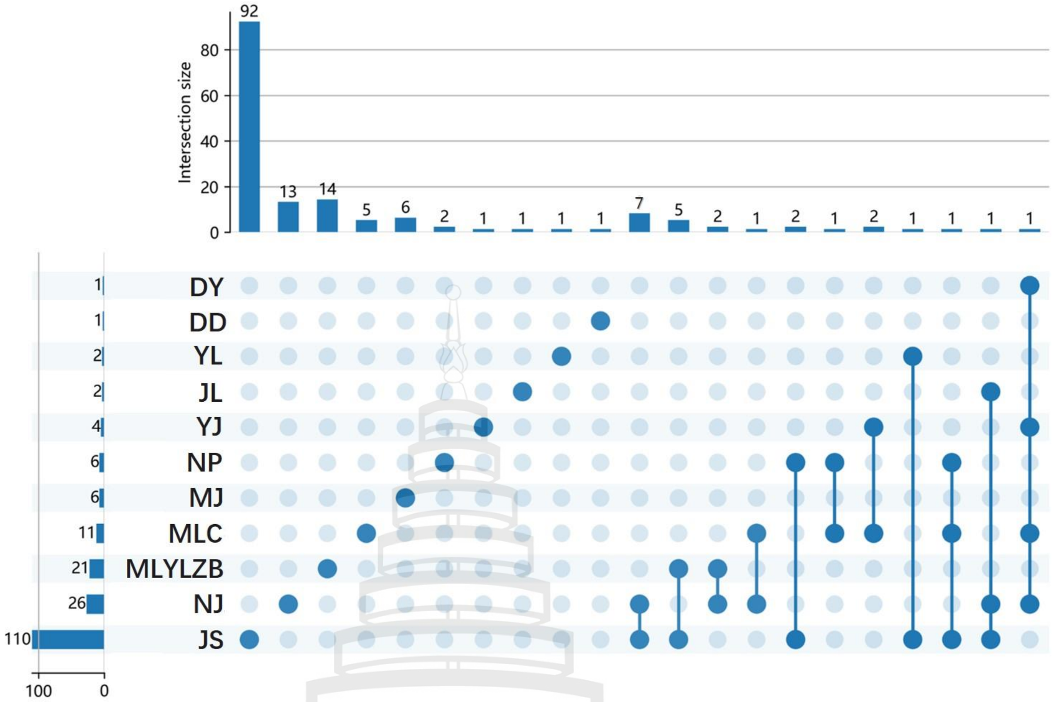
Additionally, 21 species were distributed across two basins (Figure 4.67). Among them, 7 species occurred in both the Jinsha and Nujiang River basins, and 5 species were shared between the Jinsha and Yarlung Zangbo basins. Only two species were distributed across three basins: *Protubera yunnanensis*, found in the Jinsha, middle Lancang, and Nanpan River basins within Yunnan Province; and *Tuber jinshajiangense*, found in the Jinsha, Nujiang, and Jialing River basins across Yunnan and Sichuan. One species, *Scleroderma squamulosum*, was distributed across four basins—Lancang (middle reach), Nujiang, Daying River, and Yuanjiang. Notably, all three species with multi-basin distributions are among the most commonly encountered hypogeous fungi in southwestern China. Apart from the cases mentioned above, among the 24 species with cross-basin distributions (Figure 4.67A), 12 are represented by only sparse specimen records—eight with fewer than three collections and four with fewer than six. Additionally, only six species are commonly found in southwestern China and are known for relatively high yields (Table 4.16).

This watershed-based analysis highlights several key patterns: the Jinsha River basin not only supports the richest diversity of hypogeous fungi (110 species) but also contains the largest number of endemic species (92 species). Moreover, the inter-basin distribution patterns suggest that hypogeous fungi in southwestern China exhibit strong habitat specificity and clear watershed restriction. It is uncommon for the same species to occur across multiple basins, resulting in fragmented and geographically isolated distribution patterns.



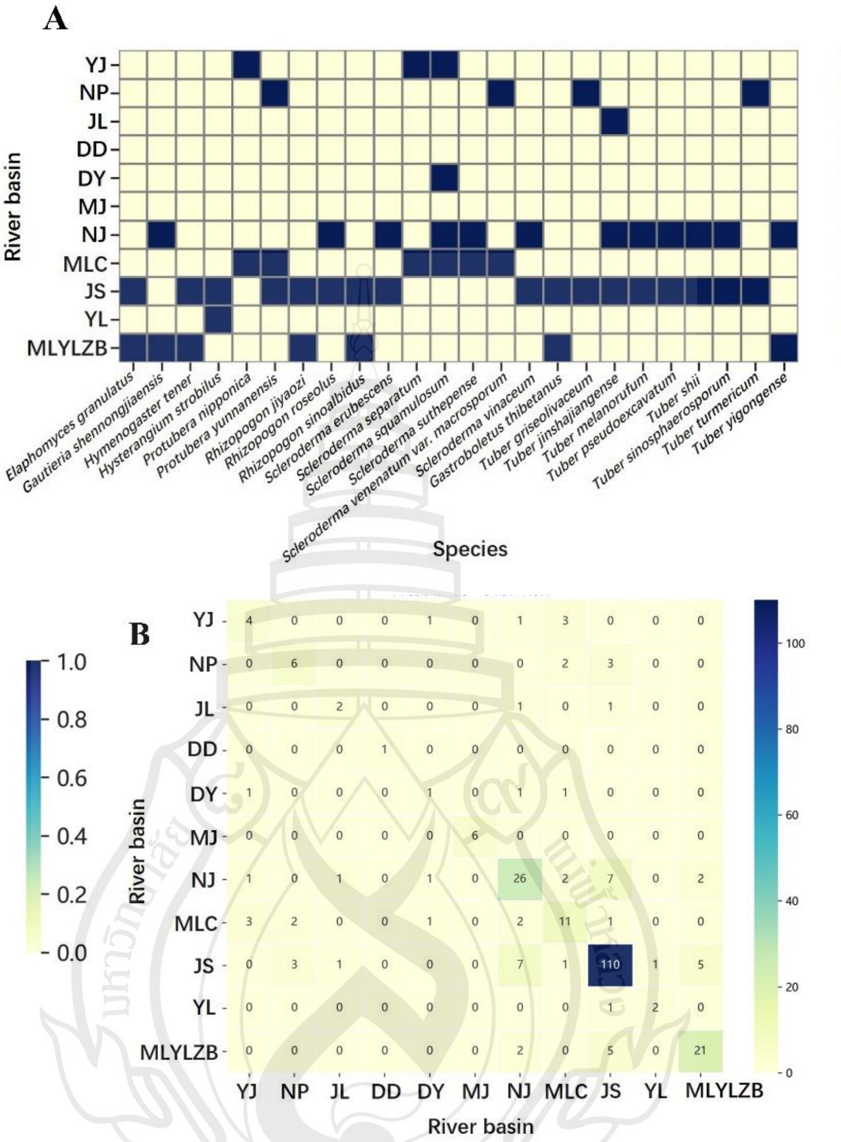
Note Each dot on the map represents a specimen collection site. The size and color of the dots carry no specific meaning and have been adjusted solely to enhance the clarity of the spatial layout.

Figure 4.65 Specimen collection sites across River Basins in southwestern China



Note River names are abbreviated in the plot as follows: DD – Dadu River; DY – Dayingjiang River (upper Irrawaddy River); JL – Jialing River; JS – Jinsha River; MJ – Min River; MLC – Middle Lancang River; MLYLZB – Middle and lower Yarlung Zangbo River; NJ – Nujiang River; NP – Nanpanjiang River (upper Pearl River); YJ – Yuanjiang River (upper Red River); YL – Yalong River.

Figure 4.66 Intersection structure of hypogeous fungal species across river basins in southwestern China (UpSet plot)



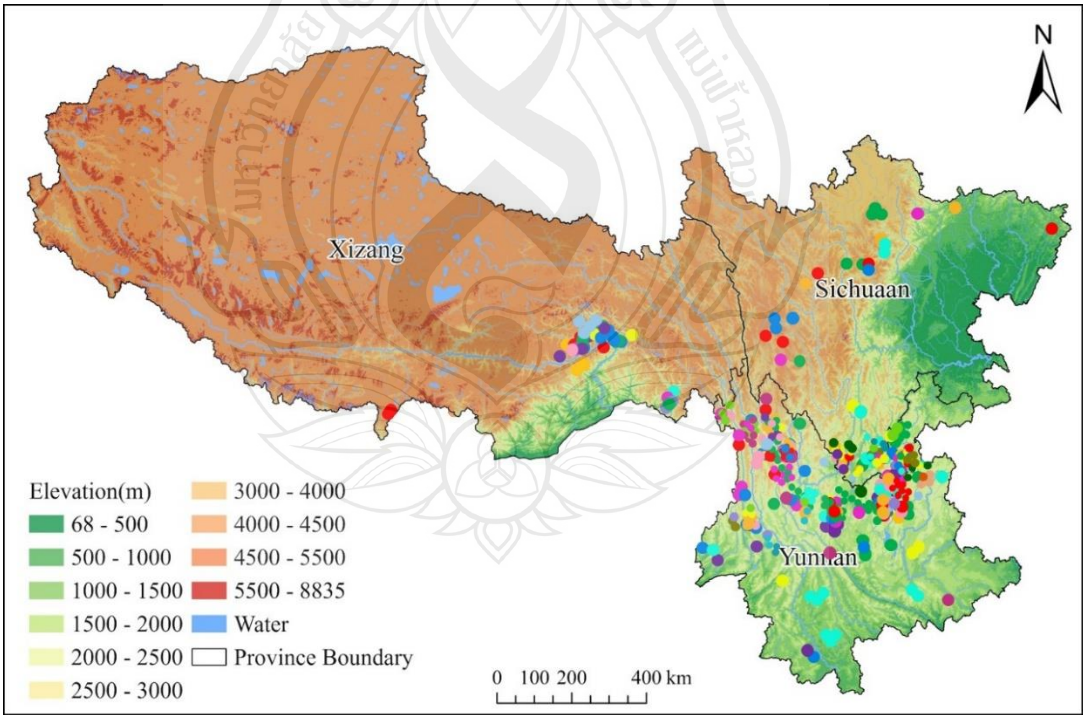
Note A. Distribution patterns of species occurring across multiple basins. B. Number of species shared between each pair of river basins.

Figure 4.67 Heatmap showing the distribution of hypogeous fungal species across river basins in southwestern China

2. Altitudinal distribution (Figure 4.68). The lowest recorded elevations for hypogeous fungi in southwestern China are found in southern Yunnan: *Turmalinea chrysocarpa* (HKAS70601) was collected at alt. 860 m in Manhao Town, Gejiu City, and *Scleroderma suthepense* (NIOHP JH2016-0727-052) at alt. 910 m in Jinghong City, Xishuangbanna. The highest records include *Choiromyces cerebriformis* (HKAS107566)

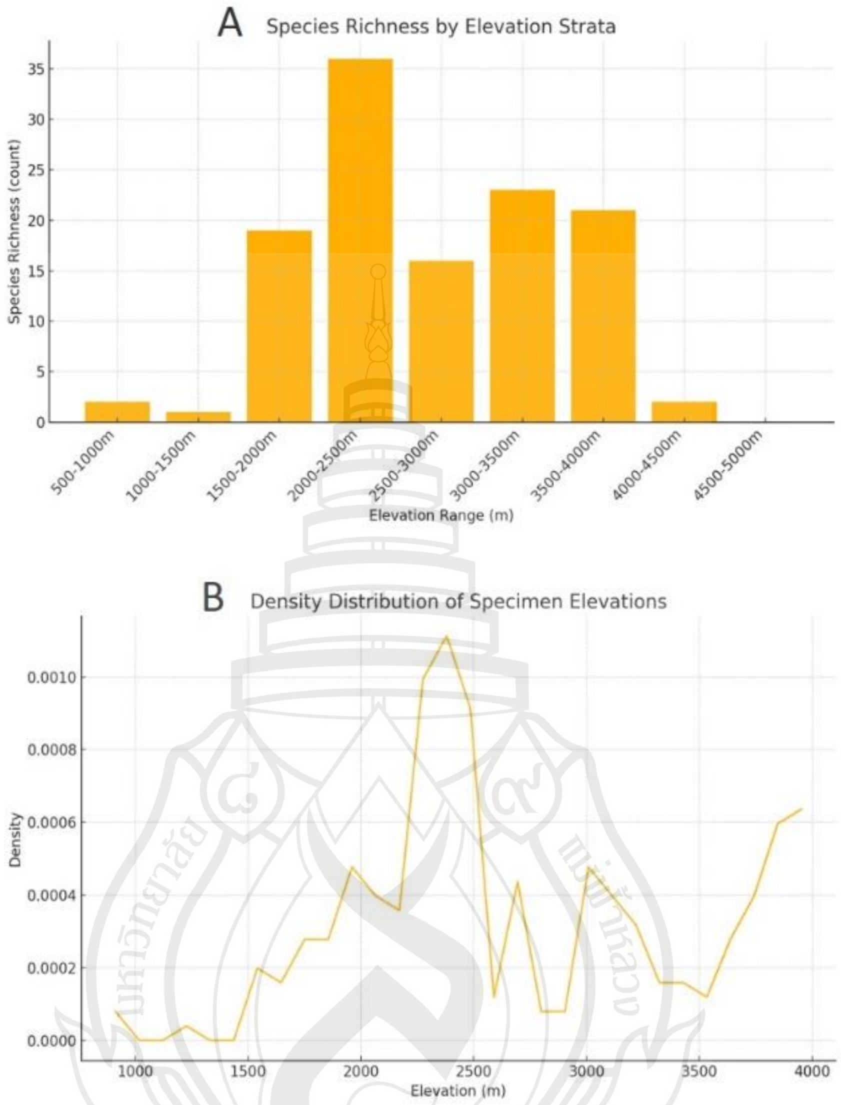
at alt. 4005 m in Shangri-La, and *Hydnotrya cubispora* (HXZE1227) at alt. 4000 m in the Sejila Mountain area of Nyingchi.

In this study, based on specimen elevation data (after excluding 36 species lacking altitude records), we performed three complementary analyses: elevational-stratum species richness, kernel density estimation of specimen elevations, and the Shannon diversity–elevation relationship. The species-richness analysis revealed a maximum of 36 species in the 2000–2500 m band, high richness across mid-high elevations (3000–4000 m), and comparatively low richness at both the lowest (500–1500 m) and highest (> 4000 m) bands (Figure 4.69A). In total, approximately 96.1 % of hypogeous fungal species were found between 1500 m and 4000 m. The elevation density curve peaked at ~2400 m, corroborating that both species richness and specimen abundance are concentrated in this band (Figure 4.69B). Finally, the diversity–elevation curve showed a "hump-shaped" pattern in Shannon index, reaching its maximum of ~3.44 at ~2500 m, with the 95 % confidence interval confirming the significance of this mid-elevation diversity peak (Figure 4.70)



Note Each dot on the map represents a specimen collection site. The size and color of the dots carry no specific meaning and have been adjusted solely to enhance the clarity of the spatial layout.

Figure 4.68 Specimen collection sites about elevation in southwestern China



Note A. Species richness by elevation strata; B. Density distribution of specimen elevations.

Figure 4.69 Altitudinal distribution of species in southwestern China

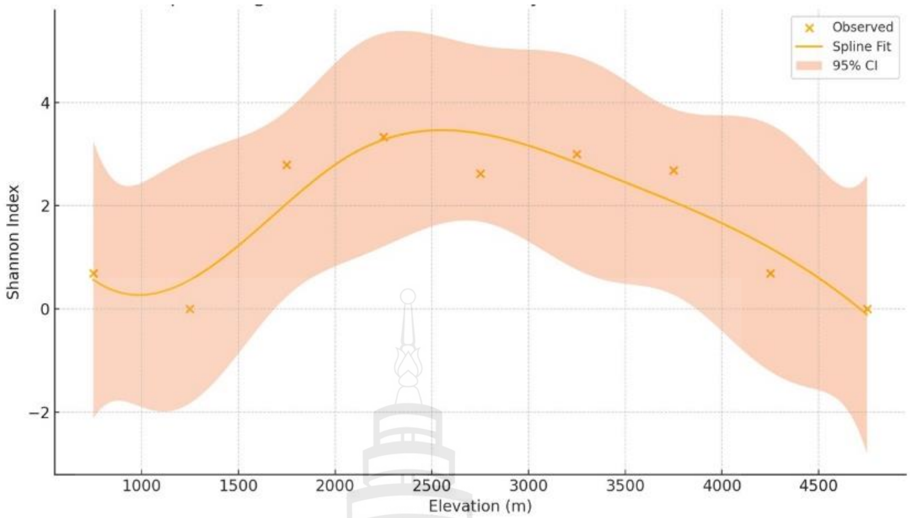


Figure 4.70 Spline regression: Shannon diversity vs elevation with 95% CI

In addition, we extracted habitat information for hypogeous fungal species distributed in southwestern China, focusing on their associated symbiotic plant data and vegetation types. Based on the statistical analysis of all hypogeous fungal specimens collected from the region, the most frequently associated host plants belong to the genus *Pinus*, which accounts for 46.19% of all records. This is followed by *Quercus* (19.80%), *Abies* (14.72%), and *Picea* (5.08%). Overall, the results indicate that the dominant symbiotic partners of hypogeous fungi in southwestern China are plants in the family Pinaceae (71.43%), followed by Fagaceae (23.98%) (Figure 4.71). Together, these two families constitute the overwhelmingly dominant hosts for hypogeous fungi in the region. Other plant families, including Salicaceae, Myrtaceae, and Fabaceae, collectively represent less than 5% of the total (Figure 4.71).

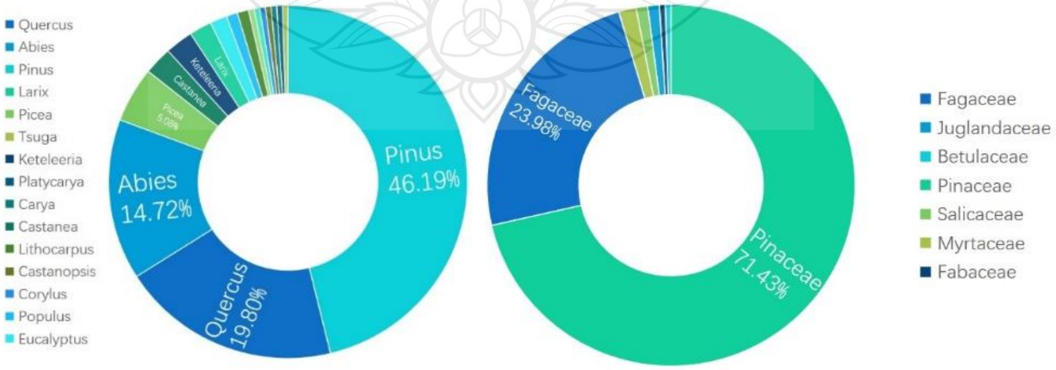
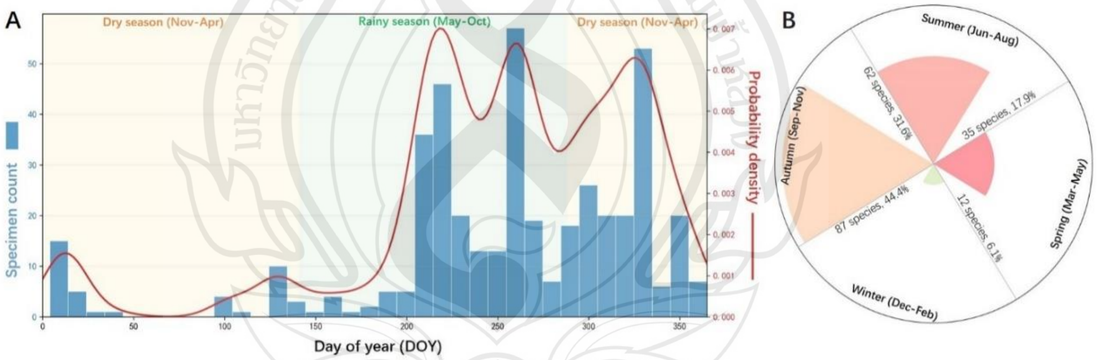


Figure 4.71 Distribution of symbiotic plant species associated with hypogeous fungi in southwestern China, based on specimen records and habitat data

In this study, we analyzed the collection dates of specimens from the 166 hypogeous fungal species summarized previously. Since hypogeous fungal specimens represent mature sporocarps, the collection date serves as a reliable proxy for the timing of fruiting body maturation.

Statistical analysis revealed a multimodal distribution of collection dates (Figure 4.72A). The primary three peaks occurred in Day of Year (DOY) 210–330 (62.3% of specimens), while a secondary peak appeared in DOY 350–365/DOY 1–30 (21.7%). The major fruiting peak (August–October) coincides with the mid-to-late rainy season, whereas the spring peak (DOY 100–160) likely reflects soil moisture from pre-monsoon rainfall (Figure 4.72 A).

The figure also shows a noticeable increase in specimen counts increase significantly from DOY 350 to the following DOY 30, corresponding to intensive harvesting of *Tuber* species post-winter solstice through early spring. Seasonal distribution shown in the circular diagram indicates (Figure 4.72 B) that significantly more species fruit in summer/autumn (DOY 210–330; P<0.001), driven by monsoonal humidity in summer and residual moisture in autumn.



Note The left y-axis shows specimen counts (bars), The right y-axis indicates probability density (KDE curve). Shaded areas denote climatic seasons. B. Number of species with sporocarp collections per season

Figure 4.72 Temporal distribution of hypogeous fungal sporocarp collections in southwestern China

4.3.3 Evolutionary Features

Based on a phylogenetic tree of Boletales constructed using a five-gene dataset, this study estimated the divergence times of hypogeous Boletales species distributed in Southwest China (Figure 4.74). Statistical analyses show that a total of 38 hypogeous Boletales species are currently recorded in this region, among which 26 species (68.4%) are endemic to Southwest China, exhibiting narrow distribution ranges and strong associations with the region's complex geography and climate. Therefore, 28 species with valid molecular data were included in the divergence time estimation analysis, leading to the following conclusions:

1. The evolution of hypogeous fruiting bodies within Boletales likely originated between the late Eocene (approximately 50 Mya) and the early Miocene (ca. 22 Mya) (Figure 4.74). Previous studies (Wu et al., 2022, 2023) also indicated that gasteromycetation in Boletales may have first emerged in the Late Cretaceous (around 74 Mya), but most divergence events associated with gasteromycetation were concentrated in the arid period spanning from the Oligocene (approximately 40 Mya) to the early Miocene (approximately 23 Mya).

2. By incorporating molecular data from hypogeous Boletales species distributed in Southwest China, this study reveals that the earliest divergence within these regional taxa began in the Eocene (e.g., *Truncocolumella pseudocolumella*, 50.09 Mya), with the majority of diversification events occurring between the Oligocene (ca. 34 Mya) and the early Pliocene (approximately 5–3 Mya) (Figure 4.73).

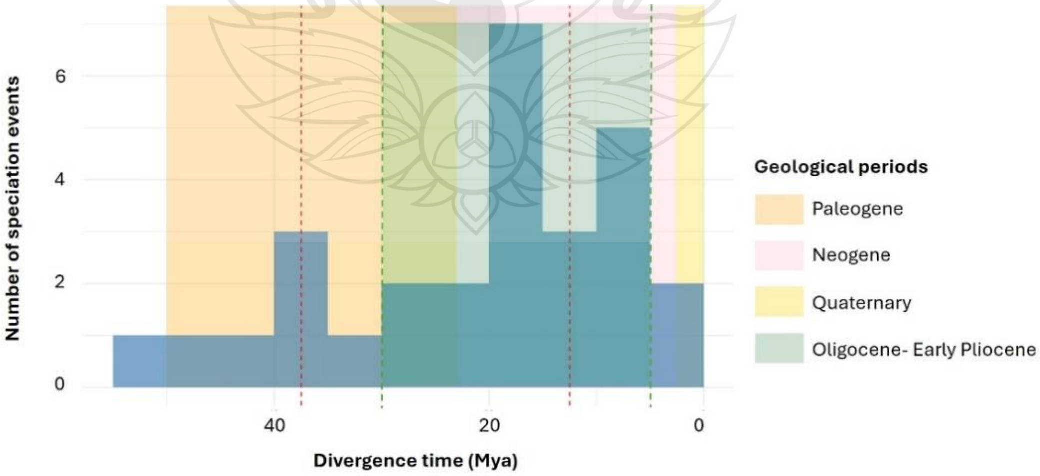


Figure 4.73 Divergence time distribution of hypogeous Boletales species distributed in southwest China

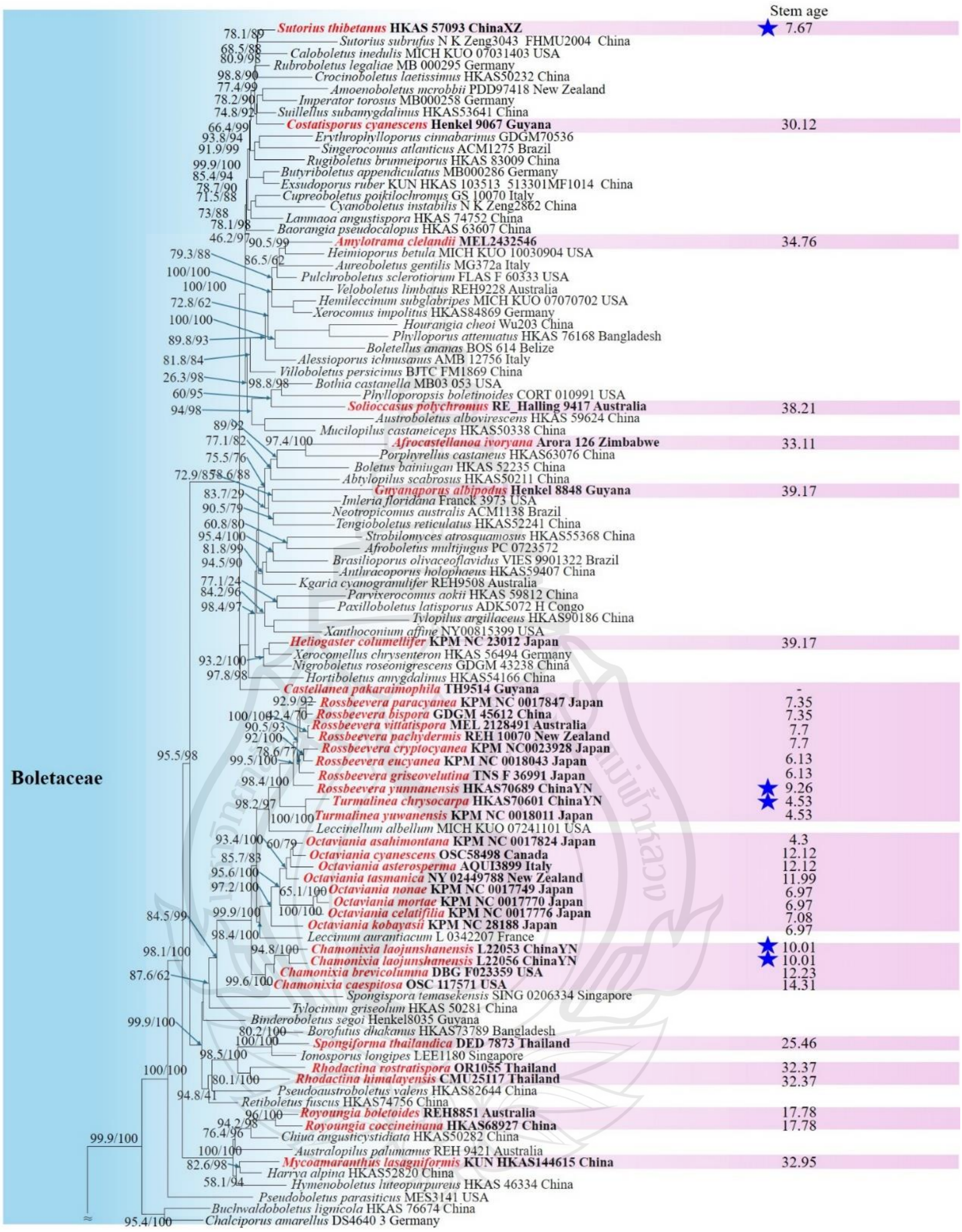


Figure 4.74 Five-gene phylogenetic tree of Boletales. The stem ages of hypogeous species are indicated on the right. Blue pentagrams denote hypogeous species from southwest China

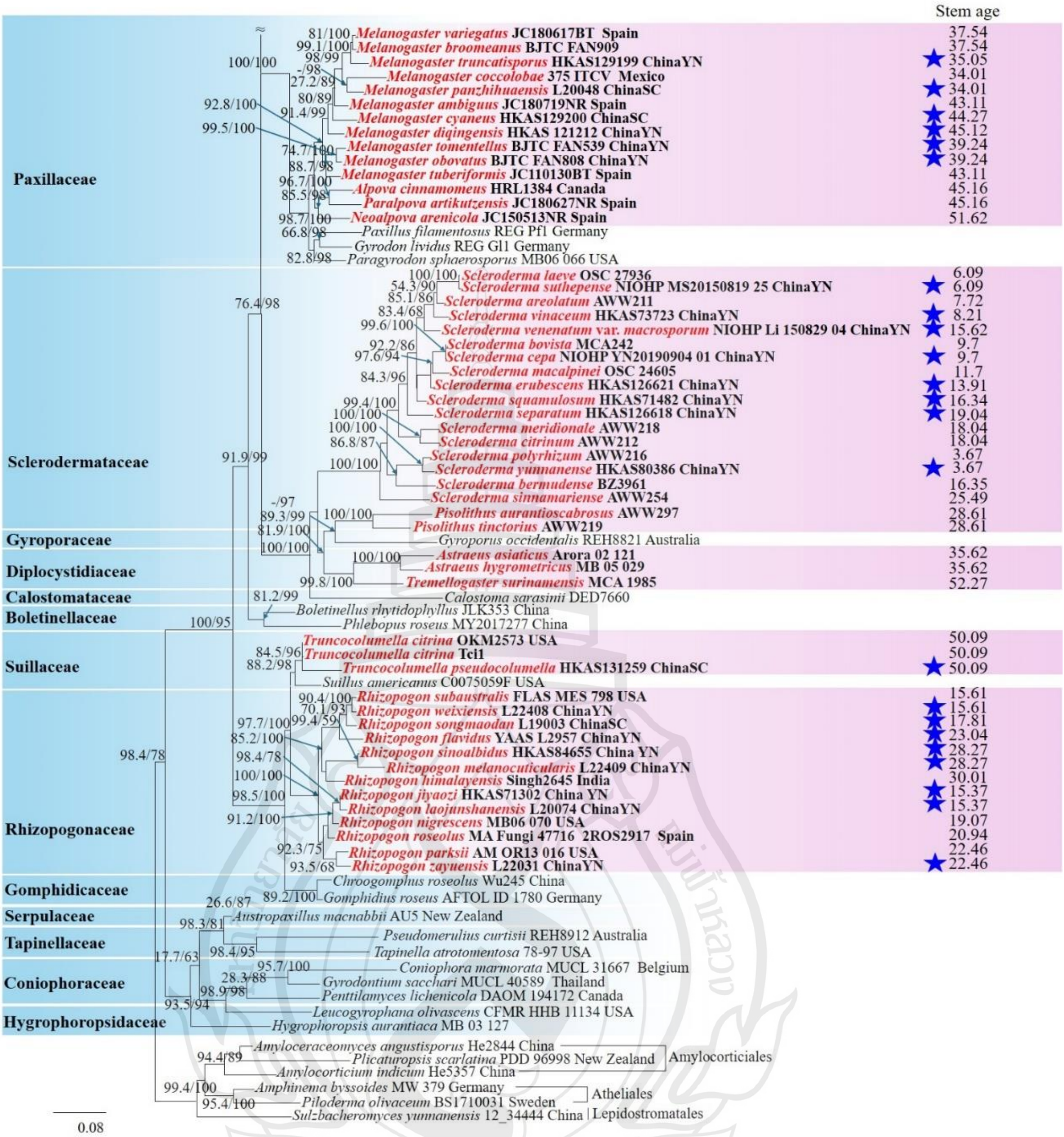


Figure 4.74 (continued)

CHAPTER 5

DISCUSSION AND CONCLUSION

5.1 Discussion

According to current statistics, a total of approximately 288 species of hypogeous fungi belonging to 51 genera are distributed across China (Fan, 2023). Based on the results of this study, 166 species in 29 genera occur in Southwest China, accounting for 57.6% of all species and 56.9% of all genera, indicating a significant advantage in regional diversity. The southwestern region of China is characterized by alternating high mountains and deep river valleys, forming a highly folded and rugged topography. For hypogeous fungi that form symbiotic relationships with specific plants and primarily rely on small animals for spore dispersal, such terrain undoubtedly increases barriers to gene flow, resulting in narrow ecological niches and promoting species differentiation (Feng & Yang, 2018). This study also supports that theory: hypogeous fungi in southwestern China not only exhibit high species diversity but also a high degree of endemism. Among the species recorded in this region, endemic species account for a substantial proportion (116 species, representing 69.9%), and rare species are also highly represented (100 species, accounting for 60.2%). Furthermore, our analysis of species distributions across river basins reveals that these fungi tend to exhibit strong habitat specificity and basin-level restriction. It is uncommon for the same species to occur across multiple basins, leading to a fragmented and geographically isolated distribution pattern. Based on current specimen records, these species typically occupy narrow habitat ranges and produce fruiting bodies in low abundance. However, their apparent rarity may be overestimated, as most have unknown edibility, are difficult to detect or collect, and have not received significant research attention. As a result, their actual distribution patterns may be underestimated.

Among the 22 relatively widespread and common hypogeous fungal species found in southwestern China, 17 are wild edible fungi that are popular and frequently encountered in local markets (Table 4.16). Due to their culinary and economic value, these species are more likely to be actively collected, increasing their chances of being discovered and recorded. In addition, human harvesting and transportation activities may have contributed, to some extent, to the expansion of their populations.

The remaining common species include four belonging to the genus *Scleroderma* and one, *Protubera yunnanensis*, all of which tend to fruit above ground, with mature sporocarps rarely buried in the soil. *Scleroderma* species are known for their low host specificity and broad host range, forming ectomycorrhizal associations with a variety of tree families, including Pinaceae, Fagaceae, and Juglandaceae (Wu et al.,, 2023). In contrast, *Protubera yunnanensis* is saprotrophic. These characteristics likely facilitate their population expansion.

According to the statistics of this study, among the 166 species of hypogeous fungi distributed in southwestern China, 39 species lack any available molecular data. Without exception, these species were collected in the early 1990s, when species identification and phylogenetic studies were limited to morphological characteristics due to restricted research conditions. Unfortunately, obtaining DNA sequences from these early specimens is challenging, and recollecting specimens from the original localities may be a feasible approach to further clarify their distribution and taxonomy.

The diversity of hypogeous fungi in southwestern China has likely been underestimated. On the one hand, most hypogeous fungi produce sporocarps underground, making them easily overlooked in conventional macrofungal surveys. On the other hand, the number of researchers dedicated specifically to hypogeous fungi remains very limited. Based on these situations, we have reason to infer that the species diversity of hypogeous fungi in Southwest China is likely much higher than the number documented in this study. Of course, a notable exception in the taxonomic research on hypogeous fungi is the genus *Tuber*, which has received considerable taxonomic attention due to its high economic value. To date, 79 *Tuber* species have been reported from China, of which 64 species occur in southwestern China (Fu & Fan, 2023; Li et al., 2024a; Yuan et al., 2025), making it one of the most intensively studied groups of hypogeous fungi in the region.

Hypogeous fungi are a unique group of macroscopic fungi that evolved as an adaptive response to arid climatic conditions. As a distinctive ecological type among macrofungi, hypogeous fungi exhibit specific distributional patterns and symbiotic associations. In southwestern China, centered in the Hengduan Mountains, these fungi—characterized by their sequestrate (truffle-like) fruiting bodies—display remarkable regularity in both geographic distribution and host plant associations.

Our study reveals that hypogeous fungi in this region are primarily distributed along major river basins, with notable concentrations in alpine and subalpine environments along a west-to-east transect encompassing the middle and lower reaches of the Yarlung Zangbo River, the Nujiang River, the middle Lancang River, the Jinsha River, and the Yalong River. These fungi are predominantly found at elevations between 1500 and 4000 meters, and 2000–4000 meters supports the highest species diversity. The altitudinal zonation of ecosystems in the region reflects the climatic characteristics of local dry-hot conditions. According to a study by Yao et al. (2010), the area between the Lancang and Yalong rivers in the Hengduan region is classified as a typical dry-hot zone, characterized by high thermal input but limited moisture availability. However, the classic dry-hot climate—defined by arid and low-rainfall conditions—is mainly confined to the low-elevation river valleys. As elevation increases, humidity also rises, and the treeline and upper limits of dark coniferous forests are distributed at higher altitudes due to improved hydrothermal conditions.

Correspondingly, the elevation distribution of hypogeous fungi in southwestern China aligns with that of their dominant symbiotic plants (*Pinaceae* and *Fagaceae*), primarily within the 1500–4000 m range. In certain mountain areas, the upper limits of dark coniferous forests extend to approximately 4000 m, resulting in a notable concentration of hypogeous fungi in high-elevation fir forests. A total of 40 hypogeous fungal species were recorded in the alpine *Abies* forests between 3000 and 4000 m elevation, including: Ascomycota: 16 species from 5 genera (*Choiromyces*, *Elaphomyces*, *Hydnobolites*, *Hydnotrya*, *Tuber*), Basidiomycota: 23 species from 12 genera (*Chamonixia*, *Gautieria*, *Gastroboletus*, *Hymenogaster*, *Hysterangium*, *Leucophleps*, *Octaviania*, *Phallogaster*, *Rhizopogon*, *Russula*, *Trappea*, *Truncocolumella*), Mucoromycota: 1 species from 1 genus (*Endogone laojunshanensis*). Such a concentrated high-elevation distribution of hypogeous fungi is rare and is clearly a result of the unique geographic and climatic conditions found in southwestern China.

The temporal distribution of specimen collection reflects the seasonal pattern of sporocarp development (i.e., maturation) in hypogeous fungi. Among specimens of hypogeous fungal species collected from southwestern China, 89% of the collections occurred between Day of Year (DOY) 150–350 (Fig. 4.72A), aligning with the region's main rainy season. This period (May–October) contributes approximately 80% of the annual precipitation in the region (Dong et al., 2018; Xu et al., 2018). Since the differentiation and growth of hypogeous sporocarps are highly dependent on soil moisture, the strong correspondence between the fruiting season of hypogeous fungi and the rainfall period in southwestern China is not surprising. Consequently, most rare and edible hypogeous fungi, such as species of *Tuber*, tend to mature during the early dry season, as the entire development process relies on sufficient precipitation. Determining appropriate harvesting periods is therefore essential for the sustainable conservation of wild hypogeous fungal resources.

While soil moisture plays a dominant role, the influence of temperature on sporocarp development should not be overlooked. However, habitat temperature data are largely absent from specimen records. Given the pronounced vertical climatic gradients across southwestern China, future long-term ecological monitoring may allow for integrated analyses of temperature and other environmental variables associated with hypogeous fungal phenology.

Based on a five-gene concatenated phylogenetic tree of Boletales, this study estimated the divergence times of hypogeous Boletales species restricted to southwestern China. The results indicate that the diversification of these fungi began in the Eocene, with *Truncocolumella pseudocolumella* diverging around 50.09 Mya, while most species diversification occurred from the Oligocene (ca. 34 Mya) to the Early Pliocene (ca. 5–3 Mya).

These temporal patterns broadly coincide with the geological history of the region. The uplift of southwestern China, centered around the Hengduan Mountains, began in the Middle Eocene (ca. 45–40 Mya) (Zhao et al., 2025). This was followed by intensified monsoonal precipitation, tectonic uplift, and river incision during the Oligocene to Miocene (ca. 34–23 Mya) (Ding et al., 2022). A major uplift phase of the Hengduan Mountains occurred from the Early to Middle Miocene (ca. 23–11.6 Mya), accompanied by increased aridification in Central Asia (Wang et al., 2018; Zhao et al., 2023). The Late Miocene to Early Pliocene (ca. 11.6–3 Mya) marked the peak of orogenic activity, leading to the formation of a rugged landscape characterized by deep valleys and high mountain ridges (Xiong et al., 2020). These tectonic and environmental changes, starting from the Middle Eocene, directly contributed to the establishment of biodiversity hotspots in the Hengduan Mountains. The increased habitat heterogeneity likely drove rapid diversification and adaptation of many montane taxa during these periods (Spicer et al., 2020; Ding et al., 2022).

Hypogeous fungi form ectomycorrhizal relationships with plants and are evolutionarily closely tied to their hosts. In southwestern China, approximately 95% of the ectomycorrhizal host plants of hypogeous fungi belong to the families Pinaceae and Fagaceae. Studies have shown that the uplift of the Himalaya–Hengduan region led to complex topography and vertical mountain climate zones, resulting in ecological niche differentiation. These conditions promoted significant increases in species diversity and accelerated evolution within Pinaceae and Fagaceae from the Miocene to Pliocene (ca. 16–3.6 Mya), with the establishment of regional diversification centers (Sun et al., 2017; Ding et al., 2020). Accordingly, the hypogeous fungi symbiotic with these plant lineages likely underwent corresponding speciation events.

As previously noted, molecular data for hypogeous fungi in Southwest China remain insufficient. This study focuses on Boletales, a group with relatively abundant species-level data, as a preliminary attempt to investigate the evolutionary patterns of hypogeous fungi endemic to this region. Taken together, the timing of geological and climatic evolution, as well as the diversification of host plants in southwestern China, closely aligns with the period of concentrated diversification observed in the region's hypogeous fungi.

Southwestern China harbors a high proportion of rare and endemic hypogeous fungal species. The inter-basin distribution patterns further indicate that these fungi exhibit strong habitat specificity and clear basin-level restrictions, resulting in a fragmented and geographically isolated distribution. To further understand the evolutionary processes of small, geographically isolated populations, more comprehensive scientific data on hypogeous fungi are urgently needed. Although this analysis does not represent the overall evolutionary history and biogeographic patterns of hypogeous fungi in Southwest China, it suggests that species with strong host specificity and animal-mediated spore dispersal are likely to exhibit heightened sensitivity to geographic and climatic changes. Their population dynamics, resource availability, and productivity are also highly responsive to environmental and anthropogenic disturbances. Many popular edible and medicinal hypogeous fungi, although currently harvested at seemingly sustainable levels to meet consumer demand, occupy narrow ecological niches and are at constant risk from destructive harvesting driven by increasing demand. Urgent measures are needed to ensure the conservation of their wild populations.

As a distinct ecological group of macrofungi, hypogeous fungi in Southwest China exhibit distribution patterns that are profoundly influenced by both environmental factors and human activities. In this region, the consumption of hypogeous fungi has a long cultural history and supports a significant trade market. This study provides a preliminary analysis of species diversity and distributional patterns of hypogeous fungi in Southwest China, offering insights that may contribute to the conservation and sustainable use of these valuable fungal resources.

5.2 Conclusion

5.2.1 Extensive collection of hypogeous fungi specimens, clarifying the species diversity and resource distribution of hypogeous fungi in southwest China.

5.2.1.1 In this study, an extensive survey of hypogeous fungi was conducted across southwestern China, encompassing 59 sampling sites, from which 209 specimens were collected.

5.2.1.2 This study revealed that a total of 166 species of hypogeous fungi are distributed across the three southwestern provinces of China. Each species was annotated with detailed specimen information, distribution and habitat data, molecular sequences, and known economic value. Among them:

Phylum Ascomycota includes two orders, five families, eight genera, and 91 species. Phylum Basidiomycota includes five orders, 13 families, 19 genera, and 72 species. Phylum Mucoromycota includes one order, one family, one genus, and three species.

5.2.1.3 Among the 166 species of hypogeous fungi recorded in southwestern China in this study, 38 are edible and 1 is medicinal. Except for the genus *Tuber*, the edibility and trade of other hypogeous fungi show marked regional and ethnic specificity. Of the 166 species distributed in this region, 100 are considered rare, while 22 are relatively widespread.

5.2.1.4 This study analyzed the geographical distribution patterns of hypogeous fungi in southwestern China.

1. The results show that these fungi are primarily concentrated in the basins of the Yarlung Zangbo River, Nujiang River, Lancang River, and Jinsha River, with the Jinsha River basin being the most prominent. This basin harbors the richest hypogeous fungal diversity, with 110 recorded species, including 92 endemics. The distribution pattern among river basins indicates that hypogeous fungi in this region exhibit high habitat specificity and strong basin-restricted distributions. Notably, 84.5% of species were confined to a single river basin, and cross-basin distributions were rare.

2. The highly folded terrain and alternating ridges and valleys of the Hengduan Mountains are likely key drivers of the geographical isolation of hypogeous fungal species. The elevational range of these fungi spans from 860 m to 4005 m, with 96.1% of species occurring in alpine and subalpine forests between 1500 and 4000 m. Species diversity peaks between 2000 and 2500 m, and 40 species are found in alpine fir (Abies) forests at elevations of 3000–4000 m.

3. It is noteworthy that 95% of hypogeous fungi in this region form ectomycorrhizal associations with trees of the families Pinaceae and Fagaceae.

4. In addition, analysis of collection dates reveals a multimodal pattern in sporocarp formation, influenced by the complex interplay of monsoonal patterns and vertical climatic zonation in mountainous areas of southwestern China. The primary fruiting peak occurs from August to October, during the mid-to-late rainy season. A secondary peak is observed from November to January—the early dry season—which corresponds to the intensive harvesting of *Tuber* species. A minor spring peak, occurring

between Day of Year (DOY) 100–160, is likely associated with increased soil moisture due to pre-monsoon rainfall.

5.2.2 Improve the classification system of hypogeous fungi.

Introduce 21 new Hypogeous fungi species (viz. *Hydnotrya oblongispora*, *H. zayuensis*, *Tuber albicavum*, *T. laojunshanense*, *T. umbilicicavatum*, *Truncocolumella pseudocolumella*, *Gautieria zixishanensis*, *Hymenogaster zayuensis*, *Chamonixia laojunshanensis*, *Rhizopogon laojunshanensis*, *R. melanocuticularis*, *R. zayuensis*, *R. weixiensis*, *Hysterangium alpinum*, *Hysterangium shibaoshanensis*, *Protubera yunnanensis*, *Russula densiobtusispora*, *R. laojunshanensis Jimgerdemannia cangshanensis*, *J. zixishanensis* and *J. laojunshanensis*,) based on morphology and phylogenetic analysis. Additionally, four new distribution records were documented.

5.2.3 Their evolutionary relationships to provide up-to-date basic information on hypogeous fungi in southwest China.

5.2.3.1 Based on a phylogenetic tree of Boletales constructed using a five-gene dataset, this study estimated the divergence times of hypogeous Boletales species distributed in Southwest China. 28 species with valid molecular data were included in the divergence time estimation analysis, leading to the following conclusions: The evolution of hypogeous fruiting bodies within Boletales likely originated between the late Eocene (approximately 50 Mya) and the early Miocene (ca. 22 Mya). By incorporating molecular data from hypogeous Boletales species distributed in Southwest China, this study reveals that the earliest divergence within these regional taxa began in the Eocene (e.g., *Truncocolumella pseudocolumella*, 50.09 Mya), with the majority of diversification events occurring between the Oligocene (ca. 34 Mya) and the early Pliocene (approximately 5–3 Mya).

5.2.3.2 The study revealed morphological evolutionary trends in the genus *Hydnotrya*, particularly in spore ornamentation, ascus characteristics, and hymenial tissue structures.

As a distinct ecological group of macrofungi, hypogeous fungi in Southwest China exhibit distribution patterns that are profoundly influenced by both environmental factors and human activities. In this region, the consumption of hypogeous fungi has a long cultural history and supports a significant trade market. This study provides a preliminary analysis of species diversity and distributional patterns of hypogeous fungi in southwestern China, offering insights that may contribute to the conservation and sustainable use of these valuable fungal resources.

5.3 Suggestions

5.3.1 Potential Benefits or Application of the Study's Outcomes

Southwestern China, centered on the Hengduan Mountains, is a global biodiversity hotspot with exceptionally rich hypogeous fungal diversity. In this study, we conducted extensive surveys and sampling of hypogeous fungi across three southwestern provinces of China, documenting a total of 166 species distributed in the region. Through taxonomic and phylogenetic analyses, we identified and described 21 new species belonging to 11 different genera. In total, 209 high-quality specimens were collected and 528 molecular sequences were obtained, providing valuable data for future studies on macrofungal systematics, evolutionary biology, and forest ecosystem research.

Many hypogeous fungi are rare wild edible and medicinal species. In southwestern China—particularly in the Jinsha River and Nujiang River valleys—there are well-known truffle-producing areas that generate considerable income for local communities each year. The region is home to more than 15 indigenous ethnic groups, and our investigation identified 38 edible species of hypogeous fungi. For many communities, these fungi are not only prized wild delicacies but also important natural resources that contribute to poverty alleviation and local livelihoods.

Through integrative taxonomic, phylogenetic, and biogeographic studies, this work clarifies the species diversity and distribution patterns of hypogeous fungi in the region. Revealing the current status and ecological characteristics of these fungal resources will support their conservation and the sustainable development of highland forest-based economies. This, in turn, will promote ethnic minority agriculture and rural revitalization, while contributing scientific data to strengthen the ecological security of southwestern China.

5.3.2 Recommendations for Further Research to More Comprehensively Address Other Aspects of the Problem

The results of this study highlight several noteworthy findings:

Among the 166 species of hypogeous fungi distributed in southwestern China, 39 species lack molecular data. Without exception, these specimens were collected in the early 1990s, a time when species identification and phylogenetic studies were constrained by limited research conditions and relied solely on morphological characteristics. Except for those described as new species, most of the remaining specimens were identified as taxa originally described from Europe or North America, which is questionable and suggests the possibility of misidentifications and homonymy. Unfortunately, obtaining DNA sequences from these early collections is challenging. Re-collecting specimens from the original localities may be a viable approach to clarify their distribution and taxonomic status.

A large proportion of the hypogeous fungi in southwestern China are rare species—100 species, accounting for 60.2%. According to our statistics, these rare species typically have narrow habitat ranges and produce sporocarps in very low quantities. This pattern may also be attributed to the hypogeous nature of these fungi, which grow underground and are difficult to detect. Except for species that are edible and traded in large quantities, most others have long been overlooked. Continued surveys and specimen collection are essential for revealing the full diversity of hypogeous fungi in the region.

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APPENDIX A

CHEMICAL REAGENTS AND MEDIA

The following reagents were used for morphological examination of fungal specimens. All reagents were freshly prepared or verified for effectiveness prior to use.

1. Melzer's Reagent

Used for testing amyloid or dextrinoid reactions in fungal tissues (Kirk et al 2008).

Composition:

Iodine crystals: 1.5 g
Potassium iodide (KI): 5.0 g
Chloral hydrate: 100.0 g
Distilled water: 100.0 mL

2. Ferric Sulfate Solution

Used for color reactions with certain fungal tissues (Buyck 1989).

Composition:

Ferric sulfate ($Fe_2(SO_4)_3$): 10.0 g
Distilled water: 100.0 mL

3. Lactophenol Cotton Blue (LCB)

A mounting and staining medium for microscopic observation of hyphae and spores (Largent et al. 1977).

Composition:

Phenol crystals: 20.0 g
Lactic acid: 20.0 mL
Glycerol: 40.0 mL
Distilled water: 20.0 mL
Cotton blue (aniline blue): 0.05 g

4. Congo Red Solution

Used as a general-purpose stain to highlight fungal structures (Brundrett et al. 1996).

Composition:

Congo red: 0.5 g

Distilled water or 50% glycerol: 100.0 mL

5. 5% Potassium Hydroxide (KOH) Solution

Used for clearing and softening fungal tissues for microscopy (Largent 1973).

Composition:

Potassium hydroxide (KOH): 5.0 g

Distilled water: 100.0 mL

APPENDIX B

ABSTRACT OF PUBLICATIONS

Journal of *Fungi*

MDPI

Article

Three New Species of *Tuber* Discovered in Alpine Fir Forests in Yunnan, China

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Abstract: Three new species of *Tuber*, *T. albicavum*, *T. laojunshanense*, and *T. umbilicicavatum* belonging to the *Puberulum* phylogroup, are described based on specimens collected in alpine *Abies* forests at 3600–4000 m, Northwest Yunnan, China. *T. albicavum* is distinguished by its ascomata with a single chamber of 0.5–1.8 cm diameter, with an apical opening of 0.2–0.6 cm in diameter, and light golden-brown alveolate reticulate ascospores up to 30 µm in length; *T. laojunshanense* is characterized by having ascomata with a slightly tomentose surface, sometimes with a white navel, a relatively thick peridium, up to 280 µm, and yellow-brown spores with alveolate reticulate ornamentation, up to 34 µm in length; *T. umbilicicavatum* is characterized by smooth ascomata with a distinct white navel, a relatively thin peridium, up to 110 µm, and golden or golden-brown alveolate reticulate ascospores, up to 40 µm in length. The molecular analysis of the internal transcribed spacer region also supports that these three new species differ from previously described *Tuber* species.

Keywords: ITS; morphological; taxonomy; truffle; Tuberaceae

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1. Introduction

The genus *Tuber* F.H. Wigg. 1780 (Tuberaceae, Pezizales, Pezizomycotina, Ascomycota) is a significant group of fungi of economic and ecological importance. *Tuber* species are primarily distributed throughout the northern hemisphere [1–5], with species within the *Puberulum* phylogroup widely distributed across Europe, Asia, North America, South America, and North Africa [6]. *Tuber* species form ectomycorrhizal associations with a variety of woody plants, including those in the Fagaceae, Betulaceae, Juglandaceae, Myrtaceae, Salicaceae, Pinaceae, and Salicaceae families [7]. These associations play an important role in these forest ecosystems [8–16]. In recent years, new species and novel ectomycorrhizal tree partners are continually being discovered in various forest ecosystems within these regions. For example, *T. elevatireticulatum* K.F. Wong & H.T. Li, 2018, was discovered in Taiwan, China, forming ectomycorrhizal associations with *Keteleeria fortunei* var. *cyclolepis* (Flous) Silba, and a *Tuber* sp. within the *Puberulum* phylogroup has been identified in Japan as forming symbiotic relationships with *Abies sachalinensis* (F. Schmidt) Mast. [17,18]. Mature *Tuber* species have a distinctive smell that attracts small animals,

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Research Article

New species of *Hydnotrya* (Ascomycota, Pezizomycetes) from southwestern China with notes on morphological characteristics of 17 species of *Hydnotrya*

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Abstract

More specimens of *Hydnotrya* have been collected from southwestern China in recent years. Morphological and molecular analyses showed that they belonged to three species of *Hydnotrya*, of which two are new to science, *H. oblongispora* and *H. zayuensis*. The third one was *H. laojunshanensis*, previously reported in 2013. The new species are described, and their relationship to other species of *Hydnotrya* is discussed. *H. laojunshanensis* is re-described in more detail. The main morphological characters of 17 species of *Hydnotrya* are compared and a key to them is provided as well.

Key words: Discinaceae, hypogeous fungi, ITS, morphological diversity, taxonomy

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Introduction

Hydnotrya Berk. & Broome is a genus of hypogeous fungi belonging to Pezizomycetes, Ascomycota. It was placed in the family Helvellaceae by Spooner (1992) and Abbott and Currah (1997) but based on the recent molecular analyses it has been shifted into the family Discinaceae (O'Donnell et al. 1997; Hansen and Pfister 2006; Tedersoo et al. 2006; Læssøe and Hansen 2007; Wang et al. 2023). Their ascomata are hollow to convoluted with simple or folded chambers, even nearly solid, lined with recognizable hymenium. *Hydnotrya* species usually forms a symbiotic relationship with both conifer and broadleaf trees and are distributed throughout the northern hemisphere (Trappe 1975; Spooner 1992; Trappe and Castellano 2000; Stielow et al. 2010; Xu et al. 2018; Slavova et al. 2021). There are 22 names listed in the Index Fungorum online database (http://www.indexfungorum.org/Names/Names.asp). However, among them, the species *H. jurana* Quél. and *H. carnea* (Corda) Zobel was synonymized with *H. tulasnei* (Berk.) Berk. & Broome (Gilkey 1954; Trappe 1969), *H. ploettneriana*

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Article

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A new false truffle species, *Gautieria zixishanensis sp. nov.* (Gomphales, Basidiomycota) found in Yunnan, China

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Abstract

A new species of false truffle was discovered in Zixishan Mountain, Yunnan Province, China. Through morphological examination and multigene phylogenetic analysis based on ITS and nrLSU sequences of the genus *Gautieria*, the species is described as *Gautieria zixishanensis sp. nov.*, belonging to *Gautieria* sect. *Hymenogastroides*. This new species is characterized by its pseudoperidial basidiomata, with a basal depression connected to abundant white basal rhizomorphs, an intact tomentose pseudoperidium that remains attached when mature, the absence of foveate-porate structures and ridges, and light golden-brown basidiospores with longitudinal ridges, measuring 17–22 µm in length.

Key words: ITS and nrLSU, morphology, taxonomy, false truffle, Gomphaceae

1. Introduction

The genus *Gautieria* Vittad. 1831 (Gomphaceae, Gomphales, Agaricomycotina, Basidiomycota, He *et al.* 2024) was established by Vittadini in 1831, with the initial description of two species from Italy, *G. morchelliformis* (type species) and *G. graveolens* (Vittadini 1831). Over the next nearly two centuries, additional species within this genus were described and reported across the Northern Hemisphere, South America and Oceania (Soehner 1951, Pilát 1958, Rauschert 1975, Gross *et al.* 1980, Halling 1981, Calonge *et al.* 1985b, 1996, Montecchi & Lazzari 1988, 1993, Calonge & Pasabán 1993, Vidal 1994, Bougher & Lebel 2001, Bau & Liu 2013, Yang *et al.* 2023, Vidal *et al.* 2023). Currently, there are 77 valid names listed in MycoBank for this genus (https://www.mycobank.org/). Vidal *et al.* (2023) conducted a morphological and phylogenetic revision of the genus *Gautieria* in Europe and the Mediterranean Basin, which clarified the four sections within the genus: sect. *Gautieria* Vittad. 1831, sect. *Hymenogastroides* J.M. Vidal 2023, sect. *Parvicellae* J.M. Vidal & States, 2023, and sect. *Glutinosiglebae* J.M. Vidal 2023. *Gautieria* is a genus of hypogeous fungi, typically forming ectomycorrhizal associations with symbiotic tree partners, such as Pinaceae, Betulaceae, and Fagaceae (Liu 1998, Vidal *et al.* 2023). Upon maturation, their basidiomata emit odors that attract small animals, which facilitate spore dispersal. The distribution of *Gautieria* species is diverse, reflecting variations

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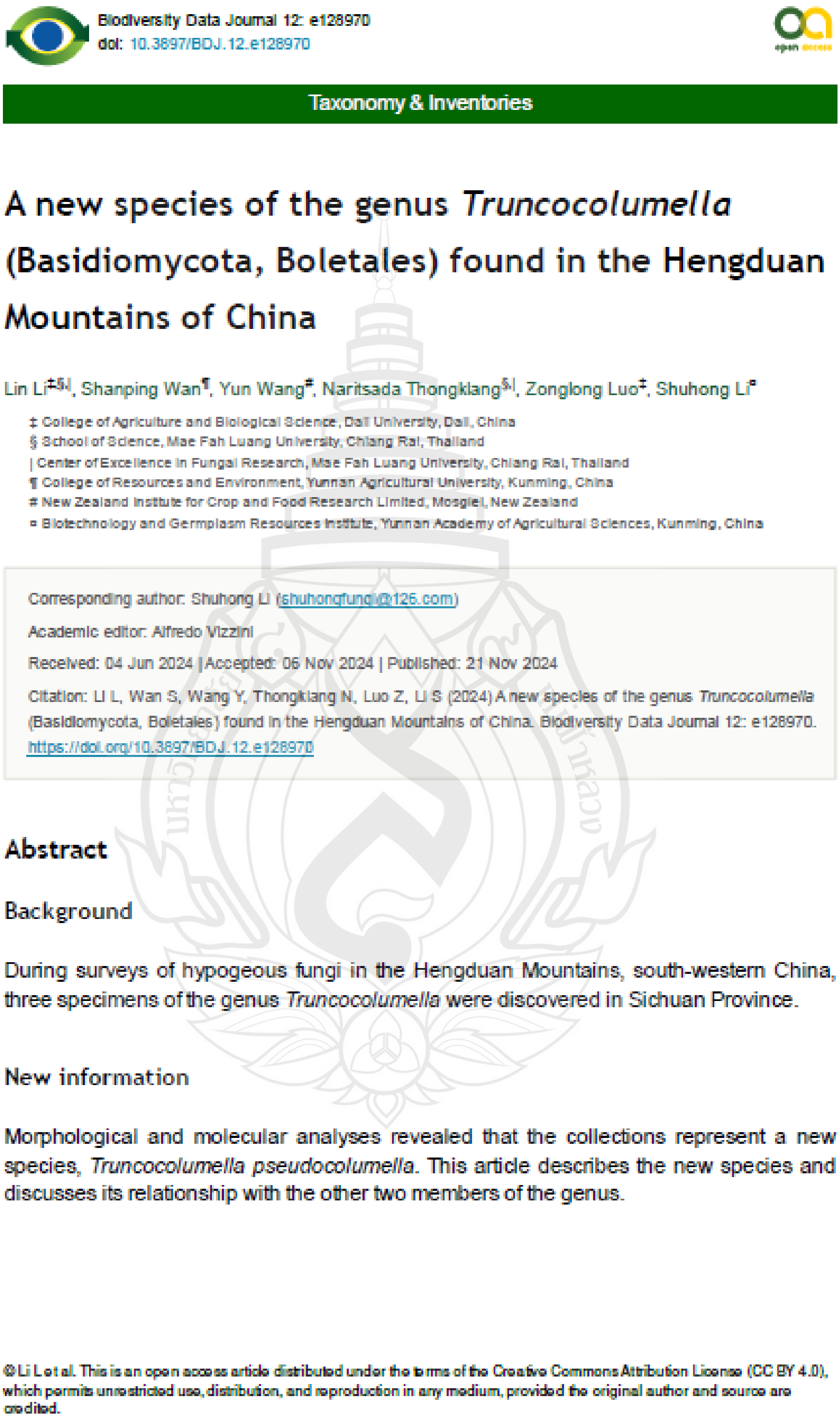

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Taxonomy & Inventories

A new species of the genus *Truncocolumella* (Basidiomycota, Boletales) found in the Hengduan Mountains of China

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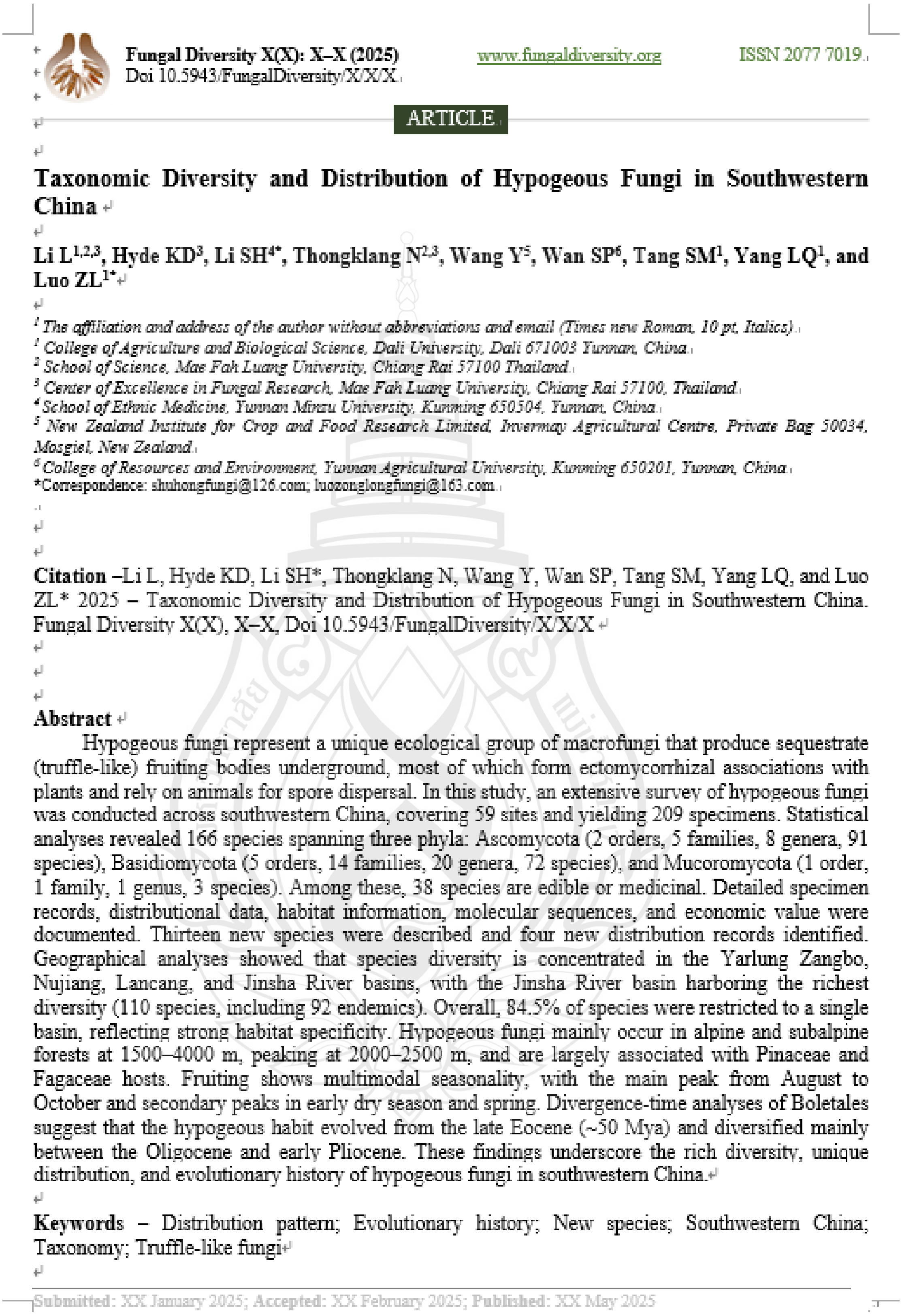
Abstract

Background

During surveys of hypogeous fungi in the Hengduan Mountains, south-western China, three specimens of the genus *Truncocolumella* were discovered in Sichuan Province.

New information

Morphological and molecular analyses revealed that the collections represent a new species, *Truncocolumella pseudocolumella*. This article describes the new species and discusses its relationship with the other two members of the genus.



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ARTICLE

Taxonomic Diversity and Distribution of Hypogeous Fungi in Southwestern China

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Abstract

Hypogeous fungi represent a unique ecological group of macrofungi that produce sequestrate (truffle-like) fruiting bodies underground, most of which form ectomycorrhizal associations with plants and rely on animals for spore dispersal. In this study, an extensive survey of hypogeous fungi was conducted across southwestern China, covering 59 sites and yielding 209 specimens. Statistical analyses revealed 166 species spanning three phyla: Ascomycota (2 orders, 5 families, 8 genera, 91 species), Basidiomycota (5 orders, 14 families, 20 genera, 72 species), and Mucoromycota (1 order, 1 family, 1 genus, 3 species). Among these, 38 species are edible or medicinal. Detailed specimen records, distributional data, habitat information, molecular sequences, and economic value were documented. Thirteen new species were described and four new distribution records identified. Geographical analyses showed that species diversity is concentrated in the Yarlung Zangbo, Nujiang, Lancang, and Jinsha River basins, with the Jinsha River basin harboring the richest diversity (110 species, including 92 endemics). Overall, 84.5% of species were restricted to a single basin, reflecting strong habitat specificity. Hypogeous fungi mainly occur in alpine and subalpine forests at 1500–4000 m, peaking at 2000–2500 m, and are largely associated with Pinaceae and Fagaceae hosts. Fruiting shows multimodal seasonality, with the main peak from August to October and secondary peaks in early dry season and spring. Divergence-time analyses of Boletales suggest that the hypogeous habit evolved from the late Eocene (~50 Mya) and diversified mainly between the Oligocene and early Pliocene. These findings underscore the rich diversity, unique distribution, and evolutionary history of hypogeous fungi in southwestern China.

Keywords – Distribution pattern; Evolutionary history; New species; Southwestern China; Taxonomy; Truffle-like fungi

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A new false truffle species, *Hysterangium shibaoshanense* sp. nov. (Hysterangiales, Basidiomycota), from northwestern Yunnan, China

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Abstract: A new species of false truffle was discovered in Shibaoshan Mountain, northwestern Yunnan Province, China. Based on morphological examination and multigene phylogenetic analyses using ITS, nrLSU, *atp6*, *rpb2*, and *tef1* sequences of the genus *Hysterangium*, the species is described as *Hysterangium shibaoshanense* sp. nov. The new species is characterized by basidiomata with a non-discoloring peridium; relatively small, light golden-yellow spores (excluding utricle, measuring only 2–4 × 0.8–1.5 μm); and utricles that are asymmetrically inflated in 5% KOH or water, reaching 0.5–1.5 μm in thickness from the spore wall.

Keywords: multigene phylogenetic analysis; morphology; taxonomy; false truffle; Hysterangiaceae

1. Introduction

Hysterangium was established by Vittadini (1831) to accommodate a hypogeous species with smooth basidiospores and a peridium that dissolves or splits at maturity. The generic concept has since been revised, and the diagnostic features of *Hysterangium* are enclosed basidiomata, an irregularly developed columella, a cartilaginous gleba, and narrowly ellipsoid to fusoid basidiospores that are smooth to rugose and covered by a membranous utricle or perisporium (Zeller & Dodge 1929; Hosaka et al. 2006). Recent molecular phylogenetic studies have demonstrated that *Hysterangium* belongs to a distinct order, Hysterangiales Hosaka & Castellano, which, together with Phallales E. Fisch., Gomphales Jülich, and Geastrales Hosaka & Castellano, is placed within the subclass Phallomycetidae Hosaka, Castellano & Spatafora (Hosaka et al. 2006; Davoodian et al. 2021, He et al. 2024, Hyde et al. 2024). The genus *Hysterangium* is species-rich and globally distributed (Zeller & Dodge 1929; Liu et al. 2005; Guevara et al. 2008; Elliott et al. 2020). Seventy-four species epithets for *Hysterangium* are listed in Index Fungorum (https://www.indexfungorum.org/names/Names.asp), all of which produce hypogeous basidiomata. Members of *Hysterangium* are known to form ectomycorrhizal associations with hosts in Fagaceae, Myrtaceae, Nothofagaceae, and Pinaceae, and they exhibit a wide geographical distribution (Malajczuk & Bougher 1987; Castellano 1988, 1999; Sulzbacher et al. 2019).

In China, 15 species of *Hysterangium* are currently recognized (Liu et al. 2005): *H. album* Zeller & C.W. Dodge 1929, distributed in Shanxi Province and Nei Mongol Autonomous Region; *H. clathroides* Vittad. 1831, *H. harknessii* Zeller & C.W. Dodge 1929, *H. neglectum* Massee & Rodway 1899, *H. occidentale* Harkn. 1899, and *H. thwaitesii* Berk. & Broome 1848, all reported from Shanxi Province; *H. calcareum* R. Hesse 1891, *H. cistophilum* (Tul. & C. Tul.) Zeller & C.W. Dodge 1929, and *H. hautu* G. Cunn. 1938, recorded from Guangdong Province; *H. microsporum* Liu & Tao 1996, reported from

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