

Dissertation Title Diversity, Taxonomy, and Chemical Evaluation of Truffles (*Tuber*) and False Truffles (*Choiromyces* and *Melanogaster*) and Commercial Boletes in Southwest China

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ABSTRACT

China, especially the southwestern region, boasts astonishing diversity of mushrooms. These mushrooms exhibit various nutrition modes, including saprotrophic, ectomycorrhizal (EcM), or parasitic. Particularly, ectomycorrhizal fungi play crucial roles in ecosystems and provide valuable resources for contemporary medicine and daily diet. This thesis focuses on hypogeous fungi and boletes in southwestern China, conducting research on the taxonomy and phylogeny of hypogeous fungi, the relationship between chemical information of boletes and species, and the exploration of chemical markers as the basis for species identification.

Hypogeous fungi are difficult to discover because their sporophores grow underground. This thesis identifies and describes the following new species through morphological and phylogenetic analysis. Two new species, *Tuber maroonense* nom. prov. and *T. conditum* nom. prov., belong to the *Latisporum* group, are described from Yunnan province of southwest China. Phylogenetic placements and morphological characteristic of these novel *Tuber* species in the *Latisporum* group are discussed. *T. maroonense* is phylogenetically closely related to *T. caoi*. Morphologically, *T. caoi* has gray and pubescens ascomata while *T. maroonense* has whitish and glabrous ascomata. *Tuber conditum* differs from *T. qujingense* and *T. songlu* by its thinner peridium (120–200 μm) and larger ascospores

(49–66.5 × 34.5–60 μm in one-spored asci). *Tuber qujingense* has a greyish white ascomata, brown snowflake-shaped gleba, prosenchymatous peridium, fusiform and *T. songlu* has dense spine-like dermatocystidia, which is completely different from each other. In addition, DNA sequence analysis revealed the similarity between two species is less than 67 % (*T. qujingense*) and 93 % (*T. songlu*) in ITS sequences, strongly supporting the separation of *T. conditum* from the Chinese species of *T. qujingense* and *T. songlu*.

Choiromyces cerebriformis sp. nov. is described and illustrated from Yunnan province, China. Both morphological evidence and sequence analyses of the internal transcribed spacer region (ITS1-5.8S-ITS2) support the species as new to science. *C. cerebriformis* differs from other *Choiromyces* species in having ascomata with larger lobes and light orange-brown gleba, and globose ascospores with short stick-like sparse spines ornamentation. Morphological differences and genetic distances with the similar species *C. helanshanensis* and *C. alveolatus* are discussed. A phenotypic key including related species is provided.

Three newly discovered *Melanogaster* species, namely *M. cyaneus* sp. nov., *M. diqingensis* sp. nov., and *M. truncatisporus* sp. nov., are introduced and illustrated based on both morphological and molecular data from Sichuan and Yunnan provinces in China. A multigene phylogenetic analysis (nrITS, nrLSU, and rpb2) was performed mainly to verify the placement of the new species in *Melanogaster*. A second, nrITS-only phylogenetic analysis comprising more *Melanogaster* species for which only ITS sequences were available, was used to infer the relationship between the new species and as many known *Melanogaster* species as possible. Specimens of *M. cyaneus*, *M. diqingensis*, and *M. truncatisporus* formed three independent clades in a phylogenetic tree inferred from the ITS data set. The robust support from ITS for these clades and genetic similarity with other species being lower than 93.2% suggest that these three species are indeed distinct from the other *Melanogaster* species in the phylogeny. Morphologically, *M. cyaneus* is characterized by its blue or bluish gleba, light brown to yellowish brown peridium,

and subglobose to globose basidiospores, $6.2\text{--}15 \times 4.6\text{--}9.0 \mu\text{m}$. *Melanogaster diqingensis* is distinguished from other *Melanogaster* species by its pale yellow to brown-yellow peridium and obovate to subglobose basidiospores, $3.0\text{--}5.1 \times 2.0\text{--}4.0 \mu\text{m}$. *Melanogaster truncatisporus* is diagnosed by its subglobose to globose or irregularly elongate-pyriform basidiomata, pale yellow to deeply orange-yellow peridium, and subglobose to globose or pyriform, truncate basidiospores.

The Agaricomycetidae (Agaricomycetes) are the most morphologically diverse Basidiomycota and they comprise two well-known orders, Agaricales and Boletales. As one of the most diverse representative species, over 500 species of boletes have been reported in China in recent years. Some of these boletes are widely consumed as delicacies, but there are still some species that are highly toxic, causing poisoning or even death (e.g. *Rubroboletus satanas* (Lenz) Kuan Zhao & Zhu L. Yang and *Heimioporus retisporus* [Pat. & C. F. Baker]) if ingested accidentally. The diversity of chemical components within boletes forms the material basis for studying their nutrition and toxicity. Currently, although species classification studies of common boletes are relatively mature, there is limited research on classifying species based on chemical information, whether there is a certain correlation between chemical information and species phylogeny and growth environment, and how the intrinsic chemical characteristics of species change. According to market research, this paper focuses on 11 common edible boletes conducts the following studies:

Here we collected 509 individuals of boletes samples on the field (belonging to 11 species identified by molecular analyses) from different geographic regions in Yunnan and Sichuan provinces of Southwest China. Those 11 species include *Boletus bainiugan* (BoB, 32 samples), *Butyriboletus pseudospeciosus* (BuP, 30 samples), *Butyriboletus roseoflavus* (BuR, 55 samples), *Lanmaoa asiatica* (LA, 56 samples), *Neoboletus obscureumbrinus* (NO, 21 samples), *Rugiboletus extremiorientalis* (RugE, 55 samples), *Rubroboletus sinicus* (RubS, 26 samples), *Retiboletus fuscus* (RetF, 60 samples), *Phlebopus portentosus* (PP, 35 samples), *Neoboletus hainanensis* (NH, 25 samples), *Neoboletus magnificus* (NM, 55 samples).

Chemical information, was gathered using near infrared spectroscopy (NIR), common peaks of high-performance liquid chromatography (HPLC) and volatile constituents. The degree of phylogenetic signal of chemical information and its relationship with ecological factors were analyzed by bioinformatics and chemometrics. We found the following: (1) The chemodiversity of boletes is widely correlated with phylogenetic signals and ecological and environmental factors. Thus, evolutionary history and ecological and environmental factors may play key roles in driving the formation of chemodiversity. (2) Phylogenetic relatedness is the best predictor of all chemical characteristics, and vice versa. Chemical information of phylogenetic conservatism is a powerful tool to identify species (addition to methods of molecular and morphological identification). (3) Chemical characteristic information strongly correlated with ecological and environmental factors could be used to identify the geographical regions of species. This study provides multiple lines of evidence that the chemodiversity of boletes is driven by both phylogeny and ecological and environmental factors. The chemical characteristic information could be used to identify species and geographical regions, which provided strong evidence for evolutionary species and ecological change in the future to explore the origin by chemical traits.

In addition, we explored the heterogeneity of volatile components (VOCs) in eleven bolete species by multivariate statistical analyses. A total of 97 VOCs were detected in 445 samples of 11 boletes species by gas chromatography-mass spectrometry, among which alkanes and esters were dominant. Principal component analysis and one-way analysis of variance revealed potential differences among the 11 boletes species. Partial least squares discriminant analysis (PLS-DA) combined with variable importance in projection results (22, VIP >1) and relative odor activity values (21, ROAV >0.1) revealed that 38 VOCs (5 common compounds) contributed to the classification of species, and identify models were effectively validated. Next, based on the analysis of the correlated heatmap combined with volcanic maps, 9 VOCs were selected as the key VOCs of 11 species. Furthermore, Fisher's linear discriminant function analysis combined with literature reports showed that 5 VOCs including methyl (9E)-9-octadecenoate, 2,6-dimethylpyrazine, 1-decen-3-one, furfural, and

methional could be used as signature compounds to identify the 11 bolete species. Ultimately, Fourier transform near infrared spectroscopy (FT-NIR) combined with partial least squares regression (PLSR) provides a fast and reliable quantitative technique for predicting the contents of the five characteristic VOCs in boletes. This study provides a strategy for effective species identification and rapid prediction of characteristic aroma substances of wild edible boletes based on qualitative and quantitative analysis of VOCs and FT-NIR spectra.

Keywords: Ectomycorrhizal Fungi, Edible Mushrooms, Chemodiversity, Pattern Recognition Research, Phylogenetics

