INTEGRATED IDENTIFICATION AND CHARACTERIZATION OF WILD EDIBLE MUSHROOMS: FROM SAMPLES TO DATA

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ABSTRACT

Wild edible mushrooms represent an ecologically and nutritionally valuable group of organisms whose importance in food security, human health, and sustainable forest management is steadily increasing. Their safe and sustainable use requires accurate identification and comprehensive characterization, which often exceed the capabilities of traditional morphology-based methods due to pronounced phenotypic plasticity and cryptic species diversity. This review presents an integrated approach to the identification and characterization of wild edible mushrooms, combining morphological, molecular, biochemical, and ecological methods. Particular emphasis is placed on the research workflow from field sampling to data processing and interpretation, including DNA barcoding, biochemical profiling, analysis of soil–mushroom interactions, and health risk assessment related to heavy metal bioaccumulation. The review also highlights key research gaps in biodiversity-rich but understudied regions and discusses future directions for the sustainable utilization and domestication of wild mushroom species.

Keywords: wild edible mushrooms, integrated identification, DNA barcoding, biochemical profiling, heavy metals, fungal ecology

INTRODUCTION

Fungi represent one of the most diverse and ecologically significant groups of eukaryotic organisms on Earth, playing indispensable roles in ecosystem functioning. As primary decomposers of organic matter, fungi drive the recycling of carbon, nitrogen, and other essential nutrients, thereby sustaining soil fertility and ecosystem productivity. In addition, numerous fungal species establish mutualistic relationships with plants, animals, and microorganisms, such as mycorrhizal associations that enhance plant nutrient uptake, or endophytic interactions that contribute to host resilience against biotic and abiotic stress. Through these ecological functions, fungi act as key regulators of biodiversity, ecosystem stability, and biogeochemical cycles. Among them, wild edible mushrooms are of particular importance due to their nutritional value, medicinal properties, and socioeconomic relevance (Sanchez, 2017; Gupta et al., 2018). Despite estimates suggesting the existence of up to 3.8–6.2 million fungal species worldwide, only a fraction have been taxonomically described and biochemically characterized (Hawksworth and Lücking, 2017; Baldrian et al., 2022). Wild edible mushrooms are rich in proteins, essential amino acids, dietary fiber, vitamins, and bioactive compounds with antioxidant, anti-inflammatory, and anticancer properties (Manzi et al., 2001; Cateni et al., 2022). However, their safe utilization depends on accurate species identification and assessment of environmental contamination, particularly with heavy metals (Širić et al., 2017; Spahić et al., 2018). Therefore, a comprehensive assessment of environmental contamination is a prerequisite for the safe consumption and commercialization of wild edible mushrooms.

Given these challenges, reliance on traditional morphological identification alone is often insufficient. An integrated identification framework that combines classical morphological characterization with molecular genetic approaches, biochemical profiling, and ecological data has become essential for reliable species delimitation. Molecular tools, such as DNA barcoding, enable the detection of cryptic diversity and improve taxonomic accuracy, while biochemical analyses provide insight into nutritional value and potential health benefits. Ecological data, including habitat preferences and substrate specificity, further contribute to a holistic understanding of species biology and distribution.

An integrated identification framework combining morphology, molecular genetics, biochemical analysis, and ecological data is therefore essential for reliable characterization and sustainable exploitation of wild edible mushrooms.

Biodiversity and Ecological Importance of Wild Edible Mushrooms

Macrofungi, characterized by visible fruiting bodies, comprise approximately 15,000 described species, of which around 2,000 are considered edible and more than 700 possess medicinal properties (Gupta et al., 2018; Anusiya et al., 2021). Ecologically, mushrooms contribute to organic matter decomposition, nutrient mobilization, and soil formation, while mycorrhizal species play a key role in plant nutrition and forest ecosystem stability (Naranjo-Ortiz and Gabaldón, 2019; Pérez-Moreno et al., 2021). In regions such as Croatia, fungal biodiversity is exceptionally high, with approximately 5,000 recorded species, representing only
an estimated 25% of the actual diversity (Tkalčec et al., 2008). Despite this richness, wild edible mushrooms remain one of the least studied organism groups, particularly regarding their genetic diversity and biochemical composition. Their sensitivity to environmental conditions also makes them valuable bioindicators of ecosystem health and pollution.

Evolutionary Background and Ecological Adaptation

The evolutionary history of mushroom-forming fungi is closely linked to major climatic and vegetation shifts. Agaricomycetes experienced rapid diversification during the Jurassic, coinciding with the expansion of coniferous forests and warm, humid climates (Varga et al., 2019). Subsequent adaptive radiations led to the emergence of characteristic pileate-stipitate morphologies, which are associated with increased diversification rates. Adaptive evolution driven by local environmental factors has been documented in species such as Lentinula edodes, where temperature adaptation contributed to genetic divergence and phenotypic differentiation (Zhang et al., 2022). Understanding these evolutionary and ecological processes is crucial for interpreting species distribution patterns and for selecting candidate species for domestication and cultivation.

Morphological Identification: Classical Foundations

Morphological identification remains the cornerstone of traditional mycology and relies on macroscopic traits (cap size, color, gill structure, stipe morphology) and microscopic features (spore size and ornamentation). While morphology is indispensable for initial field identification, it is often unreliable when used alone due to phenotypic plasticity and environmental influences (Spoerke, 2019). Consequently, morphology should be integrated with molecular tools to achieve accurate taxonomic resolution, particularly for closely related or cryptic species.

Molecular Identification and DNA Barcoding

Molecular identification has become the most reliable approach for species-level identification of wild edible mushrooms. DNA barcoding, primarily using the internal transcribed spacer (ITS) region of ribosomal DNA, is widely accepted as the universal fungal barcode (Khaund and Joshi, 2014; Ao et al., 2020).

Sequencing of ITS1 and ITS2 regions enables comparison with reference databases such as GenBank, while phylogenetic analyses provide insights into evolutionary relationships (Das et al., 2013). Complementary approaches, including MALDI-TOF MS protein profiling, offer rapid and reproducible species identification when supported by curated databases (Sugawara et al., 2016). The integration of molecular and morphological data ensures robust taxonomic assignments and reduces misidentification risks.

Biochemical and Nutritional Characterization

Wild edible mushrooms are nutritionally comparable to meat and legumes, containing 22–28% protein on a dry weight basis and all essential amino acids (Bilal, 2010; Șenilă et al., 2024). They are also rich in carbohydrates, dietary fiber, B-group vitamins, and minerals such as potassium, iron, zinc, and manganese (Kalač, 2013; Lalotra et al., 2018). Biochemical studies have identified phenolic acids, tocopherols, unsaturated fatty acids, and terpenoids responsible for antioxidant, antimicrobial, and anti-inflammatory activities (Kissanga et al., 2022). Despite their potential, comprehensive biochemical profiling of wild mushrooms remains limited in many regions, including Croatia, highlighting the need for systematic studies.

Soil - Mushroom Interactions and Microbial Communities

The chemical composition of mushrooms is strongly influenced by soil properties, microclimate, and associated microbial communities. Parameters such as soil pH, organic matter, and elemental composition affect nutrient availability and bioaccumulation processes (Tan, 2005; Rocco et al., 2016). Recent advances in next-generation sequencing have enabled detailed characterization of soil bacterial and fungal communities, revealing complex interactions that influence mushroom growth and distribution (Lu et al., 2022). Integrating soil chemistry, microbial diversity, and mushroom traits provides a holistic understanding of ecological niches and evolutionary adaptations.

Heavy Metals, Bioaccumulation, and Food Safety

Wild mushrooms are known to accumulate heavy metals such as cadmium, nickel, lead, and mercury from their environment. While many species remain safe for consumption, bioaccumulation is highly species- and site-specific (Falandysz and Borovička, 2013; Širić et al., 2022). Health risk assessment using bioaccumulation factors, dietary intake modeling, and target hazard quotient indices is essential to evaluate consumer safety and to establish guidelines for sustainable harvesting. Moreover, mushrooms serve as effective bioindicators for monitoring environmental pollution in forest ecosystems.

From Samples to Data: An Integrated Research Framework

An integrated workflow for wild edible mushroom research includes standardized field sampling, morphological and molecular identification, biochemical and nutritional analysis, soil and microbial profiling, and advanced statistical data processing. Such an approach ensures reproducibility, data reliability, and meaningful ecological interpretation. The integration of multidisciplinary datasets also facilitates data sharing through global biodiversity repositories and supports evidence-based decision-making for conservation, cultivation, and food safety policies.

Bridging Traditional Expertise and Modern Technology: A Holistic Framework

The concept described below is presented within the framework of the scientific project entitled "Integrated morphological, genetic and biochemical identification and characterization of wild edible mushrooms in Croatia – GenBioM", funded by Croatian Science Foundation. Also, the approach presented in this review differs fundamentally from conventional mycological studies in several ways. Rather than treating morphological observation, molecular analysis, and biochemical assessment as separate investigative streams, we propose their deliberate integration at every stage of the research process. This is not simply about conducting multiple tests on the same specimen; it represents a conceptual shift toward viewing wild edible mushrooms as complex biological systems that can only be understood through parallel examination of their genetic identity, chemical composition, ecological relationships, and environmental context.

Most published studies focus narrowly on either taxonomy, nutritional profiling, or contamination assessment. While such specialized work has value, it often misses the connections between these domains. For instance, knowing that a mushroom accumulates cadmium becomes far more meaningful when combined with data on its genetic lineage, soil microbiome, and evolutionary history. Similarly, biochemical variation within a species makes little sense without considering local environmental pressures and substrate characteristics. Our framework treats these relationships as primary research targets rather than secondary observations.

The workflow we describe also addresses a practical problem that has plagued field mycology for decades: the gap between specimen collection and reliable identification. Traditional approaches often separate these steps by considerable time and distance, leading to deterioration of morphological features and loss of ecological context. Through establishing protocols that link field documentation directly to laboratory analysis, we reduce this gap substantially. Photographic records, GPS coordinates, substrate samples, and preliminary morphological notes are collected simultaneously with tissue for DNA extraction and biochemical testing. This creates a permanent, multi-dimensional record for each specimen that can be revisited and reanalyzed as methods improve.

Another distinguishing work is our emphasis on soil chemistry and microbial communities as integral components of mushroom characterization. Most studies treat the substrate as a passive background factor, noting it only briefly in materials and methods sections. We argue that soil properties, pH gradients, organic matter composition, and associated bacterial and fungal taxa actively shape mushroom physiology, distribution patterns, and chemical profiles. Incorporating these measurements alongside traditional identifiers reveals how mushrooms adapt to specific microhabitats and explains why the same species can vary dramatically in nutritional content or heavy metal uptake across different locations.

The framework also recognizes regional knowledge gaps. While Western European and North American fungi have been extensively documented, areas with exceptional diversity, like Croatia, remain relatively unexplored despite harbouring thousands of species. Our approach was designed specifically to be scalable and applicable in settings where comprehensive reference databases may not exist. Through combining morphological expertise with molecular tools and building local reference collections, researchers can characterize biodiversity even in poorly studied regions.

Thus, this integrated methodology serves multiple end users simultaneously (Figure 1). Taxonomists gain more robust species delimitations, nutritionists obtain reliable compositional data tied to verified specimens, food safety authorities receive contamination assessments linked to specific taxa and collection sites, and conservation planners access population data grounded in accurate identification. Rather than forcing each stakeholder group to conduct separate studies, the framework produces datasets that address diverse needs from a single, well-documented collection effort. This efficiency becomes particularly important as climate change and habitat loss make long-term monitoring increasingly urgent.

The expected outcomes of this project include the development of an integrated framework for the accurate identification and comprehensive characterization of wild edible mushrooms in Croatia, based on combined morphological, genetic, and biochemical approaches. The project will generate reliable reference datasets encompassing taxonomic, biochemical, and heavy metal profiles, thereby improving species-level resolution and food safety assessments. By determining heavy metal concentrations and evaluating their potential implications for human health, the project will contribute to risk assessment and consumer protection. In addition, the identification of bioactive and nutritionally relevant compounds will support the sustainable use and valorization of wild edible mushroom resources, while strengthening interdisciplinary research in mycology, food science, and environmental health.

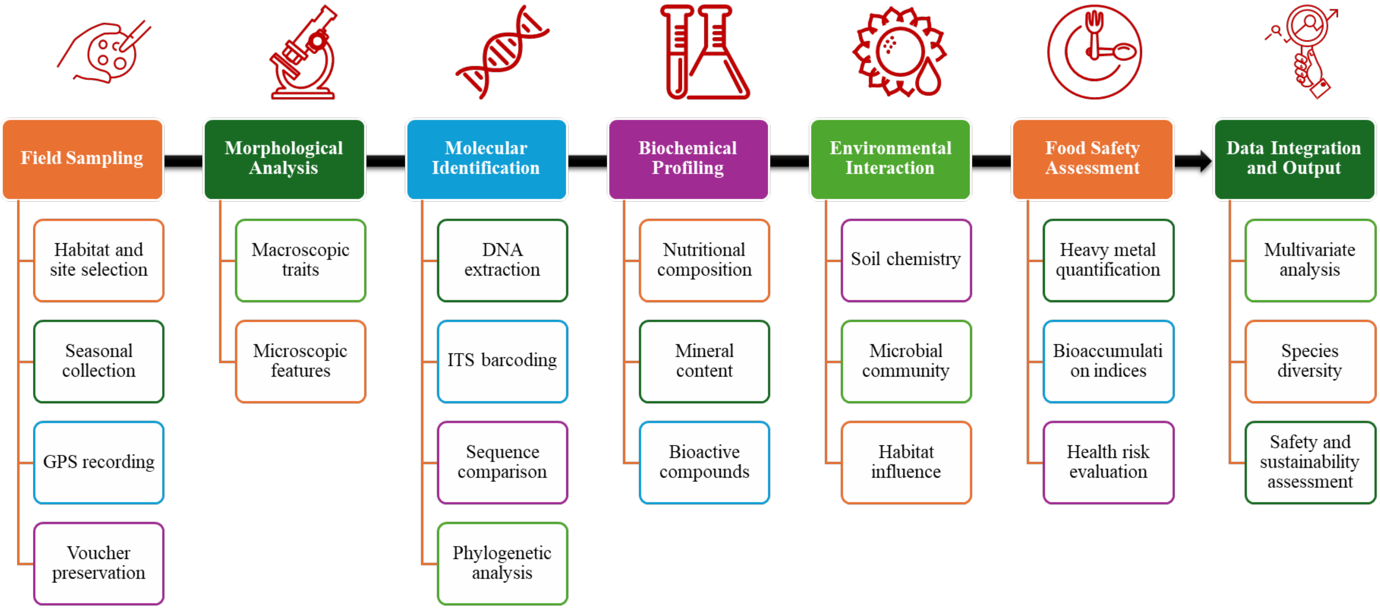

Figure 1: Conceptual overview of an integrated workflow for wild edible mushroom research, combining field sampling, morphological and molecular identification, biochemical characterization, environmental interaction analysis, and food safety assessment to support accurate species identification and sustainable utilization

CONCLUSIONS AND FUTURE PERSPECTIVES

Integrated identification and characterization approaches represent the future of wild edible mushroom research. Expanding such studies to biodiversity rich but understudied regions will enhance taxonomic resolution, uncover novel bioactive compounds, and support sustainable utilization. Future research should focus on long-term monitoring, climate change impacts, and translational applications in food, pharmaceutical, and environmental sectors.

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