Mycologia Iranica 2026, Vol. 13 (No. 1): ??–??

Short Article

First record of the genus *Saproamanita* in Iran: *Saproamanita vittadinii*

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10.22092/mi.2026.372695.1347

ABSTRACT

An extensive survey of *Amanita* species in northern and western Iran was conducted to document the diversity of this genus and related taxa. Among the collected specimens, *Saproamanita vittadinii*, a saprotrophic and edible species, is recorded for the first time in Iran, representing the first report of the genus *Saproamanita* in the country. Detailed macroscopic and microscopic examinations were performed to describe its morphological characteristics. To complete species identification, the ITS rDNA was sequenced, and phylogenetic analysis was performed. This discovery expands the known distribution of *S. vittadinii*, highlights the ecological diversity of saprotrophic *Amanita*-related taxa in Iran, and provides baseline data for future taxonomic, ecological, and phylogenetic studies.

KEYWORDS
Amanita, Edible mushrooms, ITS rDNA, *Lepidella* section, Saprotrophic.

INTRODUCTION

The genus *Amanita* Pers. (1797) includes some of the most prominent gilled mushrooms within the class *Agaricomycetes* and order *Agaricales*, characterized by white spore prints, free gills, and a well-developed universal veil that leaves remnants on the pileus and as a volva at the stipe base (Kirk et al. 2008). Most *Amanita* species are ectomycorrhizal, forming mutualistic associations with trees and playing a crucial roles in forest ecosystems. However, some species are saprotrophic and grow independently in soil and grasslands, such as *Amanita vittadinii*, *A. thiersii*, and *A. inopinata* (Bas 1969, Neville and Poumarat 2004).

Recent molecular phylogenetic studies revealed that saprotrophic *Amanita* species form a distinct clade, separate from the ectomycorrhizal *Amanita* (Moncalvo et al. 2002, Wolfe et al. 2012). To reflect these ecological and phylogenetic differences, Redhead et al. (2016) proposed the genus *Saproamanita*, with *S. vittadinii* as the type species. According to the most recent review, the genus *Saproamanita* currently includes 24 recognized species worldwide (Hyde et al. 2024).

Despite the considerable global diversity of *Amanita* and related genera, taxonomic and molecular studies on these taxa in Iran remain limited. The present study documents the occurrence of the genus *Saproamanita* in northern and western Iran. To our knowledge, this is the first report of a *Saproamanita* species from Iran and adds valuable data toward understanding the ecological and phylogenetic diversity of these fungi in the region.

MATERIALS AND METHODS

Sampling and Morphological Identification

Field surveys were conducted across the provinces of Golestan, Mazandaran, Guilan, East Azerbaijan, Kurdistan, Kermanshah, Hamedan, and Chaharmahal-va-Bakhtiari. Fruiting bodies were collected, photographed, and described based on macroscopic features including pileus shape, color, size, surface texture, margin shape and striation, lamellae attachment and color, stipe characteristics, volva and annulus morphology, spore print, and odor.

Received: 03 May 2026 Revised: 29 May 2026 Accepted: 31 May 2026 Published online: 07 June 2026

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Published by Iranian Mycological Society (IrMS)—https://mij.areeo.ac.ir

Specimens were individually packed, air-dried, and frozen at −20°C for 7–10 days. After freezing, specimens were further dried and examined microscopically. Measurements included basidiospore size and shape, amyloidity, basidia, and cystidia, with at least 20 measurements per structure (Seidmohammadi et al. 2018). Identification followed standard taxonomic keys (Hansen and Knudsen 1992, Knudsen and Vesterholt 2008).

DNA Extraction and ITS rDNA Sequencing

Genomic DNA was extracted from dried specimens using a commercial fungal DNA extraction kit (Dena Zist Asia, Iran), following the manufacturer's instructions. The internal transcribed spacer (ITS1–5.8S–ITS2) region of the nuclear ribosomal DNA was amplified using the primer pair ITS1f and ITS4. Polymerase chain reaction (PCR) products were verified by agarose gel electrophoresis and subsequently sequenced by Microsynth (Switzerland). Raw sequences were edited and assembled using BioEdit version 7, and submitted to the GenBank database.

Phylogenetic Analysis

ITS sequences were compared with reference sequences available in the GenBank database using the BLAST algorithm to confirm preliminary identifications (Table 1). Multiple sequence alignment was performed using the Clustal W algorithm. Phylogenetic relationships were inferred using the Maximum Likelihood method based on the Tamura 3-parameter substitution model. Rate heterogeneity among sites was modeled using a discrete gamma distribution with five categories. Ambiguous positions were removed using partial deletion with a 95% site coverage cutoff. Branch support was evaluated using 500 bootstrap replicates. *Limacella delicata* was used as the outgroup. All phylogenetic analyses were conducted in MEGA version 11 (Tamura et al. 2021).

RESULTS

Morphological identification of *Saproamanita vittadinii*

Among the collected specimens of *Amanita* and related taxa from northern and western Iran, seven specimens were identified as *Saproamanita vittadinii*, representing the first record of the genus *Saproamanita* in Iran.

Basidiomata were medium to large in size. The pileus measured 5–15 cm in diameter, fleshy and thick, hemispherical when young, becoming convex to slightly depressed at the center with age. The margin was non-striate, projecting, irregular, and retained remnants of the partial veil. Pileus coloration ranged from white to cream, becoming pale brown toward the center. Remnants of the universal veil were present as rough, adherent, polymorphic scales, including erect pyramidal elements and flattened patches, denser at the center and gradually flattening toward the margin; these structures were white to creamy white (Fig. 1. A, B).

Lamellae were moderately crowded, free from the stipe, white to cream, with numerous lamellulae interspersed (Fig. 1. D, E), and produced a white spore print. The stipe measured 8–15 cm in length and 2–3 cm in width, cylindrical, white, solid, and firm. A membranous, skirt-like annulus with an irregular, dentate margin was present near the pileus (Fig. 1. D). Below the annulus, the stipe surface was covered with ascending scales arranged in distinct annular zones (Fig. 1. F).

Basidia were clavate, four-sterigmate, and possessed clamp connections at the base (Fig. 1. G, H). Basidiospores were ovoid, hyaline, smooth, and amyloid, measuring 10.7–12.2 × 7.7–9 µm (Fig. 1. I). Basidiomata single to scattered, occasionally forming small rings. The species is reported as edible.

Specimens examined

IRAN, Golestan Province, 2 October 2017, leg. Mohammad Reza Asef, (IRAN 18606 F); Golestan Province, 2 October 2017, leg. Mohammad Reza Asef, (IRAN 18607 F); Golestan Province, 17 September 2015, leg. Mohammad Reza Asef, (IRAN 18611 F); Gilan Province, 1 September 2017, leg. Mohammad Reza Asef, (IRAN 18612 F); Kermanshah Province, 1 December 2016, leg. Elham Seidmohammadi, (IRAN 16988 F).

Molecular Phylogeny

The ITS sequences obtained from the Iranian specimens showed 99–100% similarity with the epitype sequence of *Saproamanita vittadinii* in GenBank (accession number: MF668693, Vizzini et al. 2017). Phylogenetic analysis placed the specimens within a well-supported clade of *Saproamanita*, clearly distinct from ectomycorrhizal *Amanita* species, as shown in the phylogenetic tree (Fig. 2). These results confirm the identity of the specimens as *S. vittadinii* and represent the first molecularly verified report of a saprotrophic *Saproamanita* in Iran.

The phylogenetic tree, constructed using the Maximum Likelihood method, demonstrates a distinct separation between *S. vittadinii* and other closely related species within the *Saproamanita* genus, including *Saproamanita nana*, and *S. codinae*. The specimens from Iran (PX647779, PX647780, PX647781, PX647782, PX647778) formed a monophyletic group, exhibiting strong bootstrap support (100%), indicating a high level of genetic consistency within this population.

Table 1. The ITS sequences retrieved from the NCBI GenBank database for phylogenetic analysis (the specimens obtained in this study are shown in bold).

Species	Section	Voucher	Country	ITS
Amanita fuliginea	*Phalloidae*	HKAS:75780	China	JX998023
Amanita rimosa	*Phalloidae*	HKAS75779	China	JX998020
Amanita augusta	*Lepidella*	-	USA	JX515564
Amanita augusta	*Lepidella*	DBB49390	USA	JQ937287
Amanita brunneomaculata	*Amidella*	HKAS70032	China	MH508279
Amanita franchetii	*Lepidella*	DBBJUS01	USA	JX515563
Amanita fritillaria	*Lepidella*	HKAS 38331	China	AY436457
Amanita lanigera	*Amidella*	HKAS97561	China	MH508421
Amanita lanigera	*Amidella*	HKAS89030	China	MH508420
Limacella delicata (out-group)		K(M):176976	United Kingdom	MZ159456
Saproamanita armillariiformis	*Lepidella*	RET 266-8	USA	HQ625012
Saproamanita codinae	*Lepidella*	AH49176	Spain	MZ493179
Saproamanita manicata	*Lepidella*	PDD_88301	New Zealand	MT863750
Saproamanita manicata	*Lepidella*	JP8	India	PP059067
Saproamanita nana	*Lepidella*	KS45	Pakistan	OR434193
Saproamanita nana	*Lepidella*	MK_SK100	Pakistan	OR177654
Saproamanita singeri	*Lepidella*	A20_ITS	Czech Republic	MK512064
Saproamanita singeri	*Lepidella*	MV 22.9679	Italy	OR354511
Saproamanita vittadinii	*Lepidella*	A1xVITT	Czech Republic	MK512062
Saproamanita vittadinii	*Lepidella*	MCVE29361	Italy	MF668693
Saproamanita vittadinii	***Lepidella***	**IRAN 18606 F**	**Iran**	**PX647779**
Saproamanita vittadinii	***Lepidella***	**IRAN 18607 F**	**Iran**	**PX647780**
Saproamanita vittadinii	***Lepidella***	**IRAN 18611 F**	**Iran**	**PX647781**
Saproamanita vittadinii	***Lepidella***	**IRAN 18612 F**	**Iran**	**PX647782**
Saproamanita vittadinii	***Lepidella***	**IRAN 16988 F**	**Iran**	**PX647778**
Saproamanita vittadinii	*Lepidella*	GK10612	Greece	ON782460
Saproamanita vittadinii	*Lepidella*	HKAS101430	China	MH508651

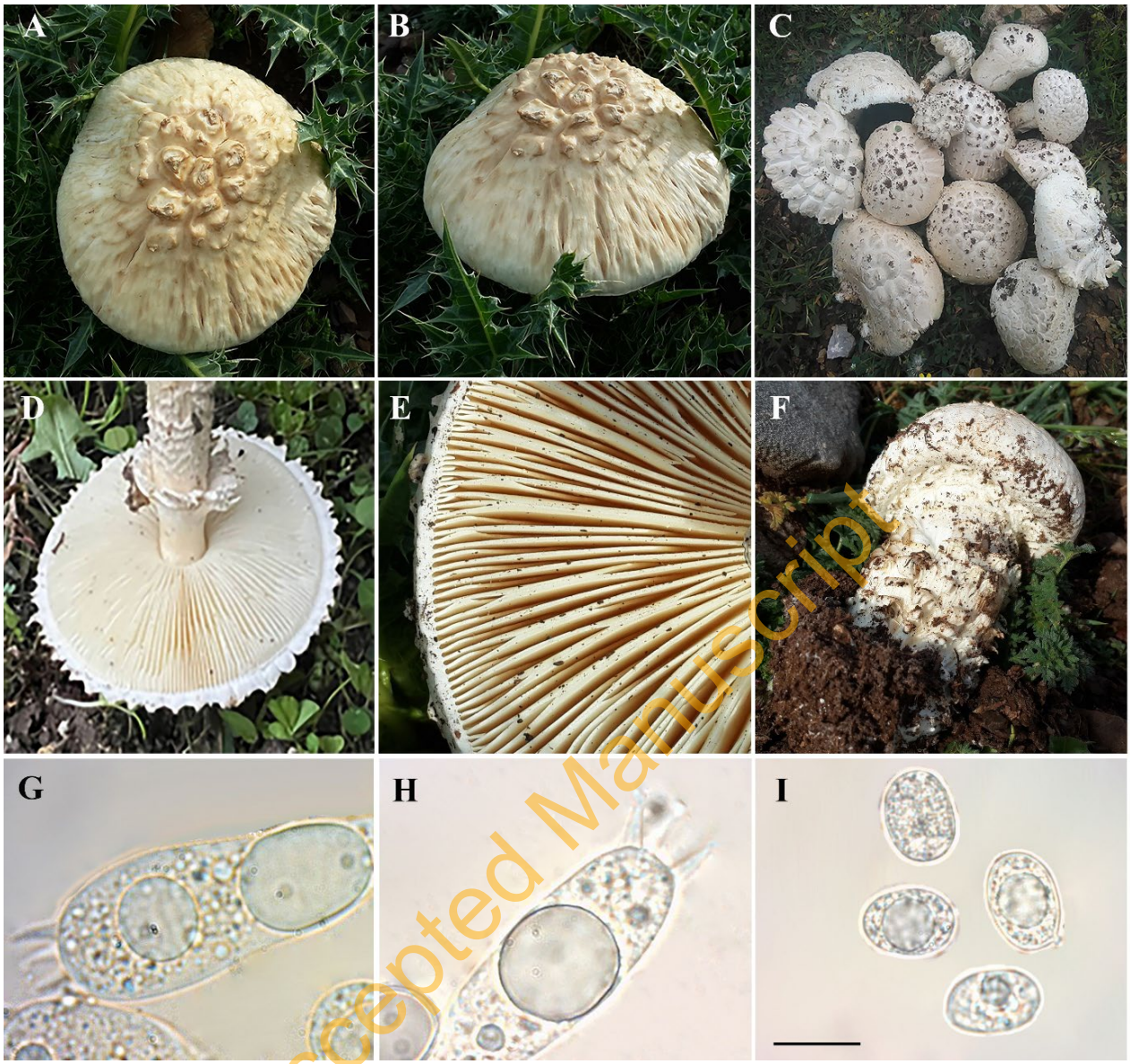

Fig. 1. *Saproamanita vittadinii* (Moretti) Redhead, Vizzini, Drehmel & Contu. (A, B) Mature basidiomata showing the pileus surface covered with rough, adherent remnants of the universal veil; these remnants appear as pyramidal to flattened scales, denser toward the center of the pileus, (C) Basidiomata at different developmental stages, (D) Lamellae free from the stipe, with a membranous, skirt-like annulus, (E) Close-up view of the lamellae showing moderate spacing and numerous lamellulae, (F) Young basidioma showing a scaly stipe base, (G, H) Clavate basidium with four sterigmata, (I) Basidiospores ovoid, smooth, hyaline, and amyloid. Scale bar = 10 µm (G–I).

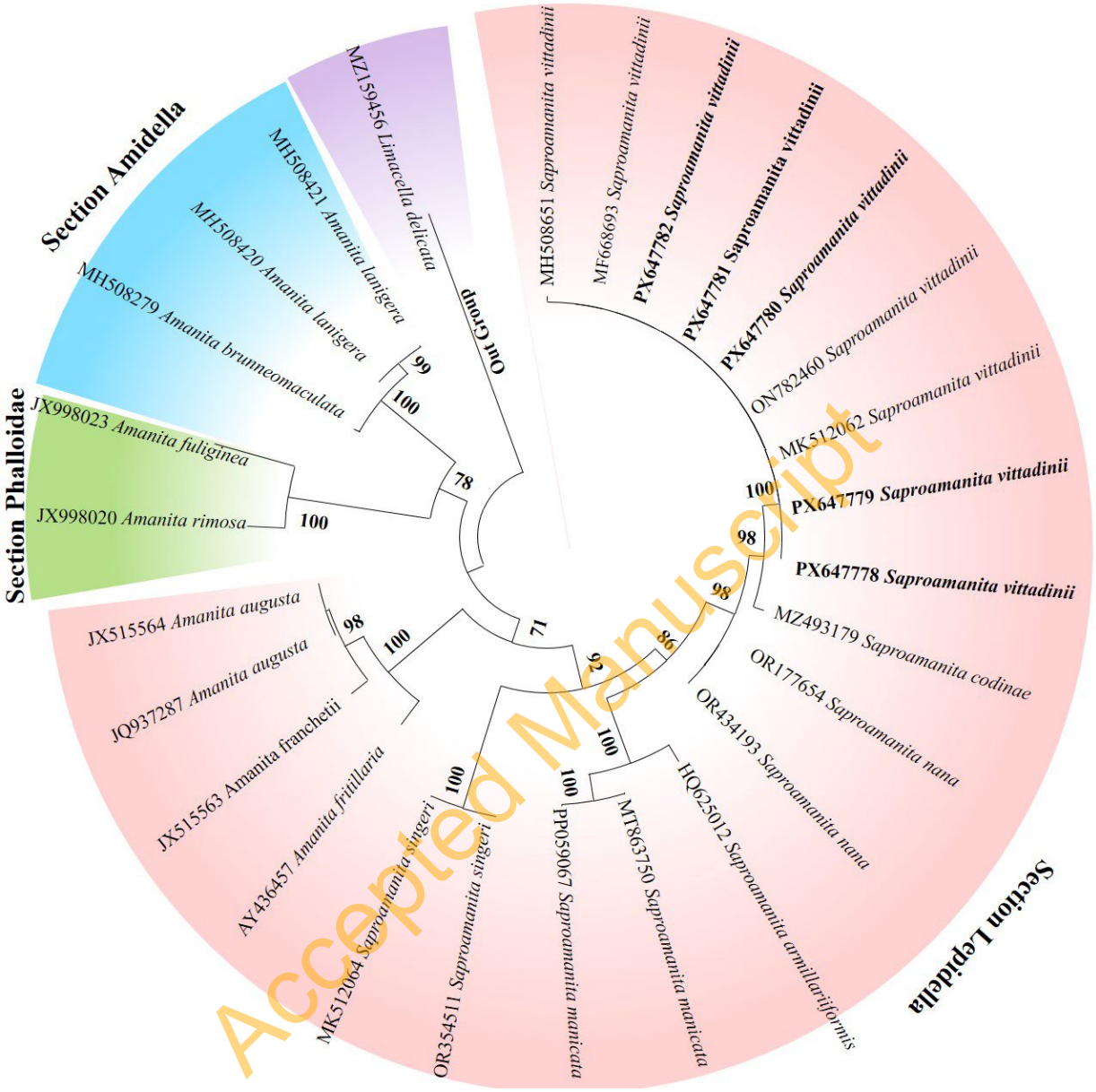

Fig. 2. Phylogenetic tree of *Saproamanita vittadinii* based on ITS-rDNA sequences. The tree was constructed using the Maximum Likelihood method, with bootstrap values from 500 replicates shown above the branches. The specimens from Iran (PX647778, PX647779, PX647780, PX647781, PX647782) cluster with other reference sequences of *S. vittadinii*, forming a well-supported monophyletic group. *Limacella delicata* was used as the outgroup. The tree was constructed in MEGA version 11 (Tamura et al. 2021).

DISCUSSION

The present study reports the first confirmed record of *Saproamanita vittadinii* from Iran based on combined morphological and molecular evidence. This finding expands the known distribution of saprotrophic *Amanitaceae* beyond well-documented regions in Europe and North America and highlights the need for broader surveys of fungal diversity in underexplored regions.

The recognition of *Saproamanita* as a distinct genus separate from ectomycorrhizal *Amanita* was formalized by Redhead et al. (2016) based on molecular phylogenetic evidence showing that saprotrophic species traditionally placed in *Amanita* sect. *Lepidella* forms a monophyletic clade distinct from ectomycorrhizal taxa. This split was based on ITS and other nuclear markers and reflects ecological divergence between saprotrophy and mycorrhizal symbiosis in the family *Amanitaceae* (Redhead et al. 2016).

Compared with the predominantly ectomycorrhizal *Amanita* species that constitute most of the family, saprotrophic *Amanitaceae* are relatively rare but provide valuable insight into evolutionary transitions in nutritional modes. A foundational study by Wolfe et al. (2012) demonstrated that the transition from saprotrophy to an ectomycorrhizal lifestyle in *Amanita* occurred only once, accompanied by the irreversible loss of two key cellulase genes. These findings confirm that mycorrhizal species have lost the ability to grow on complex organic matter and have become dependent on plant hosts for carbon acquisition (Wolfe et al. 2012).

For *Saproamanita thiersii* specifically, convincing evidence of a saprotrophic lifestyle has been provided. Wolfe et al. (2012) used stable carbon isotope analysis, transcriptomic data, and growth assays on cellulose to demonstrate that this species possesses a saprotrophic physiology, confirming its independence from plant hosts for carbon acquisition. Extensive field studies have shown that *S. thiersii* fruits in lawns, pastures, and prairie systems without association with ectomycorrhizal partners and even forms fairy rings in suitable substrates. This species, first reported in Texas in 1952, has now expanded its range to nine U.S. states, indicating its ability to establish in novel habitats (Wolfe et al. 2012).

Our phylogenetic results further confirm that *S. vittadinii* from Iran clusters within a well-supported clade of saprotrophic *Saproamanita* species, clearly separated from ectomycorrhizal lineages. This supports the use of ITS rDNA for species delimitation in these taxa and aligns with broader molecular phylogenetic studies of *Amanitaceae*. A comprehensive study using multi-locus sequence data (including nrLSU, *tef1*-α, *rpb2*, and *tub2*) and examining 1190 specimens demonstrated that the family *Amanitaceae* is a monophyletic lineage comprising five genera, with *Amanita* accounting for 95% of the species in the family (Cui et al. 2018). This study further confirmed that saprotrophic lineages are genetically distinct from ectomycorrhizal clades, and the position of subgenus *Lepidella* as the basal group within the genus is particularly important for understanding the evolution of this family (Cui et al. 2018).

Ecologically, saprotrophic species such as *S. vittadinii* presumably occupy niches in grasslands, disturbed soils, or open habitats where organic matter decomposition is the primary source of carbon and nutrients. Studies on *S. thiersii* have shown that this species appears specifically in grassy habitats without the presence of mycorrhizal host trees (Wolfe et al. 2012). Such saprotrophic life strategies contrast with the ecological roles of ectomycorrhizal *Amanita* species, which are tightly coupled to forest tree hosts and nutrient exchange mutualisms.

Our discovery suggests that *S. vittadinii* may have a broader biogeographic range than previously assumed. Similar to the range expansion pattern reported for *S. thiersii* in North America (Wolfe et al. 2012), the occurrence of this species in Iran implies either underreporting in Asia or a wider but cryptic distribution. The high diversity of *Amanitaceae* in Asia is well illustrated by Cui et al (2018), who reported 50 new species from China alone. This highlights the need for continued field surveys across diverse habitats, especially in grassland and transitional ecosystems in West Asia, to accurately map the distribution and ecological range of saprotrophic *Amanitaceae*. Finally, documenting saprotrophic taxa contributes to understanding ecosystem functioning because saprotrophic fungi play essential roles in nutrient cycling and organic matter decomposition. Highlighting their presence in a new region paves the way for further studies on ecological interactions, population structure, genetic diversity, and potential responses to environmental change.

ACKNOWLEDGMENTS

The authors would like to express their sincere gratitude to the authorities and staff of the Iranian Research Institute of Plant Protection and Razi University, where this research was conducted, for providing the facilities, scientific support, and favorable conditions necessary for successfully carrying out this study.

AUTHOR CONTRIBUTION

Mohammad Reza Asef and Saeed Abbasi planned and supervised the research project. Mohammad Reza Asef collected the specimens and performed the morphological identification. Elham Seidmohammadi conducted the microscopic examinations. Saeed Abbasi and Elham Seidmohammadi performed the molecular analyses. Elham Seidmohammadi submitted the sequences to GenBank. Saeed Abbasi prepared the first draft of the manuscript. All authors reviewed, revised, and approved the final version of the manuscript.

DATA AVAILABILITY

The ITS sequences generated during this study are available in GenBank under the accession numbers listed in the paper. All other data supporting the findings of this study are available from the corresponding author upon reasonable request.

FUNDING

This study was financially supported by the Iranian Research Institute of Plant Protection and Razi University.

DECLARATION

The authors declare no conflicts of interest.

ETHICAL APPROVAL

This study does not involve human participants or animals and therefore ethical approval and consent to participate are not applicable.

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قارچ شناسی ایران جلد ۱۳، شماره ۱، ۱۴۰۵

مقاله کوتاه

نخستین گزارش جنس *Saproamanita* در ایران: گونهٔ *Saproamanita vittadinii*

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10.22092/mi.2026.372695.1347

چکیده

یک بررسی گسترده از گونه‌های *Amanita* در شمال و غرب ایران انجام شد تا تنوع این جنس و آرایه‌های وابسته مستندسازی شود. در میان نمونه‌های جمع‌آوری‌شده، *Saproamanita vittadinii*، یک گونه ساپروتروف و خوراکی، برای اولین بار در ایران ثبت شد که نخستین گزارش از این جنس در کشور محسوب می‌شود. بررسی‌های دقیق ماکروسکوپی و میکروسکوپی برای توصیف ویژگی‌های ریخت‌شناسی این گونه انجام شد و تحلیل‌های مولکولی مبتنی بر توالی‌های ITS-rDNA هویت آن را تأیید نمود. این کشف، قلمرو جغرافیایی شناخته‌شده *S. vittadinii* را گسترش داده و تنوع بوم‌شناختی آرایه‌های مرتبط با آمانیتاهای ساپروتروف در ایران را برجسته می‌کند تا داده‌های پایه‌ای برای پژوهش‌های آتی در زمینهٔ رده‌بندی، بوم شناسی و تبارزایی فراهم آورد.

واژگان کلیدی: آمانیتا، ساپروتروف، قارچ‌های خوراکی، ITS rDNA، *Lepidella*.

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تاریخ دریافت: ۱۴۰۵/۰۲/۱۳، تاریخ بازنگری: ۱۴۰۵/۰۳/۰۸، تاریخ پذیرش: ۱۴۰۵/۰۳/۱۰، تاریخ انتشار برخط: ۱۴۰۵/۰۳/۱۷