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First records of *Onygena equina*, *Panaeolus plantaginiformis*, *Tuber* aff. *iranicum* and rediscovery of *Battarrea phalloides* in Armenia

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In this paper, we report the first occurrence in Armenia of two fungi belonging to the phylum *Ascomycota*: the keratinophilic species *Onygena equina* and a truffle species resembling the recently described *Tuber iranicum*. We also document the first report of the *Basidiomycota* species *Panaeolus plantaginiformis*, along with the rediscovery of *Battarrea phalloides*, which had previously been considered extinct in the country, with the only known record dating back to 1915. The aim of this study is to contribute to the knowledge of the geographic distribution of these fungi. The ecology and conservation status of the recorded species are also discussed. Additionally, ITS rDNA sequences were obtained from specimens of *Onygena equina*, *Panaeolus plantaginiformis*, and *Tuber* aff. *iranicum*.

Key words: *Ascomycota*, *Basidiomycota*, biodiversity, Excavatum clade, sandy stiltball, horn stalkball, South Caucasus.

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Soghoyan Y., Papikyan R., Sargsyan R., Sargsyan N., Gevorgyan M., Antashyan K., Hovsepyan R., Gasparyan A. (2025): První nálezy *Onygena equina*, *Panaeolus plantaginiformis*, *Tuber* aff. *iranicum* a znovuobjevení *Battarrea phalloides* v Arménii. – Czech Mycol. 77(2): 155–168.

Studie referuje o nových nálezech pro Arménii, kde byl poprvé zaznamenán výskyt dvou vřeckatých hub: keratinofilního druhu *Onygena equina* a lanýže připomínajícího nedávno popsaný *Tuber iranicum*. Je zde též poprvé doložen výskyt stopkovýtrusné houby *Panaeolus plantaginiformis* spolu se znovuobjevením *Battarrea phalloides*; tento druh byl v zemi považován za nezvěstný, s jediným záznamem již z roku 1915. Cílem studie je přispět k obohacení poznatků o zeměpisném rozšíření uvedených druhů, přičemž je diskutována i jejich ekologie a ochranářský status. Mimoto byly získány sekvence ITS rDNA z položek *Onygena equina*, *Panaeolus plantaginiformis* a *Tuber* aff. *iranicum*.

INTRODUCTION

Armenia, recognised as a biodiversity hotspot, hosts an impressive diversity of fungal species, with 4,577 species documented to date. Among these, a total of 2,758 belong to the phylum *Ascomycota* and 1,492 to *Basidiomycota* (Nanagulyan & Margaryan 2023). Intensive studies on the country's mycobiota, particularly *Ascomycota* and *Basidiomycota*, began in the second half of the 20th century. Major findings were compiled in two volumes of a publication on macromycetes, the Mycoflora of Armenian SSR (Melik-Khachatryan & Martirosyan 1971, Melik-Khachatryan 1980). Following the independence of Armenia, research on macrofungi has continued, improving our understanding of the country's mycobiota (Nanagulyan & Osipyan 2000, Nanagulyan 2008, Badalyan et al. 2011, Badalyan & Gharibyan 2016, Margaryan 2016, Nanagulyan et al. 2019).

Despite this progress, a number of genera remain underexplored, including *Battarrea* Pers. (*Agaricaceae*, *Agaricales*), *Onygena* Pers. (*Onygenaceae*, *Onygenales*), *Panaeolus* (Fr.) Quél. (including taxa of *Galeropsis* Velen.; *Galeropsidaceae*, *Agaricales*), and *Tuber* P. Micheli ex F.H. Wigg. (*Tuberaceae*, *Pezizales*). In recent years, intensive field research combined with occasional collections by non-specialists has led to the discovery and rediscovery of species from these underrepresented genera in Armenia.

No records of the genus *Onygena* had been published from Armenia yet. Previous studies of keratinophilic fungi in Armenia have mostly focused on microscopic soil taxa (Badalyan et al. 2002). This genus comprises fungi specialised in decomposing keratin-rich substrates such as hairs, feathers, bones, hooves, and horns (Uzun & Kaya 2019, Qiu et al. 2020). Of the accepted taxa, only *O. corvina* Alb. & Schwein. and *O. equina* (Willd.) Pers. have been validated by herbarium specimens; other taxa are considered doubtful (Kandemir et al. 2022, May et al. 2024). *Onygena equina* also serves as the type species for both the *Onygenales* order and the *Onygenaceae* family.

As for the genus *Tuber*, its presence in Armenia is documented in a publication by Badalyan et al. (2005), which mentions species such as *T. aestivum* Vittad., *T. rufum* Pollini, *T. rapaeodorum* Tul. & C. Tul., and *T. scruposum* R. Hesse. Additionally, a new species, *Tuber iranicum* F. Puliga, M. Illice, M. Iotti, D. Baldo &

A. Zambonelli (Excavatum clade), has recently been described from neighbouring Iran. Hitherto, it was known only from that country (Puliga et al. 2020). *Tuber*, commonly known as 'true truffles', is a hypogeous genus of ectomycorrhizal fungi producing belowground tuber-like ascomata and establishing symbiotic associations with trees and shrubs. It includes more than 180 species globally (Bonito et al. 2010).

In regard to the genus *Panaeolus*, belonging to the *Basidiomycota*, recent taxonomic revisions have reshaped our understanding of the genus. *Galeropsis desertorum* Velen. & Dvořák, accepted as the type species of genus *Galeropsis*, was reclassified by Malysheva et al. (2019) as *Panaeolus desertorum* (Velen. & Dvořák) E.F. Malysheva, G. Moreno, Svetash. & M. Villarreal. Additionally, *Gastrocybe iberica* G. Moreno, Illana & Heykoop has also been classified as a synonym of *Panaeolus desertorum*, while the names *Galeropsis andina* Singer and *Galeropsis bispora* Vassilkov are synonymised under *Panaeolus plantaginiformis* (Lebedeva) E.F. Malysheva.

Another genus relevant to our study is *Battarrea*, which is represented in Armenia by a single species, *Battarrea phalloides* (Dicks.) Pers., also known as the scaly-stalked puffball, sandy stiltball, or desert stalked puffball. This species is currently listed as extinct in the Red Book of Armenia (Tamanyan et al. 2010).

Therefore, in this article, we compile several collections from recent years and report the first records of three fungal species from the above-mentioned genera in Armenia: *Onygena equina*, *Panaeolus plantaginiformis*, and *Tuber* aff. *iranicum*, along with the rediscovery of the nationally extinct *Battarrea phalloides*. This study expands our knowledge of the global and regional distribution of these species and underlines Armenia's fungal diversity and its significance for nature conservation.

MATERIAL AND METHODS

Our study is based on collections of target species by K. Antashyan, R. Hovsepyan, and R. Papikyan, as well as on specimens occasionally collected by team members of the Trails for Change NGO from various regions of Armenia. The morphology of the specimens was studied on fresh material. We examined 3 fruitbodies of *T.* aff. *iranicum* including 2 samples dehydrated and metallised for SEM visualisation, and 2 fruitbodies of both *O. equina* and *P. plantaginiformis*. The microscopic features were analysed in the laboratory using an Optika B-510BF (Ponteranica, Italy) light microscope at 1000× magnification with an oil immersion lens. Measurements were taken from 30 randomly selected spores. Scanning electron microphotographs of spores were obtained using the ZEISS EVO10 (Oberkochen, Germany) scanning electron microscopy device. The examined specimens were deposited in the ERE-Fungi (Lichen and Fungi Herbarium of the Institute of Botany after A. Takhtajyan NAS RA, Yerevan, Armenia). The nomenclature of the fungi follows Index Fungorum (www.indexfungorum.org), abbreviations of the herbaria follow Index Herbariorum (https://sweetgum.nybg.org/science/ih/).

ITS rDNA sequences were obtained from the fruitbodies of *O. equina*, *P. plantaginiformis*, and *T.* aff. *Iranicum*, using ITS1/ITS4 or ITS5/ITS4 primers following the protocol in Cubero & Crespo (2002). The obtained sequences have been submitted to the NCBI GenBank database under the following accession numbers: PP804221 (*O. equina*), PX219645 (*P. plantaginiformis*), PX219514 (*T.* aff. *iranicum*).

RESULTS AND DISCUSSION

Onygena equina (Willd.) Pers.

Macroscopic features. The fruitbodies (ascocarps) of *Onygena equina* are characterised by a spherical, slightly flattened head, which is initially whitish, changing to brownish. In the mature stage, the head resembles a tiny 'button mushroom' ranging in size from 1.3 to 4.4 mm. The stipe is cylindrical, measuring 4–6.5 × 0.8–1.3 mm, with a smooth surface texture. The stipe coloration varies from white to cream-white, occasionally darkening to a very light brown (Fig. 1a–b).

Microscopic features. Asci are thin-walled, subglobose, globular, rounded, 8-spored, 15.5–20 × 11–15 µm. Hyphae are up to 5 µm broad, hyaline, thin-walled. Ascospores are broadly elliptical, hyaline, smooth, containing one or two oil drops, 7–9.5 × 4–5 µm.

Habitat and distribution. In Armenia, the species was only found in temperate mixed forest dominated by oriental beech (*Fagus orientalis*), oak (*Quercus* spp.), and hornbeam (*Carpinus betulus*) on decaying horn of domestic goat (Fig. 1a). This species has a wide global distribution range, predominantly in Europe and North America, as reported by the GBIF website (2023a). In the South Caucasus, it has only been documented in Georgia (Nakhutsrishvili 1986).

Conservation. In some countries, *O. equina* is included in national Red Lists. For instance, in the updated Red List of Estonian fungi it is assessed according to the IUCN criteria as EN A2c; D (Saar et al. 2019). Due to its rarity, the species is considered threatened in Biebrza National Park, Poland (Kujawa 2012). Taking into account its dependence on keratin as a substrate, it requires specific niche and habitat conservation strategies. In Armenia, more research on the distribution and population size of the species is necessary. However, because of restricted availability of its unusual habitat (horns, hooves, deer antlers, etc.) in forests, *O. equina* is expected to be extremely rare and has a very limited occurrence. Thus, it needs special conservation measures. We suggest assessing the conservation status of *Onygena equina* for inclusion into the Red Book of Armenia.

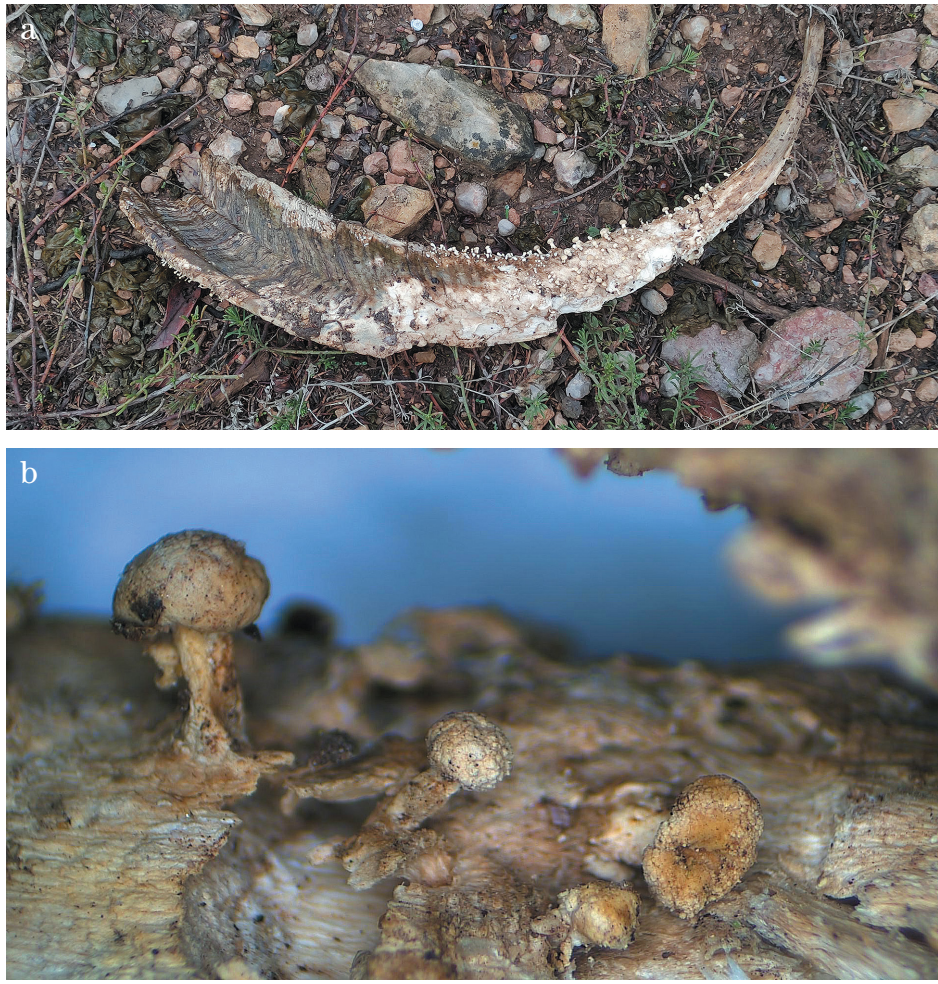

Fig. 1. *Onygena equina* (ERE-Fungi-00012). **a** – young ascocarps on decaying goat horns in natural habitat; **b** – ascocarps photographed in laboratory conditions. Photo R. Papikyan.

DNA sequence. Additionally to the traditional morphological approaches, DNA sequence-based species identification of fungi is commonly conducted, using the internal transcribed spacer (ITS) region as a primary DNA barcode (Schoch et al. 2012). Sequences for only two species within the genus, *O. equina* and *O. corvina*, are currently available in the NCBI GenBank (accessed September 2025). Presently, only 6 nucleotide sequences of *O. equina* are published in the NCBI GenBank, of which only one is an ITS sequence (MZ159461). In addition to the one ITS rDNA sequence of the species represented in the NCBI GenBank, the voucher specimen (ERE-Fungi-00012) was successfully sequenced. The BLASTn

search (https://blast.ncbi.nlm.nih.gov/Blast.cgi) showed a similarity between 98 and 99% with accessions MZ159461 (*O. equina*), PV440458, OR167982 (*O. corvina*), PX117038, and PP278378 (*Onygena* sp.). There are two more ITS sequences available for *O. equina* in the UNITE (UDB018096) and UAMH databases (UAMH03829). However, phylogenetic studies based on ITS sequences of *O. equina* and *O. corvina* have failed to resolve well-supported clades, highlighting the limitations of the current data (May et al. 2024). This shows the need for a more comprehensive dataset, incorporating additional genetic markers and broader sampling to perform a multigene analysis to clarify the taxonomy, but that is not the aim of this work. Considering the genetic and morphological results, specimen ERE-Fungi-00012 can be assigned to the *Onygena equina* complex.

Notes. *Onygena corvina*, a relatively well-studied and widely distributed species of the genus, has not yet been recorded in Armenia. This species is distinct in its substrate preferences, commonly found on owl pellets, bird carcasses, hairs, and wool. It also differs morphologically, with a taller stipe reaching up to 10 mm, as well as smaller asci (10–10.5 × 8.3–8.8 µm) and spores (5.7–5.9 × 2.6–2.7 µm) (Ribes & Pancorbo 2010).

Specimen examined

Armenia. Tavush Province, Ijevan, left bank of Aghstev river, ca 750 m a.s.l., temperate mixed forest, on decaying goat horn, 3 October 2022, leg. R. Papikyan, det. A. Gasparyan (herb. ERE-Fungi-00012).

Panaeolus plantaginiformis (Lebedeva) E.F. Malysheva

Macroscopic features. Fruitbodies differentiated into a cap (pileus) and a stem (stipe), universal and partial veils are absent. Young fruitbodies are initially undifferentiated and spherical, elongating to ovoid, and later developing into distinct cap and stem structures (Fig. 2). The pileus is 12–37 mm high and 4–8 mm wide, is cylindrical to fusiform, often twisted, and ends in a sharp point. Its surface is dry, fibrillose, striate, or finely wrinkled, and ranges in colour from straw to dull ochraceous when fresh, turning greyish brown or ash-coloured when dried. Gills are narrow, crowded, free to adnate, sometimes forked at the base, yellowish when young, becoming rufous or tobacco-brown. The spore print is brown. The stipe is 25–80 mm long and 2–3 mm thick, slender, cylindrical, straight or curved. It is pruinose, fibrillose, nearly smooth when fresh, grooved when dry, and varies in colour from white to yellowish or grey-brown.

Microscopic features. The basidiospores are ellipsoid, smooth, possessing a distinct apical germ pore. They are brown in colour and measure approximately 10–12 × 5.5–7.5 µm. Basidia are clavate (club-shaped), tetrasporic and measure about 20–27 × 7–9 µm. The pileipellis is composed of cylindrical to slightly inflated hyphae, forming a cutis.

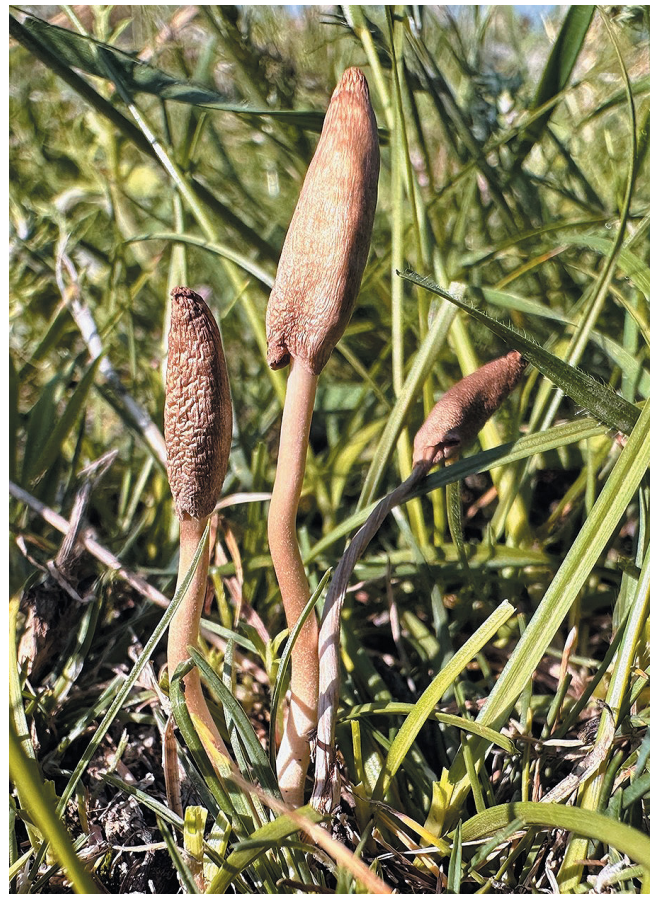

Fig. 2. *Panaeolus plantaginiformis* in its natural habitat (ERE-Fungi-00026). Photo K. Antashyan.

Habitat and distribution. Found in steppes and semi-deserts, singly or in groups, on sandy, loamy-sandy, clayey, and gravelly soils, among grass or in bare spots, in gardens, parks, etc. Ascends into the mountains up to 2,500 m elevation. In Armenia, the species has been found at a single locality on a sandy steppe. The species is known from Argentina, Peru, Caucasia, Central and East Asia, and South Siberia (Malysheva et al. 2019).

Conservation. Due to its restricted habitat, further studies on its distribution and population size at the national level are necessary. Notably, the species shares its locality with another rare fungus, *Battarrea phalloides*. Therefore, the conservation measures proposed for habitat protection of *B. phalloides* are equally applicable to *P. plantaginiformis*.

DNA sequence. The species was initially identified as *Panaeolus desertorum*. However, based on sequencing of the ITS region and considering its distribution patterns, we have re-identified it as *Panaeolus plantaginiformis* because our ITS sequence falls within the clade of *P. plantaginiformis* after Malysheva et al. (2019).

Notes. The genus *Galeropsis* had previously not been recorded in Armenia, while only two species of the genus *Panaeolus* – *Panaeolus acuminatus* (Schaeff.) Quél. and *Panaeolus papilionaceus* (Bull.) Quél. have been reported so far (Nanagulyan 2008). Both species are morphologically distinct from *Panaeolus plantaginiformis*.

Specimen examined

Armenia. Aragatsotn Province, Ashtarak Municipality, near the village of Mughni, ca 1,110 m a.s.l., steppe, in groups, on sandy soil, among grass, 28 April 2025, leg. K. Antashyan, det. K. Antashyan and A. Gasparyan (herb. ERE-Fungi-00026).

Tuber* aff. *iranicum F. Puliga, M. Illice, M. Iotti, D. Baldo & A. Zambonelli

Macroscopic features. Ascomata 10–50 mm broad, subglobose to slightly irregular in shape, often depressed, with a conspicuous basal cavity. The peridium is minutely papillose, exhibiting shades ranging from greyish brown and brown to pinkish grey. The gleba is initially pinkish grey, turning brown as it matures, marbled with cream-coloured branched veins (Fig. 3a).

Microscopic features. Asci globose, subglobose to broadly ellipsoid, 65–120 × 50–85 µm, 2–4-spored, pedicel lacking or rarely prominent, with thin wall. Ascospores broadly ellipsoid, 26–55 × 20–42 µm excluding ornamentation, spore colour red-brown, ornamented with an irregular reticulum with long crests, with commonly only 1 mesh across the spore surface (Fig. 3b–c).

Habitat and distribution. To this day, *Tuber iranicum* had only been collected in Iran, at forest sites located in the Golestan Province in the counties of Kalaleh and Ramyan (Puliga et al. 2020). The Armenian specimen was collected during the construction of a trail in the deciduous forest of Dilijan National Park, dominated by oriental beech (*Fagus orientalis*), oak (*Quercus* spp.), and hornbeam (*Carpinus betulus*), which can be potential hosts for the species.

Conservation. The species was found within a protected area (Dilijan National Park). Further intensive research is needed to better understand its population size and distribution. However, considering active mushroom foraging, local economic interest in truffles, and the development of tourism infrastructure in the species habitat, urgent conservation measures are necessary. As an immediate action, we recommend the Park's authorities avoiding sanitary cuttings at the locality. Additionally, the conservation status of the species should be

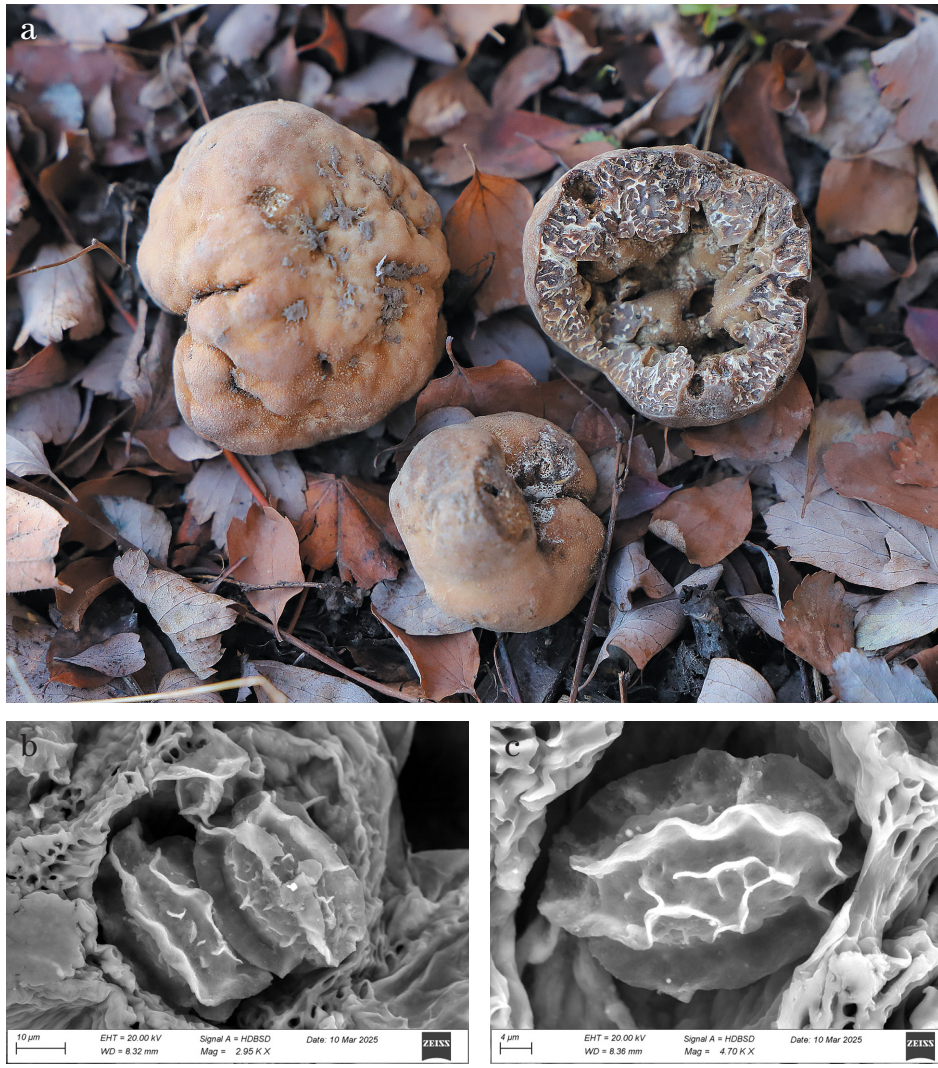

Fig. 3. *Tuber* aff. *iranicum* (ERE-Fungi-00015). **a** – ascomata; **b** – ascus with ascospores under SEM; **c** – ascospores under SEM. Scale bars = 10 µm (b), 4 µm (c). Photo Y. Soghoyan.

assessed for potential inclusion in the Red Book of Armenia. Under the Park's next management plan, its critical habitats should be strictly protected.

D N A s e q u e n c e . Advancing our understanding of the genetic diversity and phylogenetic relationships in the genus *Tuber* is important, particularly in the light of the recently described *T. iranicum*, we sequenced the ITS region. Although the collected material morphologically corresponds to *T. iranicum*, the

BLAST search results indicated a higher sequence similarity with *T. excavatum* Vittad. The latter species is known to exhibit considerable cryptic diversity, comprising at least five distinct clades (Puliga et al. 2020). However, as there is no morphological evidence supporting separation, it was provisionally retained as *T.* aff. *iranicum*. The sequenced specimen most likely represents a separate clade, yet resolving this relationship requires a more detailed phylogenetic analysis beyond the scope of the present study.

Notes. The darker colour of the gleba and the spore ornamentation are considered distinguishing features separating this species from *Tuber excavatum* (Puliga et al. 2020). To date, no other representative of the Excavatum clade has been reported from Armenia.

Specimen examined

Armenia. Tavush Province, Dilijan National Park, ca 1.5 km west of Haghartsin Monastery, ca 1,620 m a.s.l., mostly underground, 20 November 2024, leg. G. Gevorgyan, det. A. Gasparyan (herb. ERE-Fungi-00015).

Battarrea phalloides (Dicks.) Pers.

Macroscopic features. The fruitbody begins as a subterranean, spherical structure, approximately 3–6 cm in diameter. As it matures, it emerges above the ground, developing a distinct stem and spore sac, making it resemble a typical agaric mushroom, although it lacks a true cap with gills (Fig. 4). The peridium is double-layered. The exoperidium is leathery and whitish to brownish, and splits circumscissile (along a horizontal plane) at maturity. The lower part forms a cup-shaped volva at the base of the stem, while the upper part may detach to expose the spore sac. The endoperidium is smooth, nearly spherical, measuring 1.5–6 cm in height and 1.5–5 cm in diameter. This contains the gleba in the form of a powdery spore mass. The spore sac (gleba casing) sits on top of the stem, and is ovoid to spherical in shape, typically 1.0–3.5 cm in height and 3–5 cm in width. The gleba is rust-brown when mature and contains spores and a capillitium. A prominent feature is the slender, cylindrical stem, which may be slightly bulging in the middle and tapers at both ends. It has a length of 15–55 cm, occasionally up to 70 cm, and is 0.4–1.5 cm thick, greyish to ochraceous in colour, and has a woody texture. Its surface is longitudinally fibrillose, often covered with scales or remnants of the peridium. The volva is formed from the lower part of the exoperidium. It measures up to 15 cm in width and 13 cm in height and is composed of two distinct layers.

Microscopic features. Spores are spherical to broadly ovoid, typically 5.0–6.5 µm in diameter, rusty brown in water, thick-walled, densely warted (verruculose), with warts often coalescing into interconnected ridges. Capillitium consisting of two distinct types of hyphal threads: simple capillitial hyphae and elaters.

Fig. 4. *Battarrea phalloides* photographed in laboratory conditions (ERE-Fungi-00019). Photo R. Hovsepyan.

Elaters are formed by elongated, fusiform to cylindrical cells with spiral or annular thickenings.

Habitat and distribution. *Battarrea phalloides* is saprotrophic fungus found in arid, sandy environments, semi-deserts or dry steppes, often in disturbed or open areas. It is a relatively rare species, yet has a broad global distribution (Martín et al. 2013, Uzunov 2014, Ivancevic 2017, Shepherd & Cooper 2017). Records are known from Africa, Asia, Australia, Europe, North, South, and Central America, and Oceania (GBIF 2023b). In the South Caucasus, it has also been documented in Georgia and Azerbaijan (Howladar et al. 2013). The species was first mentioned for Armenia in the second volume of the Mycoflora of Armenian SSR (Melik-Khachatryan & Martirosyan 1971), which states that a fruitbody of this species was discovered in 1915 by Russian mycologist Vorontsov. He subsequently

took the specimen to Russia, leaving only a written record of its discovery in Armenia. Aside from this historical find, no other fruitbodies of *Battarrea phalloides* were recorded on the territory of Armenia until the autumn of 2003.

Conservation. The species is currently under assessment within the Global Fungal Red List Initiative. According to the species profile, it is already included in the national red lists of several countries. In Armenia, it is listed in the Red Book in category Extinct, although the recent records necessitate a reassessment of its conservation status. Land use change poses a primary threat to the species at the newly discovered localities, which are subject to active anthropogenic pressure. Further intensive research is needed to better understand its population size and distribution. While habitats of the red-listed species are legally protected, immediate conservation measures for the identified sites should be discussed with the Ministry of Environment of the Republic of Armenia.

Notes. In April 2025, K. Antashyan reported the rediscovery of this species in Aragatsotn Province, after it had been considered nationally extinct for 110 years. The report was initially shared on Facebook. Following this, his colleague, R. Hovsepyan, reported an unpublished record of the species from 2003 in Ararat Province. These finds highlight the important role of citizen science, social media communication and collaboration between amateur and professional mycologists.

Specimens examined

Armenia. Ararat Province, Aratashen Municipality, near Aratashen archaeological site during excavations, ca 850 m a.s.l., partly underground, October 2003, leg. R. Hovsepyan, det. R. Hovsepyan (herb. ERE-Fungi-00014). – Aragatsotn Province, village of Mughni, between M3 motorway and irrigation water reservoir adjacent to the village, ca 1,250 m a.s.l., 18 April 2025, leg. K. Antashyan, det. M. Gajić (herb. ERE-Fungi-00019).

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