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First molecular record of *Tuber aestivum* from Ukraine

Andrii P. Gryganskyi[1*], Mariia Pasailiuk[2], Larysa I. Kucher[3], Mykyta Peregrym[4,5], Kristen Bruce[1], Melinda Ostendorf[1], Margaryta Lomberg[6], Oksana Mykchaylova[6,7], Nina A. Bisko[6,8], Iryna M. Anishchenko[6], Antonina Vuek[9], Vira B. Kutovenko[3], Liudmyla Kava[9], Benjamin Reed Lemmond[10], Judson Van Wyk[11] & Gregory Bonito[11]

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Abstract. Truffles of the genus *Tuber* include some of the most highly prized and expensive edible fungi worldwide and are particularly valued in the cuisines of many European countries. Some of the most valuable truffle species are native to France, Italy and Spain. Ukraine consumes a lot of wild mushrooms, but truffle hunting is not common despite several historic records of truffles being collected in different Ukrainian regions and ecosystems. However, molecular data are lacking for Ukrainian truffles. In this study, we present the first molecular record, phylogenetic and genomic identification of *Tuber aestivum* in Ukraine, confirmed through a four gene phylogeny and whole genome sequencing and phylogenetic reconstructions. As this is potentially the most common and widespread economic truffle species in Ukraine, this study synthesizes important foundational information on *T. aestivum* in the region, including morphological characterization, soil analysis, biotope description and genomic resources.

Key words: ectomycorrhizal fungi, forest-steppe zone of Ukraine, phylogenomics, summer truffle, truffle biogeography, truffle habitat

Introduction

Tuber aestivum Vittad. (Monographia Tuberacearum: 38(1831) [MB#218597], *Tuberaceae*, *Pezizales*, *Pezizomycetes*, *Ascomycota*) is potentially the most widespread and commonly known truffle species in the world. It forms ectomycorrhizal associations with a broad range of deciduous and coniferous trees and is highly valued in European gastronomy due to its culinary importance (Gazo et al. 2012; Rosa-Gruszecka et al. 2017; Büntgen et al. 2019; Marathe et al. 2023; Truffles Taste).

The species has been extensively studied for its biochemical composition and bioactive compounds, which include antioxidant, antimicrobial, anti-inflammatory, and potential anticancer properties (Beara et al. 2014; Patel et al. 2017). In addition to its ecological importance, *T. aestivum* represents a significant non-timber forest resource across Europe.

Historically, natural populations have been documented across Western Europe and Northern Africa, particularly in Mediterranean regions including France, Italy, and the Iberian Peninsula. In recent decades, reports have expanded the known distribution range to Central and Eastern Europe, as well as parts of Western Asia, including the Caucasus and northern Iran (Gazo et al. 2012; Ammarellou & Alvarado 2018; Malygina et al. 2024). These findings suggest that the ecological and geographical amplitude of the species may be broader than previously assumed.

Despite this expanding distributional knowledge and numerous historical records, Eastern Europe remains comparatively underexplored with respect to molecularly confirmed findings of truffle species. In Ukraine, historical

[1] AeroVironment Inc., 4401 Dayton-Xenia Rd, Dayton, OH 45432, USA
[2] Hutsulshchyna National Nature Park, 84 Druzhby St, Kosiv, Ivano-Frankivsk Obl 78600, Ukraine
[3] Agrobiological Department, National University of Life and Environmental Sciences of Ukraine, 15 Heroiv Oborony St, Kyiv 03041, Ukraine
[4] Luhansk Taras Shevchenko National University, 3 Ivana Banka St, Poltava 36003, Ukraine
[5] Ecology and Genetics Research Unit, University of Oulu, Pentti Kaiteran katu 1, 90570 Oulu, Finland
[6] M. G. Kholodny Institute of Botany, National Academy of Sciences of Ukraine, 2 Tereshchenkivska St, Kyiv 01601, Ukraine
[7] I. Sikorsky Technical University, 37 Beresteiskyi Ave, Kyiv, Ukraine, 03056 Ukraine
[8] D. Motornyi Tavria State Agrotechnological University, 18 Bohdana Khmelnytskoho Ave, Melitopol', Zaporizhia Obl 72312, Ukraine
[9] Plant Protection Department, National University of Life and Environmental Sciences of Ukraine, 13 Heroiv Oborony St, Kyiv 03041, Ukraine
[10] Department of Plant Pathology, University of Florida, 2550 Hull Rd, Gainesville, FL 32611, USA
[11] Department of Plant Soil and Microbial Sciences, Michigan State University, 1066 Bogue St, East Lansing, MI 48824, USA
[*] Corresponding author e-mail: andrii.gryganskyi@avinc.com

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records of *T. aestivum* originate from Carpathian, central, and Crimean regions, although most of these reports are based on morphological observations without molecular verification (Bucholtz 1902; Zerova & Wasser 1972; Dudka & Wasser 1987). In addition, recent anecdotal records and non-verified reports suggest that the species may be more widespread than currently documented, although these data require confirmation.

Tuber aestivum is currently listed as rare in Ukraine, where it is subject to legal protection, although enforcement of harvesting restrictions remains limited (Zaverukha & Shelyag-Sosonko 1996; Domashlinets 2021). Across Europe, population declines have been associated with habitat disturbance, soil degradation, and increasing anthropogenic pressure, including uncontrolled harvesting in some regions.

The aim of this study was to identify collected Ukraine truffles to the species level, and describe their ecological habitat, including soil, vegetation, climatic conditions and putative microbial endosymbionts.

Materials and methods

Study area, geography, and biotope

Six truffle specimens were occasionally found while foraging in Forest-Steppe zone of Ukraine, Cherkasy Oblast, Zvenyhorod District, near the Moryntsi village in July 2021. The study site was classified according to UkrBiotop system (Didukh et al. 2011), the National Habitat Catalogue of Ukrainian Biotopes (Kuzemko et al. 2018), and the EUNIS classification (2012). Vegetation syntaxonomy followed Davydov & Shelyag-Sosonko (2019).

Soil sampling and analysis

Soil samples were collected from 15 points within a 2 m radius of the fruiting body location, including one sample directly adjacent to the truffle. Samples were taken from the upper 0–15 cm soil layer using standardized soil monoliths (5 × 5 × 10 cm; 250 cm^3). Samples were transported on ice to the laboratory of the Department of Soil Science and Soil Protection at National University of Life and Environmental Sciences of Ukraine.

Soil physicochemical properties were analyzed according to DSTU 4362:2004 (Ukraine National Standard). Soil fertility indicators were assessed following Tykhenko et al. (2024). Parameters included texture, humus content, and structural characteristics.

Fruiting body documentation and morphology

The 2021 specimens were deposited in the National Herbarium of Ukraine (KW) under voucher number KW71814. Fruiting bodies were examined at the Department of Mycology, M.G. Kholodny Institute of Botany, National Academy of Sciences of Ukraine. Morphological identification followed Zambonelli et al. (2000).

Microscopy

Light microscopy was performed using Leica EZ4 HD (Leica Microsystems, Germany) and Olympus BX51 (USA) under 5–100× magnification with bright-field illumination. Scanning electron microscopy (SEM) was conducted using a Zeiss Gemini 500 (Carl Zeiss AG, Germany) at 10,000–15,000× magnification. Images were processed using ImageJ and Fiji (NIH, USA). Length, width and length-to-width (Q parameter) ratio of 25 asci and 100 ascospores were measured. Statistical descriptors (mean, SD) were calculated in Microsoft Excel.

DNA extraction and molecular identification

Sample of intact gleba (~100 mg) from the ascocarp was aseptically transferred to 1.5 ml Eppendorf conical tubes with 1 ml CetylTrimethylAmmonium Bromide (CTAB) and stored at −20°C. Fungal biomass was mechanically disrupted in lysis buffer using sterile sand and pestle homogenization for 1 minute. Genomic DNA was extracted from stored gleba samples following Aljanabi & Martinez (1997) with minor modifications.

Target loci included ITS, LSU (28S rDNA), *rpb2*, and *EF1α*, amplified using previously published primers (Table 1). PCR conditions followed Bonito et al. (2013): Step 1. Initial Denaturation 1.30-min at 94°C. Step 2. 24 cycles of: Denaturation at 94°C for 30 sec to separate double-stranded DNA (dsDNA) into single strands (ssDNA). Annealing at 55°C for 30 sec. Extension at 72°C for 1.30 min. Step 3. Twelve cycles of: Denaturation at 94°C for 30 sec to separate double-stranded DNA (dsDNA) into single strands (ssDNA). Annealing at 45°C for 30 sec. Extension at 72°C for 2 min. Step 4. Final extension at 72°C for 10 min. Step 5. Hold at 8°C forever. Purification of PCR products was carried out using Shrimp Alkaline Phosphatase (GE HealthCare Technologies Inc., Chicago, IL, USA) and Exonuclease I (New England Biolabs, Ipswich, MA, USA), following manufacturer protocols. Amplicons were sequenced bidirectionally using ABI 3730xl DNA Analyzer (Azenta Life Sciences, Burlington, MA, USA).

Genome sequencing and assembly

Genomic DNA from dried specimen KW71814 was extracted using a modified CTAB protocol (Fauchery et al. 2018). Sequencing was performed on an Illumina NovaSeq 6000 platform (2×150 bp paired-end, S4 flow cell) at the RTSF Genomics Core Facility (Michigan State University, USA). Raw reads are available under NCBI bioproject PRJNA1112361. Assembly was generated within the pipeline described by Lemmond et al. (2025).

Metagenomic processing and microbiome analysis were performed using the standardized pipeline available at the National Microbiome Data Collaborative EDGE platform (Kelliher et al. 2024), including read classification, assembly, and binning. Taxonomic profiling was conducted using GOTTCHA2 (Freitas et al. 2015) for read-based classification. Only high-confidence taxonomic assignments were considered for interpretation. Microbiome detection workflow includes read-based taxonomic classification, metagenome assembly, and downstream binning and annotation of metagenome-assembled genomes (MAGs). To

Table 1. Primers used for molecular identification

Primer pairs, reference	Sequence 3'–5'
ITS1, ITS4 (White et al. 1990)	5'-TCC GTA GGT GAA CCT GCG G-3' 5'-TCC TCC GCT TAT TGA TAT GC-3'
LROR, LR3 (Vilgalys & Hester 1990)	5'-GTA CCC GCT GAA CTT AAG C-3' 5'-GGT CCG TGT TTC AAG AC-3'
*frpb2*_5f, *rpb2*_7r (Liu et al. 1999)	5'-Y AAY CTG ACY TTR GCY GTY AA-3' 5'-CR GTT TCC TGY TCA ATC TCA-3'
*EF1a*_Df, *EF1a* 2218r (Rehner unpublished)	5'-AAG GAY GGN CAR ACY CGN GAR CAY GC-3' 5'-AT GAC ACC RAC RGC RAC RGT YTG-3'

detect bacterial contaminants FCS-gx screening program as a part of NCBI's Foreign Contamination Screen (FCS) tool suite (Astashyn et al. 2024) was used.

Phylogenetic reconstruction

Four-gene phylogenetic analysis was conducted with a subset of data adopted from Bonito et al. (2013, Table 2). For this data subset, we selected samples that were identified morphologically to the species level. Sequences of 38 reference *Tuber* species were used to build the phylogeny as the ingroup. Sequences of the *Nothojafnea thaxteri*, *Geomorium singeri* and *G. australianum* isolates served as the outgroup. Samples with missing data were less than 20% of the whole data amount.

The data were combined into a single matrix with four partitions: one partition for each locus of the loci ITS, 28S, *rpb2* and *EF1*. The ITS dataset had eight missing records, 28S – five, *rpb2* – 26, *EF1* – 23 missing records. The ITS, 28S rDNA, *rpb2* and *EF1* genomic DNA partitions included 312, 757, 851 and 677 characters, respectively, for a combined data matrix of 1,797 characters. Sequences were aligned individually for each locus using MUSCLE (Edgar 2004). Alignments were visually inspected, and ambiguous regions were excluded using Mesquite 4.01 (Maddison & Maddison 2015). The optimized nucleotide substitution model (GTR + Γ + I) for all partitions was selected using ModelTest 3.06 (Posada & Grandal 1998). Maximum likelihood (ML) phylogenetic trees for combined 4-loci dataset were estimated using Garli-2.0 (Bazinet et al. 2014). To assess conflicting phylogenetic signals from the four loci, we searched for strong incongruence of the nodes by 1,000 ML bootstrap replicates. Statistical support with bootstrap values ≥70% was recognized as significant.

For population-level ITS comparison, ITS sequence of KW71814 was queried against the NCBI database using BLAST algorithm. The top 100 hits were retrieved and used to construct a distance-based tree using the Fast Minimum Evolution method in NCBI tools using BLAST-pairwise alignments.

Results

Study area, geography, and biotope

The studied area is a broad undulating plain with alternating rolling hills, ravines and small valleys (Fig. 1). In 2021, the average July temperature was 23.6°C, and the average temperature in January was −2.4°C, with mean annual temperature of 8.8°C and total annual precipitation 629.3 mm (Meteopost 2010).

Mostly oak-hornbeam forest grows near the village, in which the neighborhood is located where the truffles were found. Its' upper canopy is dominated by *Quercus robur* L. (projecting cover is ~20%, tree age not older than 50 years), *Carpinus betulus* L. in mid-canopy stratum (~80% cover, age 15–20 years), and insignificant presence of *Sambucus nigra* L., saplings of *Acer tataricum* L., and *A. platanoides* L. in the understory. In spring, before the trees fully develop their canopies, the soil surface is covered by *Aegopodium podagraria*, *Anemone ranunculoides*, *Corydalis solida*, *Gagea* spp., *Galeobdolon luteum*, and *Scilla bifolia*. In summer, an herbaceous layer was either absent or composed of solitary *Asarum europaeum* L. (Fig. 2). This vegetation type was assigned to the class *Carpino-Fagetea sylvaticae* Jakucs ex Passarge 1968, the alliance *Carpinion betuli* Issler 1931, and the association *Galeobdolo lutei-Carpinetum* Shevchuk, Bakalyna et Solomakha 1966.

The forest belongs to the D1.2 category: mesophilic eutrophic forests with hornbeam, oak and other broadleaved trees, subcategory D1.2.1: central European oak-hornbeam forests. Habitat type – G1.A Meso- and Eutrophic *Quercus*, *Carpinus*, *Fraxinus*, *Acer*, *Tilia*, *Ulmus* and related woodland, G1.A1 *Quercus–Fraxinus–Carpinus betulus* woodland on eutrophic and mesotrophic soils, G1.A16 Subcontinental *Quercus–Carpinus betulus* forests. This forest was categorized as G:1.215 biotopes of subcontinental hornbeam-oak forests.

Soil sampling and analysis

Soil of the studied area (Haplic Chornozem, classification WRB) is typical low-humus chornozem, coarse-silty light loam texture, developed on loess deposits (Table 3). Soil has well-structured humus horizon and moderate carbonate accumulation in the lower part of the profile as veins and carbonate mycelium. Macropores dominate, providing high aeration of these soils. Content of the particles < 0.01mm is 28,9%, and content of the coarse silt fraction is 42.1%. Humus is of the humate type.

Fruiting body morphology

Fruiting bodies of six collected truffles were hypogeous, black, mostly spherical to ellipsoid, lobed, 2.5–10 cm in diameter, two with basal cavities, two sessile, and weight from 20 to 60 g. Brownish-olivaceous-black peridium covered with large (up to 6 mm) flat-pyramidal wards

Table 2. GenBank accession numbers of the sequences used for phylogenetic reconstruction.

Species	Voucher	Country (state)	NCBI accession #			
			ITS	28S	*rpb2*	*EF1*
Nothojafnea thaxteri	N10	Argentina	JQ925638	JQ925673	JQ954482	JX022561
Geomorium singeri	MES161	Chile	JQ925656	JQ925718	JQ954529	JX022615
Geomorium australianum	OSC130601	Australia	JQ925629	JQ925663	JQ954475	JX022555
Tuber aestivum	KW71814	Ukraine	PX977765 *99–100%, **100%	PX961599 *98–100%, **98–99%	PX975893 *95–100%, **99%	PX975894 *95%, **99–100%
Tuber aestivum	GB202	Italy	GQ217542	JQ925679	JQ954487	JX022565
Tuber aestivum	JT30500	Sweden	HM485340	–	JQ954488	–
Tuber anniae	JT13209	USA (WA)	HM485338	JQ925680	–	JX022567
Tuber anniae	JT22695	USA (OR)	HM485339	JQ925681	–	JX022568
Tuber asa	M1828	Italy	HM485341	JF419278	JQ954489	JX022569
Tuber bellisporum	JT11679	USA (OR)	FJ809855	FJ809826	JQ954490	–
Tuber bellisporum	JT7270	USA (OR)	FJ809856	FJ809856	–	–
Tuber beyerlei	JT32597	USA (OR)	HM485408	JF419286	JQ954491	JX022570
Tuber borchii	GB1	Italy	FJ809852	FJ809799	JQ954492	JX022571
Tuber borchii	GB14	Italy	HM485344	JQ925682	JQ954493	JX022572
Tuber brumale	GB52	Italy	HM485345	JQ925683	JQ954494	–
Tuber brumale	GB53	Italy	FJ809852	JQ925684	JQ954495	–
Tuber californicum	JT28058	USA (CA)	HM485346	JQ925685	JQ954496	JX022574
Tuber canaliculatum	JT28215	USA (NJ)	JQ925643	–	JQ954497	JX022575
Tuber canaliculatum	OSC59072	USA (WV)	HM485347	–	JQ954498	JX022576
Tuber castellanoi	JT19924	USA (CA)	FJ809859	FJ809859	–	–
Tuber castellanoi	JT28069	USA (CA)	FJ809860	FJ809860	JQ954499	–
Tuber dryophilum	GB35	Italy	JQ925644	JQ925687	–	JX022577
Tuber dryophilum	GB37	Italy	HM485354	JQ925688	JQ954501	JX022578
Tuber excavatum	BM100	Spain	FJ748899	JQ925689	–	–
Tuber excavatum	T106A	China	**GQ217540**	–	–	–
Tuber excavatum	OSC80639	Italy	HM485355	JQ925690	–	–
Tuber ferrugineum	MA2721	Italy	–	FJ809809	–	JX022579
Tuber flavidosporum	K213	Japan	**AB553446**	AB553520	AB553560	AB553540
Tuber fulgens	M2435	Italy	HM485358	JQ925691	–	JX022580
Tuber gennadii	BM667	Spain	HM485359	–	JQ954502	JX022581
Tuber gennadii	JT9627	Spain	HM485360	JQ925692	JQ954503	JX022582
Tuber gennadii	M1904	Italy	HM485361	JQ925693	JQ954504	JX022583
Tuber gibbosum	JT26632	USA (WA)	FJ809862	FJ809862	JQ954505	JX022584
Tuber gibbosum	JT30580	USA (OR)	FJ809868	FJ809868	JQ954506	JX022585
Tuber indicum	GB236	China	FJ748906	JQ925694	JQ954507	JX022586
Tuber indicum	GB237	China	FJ748907	FJ809840	–	–
Tuber indicum	GB239	China	–	FJ809822	–	–
Tuber indicum	GB240	China	FJ748908	JQ925695	JQ954508	JX022587
Tuber indicum	JT29410	USA (OR)	FJ748902	JQ925696	JQ954509	JX022588
Tuber japonicum	K228	Japan	**AB553434**	**AB553519**	**AB553559**	**AB553539**
Tuber liaotongense	OSC87602	China	–	FJ809813	–	JX022589
Tuber lyonii	GB108	USA (GA)	FJ748910	JQ925698	JQ954510	–
Tuber lyonii	OSC58244	USA (TX)	–	JQ925699	JQ954511	–
Tuber macrosporum	JT13362	Italy	HM485373	FJ809838	–	JX022590
Tuber magnatum	GB12	Italy	JQ925645	JQ925700	JQ954512	JX022591
Tuber magnatum	GB13	Italy	JQ925646	JQ925701	JQ954513	JX022592
Tuber magnatum	JT19460	Italy	HM485374	FJ809848	–	–
Tuber malacodermum	JT32319	Spain	FJ809889	JQ925702	JQ954514	JX022593
Tuber melanosporum	GB200	Italy	–	JQ925703	JQ954515	JX022594
Tuber mesentericum	CW105	Sweden	–	JQ925704	JQ954516	JX022595
Tuber mexiusanum	ITCV181	Mexico	HM485411	JF419293	–	JX022602
Tuber miquihuanense	ITCV885	Mexico	HM485414	JF419292	–	JX022603
Tuber multimaculatum	OSC62169	Spain	HM485377	JQ925705	–	JX022596
Tuber nitidum	BM105	Spain	FJ809885	FJ809807	JQ954517	JX022597
Tuber oregonense	GB284	USA (OR)	FJ809874	FJ809835	JQ954518	–
Tuber oregonense	JT27945	USA (OR)	FJ809878	FJ809836	–	–
Tuber oregonense	JT30493	USA (OR)	JQ925647	JQ925706	JQ954519	JX022599
Tuber panniferum	JT12835	Spain	HM485380	FJ809846	–	–

Table 1. Continued.

Species	Voucher	Country (state)	NCBI accession #			
			ITS	28S	*rpb2*	*EF1*
Tuber panniferum	JT4969	France	–	JQ925707	–	–
Tuber pseudoexcavatum	CJ408	China	HM485381	FJ809816	–	–
Tuber regimontanum	ITCV909	Mexico	EU375838	EU375838	JQ954520	JX022600
Tuber rufum	GB213	Italy	–	FJ809811	–	JX022601
Tuber shearii	JT12498	USA (NC)	GQ221450	JQ925708	–	–
Tuber shearii	OSC51052	USA (NC)	HM485389	–	JQ954521	–
Tuber sphaerosporum	JT12487	USA (OR)	FJ809853	FJ809805	–	JX022609
Tuber sphaerosporum	JT19772	USA (OR)	FJ809854	FJ809806	–	–
Tuber spinoreticulatum	OSCU188	USA (MD)	FJ809884	FJ809815	JQ954527	JX022608

* % of coverage; ** % of similarity of top 10 BLAST hits in NCBI Nucleotide database;
sequences of Ukrainian specimen on earthy background; in bold sequences, not generated by the authors of this study.

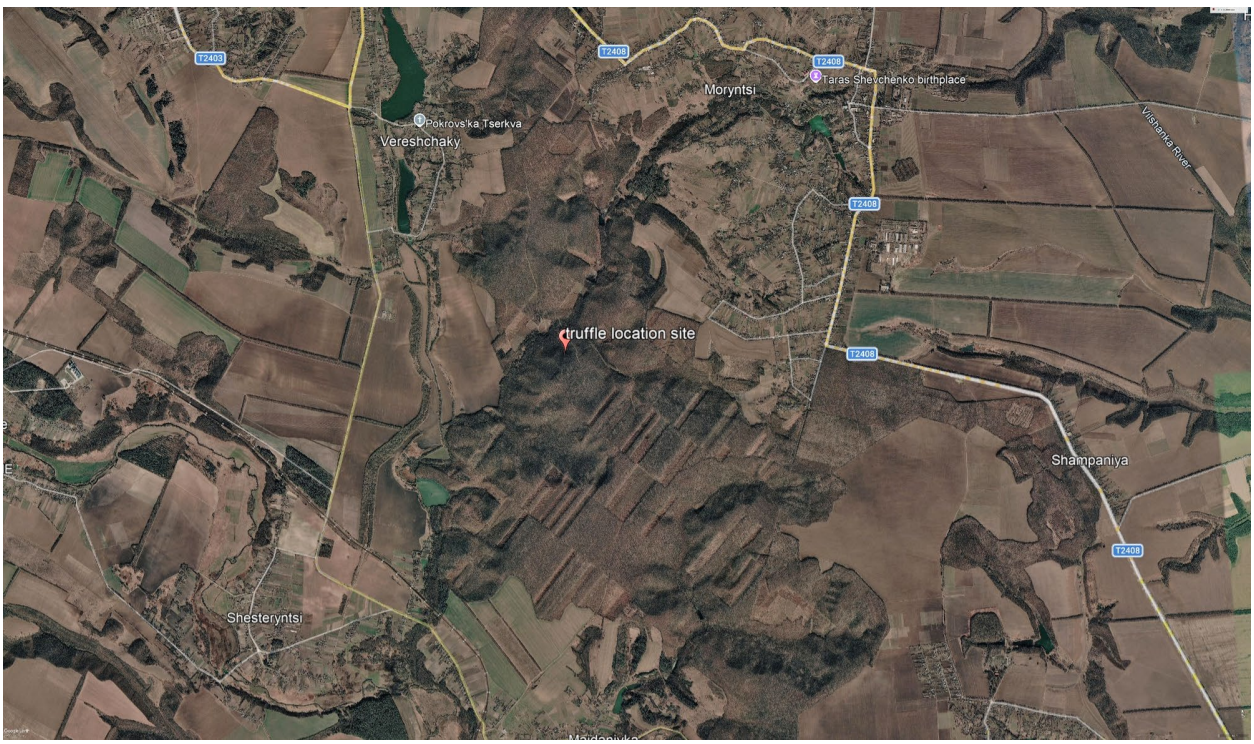

Figure 1. Fragment of the map of village Moryntsi, the birthplace of Ukrainian national poet T. Shevchenko (Cherkasy Oblast, Zvenyhorod District), with geolocation of truffle habitat and photo from Google Earth (49°14′6.33″N, 30°58′30.92″E).

and transverse streaks, almost smooth at the base. Gleba white grayish, in larger fruiting bodies yellowish-brown or brown with numerous whitish or brownish marbling veins (Fig. 3). Smell pleasant, aromatic, not very strong, slightly reminiscent of hazelnuts.

Microscopy

Prototunicate asci globose to oval/ellipsoid 60–70 µm long (SD 4.5) and 50–65 µm wide (SD 5.2), with one to six (mostly 3–4) ascospores in each ascus. Yellowish to brownish ascospores ellipsoid to broad ellipsoid, 26.0–36.0 µm long, an average of 27.5 µm, SD 4.5; and 17.0–26.0 µm wide, average width 20.9 µm, SD 5.2. Q parameter 1.3 (Table S1). Ornamentation brown, coarse alveolate-reticulate, irregular, with crests of episporium up to 3–5 µm high (Figs 4, 5).

Molecular identification

BLAST search (Altschul et al. 1990) of the sequences generated in this study both extracted from the genome assembly, as well as amplified from genomic DNA using PCR unequivocally identify the KW specimen as *T. aestivum* with high coverage and sequence similarity above 99% for all studied genes: *TEF1*, *rpb2*, ITS and 28S regions of ribosomal RNA.

ITS comparison with top 20 sequences in NCBI Nucleotide gives 99.67% to 100% identity with 100% coverage (Table 1). These top 20 sequences were obtained from the fruiting bodies of *T. aestivum*, collected mostly in the Czech Republic, but also in Greece and Poland (Fig. S2A). Sequences of *T. aestivum* from Sweden, France, Italy, Spain, Croatia, Slovenia and Iran had from 1 to 9 different nucleotide base pairs in ITS region (Fig. S2B).

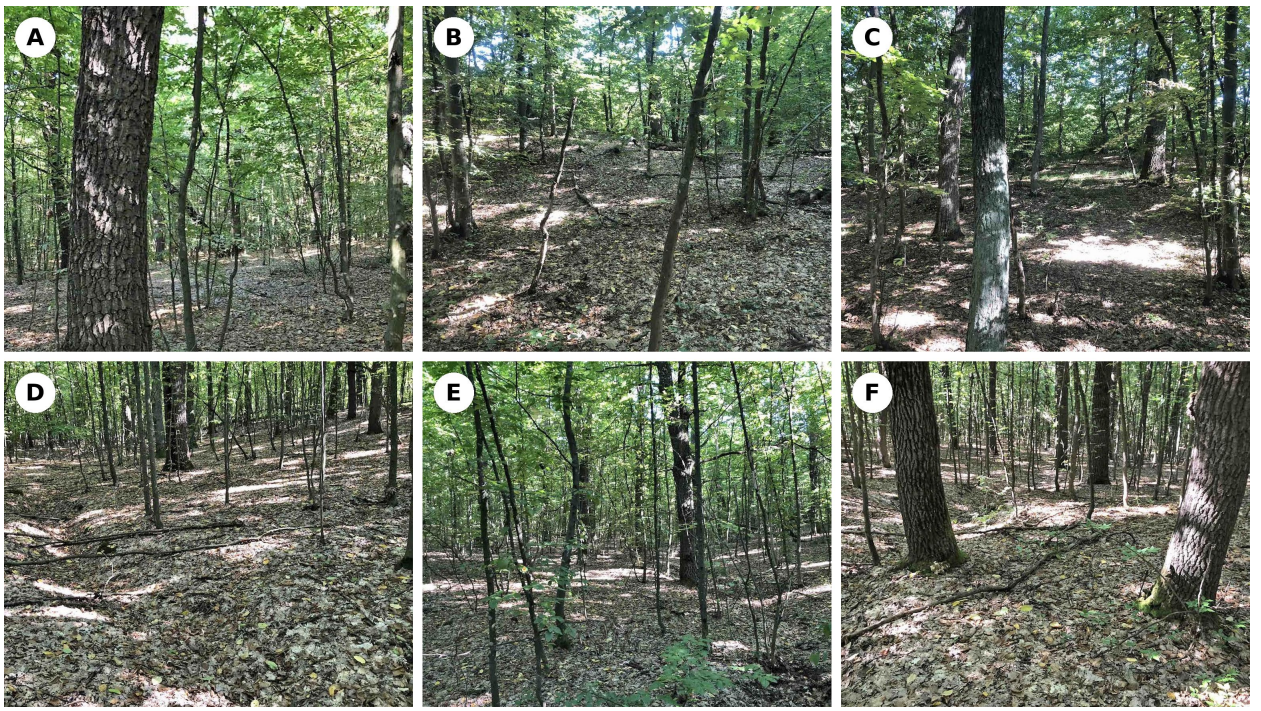

Figure 2. Vegetation structure of the oak–hornbeam forest at the *Tuber aestivum* collection site near Moryntsi, Ukraine. All six photographs (A–F) were taken within a relatively small forest area where truffle ascomata were collected, illustrating local variation in canopy structure, understory density, woody regeneration, light penetration, and forest-floor vegetation. A – multilayered forest structure with larger canopy trees and a dense lower tree stratum; B – relatively open understory with scattered saplings; C – local canopy openings allowing sunlight to reach the forest floor; D – deciduous leaf litter and sparse summer herbaceous vegetation; E – dense woody regeneration beneath the canopy; F – larger trees with relatively open spaces and scattered understory vegetation. Photos: Oleksandr Ilnytskyi.

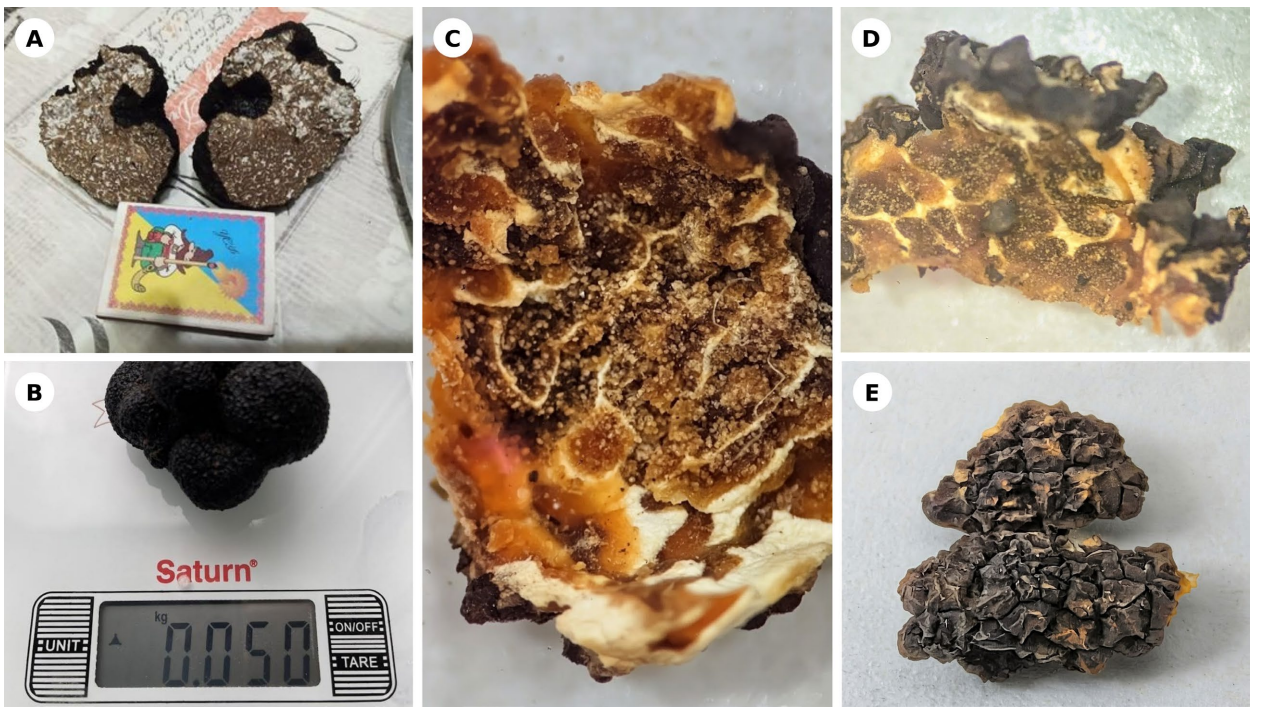

Figure 3. Macroscopic characteristics of *Tuber aestivum* ascomata collected near Moryntsi, Cherkasy Oblast, Ukraine. Individual panels show: A – longitudinally sectioned ascoma showing an internal cavity, with a matchbox for size comparison; B – intact ascoma on a weighing scale; C – sectioned ascoma showing the gleba with characteristic pale marbling veins; D – section of an ascoma showing the internal structure of the gleba and peridium; E – external surface of ascomata showing prominent polygonal peridial warts. Photos: Oleksandr Ilnytskyi.

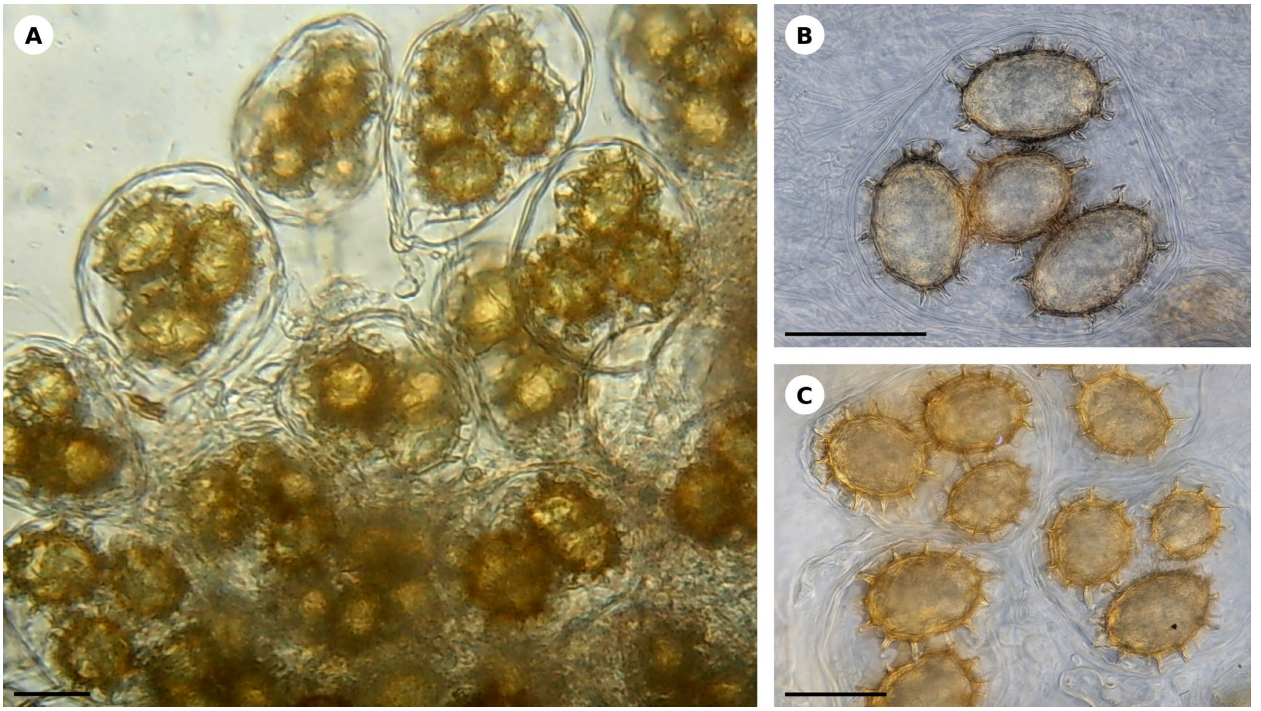

Figure 4. Microscopic characteristics of asci and ascospores of *Tuber aestivum* collected in Ukraine. Individual panels show: A – asci containing different numbers of developing ascospores; B – ascus containing four ascospores and showing a multilayered ascus wall; C – asci containing multiple ascospores at different stages of development. Magnification: ×1000. Scale = 25 µm.

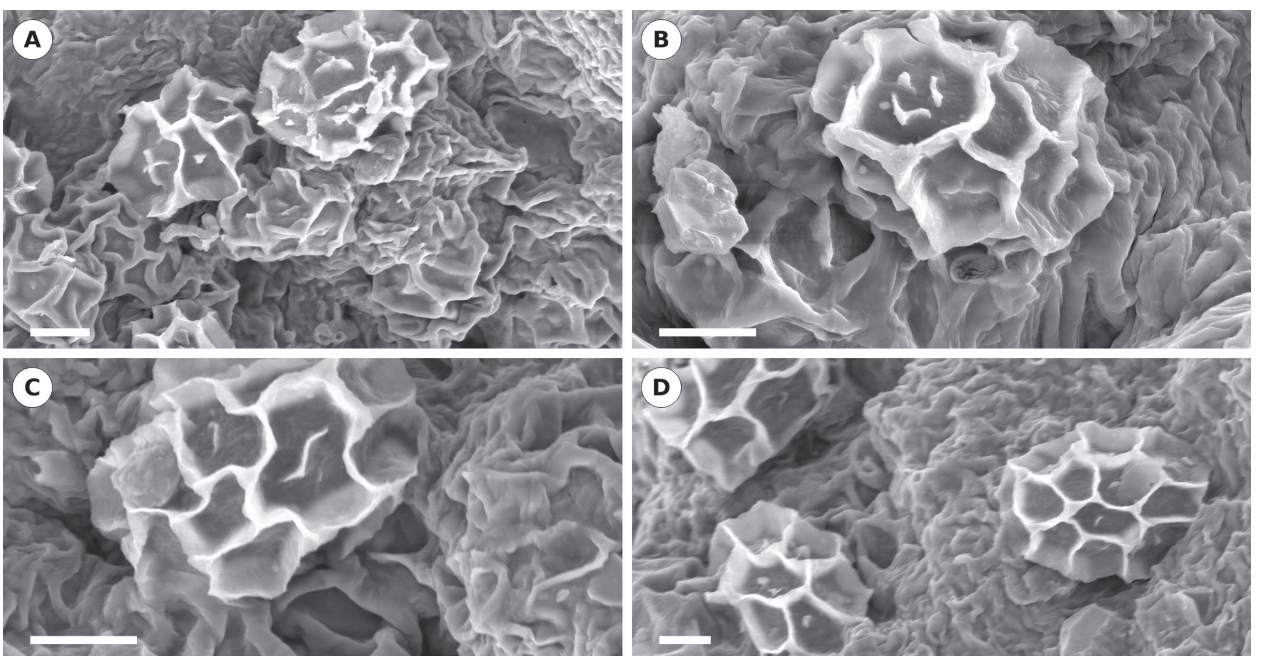

Figure 5. Scanning electron micrographs of ascospores of *Tuber aestivum* collected in Ukraine. Individual panels show: A – two ascospores with well-developed ornamentation and one ascospore partially covered by a collapsed ascus wall (×1500); B – individual ascospore showing characteristic alveolate-reticulate ornamentation (×2500); C – individual ascospore showing the structure of the alveolate-reticulate ornamentation (×2500); D – three ascospores of different sizes showing variation in the development and appearance of surface ornamentation (×1500). Scales: A, B, D = 10 µm; C = 15 µm.

Genome analysis

The 107.2 MB genome assembly for the GB846 specimen was relatively complete, with 93.4% of expected single copy Ascomycota BUSCO genes present in the assembly. The assembly contained 10,983 contigs, with an N50 of 21,502 bp. Metagenomic assembly did not result in any complete metagenome-assembled genomes (MAGs). However, read-based classification detected bacterial signatures corresponding to several *Pedobacter* and two *Bradyrhizobium* species (Fig. S2).

Phylogenetic reconstructions

Our 4-gene phylogenetic tree recognized all major clades in genus *Tuber*. All species with two or more samples group together, most with significant bootstrap values. Sample KW71814 grouped with two *T. aestivum* samples

Table 3. Agrochemical, physicochemical and hydro-physical properties of low-humus chornozem with coarse-silty light loam texture developed on loess deposits.

Parameters	Sampling depth 0–15 cm
pH $_{KCl}$ potentiometrically (1:1)	6.5 ± 0.2
Organic matter (LOI), %	6.3 ± 0.3
Nitrates (NO_3), (FIA) mg/kg	8.7 ± 0.4
Calculated degree of base saturation, %	95.0 ± 1.2
Hydrogen, (H)	14 ± 0.8
Potassium, (K)	1.0 ± 0.1
Calcium, (Ca)	69 ± 1.5
Magnesium, (Mg)	11 ± 0.6
Sodium, (Na)	0 ± 0.0
Sum of cations, mg-eq/100 g	32.2 ± 0.8
Potassium, (K), (Ac) mg/kg	197 ± 8.0
Calcium, (Ca), (Ac) mg/kg	5381 ± 142
Magnesium, (Mg), (Ac) mg/kg	468 ± 15
Sodium, (Na), (Ac) mg/kg	18 ± 1.0
Phosphorus (P), (Mehlich-3), mg·kg^{-1}	15 ± 1.2
Total porosity, calculated, %	52.0 ± 1.4
Field moisture capacity by oven-drying method, %	32.0 ± 1.1
Soil bulk density by cylinder method, g·cm^{-3}	1.12 ± 0.1
Soil particle size fractions, %	
Sand	35.50 ± 1.3
Silt	49.50 ± 1.6
Clay	15.00 ± 1.1

in single gene trees (data not presented), as well as in combined 4-loci phylogenetic reconstruction with 100% bootstrap support. The closest related species are *T. mesentericum* and *T. panniferum* (Fig. 6).

Distance tree with the 100 closest ITS sequences revealed heterogenic structure of *T. aestivum* population. KW71814 sample from Ukraine was placed in the largest clade A (Fig. 7), which contains over one third of ITS sequences. It contains the specimens from Sweden, Denmark, France, Poland, Slovakia, Czechia, Italy, Spain, Greece, and as far east as Caucasus region. The second largest clade, Clade B (over 20% of the sequences), also includes the specimen with the same geographical distribution plus several samples from Slovenia. The third largest clade, Clade C, contains samples from Iran only. Also, the tree contains several more genetically different and more diverse lineages, represented with longer branches with samples from Greece (prevailing number of sequences), Italy, France, Sweden, Iran, and Hungary.

Discussion

The occurrence of *T. aestivum* in Ukraine represents an important addition to the known eastern distribution of this species in Europe. Gourmet truffles remain rarely documented in Ukraine despite potentially suitable habitats and soils. Most previously reported records are historical and were based exclusively on morphological observations (Bucholtz 1902; Zerova & Wasser 1972; Dudka & Wasser 1987), which limits their reliability because *Tuber* species often display overlapping macromorphological and micromorphological characters and substantial intraspecific variability (Paolocci et al. 2004; Leonardi et al. 2021; Ljubojević et al. 2022). Consequently, molecular approaches provide the most reliable means for species-level identification of truffles (Bonito et al. 2010a, b; Leonardi et al. 2021; Amicucci & Ranocchi 2024). Several neighboring countries have already confirmed the presence of *T. aestivum* using DNA-based methods, including regions of Central and Southeastern Europe (Marjanović et al. 2010; Gazo et al. 2012; Rosa-Gruszecka et al. 2017; Daskalopoulos et al. 2025). Therefore, the present study fills an important

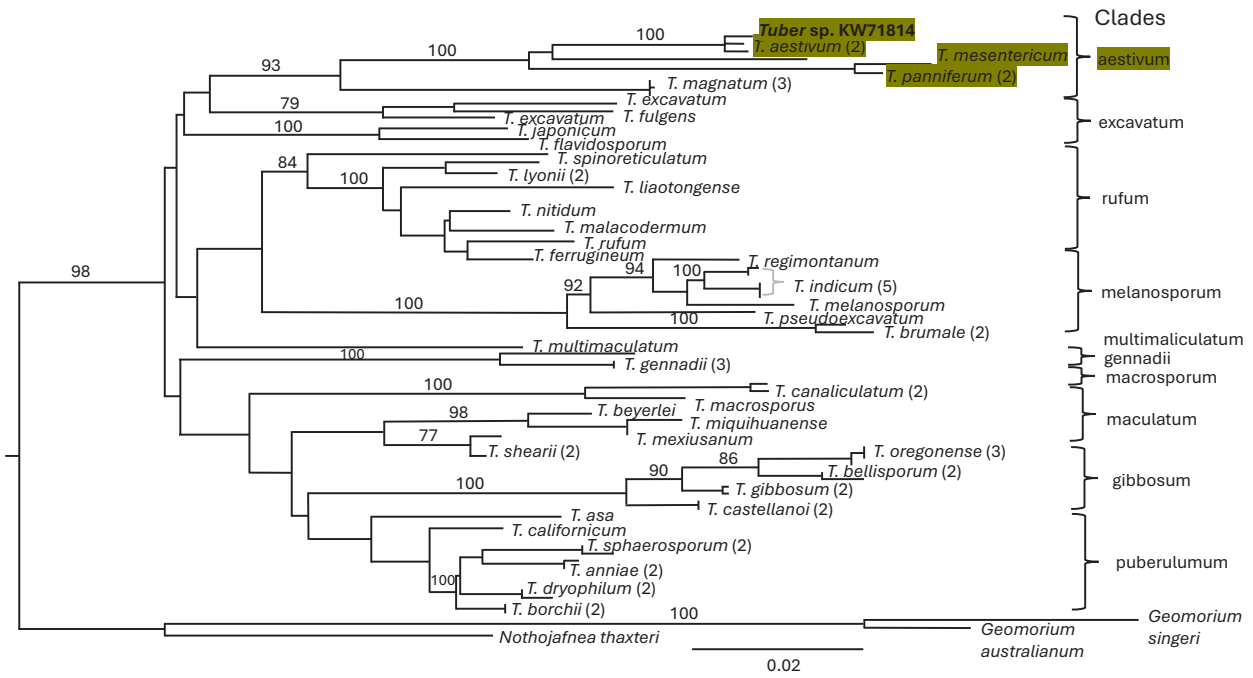

Figure 6. Maximum Likelihood tree of genus *Tuber* based on ITS, 28S, *EF1a*, *rpb2* sequences. Genus' subclades are labeled with the right brace on the right side of the picture. Aestivum clade on earthy background. Sample from Ukraine in bold on earthy background. Number of isolates included in parentheses after the species name. Bootstrap values >70 above or below corresponding nodes. Expected number of substitutions/changes per nucleotide site placed below the tree.

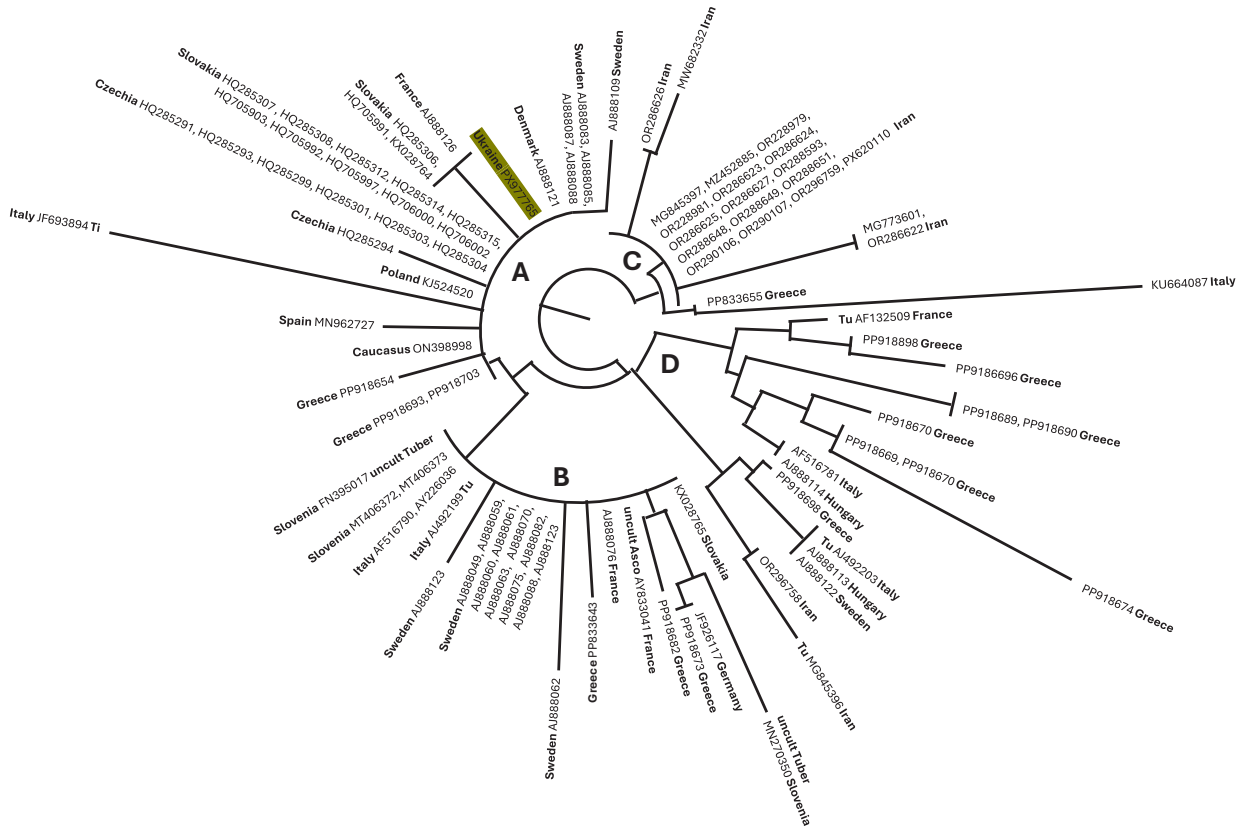

Figure 7. Distance tree of the top 100 sequences to query 3241203, ITS sequence PX977765 of KW71814 Ukrainian *Tuber aestivum* specimen. Sample from Ukraine in bold on earthy background. Ti – *Tuber indicum*, Tu – *Tuber uncinatum*, uncult Asco – uncultured Ascomycete.

biogeographic gap and provides one of the easternmost molecularly documented occurrences of *T. aestivum* together with integrative ecological characterization.

The studied habitat shares many characteristics with temperate European ecosystems which *T. aestivum* naturally inhabits. The oak–hornbeam forest community corresponds well with vegetation types commonly associated with ectomycorrhizal truffle habitats, particularly due to the dominance of *Quercus robur* and *Carpinus betulus*, both recognized as suitable host trees for *T. aestivum* (Bonito et al. 2010a; Stobbe et al. 2013). Climatic conditions recorded during the study year, including mean July temperatures of approximately 23°C, January temperatures around −2 to −3°C, mean annual temperature near 9°C, and total annual precipitation close to 630 mm, remain within the ecological tolerance range reported for *T. aestivum* (Čejka et al. 2020). Previous studies demonstrated that this species possesses considerable ecological plasticity and occupies a broader ecological niche than other commercially valuable truffle species, such as *T. magnatum* and *T. melanosporum* (Chevalier 2012; Leonardi et al. 2021). Moreover, recent work suggested that habitat properties may play a stronger role than regional climate in structuring fungal communities associated with truffle ecosystems (Piñuela et al. 2024). In this context, local microhabitat conditions, including vegetation structure, litter accumulation, soil aeration, and carbonate-rich loess substrates, may partially compensate for broader regional climatic limitations.

The analyzed soil demonstrates edaphic characteristics generally consistent with those reported from productive *T. aestivum* habitats in Europe (Hilszczańska et al. 2019; Bragato et al. 2021). The soil exhibits near-neutral pH, high organic matter content, very high base saturation, and abundant exchangeable calcium, all of which are considered favorable for truffle development and ectomycorrhizal functioning. Productive truffle ecosystems are commonly associated with calcareous soils characterized by elevated calcium availability, moderate nutrient content, and stable aggregation properties (Bragato et al. 2021). In agreement with these observations, the studied chornozem showed high porosity and well-developed structure, indicating favorable aeration and moisture dynamics for mycelial growth. At the same time, the relatively low clay content and predominance of coarse silt fractions distinguish the studied soil from some heavier textured truffle-bearing soils described in Western and Central Europe. Soil texture and aggregate stability influence water retention, microbial activity, and truffle fructification dynamics (Bragato et al. 2021). Nevertheless, the overall chemical and physical properties of the studied soil fall within the range reported for naturally productive *T. aestivum* sites and therefore appear compatible with long-term persistence of the species (Stuke 2024).

The morphology of the collected fruiting bodies and microscopic structures correspond well with published descriptions of *T. aestivum* (Bucholtz 1902; Ljubojević et al. 2022). Features such as the dark pyramidal warts of the peridium, gleba coloration, prototunicate asci, and coarse alveolate-reticulate spore ornamentation fall within the known morphological variability of the species. Minor variation in ascospore dimensions and ornamentation

patterns observed in the Ukrainian material is consistent with the substantial intraspecific variability previously reported for *T. aestivum* across its broad geographical range (Paolocci et al. 2004; Leonardi et al. 2021).

Molecular analyses unequivocally confirmed the identity of the Ukrainian specimen as *T. aestivum*. ITS, 28S, *rpb2*, and *EF1* sequence data consistently placed the specimen within the *T. aestivum* clade with strong bootstrap support, and phylogenetic reconstructions were congruent with previously published multilocus studies of the genus (Bonito et al. 2010a, b, 2013). The ITS similarity of the Ukrainian specimen to isolates from Central, Northern, and Southern Europe further supports the broad phylogeographic continuity of the species throughout Europe. At the same time, the heterogeneous structure observed in the ITS distance tree suggests substantial genetic diversity within *T. aestivum* populations, including geographically widespread clades and several more divergent lineages. The occurrence documented in Cherkasy Oblast likely represents part of a previously overlooked, but established local population rather than a recent introduction, especially considering the proximity of historical records reported from central Ukraine (Bucholtz 1902). Because *T. aestivum* is currently listed in the Red Data Book of Ukraine and known records remain fragmented, the species may be vulnerable to habitat disturbance, recreational pressure, and uncontrolled harvesting.

Preliminary genomic observations additionally provide insight into the microbial associations of the studied truffle material. Although the metagenomic assembly remained fragmented and did not yield complete metagenome-assembled genomes, read-based classification detected bacterial signatures corresponding primarily to *Pedobacter* and *Bradyrhizobium* taxa. Members of these genera are frequently associated with truffle microbiomes and may contribute to nitrogen cycling, volatile production, and ectomycorrhizal functioning (Barbieri et al. 2007; Benucci & Bonito 2016; Liu et al. 2021; Gómez-Molina et al. 2025). However, the taxonomic detection of these bacteria alone does not demonstrate functional nitrogen fixation within the studied system. Compared to the published reference genome of *T. aestivum*, the assembly generated in the present study was smaller and less contiguous, most likely reflecting the lower sequencing depth, environmental origin of the material, and filtering of potentially non-target contigs during assembly.

Eastern Europe remains comparatively underexplored with respect to hypogeous fungi despite the increasing number of recent studies on truffle ecology and diversity (Marjanović et al. 2010; Gazo et al. 2012; Rosa-Gruszecka et al. 2017; Daskalopoulos et al. 2025). The present study provides the first integrative molecularly confirmed documentation of *T. aestivum* in Ukraine based on combined ecological, morphological, phylogenetic, and genomic evidence. These findings suggest that additional populations and potentially other *Tuber* species may remain undetected in Ukrainian forest ecosystems. Future application of environmental DNA approaches and high-throughput sequencing may substantially improve detection and monitoring of hypogeous fungi in the region. Investigation of peripheral populations, such as those occurring in Ukraine, may also contribute to understanding ecological tolerance, adaptation, and future distribution dynamics of truffles under ongoing climatic change.

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Author contributions

Conceptualization: APG and GB; Methodology: all authors; Software: APG, BRL and JV; Formal Analysis: APG, JV, GB; Investigation: APG, JV, ML, OM, NAB, MP, KB, MO, LIK, AV, VIK, MP, LK, BRL and GB; Resources: APG, GB; Data Curation: APG, JV, MP, IMA, VIK, BRL and GB; Writing – Original Draft Preparation: APG, IMA; Writing – Review & Editing: all authors; Visualization: APG, IMA; Project Administration: APG, GB.

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Conflicts of interest

The authors declare no conflicts of interest.

ORCID

Nina A. Bisko https://orcid.org/0009-0006-2417-9887
Gregory Bonito https://orcid.org/0000-0002-7262-8978
Andrii P. Gryganskyi https://orcid.org/0000-0002-6037-0092
Liudmyla Kava https://orcid.org/0000-0001-5757-6738
Larysa I. Kucher https://orcid.org/0000-0002-7211-693X
Vira B. Kutovenko https://orcid.org/0000-0003-0473-1727
Benjamin R. Lemmond https://orcid.org/0000-0001-9594-1927
Margarita Lomberg https://orcid.org/0000-0001-7036-7850
Oksana Mykchaylova https://orcid.org/0000-0001-9212-5094
Mariia Pasailiuk https://orcid.org/0000-0001-6103-1247
Mykyta Peregrym https://orcid.org/0000-0001-5989-4406
Antonina Vuek https://orcid.org/0000-0002-9148-0841
Judson Van Wyk https://orcid.org/0000-0003-2137-2092

Supplementary electronic materials

Figure S1. Alignment of ITS sequences of KW71814 Ukrainian *Tuber aestivum* (query 3241203) to NCBI Nucleotide database records. A – upper sequences with no mismatches (continues grey line of 20 top sequences) or one mismatch (red cross lines in the bottom part); B – sequences with numerous mismatches (red, blue and white cross lines). Download file

Figure S2. Top 100 taxa frequencies in GB846−1_merged genome assembly of the of Ukrainian *T. aestivum* KW71814 specimen. Download file

Table S1. Fruiting bodies, ascospores and asci dimensions. Download file

References

Aljanabi, S. M. & Martinez, I. 1997. Universal and rapid salt-extraction of high quality genomic DNA for PCR-based techniques. *Nucleic Acids Research* 25: 4692–4693. https://doi.org/10.1093/nar/25.22.4692

Altschul, S. F., Gish, W., Miller, W., Myers, E. W. & Lipman, D. J. 1990. Basic local alignment search tool. *Journal of Molecular Biology* 215: 403–410. https://doi.org/10.1016/s0076-6879(96)66029-7

Amicucci, A. & Ranocchi, B. 2024. Overview of molecular methods for quick identification of *Tuber* species. *Italian Journal of Mycology* 53: 45–64. https://doi.org/10.6092/issn.2531-7342/19004

Ammarellou, A. & Alvarado, P. 2018. First report of *Tuber aestivum* var. *uncinatum* from Iran based on morphological and molecular characteristics. *Rostaniha* 18(2): 227–228. https://doi.org/10.22092/BOTANY.2018.116007

Astashyn, A., Tvedte, E. S., Sweeney, D., Sapojnikov, V., Bouk, N., Joukov, V., Mozes, E., Strope, P. K., Sylla, P. M., Wagner, L., Bidwell, S. L., Brown, L. C., Clark, K., Davis, E. W., Smith-White, B., Hlavina, W., Pruitt, K. D., Schneider, V. A. & Murphy, T. D. 2024. Rapid and sensitive detection of genome contamination at scale with FCS-GX. *Genome Biology* 25(1): 60. https://doi.org/10.1186/s13059-024-03198-7

Barbieri, E., Guidi, C., Bertaux, J., Frey-Klett, P., Garbaye, J., Ceccaroli, P., Saltarelli, R., Zambonelli, A. & Stocchi, V. 2007. Occurrence and diversity of bacterial communities in *Tuber magnatum* during truffle maturation. *Environmental Microbiology* 9(9): 2234–46. https://doi.org/10.1111/j.1462-2920.2007.01338.x

Bazinet, A. L., Zwickl, D. J. & Cummings, M. P. 2014. A Gateway for Phylogenetic Analysis Powered by Grid Computing Featuring GARLI 2.0. *Systematic Biology* 63: 812–818. https://doi.org/10.1093/sysbio/syu031

Beara, I. N., Lesjak, M. M., Četojević-Simin, D. D., Marjanović, Ž. S., Ristić, J. D., Mrkonjić, Z. O. & Mimica-Dukić, N. D. 2014. Phenolic profile, antioxidant, anti-inflammatory and cytotoxic activities of black (*Tuber aestivum* Vittad.) and white (*Tuber magnatum* Pico) truffles. *Food Chemistry* 165: 460–466. https://doi.org/10.1016/j.foodchem.2014.05.116

Benucci, G. M. N. & Bonito, G. M. 2016. The truffle microbiome. *Microbial Ecology* 72: 4–8. https://doi.org/10.1007/s00248-016-0755-3

Bonito, G., Gryganskyi, A. P., Vilgalys, R. & Trappe, J. M. 2010a. A global meta-analysis of *Tuber* ITS rDNA sequences: Species diversity, host specificity, and long-distance dispersal. *Molecular Ecology* 19: 4994–5008. https://doi.org/10.1111/j.1365-294X.2010.04855.x

Bonito, G., Trappe, J. M., Rawlinson, P., & Vilgalys, R. 2010b. Improved resolution of major clades within *Tuber* and taxonomy of species within the *Tuber gibbosum* complex. *Mycologia* 101: 1042–1057. https://doi.org/10.3852/09-213

Bonito, G., Smith, M. E., Nowak, M., Healy, R. A., Guevara, G., Cázares, E., Kinoshita, A., Nouhra, E., Domínguez, L. S., Tedersoo, L., Murat, C., Wang, Y., Moreno, B. A., Pfister, D. H., Nara, K., Zambonelli, A., Trappe J. M. & Vilgalys, R. 2013. Historical biogeography and diversification of truffles in the *Tuberaceae* and its newly identified sister lineage. *PLoS ONE* 8: e52765. https://doi.org/10.1371/journal.pone.0052765

Bragato, G., Fornasier, F., Bagi, I., Egli, S. & Marjanović, Ž. 2021. Soil parameters explain short-distance variation in production of *Tuber aestivum* Vittad. in an oak plantation in the central-northern part of the Great Hungarian Plain (Jászság region, Hungary). *Forest Ecology and Management* 479: 118578. https://doi.org/10.1016/j.foreco.2020.118578

Bucholtz, F. 1902. *Beiträge zur Morphologie und Systematik der Hypogaeen*. V. F. Hekker: Riga, Latvia.

Büntgen, U., Jäggi, M., Egli, S., Heule, M., Peter, M., Zagyva, I., Krusic, P. J, Zimermann, S. & Bagi, I. 2019. No radioactive contamination from the Chernobyl disaster in Hungarian white truffles (*Tuber magnatum*). *Environmental Pollution* 252: 1643–1647. https://doi.org/10.1016/j.envpol.2019.06.108

Čejka, T., Trnka, M., Krusic, P. J., Stobbe, U., Oliach, D., Václavík, T., Tegel, W. & Büntgen, U. 2020. Predicted climate change will increase the truffle cultivation potential in central Europe. *Scientific Reports* 10: 21281. https://doi.org/10.1038/s41598-020-76177-0

Chevalier, G. 2012. Cultivation potential of Burgundy truffle. *Acta Mycologica* 47: 127–132.

Daskalopoulos, V., Polemis, E., Konstantinidis, G., Kaounas, V., Tsilis, N., Fryssouli, V., Kouvelis, V. N. & Zervakis, G. I. 2025. The diversity of the genus *Tuber* in Greece – A new species to science in the maculatum clade and seven first national records. *Journal of Fungi* 11: 358. https://doi.org/10.3390/jof11050358

Davydov, D. A. & Shelyag-Sosonko, Yu. R. 2019. Syntaxonomia: Class *Carpino-Fagetea sylvaticae* Jakucs ex Passarge 1968, sojuz *Carpinion betuli* Issler 1931, associatia *Galeobdolo lutei–Carpinetum*. In: Dubyna, D. B., Dziuba, T. P., Yemelyanova, S. M., et al. (eds), *Prodromus of the Vegetation of Ukraine*, pp. 363. Naukova Dumka: Kyiv, Ukraine. (in Ukrainian)

Didukh, Ya. P., Fitsaylo, T. V., Korotchenko, I. A., Yakushenko, D. M., Pashkevych, N. A. & Alyoshkina, U. M. 2011. *Biotopes of the Forest and Forest-Steppe Zones of Ukraine*. M. G. Kholodny Institute of Botany, NAS of Ukraine: Kyiv, Ukraine.

Domashlinets, V. G. 2021. Order of the Ministry of Environmental Protection and Natural Resources of Ukraine dated February 15, 2021 No. 111 "Perelik vydiv Roslyn ta grybiv, zanesenych do Chervonoji knygy Ukrainy (flora)." (in Ukrainian) https://mepr.gov.ua/wp-content/uploads/2023/05/vklyuchennya-858-05.03.2021.pdf

Dudka, I. O. & Wasser, S. P. 1987. *Griby: Spravochnik mikologa i gribnika.* Naukova Dumka: Kyiv, Ukraine. (in Russian)

Edgar, R. C. 2004. MUSCLE: Multiple sequence alignment. *BMC Bioinformatics* 5: 113. https://doi.org/10.1186/1471-2105-5-113

EUNIS. Available from https://eunis.eea.europa.eu/habitats/1113 [accessed in March 2026]

Fauchery, L., Uroz, S., Buée, M. & Kohler, A. 2018. Purification of fungal high molecular weight genomic DNA from environmental samples. *Methods in Molecular Biology* 1775: 21–35. https://doi.org/10.1007/978-1-4939-7804-5_3

Freitas, T. A., Li, P. E., Scholz, M. B. & Chain, P. S. 2015. Accurate read-based metagenome characterization using a hierarchical suite of unique signatures. *Nucleic Acids Research* 43(10): e69. https://doi.org/10.1093/nar/gkv180

Gazo, J., Miko, M. & Chlpik, J. 2012. Genetic resources of summer truffle in Slovakia. *Acta Mycologica* 47: 185–189. https://doi.org/10.5586/am.2012.023

Gómez-Molina, E., Marco, P., Garcia-Barreda, S., González, V. & Sánchez, S. 2025. Effect of selected truffle-associated bacteria and fungi on the mycorrhization of *Quercus ilex* seedlings with *Tuber melanosporum*. *BioTech* 14(3): 69. https://doi.org/10.3390/biotech14030069

Hilszczańska, D., Rosa-Gruszecka, A., Sikora, K. & Szmidla, H. 2019. Soil properties conducive to *Tuber aestivum* fruiting. *Polish Journal of Environmental Studies* 28: 1713–1718. https://doi.org/10.15244/pjoes/89588

Kelliher, J. M., Xu, Y., Flynn, M. C., Babinski, M., Canon, S., Cavanna, E., Clum, A., Corilo, Y. E., Fujimoto, G., Giberson, C., Johnson, L. Y. D., Li, K. J., Li, P.-E., Li, V., Lo, C.-C., Lynch, W., Piehowski, P., Prime, K., Purvine, S., Rodriguez, F., Roux, S., Migun, S., Smith, S. Sarrafan, S., Cholia, S., McCue, L. A., Mungall, C., Hu, B. Eloe-Fadrosh, E. A. & Chain, P. S. G. 2024. Standardized and accessible multi-omics bioinformatics workflows through the NMDC EDGE resource. *Computational and Structural Biotechnology Journal* 23: 3575–3583. https://doi.org/10.1016/j.csbj.2024.09.018

Kuzemko, A. A., Didukh, Ya. P., Onyshchenko, V. A. & Sheffer, Y. 2018. *National Catalogue of the Biotopes of Ukraine*. Klymenko Print House: Kyiv, Ukraine.

Lemmond, B., Bonito, G., Healy, R., Van Wyk, J., Dawson, H. A., Noffsinger, C. R., Stephens, R., Sow, A., Trappe, J. M., Orihara, T.,

Mleczko, P., Kaounas, V. & Smith, M. E. 2025. Phylogenomics, trophic ecology, and systematics of the truffle-forming *Morchellaceae*. *Persoonia* 55: 59–91. https://doi.org/10.3114/persoonia.2025.55.02

Leonardi, M., Iotti, M., Mello, A., Vizzini, A., Paz-Conde, A., Trappe, J. M. & Pacioni, G. 2021. Typification of the four most investigated truffles. *Cryptogamie, Mycologie* 42: 149–170. https://doi.org/10.5252/cryptogamie-mycologie2021v42a9

Liu, D., Pérez-Moreno, J., He, X., Garibay-Orijel, R. & Yu, F. 2021. Truffle microbiome is driven by fruit body compartmentalization rather than soils conditioned by different host trees. *mSphere* 6(4): e0003921. https://doi.org/10.1128/mSphere.00039-21

Liu, Y. J., Whelen, S. & Hall, B. D. 1999. Phylogenetic relationships among ascomycetes: Evidence from an RNA polymerase II subunit. *Molecular Biology and Evolution* 16: 1799–1808. https://doi.org/10.1093/oxfordjournals.molbev.a026092

Ljubojević, S., Vasilišin, L., Vučić, G. & Velemir, A. 2022. Morphological characteristics of summer truffle (*Tuber aestivum* Vittad.) from Bosnia and Herzegovina. *EUREKA: Life Sciences* 2: 9–20. https://doi.org/10.21303/2504-5695.2022.002382

Maddison, W. P. & Maddison, D. R. 2015. *Mesquite: A Modular System for Evolutionary Analysis*, Version 3.03. Available from http://mesquiteproject.org [accessed in March 2026]

Malygina, E. V., Imidoeva, N. A., Belyshenko, A. Yu., Dmitrieva, M. E., Shelkovnikova, V. N., Vlasova, A. A., Telnova, T. Yu., Morgunova, M. M., Konovalov, A. S. & Axenov-Gribanov, D. V. 2024. Comparative study of mechanical and biological pretreatment for releasing spores of black truffle *Tuber aestivum*. *Mycobiology* 52: 278–286. https://doi.org/10.1080/12298093.2024.2391627

Marathe, S. J., Marozzi, G., Lorenzi, L., Donnini, D., Baciarelli-Falini, L., Bashein, A. M. & Shamekh, S. 2023. Whitish truffles in Finland. *Archives of Microbiology* 205: 111. https://doi.org/10.1007/s00203-023-03451-w

Marjanović, Ž., Grebenc, T., Marković, M., Glišić, A. & Milenković, M. 2010. Ecological specificities and molecular diversity of truffles (genus *Tuber*) originating from mid-west of the Balkan Peninsula. *Sydowia* 62(1): 67–87.

Meteopost. 2010. [accessed in May 2026]. Available from: https://meteopost.com/weather/climate/

Ormeño-Orrillo, E. & Martínez-Romero, E. 2019. A genomotaxonomy view of the *Bradyrhizobium* genus. *Frontiers in Microbiology* 10: 1334. https://doi.org/10.3389/fmicb.2019.01334

Paolocci, F., Rubini, A., Riccioni, C., Topini, F. & Arcioni, S. 2004. *Tuber aestivum* vs. *Tuber uncinatum: two morphotypes or two species? FEMS Microbiology Letters* 235: 109–115. https://doi.org/10.1111/j.1574-6968.2004.tb09574.x

Patel, S., Rauf, A., Khan, H., Khalid, S. & Mubarak, M. S. 2017. Potential health benefits of natural products derived from truffles: a review. *Trends in Food Science & Technology* 70: 1–8. https://doi.org/10.1016/j.tifs.2017.09.009

Piñuela, Y., Alday, J. G., Oliach, D. Castaño, C., Büntgen, U., Egli, S., Peña, F. M., Dashevskaya, S., Colinas, C., Peter, M. & Bonet, J. A. 2024. Habitat is more important than climate for structuring soil fungal communities associated in truffle sites. *Fungal Biology* 128: 1724–1734. https://doi.org/10.1016/j.funbio.2024.02.006

Posada, D. & Crandall, K. A. 1998. MODELTEST. *Bioinformatics* 14: 817–818. https://doi.org/10.1093/bioinformatics/14.9.817

Rosa-Gruszecka, A., Hilszczańska, D., Gil, W. & Kosel, B. 2017. Truffle renaissance in Poland. *Journal of Ethnobiology and Ethnomedicine* 13: 36. https://doi.org/10.1186/s13002-017-0163-x

Stobbe, U., Büntgen, U., Sproll, L., Tegel, W. Egli, S. & Fink, S. 2012. Spatial distribution and ecological variation of re-discovered German truffle habitats. *Fungal Ecology* 5(5): 591–599. https://doi.org/10.1016/j.funeco.2012.02.001

Stuke, A. 2024. Influence of geoecological factors on Burgundy truffle (*Tuber aestivum* var. *uncinatum*) habitat in the transition between the German Central Lowlands and Mid-Mountain Range. *Hildesheimer Geographische Studien* 14: 50–88. https://doi.org/10.18442/higs-14-3

Truffles Taste. Available from https://trufflestaste.com [accessed in March 2026]

Tykhenko, O., Martyn, A., Tykhenko, R., Openko, I., Shevchenko, O., Tsvyakh, O., Rokochynskyi, A. & Volk, P. 2024. Impact of comparative assessment of soil quality on determining the value of agricultural land (Ukraine). *Ecological Engineering & Environmental Technology* 25: 252–261. https://doi.org/10.12912/27197050/183900

Vilgalys, R. & Hester, M. 1990. Rapid genetic identification of enzymatically amplified rDNA. *Journal of Bacteriology* 172: 4238–4246. https://doi.org/10.1128/jb.172.8.4238-4246.1990

White, T. J., Bruns, T., Lee, S. & Taylor, J. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis, M. A., Gelfand, D. H., Sninsky, J. J. & White, T. J. (eds), *PCR Protocols: a Guide to Methods and Applications*, pp. 315–322. Academic Press, New York. https://doi.org/10.1016/B978-0-12-372180-8.50042-1

Zambonelli, A., Rivetti, C., Percudani, R. & Ottonello, S. 2000. TuberKey: a Delta-based tool for the description and interactive identification of truffles. *Mycotaxon* 74: 57–76. https://doi.org/10.5962/p.418567

Zaverukha, V. B. & Shelyag-Sosonko, Yu. R. 1996. Litnij triufel, jistivnyj triufel *Tuber aestivum* Vitt. *Chervona Knyga Ukrainy.* Ukrainian Encyclopedia Publishing House. (in Ukrainian)

Zerova, M. Y. & Wasser, S. P. 1972. *Jistivni ta otrujni gryby karpatskych lisiv*. Karpaty Publishing House: Uzhhorod, Ukraine. (in Ukrainian)