http://dergipark.org.tr/trkjnat
Trakya University Journal of Natural Sciences, 25(2): 203-210, 2024
ISSN 2528-9691
DOI: 10.23902/trkjnat.1475517

Research Article

Tuber magnatum Picco: a new record for the Turkish mycobiota

Hasan Hüseyin Doğan[1*], İsmail Şen[2], Hakan Allı[3]

[1] Selçuk University, Faculty of Science, Department of Biology, 42130, Konya, TÜRKİYE
[2] Demirsoy Agriculture Company, R & D Department, Seydikemer, Muğla, TÜRKIYE
[3] Muğla Sıtkı Koçman University, Faculty of Science, Department of Biology, 48000, Kötekli/Muğla, TÜRKİYE

Cite this article as:

Doğan H.H., Şen İ. & Allı H. 2024. *Tuber magnatum* Picco: a new record for the Turkish mycobiota. *Trakya Univ J Nat Sci*, 25(2): 203-210, DOI: 10.23902/trkjnat.1475517

Received: 29 April 2024, Accepted: 30 September 2024, Published: 15 October 2024

Edited by:
Boris Assyov

****Corresponding Author:***
Hasan Hüseyin Doğan
hhdogan@selcuk.edu.tr

ORCID iDs of the authors:
HHD. 0000-0001-8859-0188
İŞ. 0000-0001-5760-5535
HA. 0000-0001-8781-7029

Key words:
Ascomycota
Hypogeous fungi
Taxonomy
White truffle
Türkiye

Abstract: *Tuber magnatum* Picco, the Italian white truffle mushroom recorded for the first time in Türkiye, is a rare and economically valuable mushroom. Samples were collected from Sakarya province during field studies in 2024. The samples were identified as *T. magnatum* according to their macro- and microscopic features. To make molecular confirmation of the species, the samples' ITS1 and ITS4 gene regions were analysed and registered in Genbank with the number PP239641. The sample showed 100% compatibility with other *T. magnatum* accessions in Genbank. The distributions of *Tuber* species in Türkiye were given, and their taxonomic features were compared.

Özet: Türkiye'den ilk kez kaydedilen *Tuber magnatum* Picco, Italyan beyaz trüf mantarı, nadir bulunan ve ekonomik açıdan oldukça değerli bir mantardır. 2024 yılında Türkiye'nin Sakarya bölgesinde yapılan arazi çalışmalarında örnekler toplanmıştır. Bu örnekler makro ve mikroskobik özelliklerine göre *T. magnatum* olarak teşhis edilmiştir. Türlerin moleküler teyidini yapmak için örneklerin ITS1 ve ITS4 gen bölgeleri çalışılmış ve Genbank'a PP239641 numarası ile kayıt edilmiştir. Örnek Genbank'taki diğer *T. magnatum* kayıtlarıyla %100 uyum göstermektedir. Ayrıca Türkiye'de yayılış gösteren *Tuber* türlerinin dağılımları verilmiş ve taksonomik özellikleri karşılaştırılmıştır.

Introduction

Truffles form a symbiotic relationship with various plants, particularly with coniferous or broad-leaved trees). They develop their ascocarps in soil, resembling tubers, and may have different colours ranging from white to black. Unlike other fungal species that form fructification above soil, truffles complete their entire developmental stages in soil. As the fruiting bodies of truffle species begin to mature in soil, they begin to spread aromatic scents with different chemical structures they create in their bodies from the soil to the environment (Jeandroz *et al.* 2008). As the mushroom matures, the odour emitted gradually increases and this attractive odour affects animals. These gorgeous scents are also the main reason why truffles are valued for human consumption. Truffles' unique aroma gives them high economic value, totalling a market of nearly 3 billion euros (Lovrić *et al.* 2020).

According to Bonito *et al.* (2010) and Leonardi *et al.* (2021), approximately 200 *Tuber* species have been identified worldwide. In the Index Fungorum database (2024), 406 names related to *Tuber* genus (including synonyms) appear of which 198 are listed as accepted *Tuber* species. The addition of newly described *Tuber* species to the list in recent years is particularly noteworthy. For instance, 41 new *Tuber* species have been described worldwide within the last five years, including four species in 2019, six in 2020, nine in 2021, 15 in 2022, and seven in 2023.

The collection and consumption of truffle species dates back to ancient times (Hall *et al.* 2008). *Tuber* species grow naturally in Spain, Portugal, Italy, southern Germany, the European part of Russia, North Africa and America (California), where warm and temperate climate prevails (Alsheikh & Trappe 1983, Castellano *et al.* 2004, Wedén *et al.* 2009). Among the truffle species, those with economic value are *Tuber aestivum* (Wulfen) Spreng., *T. brumale* Vittad., *T. borchii* Vittad., *T. magnatum* Picco and *T. melanosporum* Vittad. *Tuber magnatum* and *T. melanosporum* grow in a relatively small geographical area compared to *T. aestivum*, *T. brumale* and *T. borchii*. While *T. magnatum* grows naturally only in Italy, France, Sweden, and the Balkan Peninsula, *T. melanosporum* grows in Spain, France, and Italy. In contrast throughout Europe (Castellano *et al.* 2004, Jeandroz *et al.* 2008).

The studies carried out on truffle species in Türkiye led to identification of 11 new species so far (Table 1). Öztürk *et al.* (1997) gave the first truffle record, *T. brumale*, in the country from Niğde. The same species was also recorded from Denizli, Niğde, Osmaniye, and Samsun at different times. The second record, *T. aestivum*, was reported by Gezer *et al.* (2014) from Denizli. Akata *et al.* (2022) provided the last truffle record as *T. oligospermum*.. A general consideration of the regions where truffles distribute in Türkiye, it appears that they were recorded more frequently in the Aegean and Marmara regions, most probably due to the optimal climate and vegetation these regions provide for a better development. On the other hand, when the forest presence and some suitable geographical features of other regions of Türkiye are considered, it is also possible to find more truffle species. With the special attention of targeted studies in the country, we believe that revealing the existence of truffles is very important in terms of biodiversity and in determining species with commercial value. In field studies carried out in this context, *T. magnatum* was collected for the first time in Türkiye and included in the list of Turkish mushrooms.

Materials and Methods

Macro- and microscopic investigation

Within the scope of the *Tuber* inventory project organised by the General Directorate of Forestry, some regions of Türkiye were investigated to identify truffle species. Truffle hunters Şen Kalyoncu and Onur Özmet participated in the Sakarya region with their Lagotto dogs. *Tuber* samples were checked from areas indicated by specially trained dogs digging the soil. In identifying the collected samples, the specific odours of the fresh samples and habitat characteristics were noted, and colour photographs were taken. Morphological and microscopic characteristics were studied in the laboratory using a microscope (Leica DM3000) microscope and a software measurement module (Leica) were used for microscopic examinations. Melzer's reagent, 10% KOH, methylene blue, Congo red, and distilled water were used as examination media. In determining the spore measurements, width and length of 25 different spores (n) were measured in Melzer's reagent, and the length-to-width ratio (*Q*) was determined. Spores are yellowish to yellow-brown in water, and there is no specific colour in other chemicals (Melzer's reagent or Congo red).

For the identification of the samples, the relevant literature was used (Trappe & Castellano 1991, Pegler *et al.* 1993, Montecchi & Sarasini 2000, Breitenbach & Kränzlin 1983, Trappe *et al.* 2007, Riousset *et al.* 2012). The samples were then dried at +45°C for 3-5 days. The dried samples were placed in ziplock polyethene bags, the collection number and location information was written on them, and stored at the Fungarium of Selcuk University Mushroom Application and Research Centre. The collected samples were given consecutive numbers starting with "HHD", which is the personal collection ID.

Molecular analysis

Total DNA was extracted from dried ascocarp tissue by using the DNeasy Plant Mini Kit (Qiagen, USA) following the manufacturer's protocol. The quality of the DNA was checked based on an electropherogram in 1% TBE-agarose gel. Polymerase chain reaction (PCR) amplification and sequencing amplification of the ITS region of the template DNA was performed using the primers ITS1 (TCCGTAGGTGAACCTGCGG) and ITS4 (TCCTCCGCTTATTGATATGC) (White *et al.* 1990). The PCR product was purified using A & A Biotechnology (Gdynia). A Clean-up kit was used following the manufacturer's protocol before the sequencing. The sequences of *T. magnatum* obtained in this work were deposited at GenBank (National Center for Biotechnology Information, NCBI).

Table 1. Distribution of *Tuber* species in Türkiye.

***Tuber* species**	**Collected regions**	**References**
T. aestivum	Denizli, Muğla, Konya	Gezer *et al.* 2014, Türkoğlu *et al.* 2015, Şen *et al.* 2016, Alkan *et al.* 2018, Özderin *et al.* 2018
T. borchii Vittad.	Kahramanmaraş, Aydın, Denizli, Muğla, Samsun, Tekirdağ, Konya, Gaziantep	Gezer *et al.* 2014, Elliott *et al.* 2016, Kaya *et al.* 2019, Çelik *et al.* 2020, Uzun & Kaya 2020, Çevik *et al.* 2021
T. brumale Vittad.	Niğde, Denizli, Osmaniye, Samsun	Öztürk *et al.* 1997, Gezer *et al.* 2014, Türkoğlu & Castellano 2014, Şen *et al.* 2016
T. ferrugineum Vittad.	Aydın, Muğla, Denizli, Antalya, Konya	Elliott *et al.* 2016, Şen *et al.* 2016, Çelik *et al.* 2020
T. fulgens Quél.	Kırklareli	Akata *et al.* 2020
T. mesentericum Vittad.	Denizli	Castellano & Türkoğlu 2012, Şen *et al.* 2016
T. nitidum Vittad.	Denizli, Uşak, Burdur, Burdur, Kastamonu, Osmaniye, Karaman, Konya	Türkoğlu & Castellano 2014, İleri *et al.* 2020, Çelik *et al.* 2020
T. oligospermum (Tul. & C. Tul.) Trappe	Şanlı Urfa	Akata *et al.* 2022
T. macrosporum Vittad.	Tekirdağ	Doğan 2021
T. puberulum Berk. & Broome	Denizli, Muğla, Aydın, Osmaniye, Artvin and Trabzon	Elliott *et al.* 2016, Şen *et al.* 2016, Uzun & Yakar 2018
T. rufum Pico.	Burdur, Aydın, Antalya, Bolu, Denizli, Kastamonu, Konya, Muğla and Osmaniye	Türkoğlu & Castellano 2014, Türkoğlu *et al.* 2015, Şen *et al.* 2016

For the molecular phylogeny, the Sanger reads obtained from ITS1/ITS4 were assembled using Bioedit version 7.2, and BLAST analyses were performed with the assembled sequences for the identity rate search. The assembled sequences and the nucleotide sequences of the retrieved in-group and out-group members were aligned using the ClustalW algorithm of MEGAX software (Kumar *et al.* 2018). The phylogenetic trees demonstrating the evolutionary history of HHD19491 (Genbank accession no: PP239641) were constructed using the Maximum Likelihood method and K2 nucleotide substitution model with a gamma distribution (Kimura 1980). The bootstrap method was implemented for the accuracy estimation using 1000 bootstrap replicates (Felsenstein 1985).

Results

Phylum ASCOMYCOTA (Berk.) Caval.-Sm.

Class Pezizomycetes O.E.Eriksson & Winka

Order Pezizales J.Schröt.

Family Tuberaceae Dumort.

Tuber magnatum Picco, 1788 (Figs 1, 2)

Ascocarp: Hypogeous, tuberiform, irregular in shape, lobed, gibbous, sometimes flattened, deformed, striated, angular, turbinate, usually 2–4(6) cm in diameter (Fig. 1a). Ascocarp can reach 20 cm or larger (REF). In addition, some specimens can reach to a mass value of 500 grams, representing notable dimensions for the species (Montecchi & Sarasini 2000).

Peridium: Surface smooth, white to pale yellow, yellow, light yellowish when fresh, sometimes with greyish spots, pale yellowish ochraceous to brown when dry, with small greenish papillae (Fig. 1 a); under a magnifying glass, the surface is finely grained. The rounded outline of a ribbed conical base can be observed even in young specimens, unlike other species of *Tuber*. Whitish in section, pseudo-parenchymatic (Fig. 1b).

Gleba: Solid, white when young, then pinkish yellowish, pinkish ochraceous to grey pinkish when ripe, turning brown afterwards, crossed by anastomose whitish veins. The flesh is initially compact and hard and has a somewhat soapy feel.

Smell: *Tuber magnatum* has an intense odour, fragrant with notes of methane and cheese, an intense garlic-like odour or flour-exquisite flavour, smell also like a mixture of lighting gas, fermented cheese, garlic, and shallot; it is characteristic and exquisite.

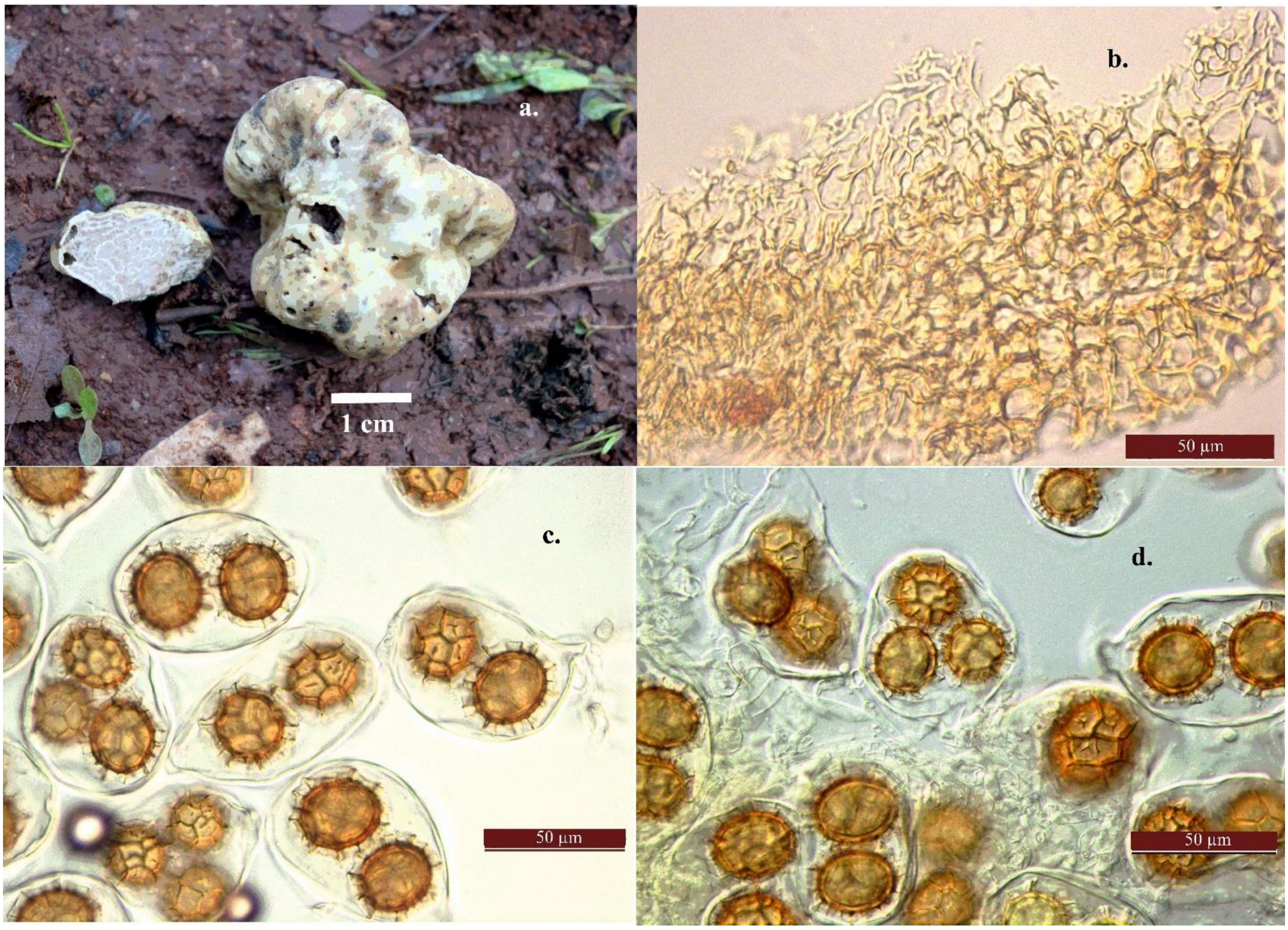

Fig. 1. a. Macroscopic view of *T. magnatum*, **b.** cross section of peridium, **c-d.** asci and ascospores.

Asci: Globose to subglobose or ellipsoid, hyaline, with thin or slightly thickened walls, stalked or non-pedunculate up to 1–2 µm, (60.81–) 65.72 × 77.83 (–80.27) µm in diameter and contain 1 to 3 spores, but could be sometimes 4-spored, (Fig 1. c-d).

Ascospores: Subglobose to broadly ellipsoid, hyaline when young, becoming light yellow to yellowish brown at maturity, with reticulo-alveolate ornamentation, with large meshes, constituting mostly of hexagonal meshes with 4–7 µm across, ornamentation 2–5 µm high. Including their alveolate-reticulate ornamentation, the ascospores measure (27.42–) 31.29–38.66(–41.27) × 30.33–30.47(–36.28) µm, *Q* = 1.031–1.27 (Fig. 1c, d).

Species examined: Sakarya-Hendek, in Poplar plantation, 40°55'03.99"N, 30°55'49.24"E, 122 m, 03.01.2024, HHD19491 (GenBank No: PP239641)

Discussion

Due to cultivation of *Tuber magnatum* is extremely difficult, it is the most expensive of the truffles and a good deal of research has been done to understand its ecology and biology. However, we still know little about its life cycle which remains currently unclear. *Tuber magnatum* is the most valued truffle because of its characteristic, exquisite, very strong smell, and particular, pleasant taste for Italians (Gori 2005, Flammer *et al.* 2013). It might be considered that a white truffle is worth two black one (*Tuber aestivum* and *T. brumale*) as it sells for twice as much and more than the Périgord truffle (*Tuber melanosporum*). It is used in thin strips and should not be heated (Gori 2005, Flammer *et al.* 2013). Its price can reach 7000 €/kg at auctions (Graziosi *et al.* 2022). It is commonly known as the "Italian white truffle," "Piedmont truffle," or "Tartufo Bianco di Alba," as it grows in a very restricted area (Riccioni *et al.* 2016). It is more common in Balkan Peninsula and European countries (Spain, France, Switzerland, Italy, Croatia, Hungary, Romania, Serbia, Bulgaria and Greece) (Hall *et al.* 2008, Marjanović *et al.* 2010, Büntgen *et al.* 2019, Graziosi *et al.* 2022). Although the distribution of *T. magnatum* is restricted in Eastern Europe, more interestingly, it was reported from Thailand based on molecular and morphological investigations (GenBank No: KY427074, KY427075 and KY427076) (Suwannarach *et al.* 2017), suggesting that its distribution is not yet fully determined, or that its natural habitat is continuously expanding due to climate change scenarios (Büntgen *et al.* 2019). *Tuber magnatum* is a whitish truffle characterized with a smooth to suede-like surface, and pale-coloured ascomata (Hall *et al.* 2008, Graziosi *et al.* 2022). It has a yellowish-clay surface and is finely papillose, the spore pattern has few large links, and the colour of the gleba is generally paler than that of other similar species. *Tuber excavatum* Vittad., *T. fulgens* Quél., and *T. dryophilum* Tul. & C. Tul. also have spores with few and large meshes partially similar to those of this species, but the spore size and number of meshes are very different. *Tuber excavatum* has a distinct cavity on the ascocarp, *T. fulgens* has more meshes (up to ten) on the spores, and *T. dryophilum* has dark gleba, globose spores and more meshes than *T. magnatum* (Mello *et al.* 2000, Bonito *et al.* 2011, Alvarado *et al.* 2012, Suwannarach *et al.* 2017).

Table 2. Accession numbers and % identities of sequences used in phylogenetic tree.

Genbank accession number	Species name	Total Score	Max Score	% Identity	Query Cover in %	Reference
PP239641	*Tuber magnatum*	1242	1242	100	-	Current **study**
JQ925646	*Tuber magnatum*	1216	1216	100	92	Bonito *et al.* (2013)
AJ002509	*Tuber magnatum*	1118	1118	100	100	Mello *et al.* (1997)
MZ423175	*Tuber magnatum*	948	948	100	100	Leonardi *et al.* (2021)
KJ524534	*Tuber excavatum*	289	289	93.37	31	Hilszczanska *et al.* (2014)
KC330228	*Tuber excavatum*	289	289	93.37	33	N/A
FM205687	*Tuber excavatum*	289	289	93.37	31	Marjanović *et al.* (2010)
JN392211	*Tuber gennadii*	384	285	95.05	37	N/A
KJ524540	*Tuber maculatum*	356	278	94.94	35	Hilszczanska *et al.* (2014)
FM205644	*Tuber maculatum*	356	278	94.94	36	Marjanović *et al.* (2010)
HM485361	*Tuber gennadii* Castellano	278	278	94.48	28	Bonito *et al.* (2013)
FM205642	*Tuber puberulum*	349	270	94.83	35	Marjanović *et al.* (2010)
FJ809882	*Tuber oregonense*	459	270	90.87	21	Bonito *et al.* (2010)
FJ809881	*Tuber oregonense*	404	270	90.87	21	Bonito *et al.* (2010)
FJ809868	*Tuber gibbosum*	402	270	91.50	19	Bonito *et al.* (2010)
AJ557541	*Tuber borchii*	270	270	94.83	30	Halász *et al.* (2005)
KP276184	*Tuber pseudomagnatum*	340	267	93.37	36	Fan *et al.* (2016)
JN392266	*Tuber oligospermum*	380	267	94.25	40	N/A
JN392259	*Tuber oligospermum*	380	267	94.25	40	N/A
FJ809870	*Tuber gibbosum*	398	267	93.85	17	Bonito *et al.* (2010)
OQ398590	*Choiromyces meandriformis*	360	261	93.71	35	Assyov & Slavova (2023)
OQ779652	*Choiromyces meandriformis*	360	261	93.71	35	N/A
JQ771192	*Tuber pseudomaganatum*	343	265	94.74	34	Fan & Cao (2013)
JF261384	*Tuber borchii*	265	265	94.25	30	Stielow *et al.* (2011)

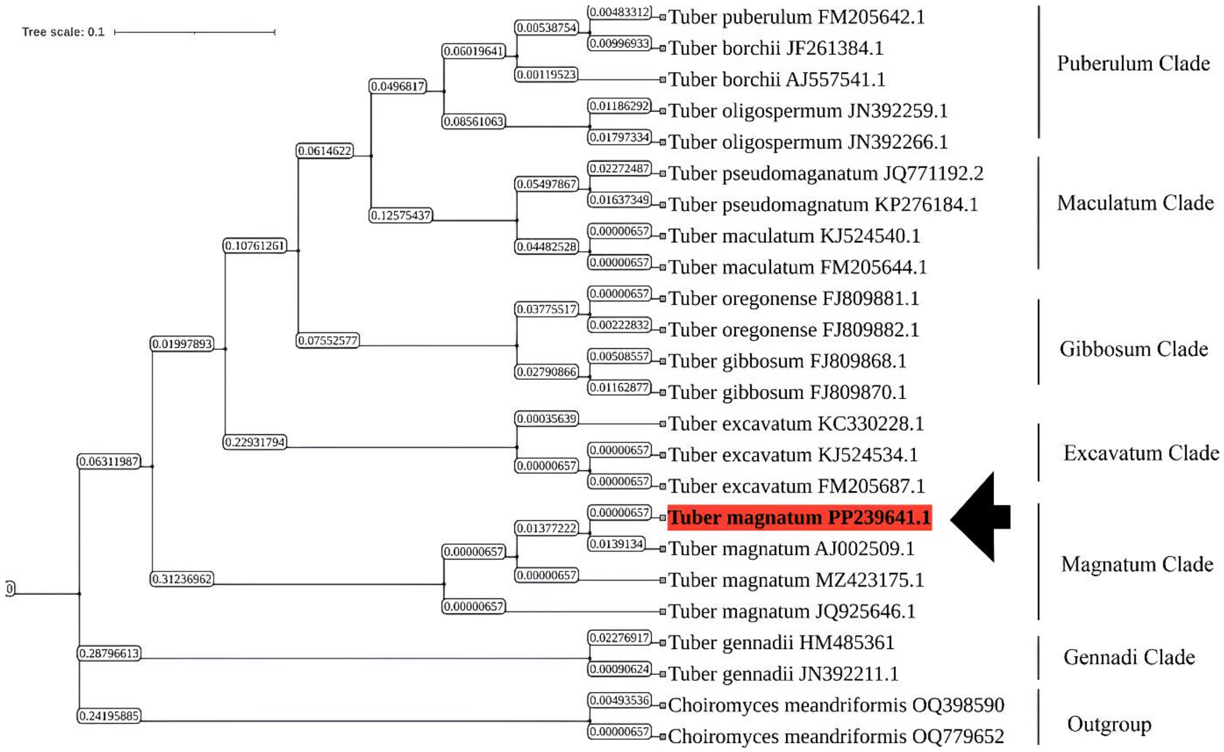

Fig. 2. The evolutionary history was inferred by using the Maximum Likelihood method and Tamura 3-parameter model (Tamura, 1992). The tree with the highest log likelihood (-6135.83) is shown. The percentage of trees in which the associated taxa clustered together is shown next to the branches. Initial tree(s) for the heuristic search were obtained automatically by applying the Maximum Parsimony method. A discrete Gamma distribution was used to model evolutionary rate differences among sites (5 categories (+G, parameter = 4.2756)). The rate variation model allowed for some sites to be evolutionarily invariable ([+I], 37.29% sites). The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. The proportion of sites where at least 1 unambiguous base is present in at least 1 sequence for each descendent clade is shown next to each internal node in the tree. This analysis involved 24 nucleotide sequences. There were a total of 1691 positions in the final dataset. Evolutionary analyses were conducted in MEGA11 (Tamura *et al.* 2021).

Tuber magnatum resembles *T. borchii*, *T. gennadii* (Chatin) Pat., *T. gibbosum* Harkn., *T. maculatum* Vittad., *T. oligospermum*, and *T. oregonense* Trappe, Bonito & P. Rawl. *Tuber magnatum* can be easily separated from others by the cells of peridium. *Tuber gennadii*, *T. maculatum*, *T. oligospermum*, and *T. oregonense* have prosenchymatous cells while *T. magnatum* has pseudoparenchymatous (Mello *et al*. 2000, Bonito *et al.* 2011, Alvarado *et al.* 2012, Suwannarach *et al.* 2017). Sometimes *T. magnatum* is macroscopically confused with *Choiromyces meandriformis* Vittad., the false meander truffle. This, however, has an intense unpleasant odour and presents round spores with digitiform warts (Montecchi & Sarasini 2000).

Tuber magnatum (PP239641) was compared phylogenetically with close species on the NCBI database. All the isolates have query covers above 90% and accession lengths are around 600 base pairs for each specimen. White *Tuber* species were divided into six main clades (Excavatum, Gennadii, Gibbosum, Maculatum, Magnatum, and Puberulum) by Bonito *et al.* (2011). An ITS-based phylogram of PP239641 (Fig. 2) places it within the monophyletic Magnatum clade with high bootstrap (100%) support and Bayesian posterior probabilities (1.0). The analysis findings demonstrated that PP239641 shared a great deal of similarity with other sequences of *T. magnatum* (Table 2 and Fig. 2). The Max scores of *T. magnatum* species are significantly higher than other close species as well as query cover of the other species is very low (Table 2). Compared to other sequences, PP239641 is undoubtedly closest to *T. magnatum* according to the BLAST algorithm and phylogenetic analysis. BLAST algorithm results are given in Table 2. Clades of *Tuber* species are distinctly separated with maximum likelihood analysis with 1000 bootstrap replicates (Fig 2).

Acknowledgement

We would like to thank Şen Kalyoncu (Sakarya, Türkiye) and Onur Özmet (Sakarya, Türkiye) for collecting the samples from the Sakarya region.

Ethics Committee Approval: Since the article does not contain any studies with human or animal subject, its approval to the ethics committee was not required.

Data Sharing Statement: All data are available within the study.

Author Contributions: Concept: H.H.D., Design: H.H.D., İ.Ş., H.A., Execution: H.H.D., H.A., Material supplying: H.H.D., Data acquisition: H.H.D., Data analysis/interpretation: H.H.D., İ.Ş., H.A., Writing: H.H.D., H.A., İ.Ş., Critical review: H.H.D.

Conflict of Interest: The authors have no conflicts of interest to declare.

Funding: The molecular part of this study was supported by Selçuk University, Scientific Research Projects Coordinating Office (BAP/20401081).

References

1. Akata, I., Sevindik, M. & Şahin, E. 2020. *Tuber fulgens* Quél. A new record for Turkish truffles. *Turkish Journal of Agriculture - Food Science and Technology,* 8(11): 2472-2475. https://doi.org/10.24925/turjaf.v8i11.2472-2475.3884
2. Akata, I., Şen, İ., Sevindik, M. & Kabaktepe Ş. 2022. Truffle checklist of Türkiye II with a new record. *Turkish Journal of Agriculture - Food Science and Technology,* 10(10): 1913-1920. https://doi.org/10.24925/turjaf.v10i10.1913-1920.5482
3. Alkan, S., Aktaş, S. & Kaşık G. 2018. Türkiye'deki *Tuber* türleri ve *Tuber aestivum* için yeni bir lokalite. *Selçuk Üniversitesi Fen Fakültesi Dergisi,* 44(1): 25-29.
4. Alsheikh, A.M. & Trappe, J.M. 1983. Taxonomy of *Phaeangium lefebvrei*, a desert truffle eaten by birds. *Canadian Journal of Botany,* 61: 1919-1925.
5. Alvarado, P., Moreno, G. & Manjón, J.L. 2012. Comparison between *Tuber gennadii* and *T. oligospermum* lineages reveals the existence of the new species *T. cistophilum* (Tuberaceae, Pezizales). *Mycologia*, 104: 894-910. https://doi.org/10.3852/11-254
6. Assyov, B. & Slavova, M. 2023. Macrofungi in stands of the endemic pine *Pinus peuce* as inferred from morphological and molecular data. *Proceedings of the Bulgarian Academy of Sciences,* 76(5): 707-715. https://doi.org/10.7546/CRABS.2023.05.06
7. Bonito, G. M., Gryganskyi, A. P., Trappe, J. M. & Vilgalys, R. 2010. A global meta-analysis of *Tuber* ITS rDNA sequences: species diversity, host associations and long-distance dispersal. *Molecular Ecology,* 19(22): 4994-5008. https://doi.org/10.1111/j.1365-294X.2010.04855.x
8. Bonito, G., Smith, M. E., Nowak, M., Healy, R. A., Guevara, G., Cazares, E., Kinoshita, A., Nouhra, E. R., Dominguez, 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 their newly identified southern hemisphere sister lineage. *PLoS One*, 8(1): e52765. https://doi.org/10.1371/journal.pone.0052765
9. Bonito, G., Trappe, J.M., Donovan, S. & Vilgalys, R. 2011. Improved resolution of major clade within *Tuber* and taxonomy of species within the *Tuber gibbosum* complex. *Mycologia*, 102: 1042-1057. https://doi.org/10.3852/09-213
10. Breitenbach, J. & Kränzlin, F. 1983. *Fungi of Switzerland: Vol. 1. Ascomycetes*. Verlag Mykologia, Luzern, 310 pp.
11. Büntgen, U., Lendorff, H., Lendorff, A., Leuchtmann, A., Peter, M., Bagi, I. & Egli, S. 2019. Truffles on the move. *Frontiers in Ecology and the Environment,* 17: 200-202. https://doi.org/10.1002/fee.2033
12. Castellano, M.A. & Türkoğlu, A. 2012. New records of truffle taxa in *Tuber* and *Terfezia* from Türkiye. *Turkish Journal of Botany*, 36: 295-218. https://doi.org/10.3906/bot-1106-10
13. Castellano, M.A., Trappe, J.M. & Luoma, D.L. 2004. Sequestrate fungi. In: G.M. Mueller, G.F. Bills and M.S. Foster (eds.). *Biodiversity of Fungi: inventory and monitoring methods*, Elsevier, Burlington, 197-213.
14. Çelik, A., Uzun, Y. & Kaya, A. 2020. Macrofungal biodiversity of Güneysınır district (Konya-Türkiye). *Mantar Dergisi,* 11(1): 75-83. https://doi.org/10.30708/mantar.662015
15. Çevik, F.T., Uzun, Y. & Kaya A. 2021. Ereğli (Konya) yöresinde belirlenen makromantarlar. *Mantar Dergisi*, 12(2): 138-178. https://doi.org/10.30708/mantar.960666
16. Doğan, H.H. 2021. A new truffle species addition, *Tuber macrosporum* Vittad., to Turkish mycota. *Trakya University Journal of Natural Sciences,* 22(2): 139-146. https://doi.org/10.23902/trkjnat.873651
17. Elliott T.F., Türkoğlu A., Trappe J.M. & Yaratanakul Güngör M. 2016. Turkish truffles 2: eight new records from Anatolia. *Mycotaxon,* 131: 439-453. https://doi.org/10.5248/131.439
18. Fan, L., & Cao, J.Z. 2013. Two new species of white truffle from China. *Mycotaxon*, 121(1): 297-304.
19. Fan, L., Han, L., Zhang, P.R. & Yan, X.Y. 2016. Molecular analysis of Chinese truffles resembling *Tuber californicum* in morphology reveals a rich pattern of species diversity with emphasis on four new species. *Mycologia*, 108(2): 344-353.
20. Felsenstein, J. 1985. Confidence limits on phylogenies: An approach using the bootstrap. *Evolution*, 39: 783-791. https://doi.org/10.2307/2408678
21. Flammer, R., Flammer, T. & Reil P. 2013. *Les truffes*, IHW Verlag. 80 pp.
22. Gezer, K., Kaygusuz, O., Çelik, A. & Işıloğlu M. 2014. Ecological characteristics of truffles growing in Denizli province, Türkiye. *Journal of Food Agriculture & Environment*, 12(2): 1105-1109.
23. Gori, L. 2005. *Funghi Ipogei Della Lucchesia di Aitre Province Italiane e dall' Estero*. Pacini Fazzi, 316 pp.
24. Graziosi, S., Hall, I.R. & Zambonelli, A. 2022. The mysteries of the white truffle: Its biology, ecology and cultivation. *Encyclopedia*, 2: 1959-1971. https://doi.org/10.3390/encyclopedia2040135
25. Halász, K., Bratek, Z., Szegő, D., Rudnóy, S., Rácz, I., Lásztity, D. & Trappe, J.M. 2005. Tests of species concepts of the small, white, European group of *Tuber spp.* based on morphology and rDNA ITS sequences with special reference to *Tuber rapaeodorum*. *Mycological Progress*, 4: 281-290.

26. Hall, I., Brown, G. & Zambonelli, A. 2008. *Taming The Truffle*: *The History, Lore and Science of The Ultimate Mushroom*. Timber Press, Portland, 304 pp.
27. Hilszczanska, D., Rosa-Gruszecka, A., Sikora, K. & Szmidla, H. 2014. Soil factors determining the production of *Tuber* spp. ascocarps in natural stands. Database: http://www.ncbi.nlm.nih.gov/nuccore/KJ524536
28. İleri, R., Uzun, Y. & Kaya, A. 2020. Macromycetes of Karadağ (Karaman) and its environs. *Mantar Dergisi,* 11(1): 57-63. https://doi.org/10.30708/mantar.654111
29. Index Fungorum, (2024). https://www.indexfungorum.org/names/Names.asp
30. Jeandroz, S., Murat, C., Wang, Y., Bonfante, P & Le Tacon, F. 2008. Molecular phylogeny and historical biogeography of the genus *Tuber*, the 'True Truffles'. *Journal of Biogeoprahy,* 35(5): 815-829. https://doi.org/10.1111/j.1365-2699.2007.01851.x
31. Kaya, A., Uzun, Y., Karacan, H.İ. & Yakar, S. 2019. Contributions to the macromycota of Gaziantep province. *Kastamonu Universitesi Orman Fakültesi Dergisi,* 19(3): 329-341. https://doi.org/10.30616/ajb.749820
32. Kimura, M. 1980. A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. *Journal of Molecular Evolution,* 16(2): 111-1120. https://doi.org/10.1007/BF01731581
33. Kumar, S., Stecher, G., Li, M., Knyaz, C. & Tamura, K. 2018. MEGA X: Molecular evolutionary genetics analysis across computing platforms. *Molecular Biology and Evolution,* 35(6): 1547-1549. https://doi.org/10.1093/molbev/msy096
34. Leonardi, M., Iotti, M., Mello, A., Vizzini, A., Paz-Conde, A., Trappe, J. & Pacioni, G. 2021. Typification of the four most investigated and valuable truffles: *Tuber aestivum* Vittad., *T. borchii* Vittad., *T. magnatum* Picco and *T. melanosporum* Vittad. *Cryptogamie Mycologie*, 42(9): 149-170. https://doi.org/10.5252/cryptogamie-mycologie2021v42a9
35. Lovrić, M., Da Re, D., Vidale, E., Prokofieva, I., Wong, J., Pettenella, D., Verkerk, P.J. & Mavsar, R. 2020. Non-wood forest products in Europe – a quantitative overview. *Forest Policy and Economics,* 116: 102175. https://doi.org/10.1016/j.forpol.2020.102175
36. Marjanović, Ž., Grebenc T., Marković, M., Glišić, A. & Milenković, M. 2010. Ecological specificity and molecular diversity of truffles (Genus *Tuber*) originating from mid-west of the Balkan Peninsula. *Sydowia,* 62: 67-87.
37. Mello, A., Garnero, L., Meotto, F. & Bonfante, P. 1997. Specific primers for rapid typing of *Tuber borchii* mycorrhizal roots. *Symposium on Plant Biotechnology as a tool for the Exploitation of Mountain Lands*, ISHS Acta Horticulturae 457. https://doi.org/10.17660/ActaHortic.1998.457.28
38. Mello, A., Vizzini, A., Rollo, F., Bonfante, P. & Trappe, J.M. 2000. *Tuber borchii* versus *Tuber maculatum*: neotype studies and DNA analysis. *Mycologia,* 92: 326-331. https://doi.org/10.2307/3761569
39. Montecchi, A. & Sarasini, M. 2000. *Funghi Ipogei d'Europa*. Associazione Micologica Bresadola, Trento, 714 pp.
40. Özderin, S., Yılmaz, F. & Allı, H. 2018. Determining mycorrhiza rate in some oak species inoculated with *Tuber aestivum* Vittad. (Summer Truffle), *Turkish Journal of Forestry,* 19(3): 226-232. http://dx.doi.org/10.18182/tjf.435372
41. Öztürk, C., Kaşık., G. & Toprak, E. 1997. Ascomycetes makrofunguslarından Türkiye için iki yeni kayıt. *Ot Sistematik Botanik Dergisi,* 4(1): 53-56.
42. Pegler, D.N., Spooner, B.M. & Young, T.W.K. 1993. *British Truffles*: *A revision of British Hypogeous fungi*. The Royal Botanic Gardens, Kew, 216 pp.
43. Riccioni, C., Rubini, A., Belfiori, B., Gregori, G. & Paolocci, F. 2016. *Tuber magnatum:* the special one. What makes it so different from other *Tuber spp.* In: Zambonelli, A., Iotti, M., & Murat, C. (eds.). *True truffle (Tuber spp.) in the world. Soil ecology, systematics and biochemistry*. Springer, Switzerland. 87-103.
44. Riousset, G., Riousset, L., Chevalier, G. & Bardet, M.C. 2012. *Truffe d'Europe et de Chine*. INRA, Paris.
45. Şen, İ., Allı, H. & Civelek, H.S. 2016. Checklist of Turkish truffles. *Türk Yaşam Bilimleri Dergisi,* 1(2): 103-109.
46. Stielow, B., Bratek, Z., Orczán, A.K.I, Rudnóy, S., Hensel, G., Hoffmann, P., Klenk, H.P. & Göker, M. 2011. Species delimitation in taxonomically difficult fungi: the case of *Hymenogaster. Plos One,* 6(1): e15614. https://doi.org/10.1371/journal.pone.0015614
47. Suwannarach, N., Kumla, J., Meerak, J. & Lumyong, S. 2017. *Tuber magnatum* in Thailand, a first report from Asia. *Mycotaxon,* 132: 635-642. https://doi.org/10.5248/132.635
48. Tamura, K. 1992. Estimation of the number of nucleotide substitutions when there are strong transition-transversion and G + C-content biases. *Molecular Biology and Evolution*, 9: 678-687.
49. Tamura, K., Stecher, G. & Kumar, S. 2021. MEGA 11: Molecular Evolutionary Genetics Analysis Version 11. *Molecular Biology and Evolution*, 38(7): 3022-3027. https://doi.org/10.1093/molbev/msab120
50. Trappe, J.M. & Castellano M.A. 1991. Keys to the genera of truffles *(Ascomycetes). McIlvanea*, 10: 47-65.
51. Trappe, M., Evans, F. & Trappe, J. 2007. *Field Guide to North American Truffles*: *Hunting, Identifying, and Enjoying the World's Most Prized Fungi*. Ten Speed Press, Berkeley, Toronto, 136 pp.
52. Türkoğlu, A. & Castellano, M.A. 2014. New records of some Ascomycete truffle fungi from Türkiye. *Turkish Journal of Botany*, 38: 406-416. https://doi.org/10.3906/bot-1303-24
53. Türkoğlu, A., Castellano, A.M., Trappe, M.J. & Güngör-Yaratanakul, M. 2015. Turkish truffles I: 18 new records for Türkiye. *Turkish Journal of Botany,* 39: 359-376. https://doi.org/10.3906/bot-1406-42
54. Uzun, Y. & Kaya, A. 2020. The Checklist of the macromycetes determined in Gaziantep province. *Anatolian Journal of Botany,* 4(2): 106-115. https://doi.org/10.18016/ksutarimdoga.vi.856014
55. Uzun, Y. & Yakar, S. 2018. New locality record for two *Tuber* species in Türkiye. *Anatolian Journal of Botany,* 2(2): 88-92. https://doi.org/10.18016/ksutarimdoga.vi.857201

56. Wedén, C., Pettersson, L. & Danell, E. 2009. Truffle cultivation in Sweden: Results from *Quercus robur* and *Corylus avellana* field trials on the island of Gotland. *Scandinavian Journal of Forest Research*, 24(1): 37-53. https://doi.org/10.1080/02827580802562056

57. White, T.J., Bruns, T., Lee, S. & Taylor, J. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. Pp. 315-322. In: Innis M.A., Gefland D.H., Sninsky J.J. & White T.J. (eds). *PCR Protocols: A Guide to Methods and Applications.* Academic Press Inc., New York, 482 pp.