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Hidden treasure demystified: *Tuber asiaticum* sp. nov., a true truffle species from Indian Himalaya

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ABSTRACT: The present study from the state of Himachal Pradesh (India) describes a new species of true truffle: *Tuber asiaticum* (Tuberaceae, Ascomycota). This is the first report of a *Tuber* from this country supported by morphological and molecular data. This species was found to grow in a temperate to subalpine coniferous forest in Himalaya. Detailed morphological features, illustrations, micromorphological drawings and molecular phylogenetic estimation are presented for this species.

KEY WORDS: Angiocarpic fungus, molecular phylogeny, nrITS, *Pinaceae*, *tef* 1-α, *Tuber mohedanoi, Tuber zambonelliae*.

INTRODUCTION

Truffles belong to one of the fascinating groups of angiocarpic macrofungi (mushrooms) that includes a polyphyletic group in the Kingdom Fungi and exhibit fruiting bodies (Ascomata or Basidiomata) below ground or at the soil surface keeping their spores unexposed and covered inside (Trappe *et al.*, 2009). It is realized that during the course of evolution they have appeared independently numerous times (Tedersoo *et al.*, 2010). They are important food sources for wild animals in different parts of the world. Due to their distinct and pleasant flavor, truffles are well appreciated by mushroom hunters and regional communities and are taken with delicacy across the continents. Various truffle species are rich in essential nutrients, including carbohydrates, proteins, fiber, minerals, fatty acids, and amino acids (Yan *et al.,* 2017; Wu *et al.,* 2021; Khan *et al.,* 2022). Besides their nutritional significance, they possess considerable potential as therapeutic agents (Lee *et al.*, 2020; Tejedor-Calvo *et al.*, 2021). Species of the genus *Tuber* P. Micheli ex F.H. Wigg., belonging to the family Tuberaceae (Ascomycota), are known for their significant ecological and economic values. The hypogeous ascocarps produced by *Tuber* spp. are referred to as the "true truffles". *Tuber* species are predominantly found across the northern hemisphere (Zambonelli *et al.*, 2016). All species within the genus *Tuber* establish ectomycorrhizal associations with numerous gymnosperms and angiosperms, such as *Pinus*, *Picea*, *Abies*, *Larix*, *Salix*, *Alnus*, *Carpinus*, *Corylus*, *Quercus*, *Ostrya*, *Castanea*, *Carya*, *Tilia*, *Cistus*, *Betula*, *Populus*, and *Fagus* (Mello *et al.*, 2001; Geng *et al.*, 2009; Bonito *et al.*, 2010; Benucci *et al.*, 2012; Gryndler, 2016; Nahberger *et al.*, 2021). Some *Tuber* species are distributed worldwide such as *Tuber melanosporum* Vittad., *T. aestivum* (Wulfen) Spreng., *T. borchii* Vittad., and *T. magnatum* Picco are highly sought after for both culinary and medicinal uses.

The diversity of *Tuber* species is relatively well-studied in Europe (Zambonelli *et al.*, 2016), North America (Guevara *et al.*, 2013), and China (Fan *et al.*, 2022), whereas species diversity in other regions remains seriously underexplored. From Asia, the first known species in this genus was described from India as *T. indicum* by Cooke and Massee (1892) and subsequently, *Tuber himalayense* B.C. Zhang & Minter (1988) was described. While a few additional instances of locally consumed *Tuber* species have been noted in the Indian Himalayan region (Parkash *et al.*, 2023), comprehensive documentation remains limited. There are also a few local reports of European and North American species, including *T. aestivum*, *T. cibaricum* Bull., *T. melanosporum*, and *T. rufum* Picco (Panda and Tayung, 2015; Vishwakarma *et al.*, 2016; Atri *et al.*, 2019), although their taxonomic identities are not established with phylogenetic support. A recent study by Tapwal and Sharma (2024) revealed a dominance of *Tuber* species in the mycorrhizal roots of *Abies pindrow* through ITS metabarcoding analysis. In recent years, there has been a continual discovery of new species and novel ectomycorrhizal tree partners across various forest ecosystems worldwide. As of now, Index Fungorum lists 263 recognized species within the genus *Tuber.*

The Himalayan region is home to genera such as *Pinus*, *Abies*, *Picea*, *Quercus*, *Betula*, *Salix*, and *Populus*, which are documented to establish ectomycorrhizal associations with species of the genus *Tuber*. As a result, the occurrence of *Tuber* species in this area is highly plausible. Recently, while undertaking routine survey of ectomycorrhizal fungi, two of the authors (NS & AT) have come across some specimens of truffles which after thorough examination through morphology and molecular phylogeny (Fig. 1) appear to be a novel species of *Tuber* and described herein as *T. asiaticum* sp. nov. with morphological details and molecular phylogenetic inference.

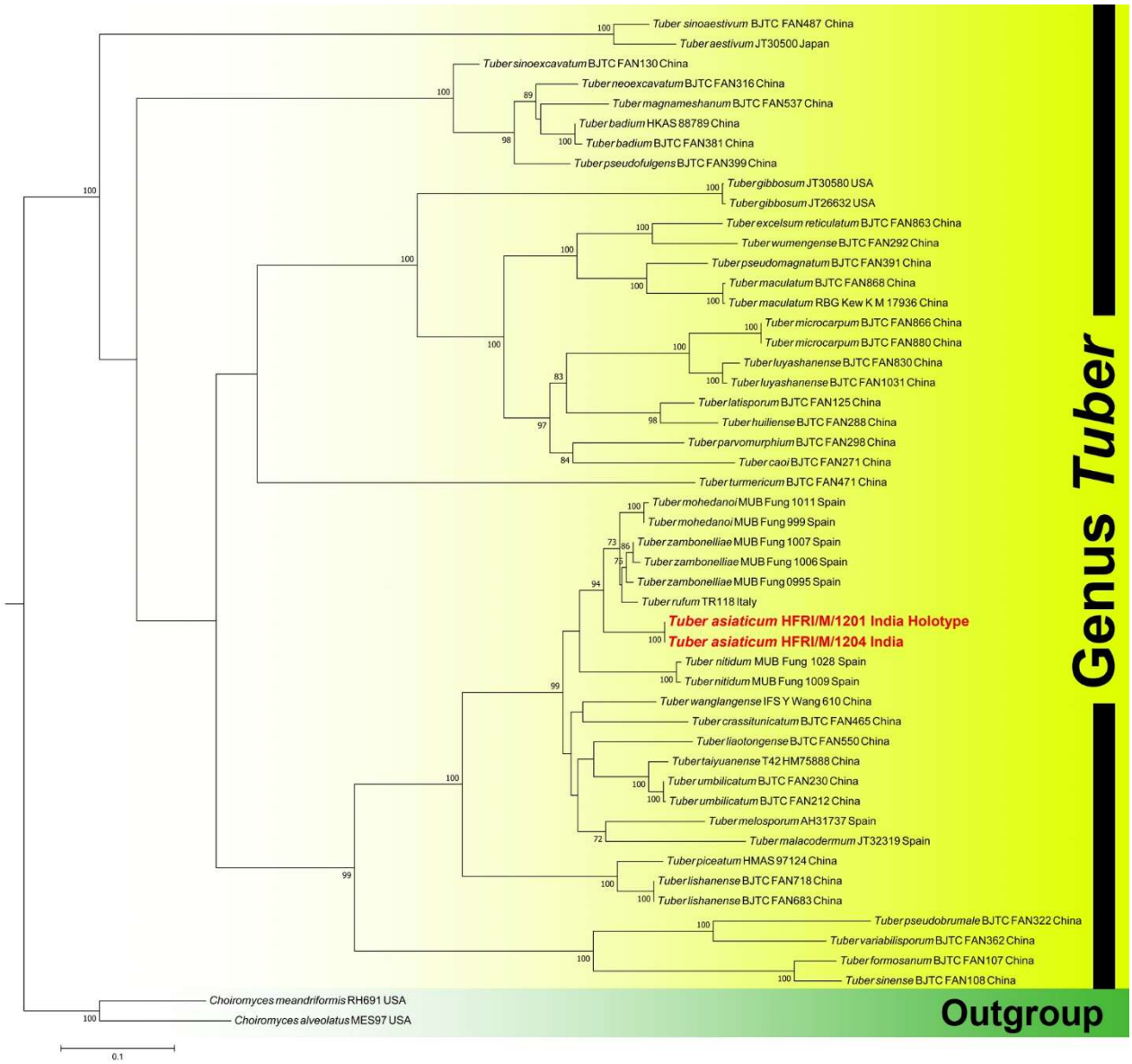

Fig. 1. Phylogram generated by Maximum Likelihood analysis based on two locus (ITS and *tef* 1-α) sequence data for ***Tuber asiaticum*** and allied species. Maximum likelihood bootstrap support values (MLbs) ≥70% are shown above or below the branches at nodes. ***T. asiaticum*** is placed in bold red font to highlight its phylogenetic position in the tree.

MATERIALS AND METHODS

Morphology

Macrofungal surveys were frequently undertaken to *Abies* dominated forests of Narkanda and surrounding forests of Shimla district in Himachal Pradesh. Fresh below ground ascomata were collected during rainy seasons of 2023 (September). Macromorphological and field characters were duly recorded in the forest or in the basecamp. Images of the fresh and dissected ascomata were captured with Canon Power Shot SX50 HS and Canon Power Shot SX220 HS cameras. Colour codes and terminologies primarily are after Kornerup and Wanscher (1978). After recording all the macromorphological characters, dissected samples were placed for drying in an field drier. Micromorphological characters were observed in the laboratory after mounting the freehand sections of dried samples in a solution of 5% KOH, 1% phloxine, and 1% ammoniacal Congo red under an Olympus CX 41 compound microscope. Drawings of the micromorphological features were made with the help of a drawing tube at 1000× magnification. Microscopic images were captured with a camera attached to an Olympus BX 53 microscope. The ascospores were measured in lateral view. Ascospore measurements and length/width ratios (Q) are recorded as: minimum–mean–maximum. Ascus length was measured. The herbarium acronym CAL follow Thiers (https://sweetgum.nybg.org/science/ih/), while HFRI/M refers to.

Table 1. ***Tuber*** and allied sequences used in the phylogenetic analysis of this study. Newly barcoded collections are in bold.

Sl. no.	Name of the species	Voucher No.	Country	ITS	tef 1-α
1.	*Choiromyce salveolatus*	MES97	USA	HM485332	
2.	*Choiromyces meandriformis*	RH691	USA	HM485330	
3.	*Tuber aestivum*	JT30500	Japan	HM485340	
4.	***Tuber asiaticum***	**HFRI/M/1201 (Type)**	**India**	**PQ483097**	**PV146254**
5.	***Tuber asiaticum***	**HFRI/M/1204**	**India**	**PQ483098**	**PV146255**
6.	*Tuber badium*	HKAS 88789	China	NR_155922	
7.	*Tuber badium*	BJTC FAN381	China	OM256748	OM649598
8.	*Tuber caoi*	BJTCFAN271	China	KP276183	KP276216
9.	*Tuber crassitunicatum*	BJTCFAN465	China	MH115295	OM649610
10.	*Tuber excelsum-reticulatum*	BJTC FAN863	China	NR_182412	OM649631
11.	*Tuber formosanum*	BJTCFAN107	China	MF621549	OM649564
12.	*Tuber gibbosum*	JT30580	USA	FJ809868	JX022585
13.	*Tuber gibbosum*	JT26632	USA	FJ809862	JX022584
14.	*Tuber huiliense*	BJTC FAN288	China	OM256781	OM649585
15.	*Tuber latisporum*	BJTC FAN125	China	KT067676	KT067725
16.	*Tuber liaotongense*	BJTC: FAN550	China	MH115302	OM649618
17.	*Tuber lishanense*	BJTCFAN683	China	MH115305	OM649621
18.	*Tuber lishanense*	BJTC:FAN718	China	MH115303	OM649622
19.	*Tuber luyashanense*	BJTCFAN830	China	OM256821	OM649628
20.	*Tuber luyashanense*	BJTC FAN1031	China	OM256769	OM649637
21.	*Tuber maculatum*	RBG Kew K(M)17936	UK	EU784428	
22.	*Tuber maculatum*	BJTC FAN868	China	OM265274	OM649634
23.	*Tuber magnameshanum*	BJTC FAN537	China	OM256767	OM649617
24.	*Tuber malacodermum*	JT32319	Spain	FJ809889	JX022593
25.	*Tuber melosporum*	AH31737	Spain	JN392144	
26.	*Tuber microcarpum*	BJTC FAN880	China	OM256832	
27.	*Tuber microcarpum*	BJTC FAN866	China	OM256770	OM649632
28.	*Tuber mohedanoi*	MUB_Fung-999	Spain	OM756741	
29.	*Tuber mohedanoi*	MUB_Fung-1011	Spain	OM756743	
30.	*Tuber neoexcavatum*	BJTC FAN316	China	OM256741	OM649589
31.	*Tuber nitidum*	MUB:Fung-1028	Spain	PQ010063	
32.	*Tuber nitidum*	MUB:Fung-1009	Spain	PQ010060	
33.	*Tuber parvomurphium*	BJTC FAN298	China	KP276186	KP276214
34.	*Tuber piceatum*	HMAS: 97124	China	MH115320	
35.	*Tuber pseudobrumale*	BJTC FAN322	China	OM287839	OM649591
36.	*Tuber pseudofulgens*	BJTC FAN399	China	NR_182567	OM649601
37.	*Tuber pseudomaganatum*	BJTC FAN391	China	OM265244	OM649600
38.	*Tuber rufum*	TR118	Italy	MT351082	
39.	*Tuber sinense*	BJTC FAN108	China	MF627968	OM649565
40.	*Tuber sinoaestivum*	BJTC FAN487	China	OM256773	OM649614
41.	*Tuber sinoexcavatum*	BJTC FAN130	China	JX458717	OM649568
42.	*Tuber taiyuanense*	T42_HM75888	China	GU979033	
43.	*Tuber turmericum*	BJTC FAN471	China	KT758835	OM649613
44.	*Tuber umbilicatum*	BJTC FAN212	China	OM311201	OM649577
45.	*Tuber umbilicatum*	BJTC FAN230	China	OM311205	OM649582
46.	*Tuber variabilisporum*	BJTC FAN362	China	OM287845	OM649595
47.	*Tuber wanglangense*	IFS Y. Wang 610	China	DQ478637	
48.	*Tuber wumengense*	BJTC FAN292	China	KT067683	KT067716
49.	*Tuber zambonelliae*	MUB_Fung-1006	Spain	MW632954	
50.	*Tuber zambonelliae*	MUB_Fung-1007	Spain	MW632955	
51.	*Tuber zambonelliae*	MUB_Fung-995	Spain	MW632951	

DNA extraction, polymerase chain reaction (PCR) and sequencing

Genomic DNA was isolated from 100 mg of dried fruiting body with the HiPurA Fungal DNA Purification Kit (HIMEDIA) following the manufacturer's instructions provided in the kit itself. The PCR amplifications of two nuclear loci, the internal transcribed spacer (ITS1-5.8S-ITS2 = ITS), and the gene for the

translation elongation factor 1-α (*tef* 1-α) were done using the primer pairs ITS1-F and ITS4 and ef1-983F and ef1-1567R, respectively (White *et al.*, 1990; Gardes and Bruns, 1993; Rehner and Buckley, 2005). PCR amplification for these loci was undertaken in a ProFlex PCR system (Applied Biosystems) programmed for an initial denaturation at 94 °C for 3 min, followed by 35 cycles of denaturation at 94 °C for 1 min, annealing at 50 °C for 30 sec, and extension at 72 °C for 1 min. The final extension was placed at 72 °C for 7 min. The PCR product was purified using the QIAquick PCR purification kit (QIAGEN, Germany). The cycle sequencing products were run on ABI 3500 automated DNA analyzer (Applied Biosystems, USA). The quality of the sequences was checked using Sequence Scanner Software ver. 1 (Applied Biosystems). Sequence alignment, required editing and contig preparation of the obtained sequences were carried out using Geneious Pro ver. 5.1 (Drummond *et al*., 2010). In this study, four sequences (two each for ITS and *tef* 1-α) were generated from two separate collections of our species of *Tuber* (voucher nos. HFRI/M/1201 and HFRI/M/1204) and subsequently deposited in GenBank.

Phylogenetic analysis

The nrITS and *tef* 1-α sequences of the newly described *Tuber* species plus close relatives were retrieved from BLASTn search against GenBank (https://www.ncbi.nlm.nih.gov/genbank) and relevant published phylogenies (Crous *et al.* 2021; Tan *et al.* 2022; Li *et al*., 2024). Two raw datasets (ITS and *tef* 1-α) were prepared separately (Table 1). The dataset (nrITS- and *tef* 1-α-based) were aligned using the online version of the multiple sequence alignment program MAFFT v. 7 (https://mafftuber.cbrc.jp/alignment/software/) with L-INS-i strategy. The alignment was checked and trimmed with the conserved motifs manually with MEGA v. 7 (Kumar *et al*., 2016). Furthermore, two alignments (ITS and *tef* 1-α) were concatenated using BioEdit v. 7.0.9 (Hall, 1999) and processed for the phylogenetic analyses. In the two-locus dataset (ITS + *tef* 1α) of *Tuber*, sequence lengths were determined to be 889 bp for ITS and 829 bp for *tef* 1-α. This aligned dataset was used for the phylogenetic analyses using maximum likelihood (ML) method. The ML analysis was performed using raxmlGUI 2.0 (Edler *et al.* 2021) with the GTRGAMMAI substitution model. This analysis was executed applying the rapid bootstrap algorithm with 1000 replicates to obtain nodal support values. Our novel taxon (with two collections) is highlighted in the combined phylogenetic tree using bold red font (Fig. 1).

RESULTS

Phylogenetic inferences

In our present phylogenetic analysis, ITS and *tef* 1-α based dataset was consisted of 49 sequences of *Tuber* as ingroup with two taxa of *Choiromyces alveolatus* (Harkn.) Trappe and *C. meandriformis* Vittad. as outgroup. Based on the molecular phylogenetic analysis (Fig. 1), our species represented by two samples (HFRI/M/1201 and HFRI/M/1204) formed a solitary lineage clustering with the clade bearing three sister species *Tuber mohedanoi*, *T. zambonelliae* and *T. rufum*. However, our specimens have strongly recovered as a distinct clade receiving a strong support value (MLbs = 94%) and thus suggesting as a phylogenetically unknown species.

TAXONOMIC TREATMENT

Tuber asiaticum N. Sharma & Tapwal, ***sp. nov.***

Figs. 2 & 3

***MycoBank*:** MB856297; ***GenBank*:** PQ483097 (ITS, from holotype), PQ483098 (ITS); PV146254 (*tef* 1-α, from holotype), PV146255 (*tef* 1-α).

***Type*:** INDIA, HIMACHAL PRADESH, Narkanda, in temperate to subalpine forests under *Abies pindrow*, 02 September 2023, alt. 2708 m, N 31.27553279, E 77.45419070, *Neha Sharma* and *Ashwani Tapwal*, *HFRI/M/1201* (holotype! CAL 2117).

***Diagnosis*:** *Tuber asiaticum* can be separated micromorphologically from *T. zambonelliae* and *T. mohedanoi* by possessing two-layered peridium, occurrence under coniferous tree *Abies pindrow*, longer pointed spines (4–5.5 µm long) on ascospores and sequence data of ITS and *tef* 1-α

***Description*:** Ascomata 20–30 × 40–60 mm, ellipsoid to irregular, slightly lobed, golden blonde to Pompeian yellow (5C4–6) at maturity, becoming cinnamon to light khaki (6C7) when dry; peridium surface slightly rough with small-narrow fissures. Gleba solid, firm, with poor elasticity, brittle, white when immature but concolourous to the peridium with maturity, marbled with bright white mycelium. Odour pleasant with earthy aroma.

Peridium 380–520 µm thick, composed of two layers. Outer layer 300–420 µm thick, pseudoparenchymatous, composed of globose, subglobose to subangular or irregular cells (4.8–29 × 4.8–10.5 µm), cells hyaline to brown pigmented, thick-walled (wall up to 2.2 µm thick). Inner layer 80–100 µm thick, composed of hyaline parallel or slightly interwoven hyphae arranged compactly; hyphae 3.5–4.8 µm wide, septate, slightly thin- to thick-walled. Gleba with asci embedded in trama; asci 44–73.5 × 34.5–54.6 µm, pyriform, broadly clavate or subglobose to shape of a table tennis racket, often with short stalk (12–18 × 5–6 µm), thik-walled (wall up to 2 µm thick), 1–5(–6)-spored. Ascospores 19.3–28.36–35.5 × 19–23.66–30.0 µm (Q = 1.00–1.25–1.52), globose to broadly ellipsoid to ellipsoid, thick-walled (wall up to 4.6 µm thick), at first hyaline, becoming light yellowish-brown to brown at maturity; ornamentations spinoid; composed of densely arranged isolated straight to curved

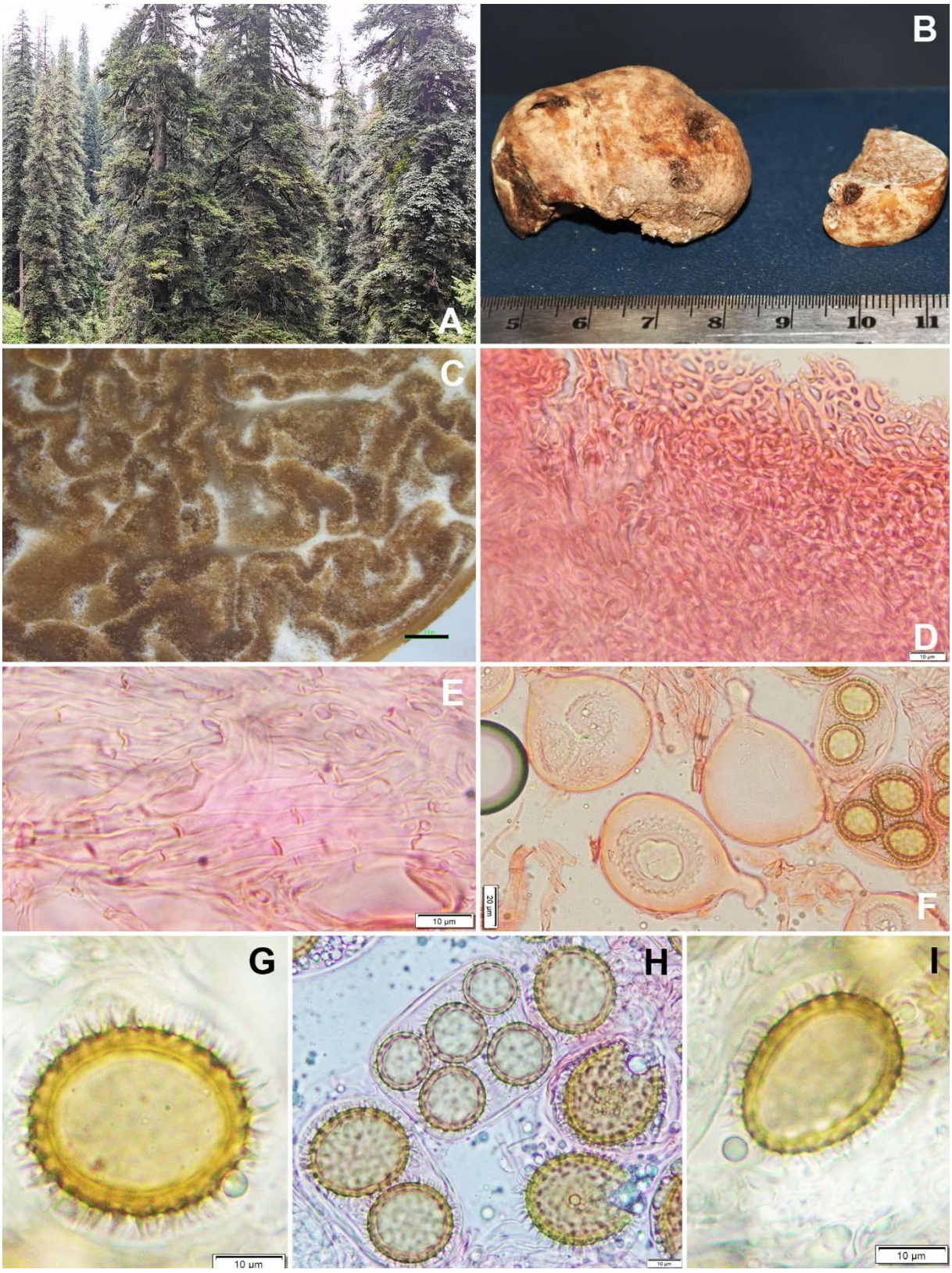

Fig. 2. Photographic illustrations of ***Tuber asiaticum*** (from CAL 2117, holotype) **A**: Habitat showing ***Abies*** dominated temperate forest. **B**: Fresh Ascomata. **C**: Gleba. **D**: Pseudoparenchymatous outer layer of peridium. **E**: Hyphal inner layer of peridium. **F**. Asci with one to three ascospores under light microscope. **G** & **I**. Ascospore. **H**. Asci with two or five ascospores. Scale bars: **C** = 1 mm, **D–E** & **G–I** = 10 µm, **F** = 20 µm.

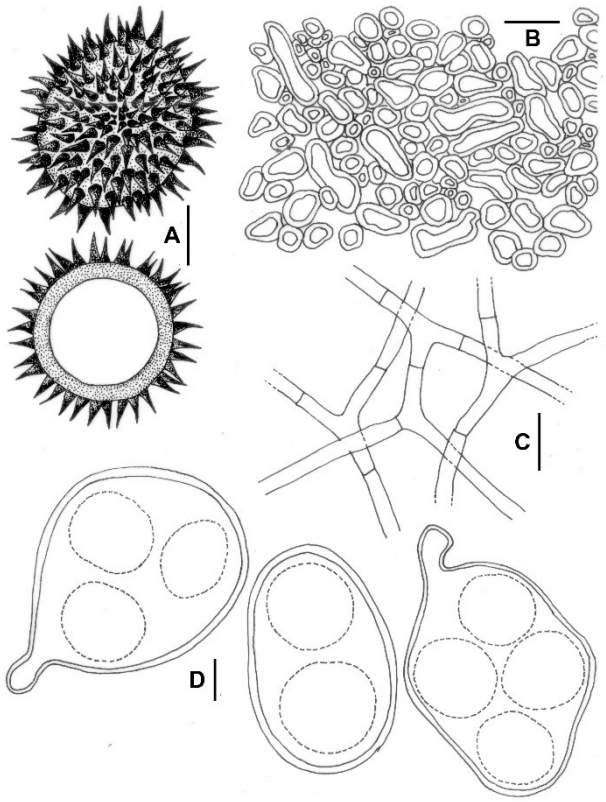

Fig. 3. Drawing illustrations of ***Tuber asiaticum*** (from CAL 2117, holotype) **A**: Ascospores. **B**: Pseudoparenchymatous outer layer of peridium. **C**: Hyphal inner layer of peridium. **D**: Asci with young ascospores. Scale bars: **A–D** = 10 µm.

pointed spines (4–5.5 µm high), never forming any reticulum. Glebal hyphae (2.7–4 µm wide), arranged loosely in interwoven pattern, branched and septate.

***Etymology*:** Referring to the continent Asia where the type locality is present

Ecology and distribution: Hypogeous, solitary, or in groups in the soils under *Abies pindrow* (Royle ex D.Don) Royle, in temperate to subalpine Himalaya during August to September.

***Additional specimen examined*:** INDIA, HIMACHAL PRADESH, Narkanda, in temperate to subalpine forests under *Abies pindrow*, 03 September 2023, alt. 2708 m, N 31.27553279, E 77.45419070. *Neha Sharma* and *Ashwani Tapwal*, HFRI/M/1204 (CAL 2118).

***Discussion*:** Macromorphological or field characters are hardly sufficient to separate similar truffle species. Even microscopical characters are not always enough to distinguish species. Therefore, molecular phylogeny in combination with the morphological evidences is the main requirement to identify the species or to establish the conspecificity in the genus *Tuber*. *T. asiaticum*, the present novel species is phylogenetically quite close to *T. zambonelliae* Ant. Rodr. & Morte and *T. mohedanoi* Ant. Rodr. & A. Morte, and is similar in possessing smooth peridium, brown gleba marbled with white and dark veins and spiny spores. Both *T. zambonelliae* and *T. mohedanoi* (originally reported from Europe) resemble the present species but differ in possessing unilayered peridium composed of hyaline, agglutinated, interwoven hyphae and growing in association with broadleaf *Quercus* trees (Fagaceae). Moreover, the spores of *T. zambonelliae* are ornamented with distinctively short spines (1–2 µm long), often connected by lower ridges, forming an irregular and incompletely spiny reticulum (Crous *et. al.*, 2021), whereas, the present species has isolated, straight to curved, much longer pointed spines (4–5.5 µm long) that never forms a reticulum. Similarly, the spores of *T. mohedanoi* also have shorter spines 2–3 µm (Tan *et. al.*, 2022). The genetic differences along with the above morphological characters clearly distinguish *T. asiaticum* as a distinct species. *T. nitidum* Vittad. and *T. rufum* Pollini are two other close European species, however, *T. nitidum* differs by possessing a basal cavity (in ascomata), asci with 1–4 spores (up to 6 in *T. asiaticum*), shorter (1–2 µm) spines and occurrence under broadleaf trees; while, *T. rufum* has reddish to reddish-brown ascomata, reddish-violet gleba, asci with 1–4 spores and shorter (1–2 µm) spines (Ceruti *et al.*, 2003; Moreno-Arroyo *et al.*, 2005).

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LITERATURE CITED

Atri, N.S., Sharma, Y.P., Kumar, S., Mridu 2019 Wild edible mushrooms of North West Himalaya: Their nutritional, nutraceutical, and sociobiological aspects. In: Satyanarayana, T., Das, S., Johri, B. (eds), Microbial Diversity in Ecosystem Sustainability and Biotechnological Applications, Soil and Agroecosystem **2**: 533–563.

Bonito, G., Smith, M.E., Nowak, M., Healy, R.A., Guevara, G., Cázares, E., Kinoshita, A., Nouhra, E.R., 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 their newly identified Southern Hemisphere sister lineage. PLoS ONE **8(1)**: e52765.

Benucci, G.M.N., Bonito, G., Falini, L.B., Bencivenga, M. 2012 Mycorrhization of Pecan trees (*Carya illinoinensis*) with commercial truffle species: *Tuber aestivum* Vittad. and *Tuber borchii* Vittad. Mycorrhiza **22(5)**: 383–392.

Ceruti, A., Fontana, A., Nosenzo, C. 2003 Le Specie Europee del genere Tuber. Una Revisione Storica. Museo Regionale de Scienze Naturali, Torino.

Cooke, M.C., Massee, G. 1892 Himalayan truffles. Grevillea **20**: 67.

Crous, P.W., Cowan, D.A., Maggs-Kölling, G., *et al.* 2021 Fungal planet description sheets: 1182–1283. Persoonia **46**: 313–528.

Drummond, A.J., Ashton, B., Buxton, S., Cheung, M., Cooper, A., Heled, J., Kearse, M., Moir, R., Stones-Havas, S., Sturrock, S. *et al.*, Geneious v 5.1. 2010. Available online: https://www.geneious.com.

Edler, D., Klein, J., Antonelli, A., Silvestro, D. 2021 raxmlGUI 2.0: A graphical interface and toolkit for phylogenetic analyses using RAxML. Methods Ecol. Evol. **12(2)**:373–377.

Fan, L., Li, T., Xu, Y.Y., Yan, X.Y. 2022 Species diversity, phylogeny, endemism, and geography of the truffle genus *Tuber* in China based on morphological and molecular data. Persoonia **48(1)**: 175–202.

Gardes, M., Bruns, T.D. 1993 ITS primers with enhanced specificity for basidiomycetes-application to the identification of mycorrhizae and rusts. Mole. Ecol. **2(2)**: 113–118.

Geng, L.Y., Wang, X.H., Yu, F.Q., Deng, X.J., Tian, X.F., Shi, X.F., Xie, X.D., Liu, P.G., Shen, Y.Y. 2009 Mycorrhizal synthesis of *Tuber indicum* with two indigenous hosts, *Castanea mollissima* and *Pinus armandii*. Mycorrhiza **19(7)**: 461–467.

Gryndler, M. 2016 True truffle host diversity. In: Zambonelli, A., Iotti, M., Murat, C. (eds), True Truffle *(Tuber* spp.*)* in the World: Soil Ecology, Systematics and Biochemistry. Springer, pp. 267–281.

Guevara, G., Bonito, G., Trappe, J.M., Cázares, E., Williams, G., Healy, R.A., Schadt, C., Vilgalys, R. 2013 New North American truffles (*Tuber* spp.) and their ectomycorrhizal associations. Mycologia **105(1)**: 194–209.

Hall, T.A. 1999 BioEdit: a user-friendly biological sequence alignment editor and analyses program for Windows 95/98/NT. Nucleic Acids Symposium Series **41**: 95–98.

Khan, F.S., Hussain, I., Akram, M., Owaid, M.N. 2022 Truffles: The cultivation and health benefits. In Arya, A., Rusevska, K. eds. Biology, Cultivation and Applications of Mushrooms. Springer, pp. 285–300.

Kumar, M., Harsh, N.S.K., Prasad, R., Pandey, V.V. 2017 An ethnomycological survey of Jaunsar, Chakrata, Dehradun, India. J. Threat. Taxa **9(9)**: 10717–10725.

Kumar, S., Stecher, G., Tamura, K. 2016 MEGA7: Molecular Evolutionary Genetics Analysis version 7.0 for bigger datasets. Mol. Biol. Evol. **33(7)**: 1870–1874.

Lee, H., Nam, K., Zahra, Z., Farooqi M.Q.U. 2020 Potentials of truffles in nutritional and medicinal applications: A review. Fungal Biol. Biotechnol. **7**: 9.

Li, L., Wan, S., Wang, Y., Thongklang, N., Yang, M., Liu, C., Luo, Z., Li, S. 2024 Three new species of *Tuber* discovered in alpine fir forests in Yunnan, China. J. Fungi **10(7)**: 453.

Mello, A., Fontana, A., Meotto, F., Comandini, O., Bonfante, P. 2001 Molecular and morphological characterization of *Tuber magnatum* mycorrhizas in a long-term survey. Microbiol. Res. **155(4)**: 279–284.

Moreno-Arroyo, B., Gómez, J., Pulido, E. 2005 *Tesoros de nuestro montes. Trufas de Andalucía*. Consejería de Medio Ambiente, Junta de Andalucía, Córdoba, España.

Nahberger, T.U., Benucci, G.M.N., Kraigher, H., Grebenc, T. 2021. Effect of earthworms on mycorrhization, root morphology, and biomass of silver fir seedlings inoculated with black summer truffle (*Tuber aestivum* Vittad.). Sci. Rep. **11(1)**: 6167.

Panda, M.K., Tayung, K. 2015 Documentation and ethnomedicinal knowledge on wild edible mushrooms among ethnic tribes of Northern Odisha, India. Asian J. Pharm. Clin. Res. **8(4)**: 139–143.

Parkash, V., Kaundal, R., Paul, S., Chauhan, A., Aggarwal, A. 2023 Some uncommon traditionally and medicinally important wild macrofungi of the Garhwal Himalayas, Uttarakhand, India. In: Semwal, K.C., Stephenson, S.L., Husen, A. (eds.) Wild Mushrooms and Health: Diversity, Phytochemistry, Medicinal Benefits, and Cultivation, CRC Press, pp. 261–273.

Rehner, S.A., Buckley, E.A. 2005 *Beauveria* phylogeny inferred from nuclear ITS and EF1-a sequences: Evidence for cryptic diversification and links to *Cordyceps* teleomorphs. Mycologia **97(1)**: 84–98.

Tan, Y.P., Bishop-Hurley, S.L., Shivas, R.G. *et al.* 2022 Fungal Planet description sheets: 1436–1477. Persoonia **49(1)**: 261–350.

Tapwal, A., Sharma, N. 2024 Characterization of ectomycorrhizal fungal community of *Abies pindrow* using sporocarp sampling, morphotyping, and metabarcoding through next-generation sequencing. Int. J. Microbiol. **28**: 69–79.

Tedersoo, L., May, T.W., Smith, M.E. 2010 Ectomycorrhizal lifestyle in fungi: Global diversity, distribution, and evolution of phylogenetic lineages. Mycorrhiza **20(4)**: 217–263.

Tejedor-Calvo, E., Amara, K, Reis, F. S., Barros, L., Martins, A., Calhelha, R.C., Ferreira, I.C. 2021 Chemical composition and evaluation of antioxidant, antimicrobial, and antiproliferative activities of *Tuber* and *Terfezia* truffles. Food Res. Int. **140**: 110071.

Trappe, J., Molina, R., Luoma, D., Cazares, E., Pilz, D., Smith, J.E., Castellano, M., Miller, S., Trappe, M. 2009 Diversity, ecology, and conservation of truffle fungi in forests of the Pacific Northwest. Pacific Northwest General Technical Report - PNW-GTR-772. United States Department of Agriculture, Pacific Northwest Research Station, Portland, OR. pp. 194.

Vishwakarma, P., Singh, P., Tripathi, N.N. 2016 Nutritional and antioxidant properties of wild edible macrofungi from North-Eastern Uttar Pradesh, India. Indian J. Tradit. Knowl. **15(1)**: 143–148.

White, T.J., Bruns, T., Lee, S., Taylor, J. 1990 Amplification and direct sequencing offungal ribosomal RNA genes for phylogenetics. In: Innis, M.A., Gelfand, D.H., Sninsky, J.J., White, T.J. (eds.), PCR Protocols: a guide to method and applications. Academic Press, San Diego. pp. 315–322.

Wu, Z., Meenu, M., Xu, B. 2021 Nutritional value and antioxidant activity of Chinese black truffle (*Tuber indicum*) grown in different geographical regions in China. LWT – Food Science and Technology **135**: 110226.

Yan, X., Wang, Y., Sang, X., Fan, L. 2017 Nutritional value, chemical composition, and antioxidant activity of three *Tuber* species from China. AMB Express **7**: 136.

Zambonelli, A., Lotti, M., Murat, C. 2016 True Truffle (*Tuber* spp.) in the World: Soil Ecology, Systematics and Biochemistry. Ser. Soil Biology. Springer International Publishing, Switzerland.

Zhang, B.C., Minter, D.W. 1988 *Tuber himalayense* sp. nov. with notes on Himalayan truffles. Trans. Br. Mycol. Soc. **91(4)**: 593–597.