

Phylogenetic and morphological analyses reveal *Tuber chuxiongense* (Tuberaceae, Pezizales), a new species from Yunnan, China

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Abstract

During a survey of the high-value fungal diversity in Yunnan, China, a new species of *Tuber* was identified on the basis of its morphological characteristics and combined molecular analyses of the internal transcribed spacer (ITS), nuclear large subunit ribosomal RNA (nrLSU), translation elongation factor 1- α (*tef1- α*), and RNA polymerase II subunit (*rpb2*) data. The novel species is characterized by small ascomata with a distinct cavity. The ascospores are covered with free apical spines and low ridges, forming an irregular reticulum 5–7 meshes across the spore width. Phylogenetic analyses using maximum likelihood and Bayesian inference methods showed a distinct position of the new species among known species belonging to the group *Melanosporum*, with significant statistical support. Color photographs of the ascomata, micromorphological structures based on scanning electron microscopy (SEM) of the ascospores, and an updated phylogenetic tree of *Tuber* are also provided in this study.

Keywords: Ectomycorrhizal fungi, Multi-gene, Taxonomy, Truffle, Tuberaceae

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Introduction

Species of the genus *Tuber* P. Micheli ex F.H. Wigg. belong to the family Tuberaceae (Ascomycota). The fruiting bodies of *Tuber* species are known as "truffles", which are generally hypogeous sporocarps and are regarded as one of the prized edible fungi owing to their unique flavor^[1]. *Tuber* species establish obligate mycorrhizal associations and promote the growth of major forest tree species such as *Betulaceae*, *Cistaceae*, *Corylaceae*, *Fagaceae*, and *Pinaceae*^[2]. These fungi are ecologically and economically significant owing to their role in maintaining forest ecosystems and their value in gourmet markets^[3]. To date, Species Fungorum includes 293 species of *Tuber* (www.speciesfungorum.org/Names/Names.asp, accessed on 16 February 2026), while the molecular data of around 192 species are available in GenBank (www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?id=36048, accessed on 16 February 2026). The biodiversity hotspots of *Tuber* are distributed along the Alps in Europe, the Himalayas, and the Hengduan Mountains in Asia, and certain regions of North America^[4].

The study on Chinese *Tuber* species began in 1985, which led to the discovery of *T. taiyuanense*^[5]. Wang et al.^[6] and García-Montero et al.^[7] presented a comprehensive review of Chinese *Tuber* species. Liu et al.^[8] documented 62 species from China with their complete description. Yunnan, China, covers a major part of the Hengduan Mountains and is recognized as a global biodiversity hotspot, harboring exceptional fungal diversity. Recent studies have revealed several new *Tuber* species in this region over the past decade, such as *T. baoshanense*, *T. caoi*, *T. crassitunicatum*, *T. humilireticulatum*, *T. laojunshanense*, *T. shidianense*, *T. wumengense*, and

T. yunnanense^[9–16]. Despite the discoveries, many areas in Yunnan remain unexplored, and cryptic *Tuber* lineages are yet to be investigated and described.

Traditionally, identification of *Tuber* species is based on their morphological characteristics. Macroscopically, the surface ornamentation and color of the peridium, as well as the presence of cavities and the marbled patterning of the gleba, serve as important diagnostic criteria^[7,17]. Microscopically, key taxonomic characteristics for distinguishing different species include the structure of the peridium (viewed in cross-section), the structure of the asci (particularly the number of ascospores per ascus), and the color, size, shape, and ornamentation of the mature ascospores, specifically the morphology of the alveolate reticulum^[7,18,19]. However, the taxonomic and systematic assessment of *Tuber* has been challenging because of its many cryptic species and a limited number of diagnostic characteristics. In the past two decades, modern taxonomic approaches integrating molecular phylogenetics (such as combined internal transcribed spacer [ITS], nuclear large subunit ribosomal RNA [nrLSU], RNA polymerase II subunit [*rpb2*], and translation elongation factor 1- α [*tef1- α*]) and morphological analysis were conducted to refine the taxonomy for species delimitation of *Tuber* in China^[9]. In this study, we introduce and illustrate a novel *Tuber* species collected from the forest ecosystems of Yunnan using a combination of macro- and micromorphological characteristics and multi-locus phylogenetic analyses. This discovery expands the known diversity of Asian truffles and underscores the importance of Yunnan as a reservoir of undescribed fungal taxa.

Materials and methods

Specimen collection and morphological study

Fresh samples were collected from Dayao County, Chuxiong Yi Autonomous Prefecture, Yunnan, China, and they were desiccated at 45 °C for 2 days. Morphological description was based on fresh materials and microscopic examination of dry materials following the methods of Wang et al.^[12] Hand-cut sections were mounted in 10% (w/v) KOH solution and examined under a Nikon ECLIPSE Ni-U complex microscope with differential interference contrast (DIC) and phase contrast (PC) illumination. Key colors were obtained from the work of Kornerup and Wanscher^[20]. To evaluate the range of spore size, at least 30 ascospores were measured for each collection. In the description of the ascospores, the abbreviation "Q" represents the ratio range of spore length to spore width calculated for each spore; "Qm" refers to the average Q of all ascospores $\pm$ standard deviation; "n" refers to the number of spores measured. Photo plates and measurements were processed using Adobe Photoshop 2021 (Adobe Systems, CA, USA). For scanning electron microscopy (SEM), spores were scraped from the dried gleba into a sterile centrifuge tube, followed by immersion in distilled water for 1 h. Subsequently, the hydrated spores were inverted onto a sterile filter paper. After drying, spores were scraped from the filter paper onto a SEM stub with a double-sided tape, coated with gold-palladium, and photographed using a JSM-5600LV SEM (JEOL, Tokyo, Japan). The specimens were deposited in the Herbarium of Cryptogams, Kunming Institute of Botany, Chinese Academy of Sciences (HKAS), China.

DNA extraction, PCR amplification, and sequencing

Genomic DNA was directly extracted from the ascoma using a Forensic DNA Kit (Omega®, USA) following the manufacturer's protocol. The following primer pairs were used for polymerase chain reaction (PCR) amplification and sequencing: ITS1F/ITS4^[21] for the ITS rDNA region; LR0R/LR5^[22] for the nrLSU; EF1-983F/EF1-2218R^[23] for the *tef1- α* ; and RPB2-6F/bRPB2-7.1R^[24] for the *rpb2*. PCR was performed in each region as described by Fan et al.^[9] The PCR products were sent to Sangon Biotech Co., Ltd. (Shanghai, China) for purification and sequencing. The newly generated sequences were deposited in GenBank.

Alignment and phylogenetic analyses

Sequences (ITS, nrLSU, *tef1- α* , and *rpb2*) from the studied specimens were compiled along with sequences from the reference taxa curated in GenBank (www.ncbi.nlm.nih.gov, accessed on 10 May 2025). A total of 147 taxa, including holotypes and two new strains from this study, were analyzed (Table 1). On the basis of previous research, *Choioomyces alveolatus*, *Choioomyces meandriformis*, and *Labyrinthomyces* sp. were selected and used as outgroups^[9]. Sequences generated in this study were assembled, trimmed, and manually analyzed using Geneious 9.1.2^[25]. Phylogenetic analysis was conducted using the One-click Fungal Phylogenetic Tool (OFPT) for establishing a combined maximum likelihood (ML) tree^[26]. Using ModelFinder^[27] via the OFPT^[26], the best-fit model of substitution for analysis was estimated for each gene, based on the Bayesian Information Criterion (BIC). The ML analysis was performed using ultrafast bootstrap approximation with 1,000 replicates^[28]. The consensus tree was summarized using the extended majority rule. The Bayesian Inference (BI) was performed using MrBayes v3.2.7^[29],

in which two parallel Metropolis-coupled Markov chain Monte Carlo (MCMC) chains were sampled every 100 generations starting from a random tree. Tree samples from the different runs were compared every 1,000 generations, and the run was stopped automatically when the average standard deviation of split frequencies fell below 0.01. The consensus tree was summarized after discarding the first 25% samples. A clade was considered to be strongly supported if it showed a bootstrap support (BS) value $\geq$ 80% and/or a posterior probability (PP) value $\geq$ 0.90. The resulting tree was visualized using FigTree v1.4.0 and edited using Adobe Illustrator 2024. The finalized alignment and tree were deposited in Zenodo under submission DOI: 10.5281/zenodo.17355046 (<https://zenodo.org>).

Results

Phylogenetic analyses

A total of 536 sequences (146 ITS, 140 nrLSU, 132 *tef1- α* , and 118 *rpb2* sequences) from 147 taxa (144 *Tuber* species with 3 outgroups) were used in the phylogenetic analysis, and 3,737 characteristics (443 for ITS, 1,459 for nrLSU, 785 for *tef1- α* , and 1,050 for *rpb2*) were analyzed. The RaxML analysis of the dataset yielded a best-scoring tree with a final ML optimization likelihood value of $-43,852.917060$. The Bayesian analysis resulted in 11,139 trees after 807,000 generations. The first 2,785 trees, representing the burn-in phase of the analysis, were discarded, while the remaining 8,354 trees were used for calculating posterior probabilities in the majority-rule consensus tree. The final average standard deviation of split frequencies was 0.009924. The best-fit models for each gene selected by BIC are as follows: TIM2+F+I+G4 (ITS), SYM+I+G4 (nrLSU), K3P+I+G4 (*rpb2*), and TNe+I+G4 (*tef1- α*). The final Bayesian analyses yielded similar tree topologies to the ML tree; the tree from the ML analysis is shown in Fig. 1. The ML tree had 12 distinct sections. *T. chuxiongense* was placed clearly in the *Melanosporium* section, within which it formed a clade with nine species, viz. *T. brumale*, *T. formosanum*, *T. longispinosum*, *T. melanosporum*, *T. pseudobrumale*, *T. pseudohimalayaense*, *T. sinense*, *T. variabilisporum*, and *T. yigongense*, with a strong BS (= 98). Phylogenetic analyses revealed that the new sequences generated in this study were sisters to *T. pseudohimalayaense* G. Moreno, Manjón, J. Diez & García-Mont with a strong BS (= 99) (PP = 0.91). Pairwise sequence comparison between *T. chuxiongense* (HKAS 148947) and *T. pseudohimalayaense* (BJTC FAN122) revealed base pair differences (excluding gaps) of 31/770 (4.03%), 7/836 (0.84%), 11/614 (1.79%), and 6/483 (1.24%) for the ITS, nrLSU, *tef1- α* , and *rpb2* regions, respectively. The base pair comparison between *T. chuxiongense* (HKAS 148948) and *T. pseudohimalayaense* (BJTC FAN122) revealed base pair differences (excluding gaps) of 23/791 (2.40%), 7/830 (0.84%), and 5/651 (0.77%) for the ITS, nrLSU, and *tef1- α* regions, respectively.

Taxonomy

Tuber chuxiongense Q.Q. Tao, Y.W. Hu, H.B. Jiang & J.F. Li, sp. nov. (Fig. 2).

Mycobank no.: 860999

Etymology: In reference to Chuxiong Yi Autonomous Prefecture, the type locality.

Holotype: CHINA, Yunnan Prov., Chuxiong Yi Autonomous Prefecture, Dayao County, in soil under forest dominated by *Pinus* sp., 26 December 2024, Junfu Li & Hongbo Jiang, T12-2 (HKAS 148947).

Description: *Ascomata* hypogeous, irregular to subglobose with a distinct cavity, and generally depressed, 1.6–4 cm in diameter, dark

Table 1. Taxa information and GenBank accession numbers of the *Tuber* specimens used in the phylogenetic study.

Species name	Voucher/strain	GenBank accession numbers				Ref.
		nrLSU	ITS	<i>tef1-α</i>	<i>rpb2</i>	
<i>Choiromyces alveolatus</i>	MES97	JQ925660	HM485332	–	JQ954470	[30]
<i>Choiromyces meandriformis</i>	RH691	–	HM485330	JX022550	JQ954471	[31]
<i>Labyrinthomyces</i> sp.	JT27750	JQ925670	HM485335	–	JQ954480	[31]
<i>Tuber aestivum</i>	GB202	JQ925679	–	JX022565	JQ954487	[30]
<i>Tuber aestivum</i>	JT30500	–	HM485340	–	JQ954488	[31]
<i>Tuber anniae</i>	BJTC FAN640	OM366215	OM286868	OM649620	OM584274	[9]
<i>Tuber anniae</i>	JT13209, holotype	JQ925680	HM485338	JX022567	–	[30]
<i>Tuber anniae</i>	JT22695	JQ925681	HM485339	JX022568	–	[30]
<i>Tuber badium</i>	BJTC FAN371	OM366192	OM256747	OM649597	OM584255	[9]
<i>Tuber badium</i>	BJTC FAN381	OM366193	OM256748	OM649598	OM584256	[9]
<i>Tuber baoshanense</i>	BJTC FAN400	OM366197	OM256791	OM649602	OM584260	[9]
<i>Tuber beyeleri</i>	JT32597, holotype	JF419286	HM485408	JX022570	JQ954491	[31]
<i>Tuber bomiense</i>	BJTC FAN467	OM366206	OM265247	OM649611	–	[9]
<i>Tuber borchii</i>	BJTC FAN217	KT067706	KT067681	KT067717	OM584229	[9,10]
<i>Tuber borchii</i>	GB1/GB32	FJ809799	FJ809852	JX022571	JQ954492	[30,32]
<i>Tuber brumale</i>	GB52	JQ925683	HM485345	–	JQ954494	[30,31]
<i>Tuber brumale</i>	GB53	JQ925684	FJ748900	–	JQ954495	[30,33]
<i>Tuber californicum</i>	JT28058	JQ925685	HM485346	JX022574	JQ954496	[30,31]
<i>Tuber calosporum</i>	BJTC FAN301	OM366182	OM246568	OM649586	OM584242	[9]
<i>Tuber calosporum</i>	BJTC FAN418	OM366202	OM246573	OM649607	OM584265	[9]
<i>Tuber canaliculatum</i>	JT28215	–	JQ925643	JX022575	JQ954497	[30]
<i>Tuber canaliculatum</i>	OSC59072	–	HM485347	JX022576	JQ954498	[30,31]
<i>Tuber caoi</i>	BJTC FAN271, holotype	KP276197	KP276183	KP276216	OM584237	[9,11]
<i>Tuber caoi</i>	BJTC FAN293	KP276198	KP276182	KP276217	OM584240	[9,11]
<i>Tuber chuxiongense</i>	HKAS 148947, holotype	PX247850	PX245746	PX512137	PX310545	This study
<i>Tuber chuxiongense</i>	HKAS 148948	PX247851	PX245747	PX512138	–	This study
<i>Tuber crassitunicatum</i>	BJTC FAN465, holotype	OM366205	MH115295	OM649610	OM584268	[9,13]
<i>Tuber depressum</i>	BJTC FAN340	OM366187	OM256744	OM649592	OM584250	[9]
<i>Tuber depressum</i>	BJTC FAN534	OM366211	OM256764	OM649616	–	[9]
<i>Tuber dryophilum</i>	GB37	JQ925688	HM485354	JX022578	JQ954501	[30,31]
<i>Tuber elevatireticulatum</i>	XTAM3, holotype	LC425121	MF540618	–	–	[34]
<i>Tuber excavatum</i>	A-OSC80639	JQ925690	HM485355	–	–	[30,31]
<i>Tuber excelsum-reticulatum</i>	BJTC FAN755, paratype	OM366217	OM265262	OM649624	OM584278	[9]
<i>Tuber excelsum-reticulatum</i>	BJTC FAN758	OM366218	OM265264	OM649625	OM584279	[9]
<i>Tuber excelsum-reticulatum</i>	BJTC FAN863, holotype	OM366224	OM265272	OM649631	OM584281	[9]
<i>Tuber flavidosporum</i>	K213, holotype	AB553520	AB553446	AB553540	AB553560	[35]
<i>Tuber formosanum</i>	BJTC FAN107	OM366159	MF621549	OM649564	OM584210	[9]
<i>Tuber formosanum</i>	BJTC FAN356	OM366189	MF627986	OM649594	OM584252	[9]
<i>Tuber fulgens</i>	M2435	JQ925691	HM485358	JX022580	–	[30,31]
<i>Tuber gennadii</i>	BM667	–	HM485359	JX022581	JQ954502	[30,31]
<i>Tuber gennadii</i>	JT9627, paratype	JQ925692	HM485360	JX022582	JQ954503	[31]
<i>Tuber gennadii</i>	M1904	JQ925693	HM485361	JX022583	JQ954504	[31]
<i>Tuber gibbosum</i>	JT26632	FJ809862	FJ809862	JX022584	JQ954505	[30,32]
<i>Tuber gibbosum</i>	JT30580	FJ809868	FJ809868	JX022585	JQ954506	[32]
<i>Tuber glabrum</i>	BJTC FAN228, holotype	OM366177	KF002731	OM649581	OM584234	[9]
<i>Tuber glabrum</i>	BJTC FAN232, paratype	OM366179	KF002727	OM649583	OM584236	[9]
<i>Tuber griseolivaceum</i>	BJTC FAN469, holotype	OM366207	KY428921	OM649612	–	[36]
<i>Tuber huidongense</i>	BJTC FAN101	OM366156	OM311172	OM649562	OM584208	[9]
<i>Tuber huidongense</i>	BJTC FAN104	OM366158	JF921163	OM649563	OM584209	[9]
<i>Tuber huiliense</i>	BJTC FAN288	OM366181	OM256781	OM649585	OM584238	[9]
<i>Tuber huizeanum</i>	BJTC FAN186, holotype	OM366170	JQ910651	OM649575	–	[9]
<i>Tuber huizeanum</i>	BJTC FAN313	KT067691	KT067684	KT067715	OM584245	[9,10]
<i>Tuber huizeanum</i>	BJTC FAN314	KT067692	KT067685	OM649588	OM584246	[10]
<i>Tuber humilireticulatum</i>	BJTC FAN174, holotype	OM366168	KT067677	KT067724	OM584224	[10]
<i>Tuber humilireticulatum</i>	BJTC FAN189	OM366171	KT067689	KT067718	OM584226	[10]
<i>Tuber japonicum</i>	K228, paratype	AB553519	AB553434	AB553539	AB553559	[35]
<i>Tuber jinshajiangense</i>	BJTC FAN406	OM366199	KX575841	OM649604	OM584262	[37]
<i>Tuber jinshajiangense</i>	BJTC FAN407	OM366200	KX575842	OM649605	OM584263	[37]
<i>Tuber huidongense</i>	SDBR-CMU-MTUF006, holotype	KU207732	KT758730	–	–	Unpublished
<i>Tuber latisporum</i>	BJTC FAN125	KT067695	KT067676	KT067725	OM584214	[10]
<i>Tuber latisporum</i>	BJTC FAN126	KP276204	KP276189	KP276205	OM584215	[11]
<i>Tuber liaotongense</i>	BJTC FAN550	OM366213	MH115302	OM649618	OM584272	[13]

(to be continued)

Table 1. (continued)

Species name	Voucher/strain	GenBank accession numbers				Ref.
		nrLSU	ITS	<i>tef1-α</i>	<i>rpb2</i>	
<i>Tuber lijiangense</i>	BJTC FAN307	KP276203	KP276188	KP276206	OM584244	[11]
<i>Tuber lishanense</i>	BJTC FAN718, holotype	MH115304	MH115303	OM649622	OM584276	[13]
<i>Tuber lishanense</i>	BJTCFAN683	MH115306	MH115305	OM649621	OM584275	[13]
<i>Tuber liyuanum</i>	BJTC FAN162, holotype	KT067698	JQ771191	KT067710	OM584218	[38]
<i>Tuber longispinosum</i>	K225	AB553518	AB553414	AB553538	AB553558	[35]
<i>Tuber luyashanense</i>	BJTC FAN1031, holotype	OM366157	OM256769	OM649637	—	[9]
<i>Tuber luyashanense</i>	BJTC FAN776	OM366219	OM256803	OM649626	—	[9]
<i>Tuber luyashanense</i>	BJTC FAN803, paratype	OM366220	OM256771	OM649627	—	[9]
<i>Tuber luyashanense</i>	BJTC FAN830	OM366221	OM256821	OM649628	—	[9]
<i>Tuber lyonii</i>	GB108	JQ925698	FJ748910	—	JQ954510	[30,33]
<i>Tuber macrosporium</i>	JT13362	FJ809838	HM485373	JX022590	—	[32]
<i>Tuber maculatum</i>	BJTC FAN868	OM366227	OM265274	OM649634	OM584283	[9]
<i>Tuber maculatum</i>	BJTC FAN876	OM366228	OM265278	OM649635	OM584284	[9]
<i>Tuber magnameshanum</i>	BJTC FAN537	OM366212	OM256767	OM649617	—	[9]
<i>Tuber magnatum</i>	GB12	JQ925700	JQ925645	JX022591	JQ954512	[30]
<i>Tuber magnatum</i>	GB13	JQ925701	JQ925646	JX022592	JQ954513	[30]
<i>Tuber malacodermum</i>	JT32319	JQ925702	FJ809889	JX022593	JQ954514	[32]
<i>Tuber melanosporum</i>	GB200	JQ925703	FJ748904	JX022594	JQ954515	[32]
<i>Tuber mexiusanum</i>	ITCV181	JF419293	HM485411	JX022602	—	[31]
<i>Tuber microcarpum</i>	BJTC FAN848	OM366223	OM256829	OM649630	—	[9]
<i>Tuber microcarpum</i>	BJTC FAN866, holotype	OM366225	OM256770	OM649632	OM584282	[9]
<i>Tuber microcarpum</i>	BJTC FAN867	OM366226	OM256831	OM649633	—	[9]
<i>Tuber miquihuanense</i>	ITCV885	JF419292	HM485414	JX022603	—	[31]
<i>Tuber multumaculatum</i>	OSC62169, paratype	JQ925705	HM485377	JX022596	—	[31]
<i>Tuber neoexcavatum</i>	BJTC FAN184, holotype	OM366169	JX458715	OM649574	—	[9]
<i>Tuber neoexcavatum</i>	BJTC FAN316	OM366184	OM256741	OM649589	OM584247	[9]
<i>Tuber nitidum</i>	BM105	FJ809807	FJ809885	JX022597	JQ954517	[32]
<i>Tuber oligospermum</i>	AH38984	JN392320	JN392261	—	—	Unpublished
<i>Tuber oregonense</i>	GB284, holotype	FJ809874	FJ809874	—	JQ954518	[32]
<i>Tuber parvomurphium</i>	BJTC FAN298, holotype	KP276190	KP276186	KP276214	OM584241	[11]
<i>Tuber pseudobrumale</i>	BJTC FAN306	OM366183	OM287838	OM649587	OM584243	[9]
<i>Tuber pseudobrumale</i>	BJTC FAN322	OM366186	OM287839	OM649591	OM584249	[9]
<i>Tuber pseudofulgens</i>	BJTC FAN368	OM366191	OM256745	OM649596	OM584254	[9]
<i>Tuber pseudofulgens</i>	BJTC FAN388	OM366194	OM256755	OM649599	OM584257	[9]
<i>Tuber pseudofulgens</i>	BJTC FAN399, holotype	OM366196	OM256757	OM649601	OM584259	[9]
<i>Tuber pseudohimalayaense</i>	BJTC FAN122	OM366162	MF627983	OM649567	OM584213	[9]
<i>Tuber pseudomaganatum</i>	BJTC FAN163, holotype	KP276192	JQ771192	KP276208	OM584219	[11]
<i>Tuber pseudomaganatum</i>	BJTC FAN391	OM366195	OM265244	OM649600	OM584258	[9]
<i>Tuber pseudosphaerosporum</i>	BJTC FAN250, holotype	OM366180	KF744063	OM649584	—	[39]
<i>Tuber shearii</i>	OSC51052	JF419280	HM485389	—	JQ954521	[31]
<i>Tuber shii</i>	BJTC FAN405	OM366198	KX555453	OM649603	OM584261	[37]
<i>Tuber shii</i>	BJTC FAN409, holotype	OM366201	KX555454	OM649606	OM584264	[37]
<i>Tuber shii</i>	BJTC FAN431	OM366204	KX555462	OM649609	OM584267	[37]
<i>Tuber sinense</i>	BJTC FAN108	OM366160	MF627968	OM649565	OM584211	[9]
<i>Tuber sinense</i>	BJTC FAN110	OM366161	MF627970	OM649566	OM584212	[9]
<i>Tuber sinoaestivum</i>	BJTC FAN487	OM366209	OM256773	OM649614	OM584270	[9]
<i>Tuber sinoaestivum</i>	BJTC FAN522	OM366210	OM256774	OM649615	OM584271	[9]
<i>Tuber sinoborchii</i>	BJTC FAN169	OM366166	OM286800	OM649572	OM584222	[9]
<i>Tuber sinoborchii</i>	BJTC FAN171, holotype	OM366167	OM286802	OM649573	OM584223	[9]
<i>Tuber sinoexcavatum</i>	BJTC FAN130, holotype	OM366163	JX458717	OM649568	OM584216	[9]
<i>Tuber sinoexcavatum</i>	BJTC FAN166	OM366165	JX458718	OM649571	OM584221	[9]
<i>Tuber sinosphaerosporum</i>	BJTC FAN135, holotype	KP276195	JX092086	OM649569	OM584217	[11]
<i>Tuber</i> sp. 1_Japan	K201	AB553512	AB553344	AB553532	AB553552	[35]
<i>Tuber</i> sp. 11_Japan	K131	—	AB553456	AB553542	AB553562	[35]
<i>Tuber</i> sp. 12_Japan	K184	AB553523	AB553458	AB553543	AB553563	[35]
<i>Tuber huidongense</i>	K217	AB553514	AB553372	AB553534	AB553554	[35]
<i>Tuber</i> sp. 4_Japan	K109-2	AB553515	AB553379	AB553535	AB553555	[35]
<i>Tuber</i> sp. 79	ITCV910	JQ925710	JQ925649	JX022612	JQ954522	[30]
<i>Tuber</i> sp. CHN-1	BJTC FAN190	OM366172	OM256778	OM649576	OM584227	[9]
<i>Tuber</i> sp. CHN-16	BJTC FAN986	OM366229	OM311257	OM649636	OM584285	[9]
<i>Tuber</i> sp. 14_Japan	K186	AB553525	AB553464	AB553545	AB553565	[35]
<i>Tuber</i> sp. 16_Japan	K221	AB553527	AB553466	AB553547	AB553567	[35]

(to be continued)

Table 1. (continued)

Species name	Voucher/strain	GenBank accession numbers				Ref.
		nrLSU	ITS	<i>tef1-α</i>	<i>rpb2</i>	
<i>Tuber</i> sp. 17_Japan	N35	AB553528	AB553467	AB553548	AB553568	[35]
<i>Tuber sphaerospermum</i>	AH39184	JN392306	JN392247	—	—	[40]
<i>Tuber sphaerosporum</i>	JT12487	FJ809853	FJ809853	JX022609	—	[32]
<i>Tuber spinoreticulatum</i>	U188, isotype	FJ809815	FJ809884	JX022608	JQ954527	[32]
<i>Tuber subglobosum</i>	BJTC FAN222	OM366175	KF002728	OM649579	OM584232	[41]
<i>Tuber taiyuanense</i>	BJTC FAN164	OM366164	OM311182	OM649570	OM584220	[9]
<i>Tuber taiyuanense</i>	BJTC FAN220	OM366174	MH115315	OM649578	OM584231	[13]
<i>Tuber taiyuanense</i>	BJTC FAN225	OM366176	MH115325	OM649580	OM584233	[13]
<i>Tuber turmericum</i>	BJTC FAN471	OM366208	KT758835	OM649613	OM584269	[42]
<i>Tuber umbilicatum</i>	BJTC FAN212	OM366173	OM311201	OM649577	OM584228	[42]
<i>Tuber umbilicatum</i>	BJTC FAN230	OM366178	OM311205	OM649582	OM584235	[9]
<i>Tuber umbilicatum</i>	BJTC FAN317	OM366185	OM311216	OM649590	OM584248	[9]
<i>Tuber umbilicatum</i>	BJTC FAN344	OM366188	OM311220	OM649593	OM584251	[9]
<i>Tuber umbilicatum</i>	BJTC FAN428	OM366203	OM311229	OM649608	OM584266	[9]
<i>Tuber variabilisporum</i>	BJTC FAN362, holotype	OM366190	OM287845	OM649595	OM584253	[9]
<i>Tuber verrucosivolum</i>	HKAS 88863, holotype	KY013650	KX904887	—	—	[14]
<i>Tuber wenchuanense</i>	BJTC FAN833	OM366222	OM311256	OM649629	OM584280	[9]
<i>Tuber wumengense</i>	BJTC FAN218A, holotype	KT067707	KT067682	KT067714	OM584230	[10]
<i>Tuber wumengense</i>	BJTC FAN292	KT067709	KT067683	KT067716	OM584239	[9]
<i>Tuber xuanhuaense</i>	BJTC FAN618	OM366214	MK045627	OM649619	OM584273	[9]
<i>Tuber yigongense</i>	BJTC FAN729	—	MF663716	OM649623	OM584277	[9]
<i>Tuber yigongense</i>	BJTC FAN731, holotype	OM366216	MF663714	—	—	[9]
<i>Tuber zhongdianense</i>	BJTC FAN178	KT067701	KT067679	KT067722	OM584225	[10]

Taxa information of the newly produced sequences in this study is shown in bold.

brown (6F8). The surface has grooves and is covered by brown, loose-textured, irregular pyramidal warts when fresh. The gleba is marbled, with white sterile veins and yellowish brown to blackish beige fertile tissue. Odor mild, taste not recorded. The peridium is composed of two layers and is pseudoparenchymatous; the outer cortical layer is $\sim 338.6 \mu\text{m}$ thick, composed of subglobose to subangular cells, $7.7\text{--}34 \times 5.4\text{--}20 \mu\text{m}$, $Q = 1.0\text{--}2.4$, $Q_m = 1.3 \pm 0.33$ ($n = 51$), brown or hyaline; the inner layer is $71.4\text{--}140 \mu\text{m}$ thick, composed of intricately interwoven, hyaline, thin-walled hyphae. Asci are irregularly spherical, $43\text{--}84.5 \times 35\text{--}80.0 \mu\text{m}$ ($n = 91$), with size and shape varying depending on the number of ascospores, with (1)–3–6(–7) ascospores. Ascospores are olive yellow (3D7) in color, ellipsoid or subglobose. Spore sizes are as follows: in 1-spored asci: $(20\text{--})22.2\text{--}38(44) \times (14\text{--})16.3\text{--}25.5(31) \mu\text{m}$, $Q = 1.2\text{--}1.6$, $Q_m = 1.4 \pm 0.13$ ($n = 9$); in 2-spored asci: $(17.7\text{--})19\text{--}33.7(35.3) \times (14.5\text{--})15.4\text{--}22.0(24) \mu\text{m}$, $Q = 1.2\text{--}1.7$, $Q_m = 1.4 \pm 0.17$ ($n = 10$); in 3-spored asci: $(20.4\text{--})21.7\text{--}34.0(37.8) \times (15.1\text{--})15.5\text{--}23.6(25.6) \mu\text{m}$, $Q = 1.2\text{--}1.9$, $Q_m = 1.5 \pm 0.15$ ($n = 46$); in 4-spored asci: $(16.8\text{--})16.9\text{--}30(30.6) \times (11.7\text{--})12.5\text{--}21(21.2) \mu\text{m}$, $Q = 1.1\text{--}2.0$, $Q_m = 1.5 \pm 0.21$ ($n = 52$); in 5-spored asci: $(15.8\text{--})16.5\text{--}31(32.7) \times (12.4\text{--})12.6\text{--}24.5(25.8) \mu\text{m}$, $Q = 1.1\text{--}1.7$, $Q_m = 1.3 \pm 0.16$ ($n = 71$); in 6-spored asci: $(15.6\text{--})17.4\text{--}26.7(27) \times (12.6\text{--})13.5\text{--}19.5(19.8) \mu\text{m}$, $Q = 1.1\text{--}1.7$, $Q_m = 1.4 \pm 0.12$ ($n = 58$); in 7-spored asci: $(10.8\text{--})14.8\text{--}26.3(27) \times (9.4\text{--})11.9\text{--}19.7(20.6) \mu\text{m}$, $Q = 1.2\text{--}1.6$, $Q_m = 1.3 \pm 0.13$ ($n = 33$). Spines on the outer surface of each spore are 2–4 μm high; the spine apices are free, blunt to slightly acute, and some bear apical hooks; the broadened bases connect to form low ridges (0.3–1.3 μm high), creating a reticulate ornamentation, and some meshes occasionally exhibit discrete spines; mostly 5–6(–7) meshes are present across the spore width.

Ecology and distribution: hypogeous, gregarious, in soil under forest dominated by *Pinus* spp.

Additional material examined (paratype): CHINA, Yunnan Prov., Chuxiong Yi Autonomous Prefecture, Dayao County, in soil under forest dominated by *Pinus* sp., 26 December 2024, Junfu Li & Hongbo Jiang, T18-2 (HKAS 148948).

Notes: *Tuber chuxiongense* differs from *T. pseudohimalayense* primarily in its ascospore reticulum (5–6(–7) irregular meshes across width vs. 3–4 regular meshes) and from *T. variabilisporum* by a higher mesh count (5–6(–7) vs. 4–5 meshes/width). Although morphologically close to *T. pseudohimalayense*, phylogenetic analyses based on combined ITS, nrLSU, *tef1-α*, and *rpb2* sequences placed *T. chuxiongense* in a distinct clade within the *Melanosporum* section (Fig. 1) (100% ML, 0.91 BYPP). The ITS rDNA sequence analysis showed that the similarity between *T. chuxiongense* and *T. pseudohimalayense* was 96.49%.

Discussion

The morphological differences in the genus *Tuber* likely reflect evolutionary adaptations in their life history and ecology[30]. Mature ascomata of *Tuber* species, which develop from underground ectomycorrhizae and enclose ascospores in the gleba, attract animal dispersers via specific volatiles (e.g., dimethyl disulfide)[43,44], thus facilitating spore dispersal and negating the need for aerial discharge. The delineation of sections in the truffle phylogeny shows a strong correlation with the characteristics of the ascomatal surface, including color, and the presence of warts, pubescence, texture, as well as ascospore traits such as size, shape, and ornamentation[8]. For example, most *Tuber* species from the *Melanosporum* section have dark, rough ascomata with distinct cavities on the surface, and ascospores bearing a spinose-reticulate ornamentation[9,12].

The results of phylogenetic analysis placed our species in the *Melanosporum* section[12] (Fig. 1). Within the *Melanosporum* section, *T. brumale* and *T. pseudobrumale* are separated by an ascomata surface covered with pyramidal warts; the surface of *T. brumale* is covered with easily detachable, penta- or hexagonal flat warts[45], while sharp brownish-yellow cones are present on *T. pseudobrumale*[12]. The new species has distinct cavities that can



Fig. 1 Maximum likelihood phylogram of *Tuber* inferred from a dataset (ITS-nrLSU-*tef1- α -rpb2*), showing the phylogenetic relationship of the new species described in this study. Bootstrap support values over 80% of maximum likelihood (BS, left) and Bayesian posterior probabilities over 0.90 (PP, right) are mentioned on the nodes. The scale bar represents the number of mutations per site. The new species is shown in bold.

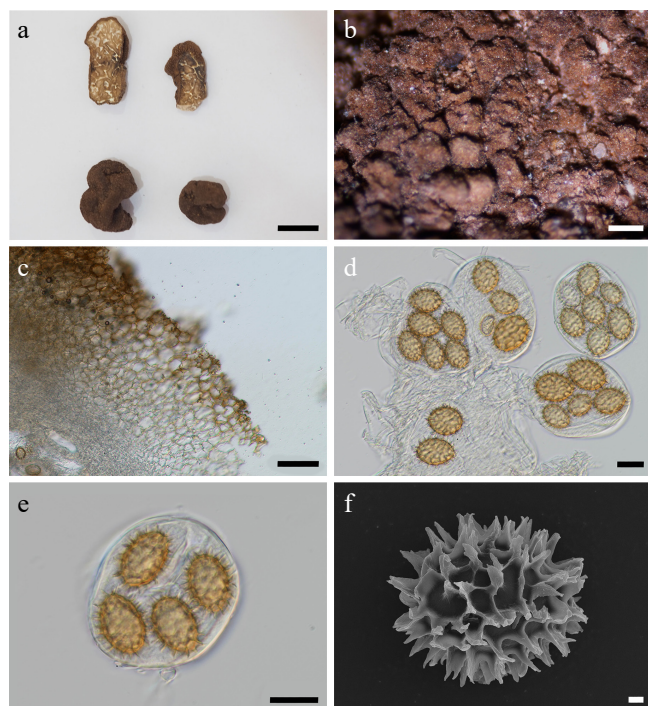


Fig. 2 *Tuber chuxiongense* (HKAS 148947, holotype). (a) Gleba and ascoma appearance. (b) Surface ascomata after drying. (c) Peridium hyphal arrangements in 10% KOH. (d, e) Asci and ascospores in 10% KOH. (f) Ascospore under scanning electron microscopy. Scale bars: a = 1 cm, b = 1 mm, c = 100 μ m, d and e = 20 μ m, f = 2 μ m.

be easily separated from *T. formosanum*, *T. longispinosum*, and *T. yigongense*, which have slightly lobed ascomata^[9,46–48]. The morphology of *T. variabilisporum* has recently undergone a revision. Originally described by Fan et al.^[9] as lacking basal cavities and possessing 1–5-spored asci with yellow-brown mature ascospores, the description was later amended by Wang et al.^[12] to include the frequent occurrence of surface depressions. Our specimens are distinguished from *T. variabilisporum* by their olive-yellow (3D7) mature spores (vs. yellow-brown), a broader ascospore number range of 1–7 per ascus (vs. 1–6), and a higher mesh count on the spore reticulum. The olive yellow ascospores of *T. chuxiongense* also distinguish it from *T. melanosporum* (blackish spiny ascospores) and *T. sinense* (red-brown to blackish brown ascospores)^[46]. *T. chuxiongense* is difficult to distinguish from *T. pseudohimalayense* in the fresh state. Moreno et al.^[49] first described the morphological characteristics of *T. pseudohimalayense*, with the observation that its ascospores covered a regular and completely spinose-reticulate ornamentation. The ascospore ornamentation of *T. pseudohimalayense* was documented by Liu^[50] as having 3–4 meshes along the transverse diameter. The discovery of *T. chuxiongense* adds to the growing inventory of endemic *Tuber* species in southwestern China. Further surveys are needed to determine the host associations and ecological preferences of this newly described taxon.

Conclusions

Based on both the morphological characteristics and phylogenetic analysis of combined ITS, nrLSU, *rpb2*, and *tef1- α* sequence data, this study describes *T. chuxiongense* as a new species of truffle from the pine forests of Yunnan, China, belonging to the *Melanosporum* group. These data will be useful for taxonomic, biogeographical, and agroforestry studies in the future.

Author contributions

The authors confirm their contributions to the paper as follows: study conception and design: Tao Q, Hu Y, Jiang H; data collection: Tao Q, Hu Y, Jiang H, Li J; analysis and interpretation of the results: Tao Q, Hu Y, Jiang H, Li J, Ye L, Xu J, Jeewon R; draft manuscript preparation: Tao Q, Hu Y, Jiang H. All authors reviewed the results and approved the final version of the manuscript.

Data availability

All sequences generated in this study were submitted to GenBank. In addition, the new taxon was registered in MycoBank, and the final alignment of phylogeny based on the ITS-nrLSU-*tef1- α* -*rpb2* dataset in this study was deposited in Zenodo.

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Conflict of interest

The authors declare that they have no conflict of interest.

Dates

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