

Report on a New Truffle Species, *Tuber koreanum* sp. nov., from Korea

Hyeok Park^{a,b} , Ju-Hui Gwon^a , Jong-Chul Lee^a  and Ahn-Heum Eom^a 

^aDepartment of Biology Education, National University of Education, Cheongju, Republic of Korea; ^bDivision of Bioresource Bank, Honam National Institute of Biological Resources, Mokpo, Republic of Korea

ABSTRACT

The truffle and ectomycorrhizal roots formed by *Tuber* sp. were collected from the rhizosphere of *Quercus aliena* in Korea. The morphological characteristics of the ascoma, and molecular phylogenetic analysis using sequences from the internal transcribed spacer (ITS) and large subunit (LSU) of ribosomal DNA, translation elongation factor 1-alpha (TEF), and RNA polymerase second largest subunit (RPB2) regions confirmed the distinct morphology of the truffle. This truffle belongs to a monophyletic clade among the other *Tuber* species in the phylogeny. This study describes the truffle, *Tuber koreanum*, as a new species reported from Korea.

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1. Introduction

The genus *Tuber* P. Micheli ex F. H. Wigg. belongs to the family Tuberaceae, order Pezizales, and division Ascomycota. *Tuber* species produce hypogeous fruiting bodies called truffles. Truffles have several ascospores in a hyaline ascus, and the ascospores have an alveolate–reticulate or a spino-reticulate shaped surface [1]. Some truffles are edible and have commercial value because of their unique aroma [2]. *Tuber* species have an ectomycorrhizal (ECM) relationship with the roots of some woody plants belonging to the families Betulaceae, Cistaceae, Corylaceae, Fagaceae, and Pinaceae [3,4].

More than 180 *Tuber* species have been recorded in the MycoBank database (<http://mycobank.org>) and new species are continuously being reported worldwide [5]. However, *Tuber* species have been poorly studied and only five species have been reported till date in Korea: *T. aestivum* subsp. *uncinatum*, *T. borchii*, *T. himalayense*, *T. huidongense*, and *T. indicum* [6–9]. During a survey of *Tuber* species in Korea, *Tuber* ascoma were collected from the rhizosphere of oak trees. Based on their morphological characteristics and molecular analysis, the ascoma did not represent any previously described species of the genus *Tuber*. Therefore, in this study, we report a novel *Tuber* species from Korea.

2. Materials and methods

2.1. Sampling

Fresh specimens of fruiting bodies and ECM roots were collected from the rhizosphere of oak trees,

Quercus aliena Blume., in Gyeongju (N35°43'10.8", E129°20'36.3") in September 2020. The specimens were transported to the laboratory, where the morphological characteristics of ascoma and ascospores were observed under a dissecting microscope and an optical microscope. The ascocarps examined were deposited in the Herbarium of Korea National University of Education, Cheongju, Korea.

2.2. Morphological analysis

Mycorrhizal roots were observed under a microscope, and the mycorrhizal cross sections were obtained using a microtome (Leica Microsystems Nussloch GmbH, Nussloch, Germany). Morphological characteristics such as the color, branching systems, texture, and hyphae of the mycorrhiza were recorded [10].

2.3. Phylogenetic analysis

The genomic DNA of the ECM root tips and ascomata were extracted and amplified using PCR. ITS1F/ITS4 primers were used to amplify the internal transcribed spacer (ITS) region of ribosomal DNA (rDNA) [11]. In addition, LR0R/LR16 primers to amplify the large subunit (LSU) region [12], EF1 α Tuber-f/EF1 α Tuber-r that amplifies translation elongation factor 1-alpha (TEF) region [5], and fRPB2-5F/fRPB2-7cR primers that amplify RNA polymerase second largest subunit (RPB2) region [13] were used for ascomata. After PCR, each DNA band was confirmed through polymerase gel electrophoresis and sent for DNA sequencing (SolGent Co. Ltd., Daejeon, Korea). The DNA sequence

information was used to find similarities using the BLAST search program from the National Center for Biological Information (NCBI). The phylogenetic trees of aligned DNA sequences (Table 1) were constructed using maximum likelihood (ML) method by Kimura 2-parameter (K2P) substitution model [14]. The ambiguous characters were excluded from the analysis, and the gaps were treated as missing data. The ML bootstrap replicates (1000) were computed for the best scoring ML tree. Bayesian analysis [15] was performed for calculating posterior probabilities (>50% majority rule consensus trees) on the branches of phylogenetic tree.

3. Results and discussion

3.1. Taxonomy

Tuber koreanum H. Park & A. H. Eom, sp. nov. [#MB840072] (Figure 1).

Type: KOREA. Gyeongsangbukdo: Gyeongju-si, in rhizosphere soil under *Quercus aliena*, 9 September 2020, collected by Hyeok Park, GB20004 (holotype), GB20011 and GB20046 (isotype). GenBank no.: OK275104 (ITS), OK275105 (LSU).

Etymology: The first new truffle species discovered in Korea

ASCOMATA globose to subglobose, rarely ovoid, irregularly rugged surface, bright white to yellowish beige, (5–20) × (4–15) mm in diameter. GLEBA grayish brown to yellowish brown, bright white mycelia mixed in partially, pale brown in the part with mature ascospores. PERIDIUM (61.0–)71.2(–92.8) µm in thickness, dark beige to grayish brown, divided into two layers, outer layer yellowish brown and thicker than inner layer, inner layer mucoid, dark brown, white mycelia distributed in several places. ASCI hyaline, squashed, ellipsoid to conical, with smooth margin, aseptate, size differing based on the number of ascospores (2–4 spores) in each ascus, (22.6–)38.5(–51.2) × (20.1–)29.8(–34.8) µm in diameter. ASCOSPORE initially bright ivory or pale gold, reddish brown to pale brown in mature spore, glittering, subglobose to ovoid, sometimes ellipsoid (13.2–)17.2(–21.9) × (13.2–)15.9(–19.6) µm in diameter. The polygonal pieces on the surface of the mature ascospore form a reticulate ornament with several spines on the external surface; spines are usually curved, with sharp end, (1.9–)2.7(–3.4) µm high.

Table 1. List of *Tuber* sequences used in the phylogenetic analysis.

Species	Isolate/voucher	ITS	GenBank accession No.		
			LSU	TEF	RPB2
<i>Tuber koreanum</i>	GB20011	OK275104	OK275105		
<i>Tuber asa</i>	M1828			JX022569	
<i>Tuber beyerlei</i>	JT32597			JX022570	
<i>Tuber bernardinii</i>	M44				KY420132
<i>Tuber borchii</i>	AH39139	JN392230	JN392291	JX022571	
<i>Tuber brennemanii</i>	RH1279			MH159204	MH032561
<i>Tuber californicum</i>	JT28058			JX022574	JQ954496
<i>Tuber canaliculatum</i>	OSC59072				JQ954498
<i>Tuber cistophilum</i>	AH39275	JN392231	JN392293		
<i>Tuber dryophilum</i>	GB35	JQ925644	JQ925687	JX022578	JQ954501
<i>Tuber flavidosporum</i>	K213	AB553446	AB553520		AB553560
<i>Tuber floridanum</i>	MES654				MH032563
<i>Tuber gennadii</i>	BM667				JQ954502
<i>Tuber gibbosum</i>	JT26632	FJ809862	FJ809834		JQ954505
<i>Tuber huizeanum</i>	BJTC FAN186	JQ910651	KT067703	KT067720	
<i>Tuber japonicum</i>	K228	AB553434	AB553519		AB553559
<i>Tuber jinshajiangense</i>	BJTC FAN451	KX575845	KX575849		
<i>Tuber latisporum</i>	BJTC FAN125			KT067725	
<i>Tuber lijiangense</i>	BJTC FAN307	KP276188	KP276203		
<i>Tuber liyuanum</i>	BJTC FAN162	JQ771191	KT067698	KT067719	
<i>Tuber lyonii</i>	GA21				JQ954510
<i>Tuber melanosporum</i>	GB200	KM659874	JQ925703		
<i>Tuber mexiusanum</i>	ITCV181			JX022602	
<i>Tuber nitidum</i>	BM105				JQ954517
<i>Tuber oregonense</i>	GB284	FJ809874	FJ809835		JQ954519
<i>Tuber pseudomagnatum</i>	BJTC FAN315			KT067711	
<i>Tuber puberulum</i>	ZB1077	JF261376	JF261339		
<i>Tuber shearii</i>	OSC51052				JQ954521
<i>Tuber sinosphaerosporum</i>	BJTC FAN136	JX092087	KP276196		
<i>Tuber sphaerosporum</i>	JT12487			JX022609	
<i>Tuber subglobosum</i>	BJTC FAN222	KF002728	MH115324		
<i>Tuber wumengense</i>	BJTC FAN292			KT067716	
<i>Tuber zhongdianense</i>	BJTC FAN176	KP276178	KP276201	KT067723	
<i>Venturia pyrina</i>	CBS 120825	MH863093	MH874652		
<i>Morchella americana</i>	IN15-13			KY637207	
<i>Epicoccum laticollum</i>	ZJB380037				MN991305

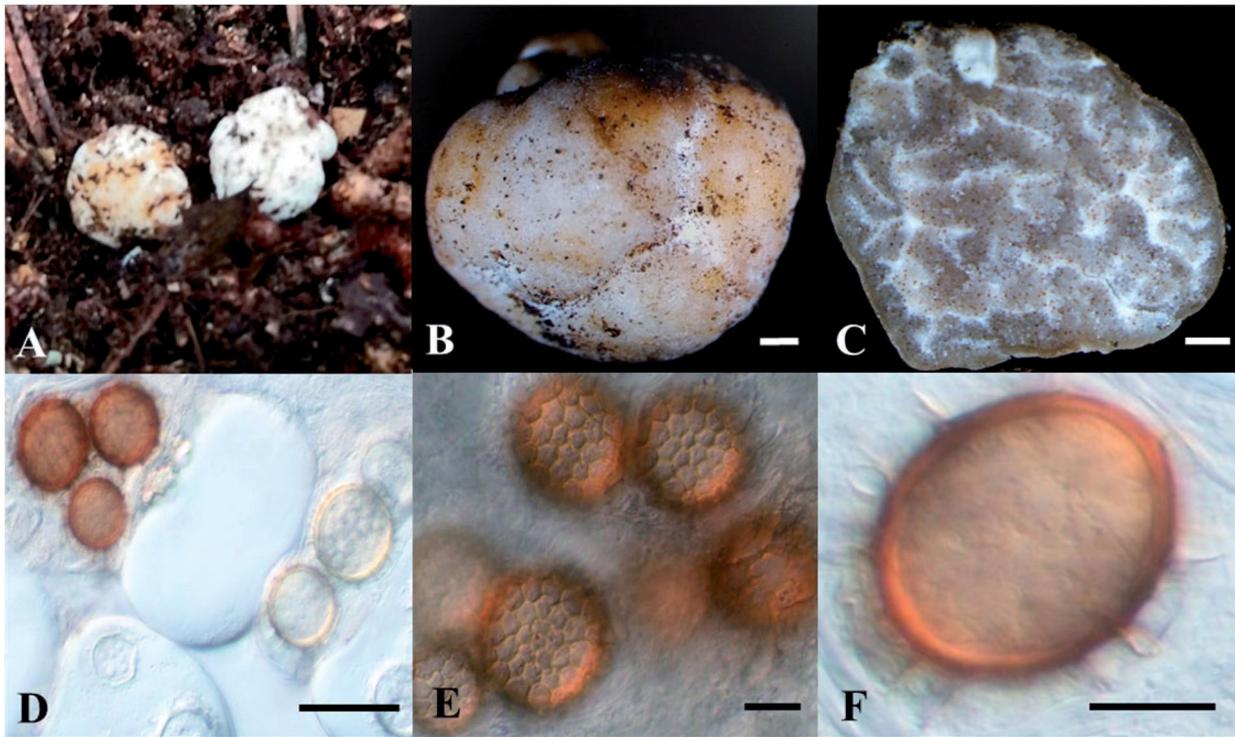


Figure 1. Morphological characteristics of *Tuber koreanum*. Ascoma (A, B), gleba and peridium (C), asci (D), and ascospores (E, F) (scale bars: B, C = 1000 μ m, D = 100 μ m, E, F = 10 μ m).

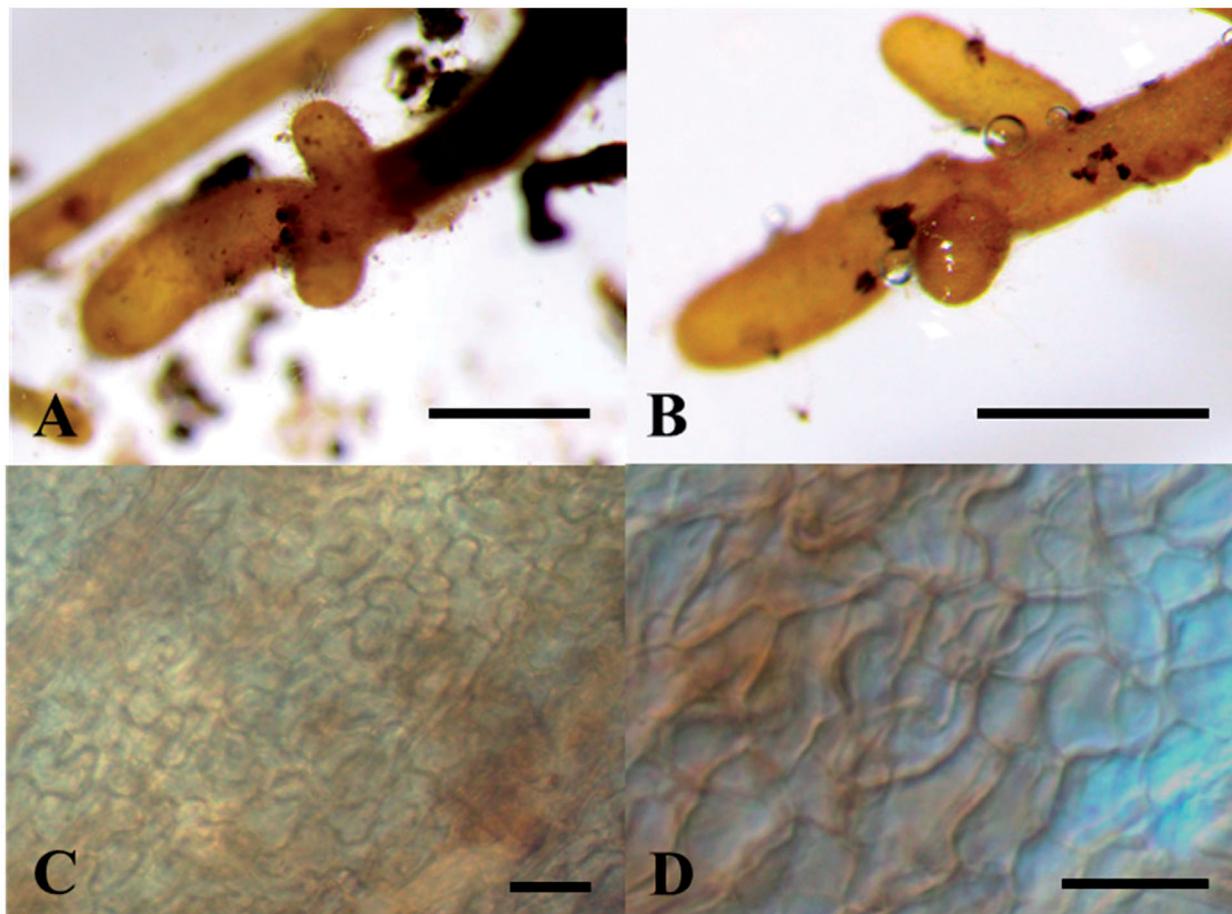


Figure 2. Morphological characteristics of ectomycorrhiza colonized by *Tuber koreanum* from root of *Quercus aliena*. Mycorrhizal root tips (A, B); Fungal mantle layer (E) (scale bars: A, B = 500 μ m, C, D = 20 μ m).

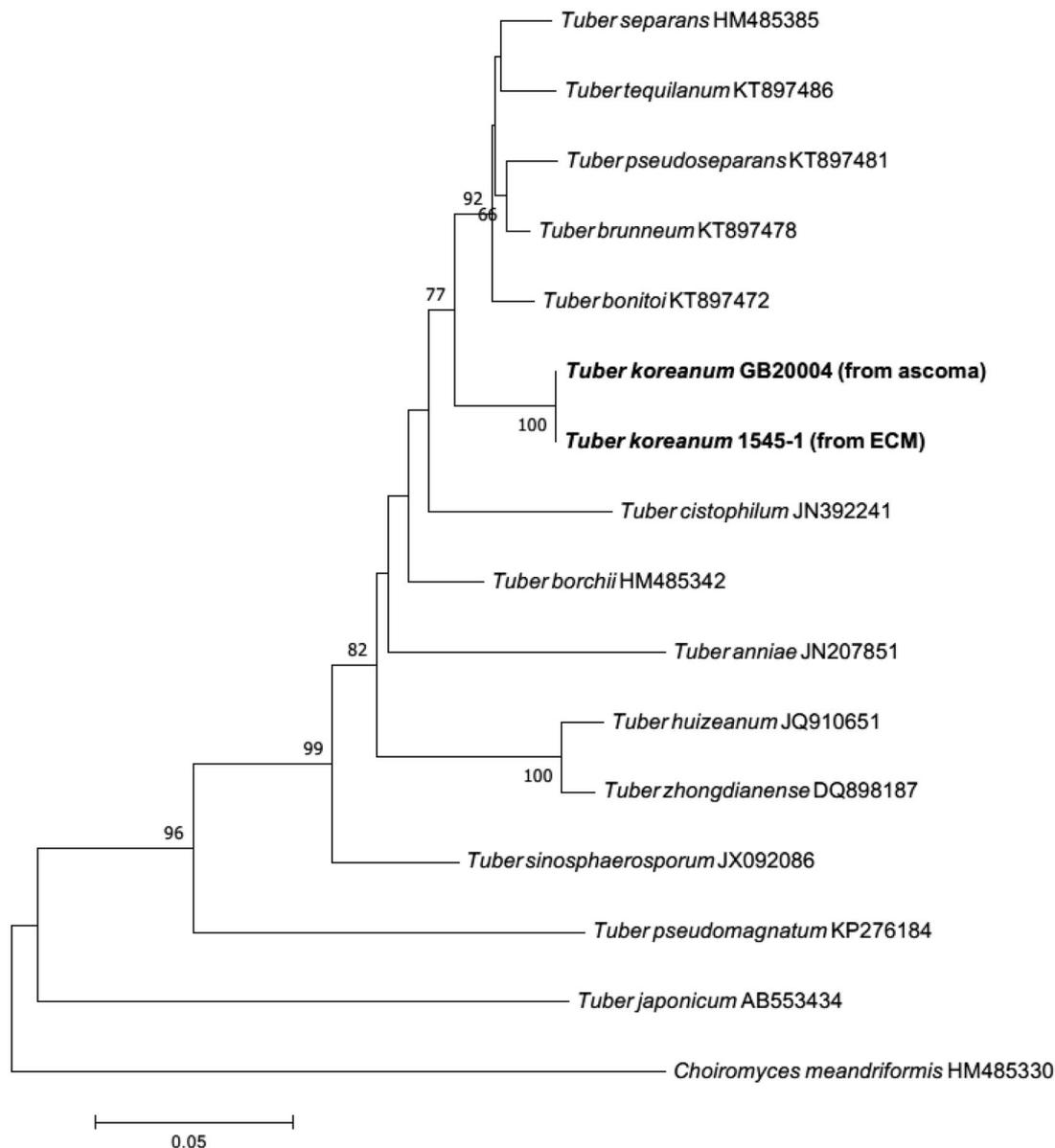


Figure 3. Maximum-likelihood phylogenetic tree of *Tuber koreanum* based on the alignment of the internal transcribed spacer (ITS) rDNA sequences obtained from ectomycorrhizal root tip. *Choioomyces meandriformis* was used as an outgroup. Numbers on branches indicate bootstrap values (1,000 replicates). Sequences from the present study were in bold.

Mycorrhiza: The ECOM root tips were straight, rarely curved, pale yellow to ivory, vertically branched, and the length of the branch from the side was generally shorter than that of the forward branch (Figure 2). The fungal mantle layer showed an interlocking-synenchyma structure (Figure 2). The ITS rDNA sequence of ECM root tip showed a coincidence with the sequences of the ascoma and the mycelium. Furthermore, they formed a monophyletic group on the phylogenetic tree (Figure 3).

The morphological characteristics of GB20004 ascoma were compared with the other truffle species that have similar appearances (Table 2). The GB20004 ascoma have beige or light white peridium, light grayish-brown or yellowish-brown gleba, and conical or irregular oval ascus, whereas

Tuber borchii showed reddish-brown or dark brown peridium, dark brown gleba, and an oval-shaped ascus tapering at the base [16]. Some characteristics of GB20004 ascoma were similar to *Tuber flavidosporum* or *Tuber japonicum*, a truffle discovered in Japan [17]. The ascospores of GB20004 were mainly ovoid, and sometimes ellipsoid, while the ascospores of *T. flavidosporum* or *T. japonicum* were close to perfect globose shaped. The color of GB20004 ascospores was ivory or light gold, initially but changed to reddish-brown or pale brown as the spores matured. *T. flavidosporum* and *T. japonicum* showed white or pale yellow ascospores [17]. In addition, the peridium thickness of *T. flavidosporum* and *T. japonicum* was more than 200 μm [17], while that of GB20004 was less than 100 μm . GB20004 showed

Table 2. Morphological characteristics of *Tuber koreanum* with the allied *Tuber* species.

	<i>T. koreanum</i> GB20004	<i>T. borchii</i> [16]	<i>T. flavidosporum</i> [17]	<i>T. japonicum</i> [17]
Ascoma	Globose to subglobose, rarely ovoid, irregularly rugged surface, bright white to yellowish beige	Subglobose to irregular, surface dry, glabrous, initially paler, darkening to brown, rarely with red-brown patches	Subglobose, light brown to brown	Subglobose or lobed, firm, whitish to pale yellow
Size	(5–20) × (4–15) mm in diam.	25 × 5 mm in diam.	20 mm in diam.	10–40 mm in diam.
Gleba	Grayish brown to yellowish brown, bright white mycelia mixed in partially, pale brown in the part with mature ascospores	Grey when young, becoming dark brown with maturity, marbled throughout with irregularly branching white veins	Whitish to pale yellow	Whitish to pale yellow, marbled with white sterile veins
Peridium	(61.0–)71.2(–92.8) μm in thickness, 2-layers, dark beige to grayish brown	140–260 μm thick, hyaline, 2-layers	Smooth, 2-layers. 200–250 μm thick	Smooth, 2-layers, 240–280 μm thick
Asci	Hyaline, ellipsoid to conical, with smooth margin, 2–4 spored, (22.6–)38.6(–51.3) × (20.2–)29.9(–34.8) μm in diam.	Subglobose to ellipsoid, tapering toward base, 1–4 spored, 76–99 × 63–85 μm in diam.	obovate to broadly ellipsoid, 1–2 spored, (73–97) × (56–77) μm in diam.	spindle to obovate, (1–)2(–3) spored, (75–133) × (47–106) μm in diam.
Ascospores	Initially bright ivory or pale gold, reddish brown to pale brown in mature, glittering, subglobose to ovoid, sometimes ellipsoid, reticulate ornaments, (13.2–)17.2(–21.9) × (13.18–)15.89(–19.61) μm in diam.	Subglobose to ellipsoid, yellow-brown, ornamentation densely reticulate-alveolate (23–)27–47(–55) × (19–)21–41(–44) μm in diam.	globose, reticulate, light yellow, reticulate ornaments, 32–45 μm in diam.	Globose, whitish to yellow, reticulate ornamentation, 25–50 μm in diam.

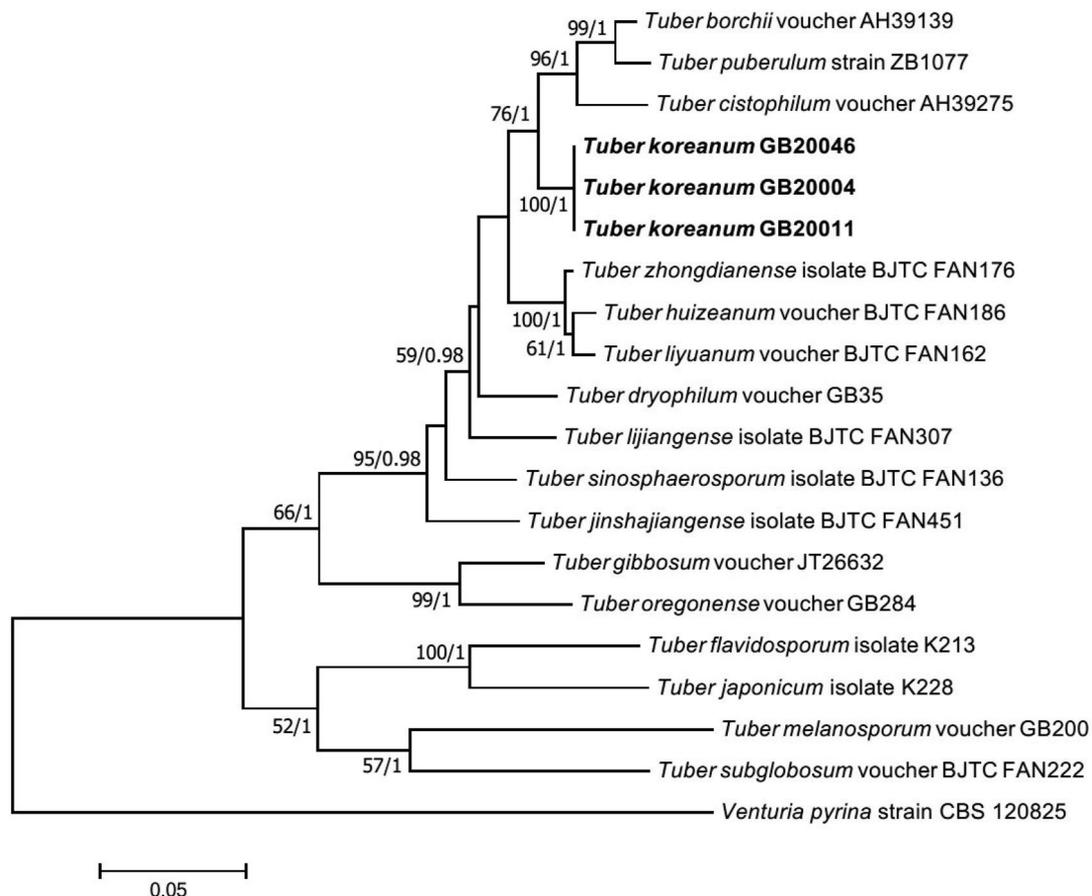
**Figure 4.** Phylogenetic tree of *Tuber koreanum* ascoma inferred using the maximum likelihood method based on alignment of ITS and LSU DNA sequences. *Venturia pyrina* was used as an outgroup. Strains used in this study are in bold.



Figure 5. Phylogenetic tree of *Tuber koreanum* ascoma inferred using the maximum likelihood method based on alignment of TEF DNA sequences. Bootstrap values and Bayesian posterior probabilities are indicated below branches. *Epicoccum laticollum* was used as an outgroup. Strains used in this study are in bold.

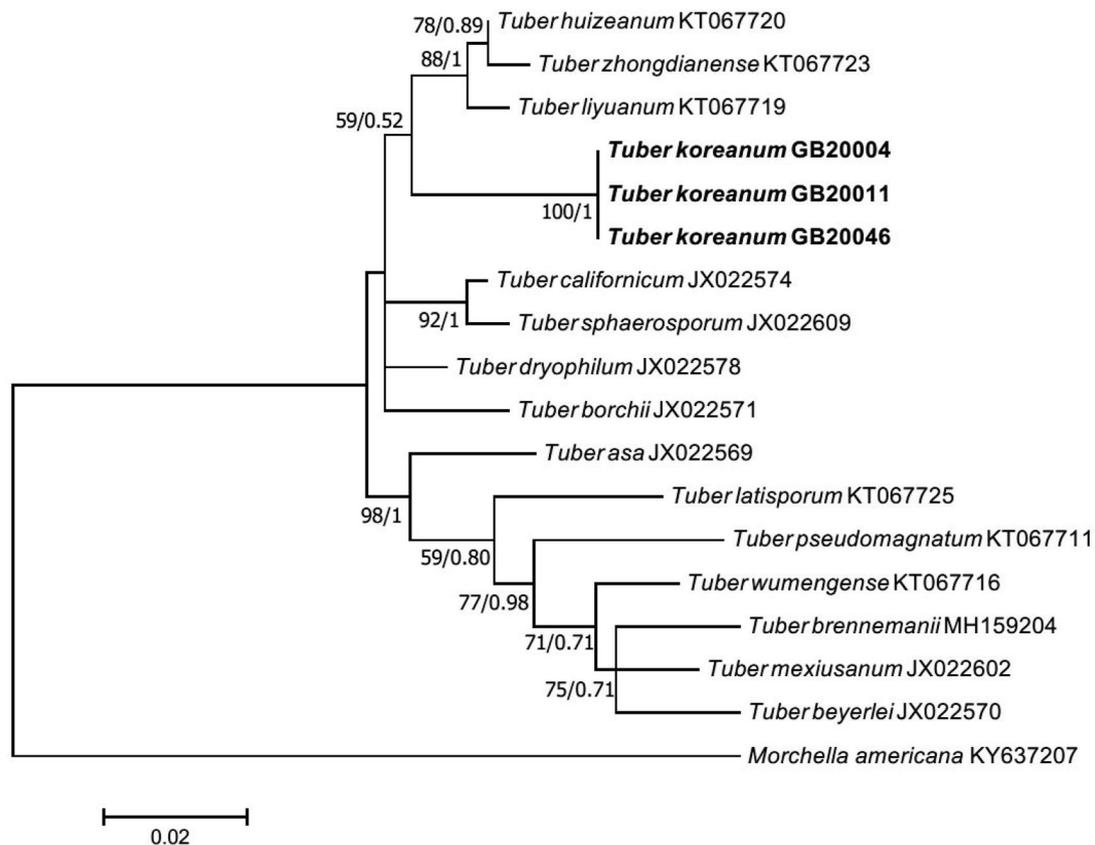


Figure 6. Phylogenetic tree of *Tuber koreanum* ascoma inferred using the maximum likelihood method based on alignment of RPB2 DNA sequences. Bootstrap values and Bayesian posterior probabilities are indicated below branches. *Morchella americana* was used as an outgroup. Strains used in this study are in bold.

morphological characteristics that were clearly distinguishable from *T. borchii*, *T. flavidosporum*, and *T. japonicum*.

On the ML phylogenetic tree, GB20004 formed a monophyletic group distinct from other species. The analysis of the ITS-LSU region and the TEF DNA sequences showed that GB20004 was closer to *Tuber liyuanum*, *Tuber huizeanum*, and *Tuber zhongdianense* than to *T. borchii*, *T. flavidosporum* and *T. japonicum* (Figures 4 and 5). An analysis of the RPB2 DNA sequence also showed that GB20004 was clearly located apart from *T. borchii*, *T. flavidosporum* and *T. japonicum* (Figure 6), implying that GB20004 is distinctly different from these three species both morphologically and molecularly. Based on these results, we determined *Tuber koreanum* GB20004 as a novel truffle species that has not been recorded yet.

Disclosure statement

No potential conflict of interest was reported by the authors.

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ORCID

Hyeok Park  <http://orcid.org/0000-0003-1387-4201>
 Ju-Hui Gwon  <http://orcid.org/0000-0003-4856-2101>
 Jong-Chul Lee  <http://orcid.org/0000-0003-0581-8154>
 Ahn-Heum Eom  <http://orcid.org/0000-0002-6821-1088>

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