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, and T. huidongense [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 asccarps 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 [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_, September 2020, collected by Hyeok Park, GB20046 (holotype), GB20004 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. PERIDIIUM (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 | GenBank accession No. |
|--------------------------|-----------------|-----------------------|
| _Tuber koreanum_         | GB20011         | OK275104, OK275105    |
| _Tuber asa_              | M1828           | JX022569              |
| _Tuber beyerlei_         | JT32597         | JX022570              |
| _Tuber bernardii_        | M44             | JX022571              |
| _Tuber borchii_          | AH39139         | JN392230              |
| _Tuber brennemanii_      | RH1279          | JN392249              |
| _Tuber californicum_     | JT28058         | JX022574              |
| _Tuber canalicularatum_  | OSC59072        | JQ954498              |
| _Tuber cistophilum_      | AH39275         | JN392231              |
| _Tuber dryophilum_       | GB35            | JQ925644              |
| _Tuber flavidosporum_    | K213            | AB553446, AB553520    |
| _Tuber floridanum_       | MES654          | JQ954502              |
| _Tuber gennadii_         | BM667           | JX022578              |
| _Tuber gibbosum_         | JT26632         | FJ809862, FJ809834    |
| _Tuber huizeanum_        | BJTC FAN186     | JQ910651, KT067703, KT067720 |
| _Tuber japonicum_        | K228            | AB553434              |
| _Tuber jinshajiangense_  | BJTC FAN451     | AB553519              |
| _Tuber latisporum_       | BJTC FAN125     | AB553584              |
| _Tuber lisanjense_       | BJTC FAN307     | KP276203              |
| _Tuber liyani_           | BJTC FAN162     | JO771191, KT067698, KT067719 |
| _Tuber liyianum_         | GA21            | AB553546              |
| _Tuber melanosporum_     | GE200           | KM659874, JO925703    |
| _Tuber mexicanum_        | ITCV181         | JO925410              |
| _Tuber nitidum_          | BM105           | JX022602              |
| _Tuber oregonense_       | GB284           | FJ809874              |
| _Tuber pseudomagnatum_   | BJTC FAN315     | JF261376, JF261339    |
| _Tuber puberulum_        | ZB1077          | JF261376              |
| _Tuber shearii_          | OSC51052        | JT067711              |
| _Tuber sinosphearaeoporum_ | BJTC FAN136  | KP276196              |
| _Tuber sphaerooporum_    | JT12487         | KP276196              |
| _Tuber subglobosum_      | BJTC FAN222     | KP276196              |
| _Tuber wumengense_       | BJTC FAN292     | KP276196              |
| _Tuber zhongdianense_    | BJTC FAN176     | KP276196              |
| _Venturia pyrina_        | CBS 120825      | MH863093              |
| _Morchella americana_    | IN15-13         | KY637207              |
| _Epicoccum latusicollum_ | JZB380037       | 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 μm, D = 100 μm, E, F = 10 μm).

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 μm, C, D = 20 μm).
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

![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. Choiromyces meandriformis was used as an outgroup. Numbers on branches indicate bootstrap values (1,000 replicates). Sequences from the present study were in bold.](image-url)
Table 2. Morphological characteristics of *Tuber koreanum* with the allied *Tuber* species.

|                        | *T. koreanum* GB20004 | *T. borchii* [16] | *T. flavidosporum* [17] | *T. japonicum* [17] |
|------------------------|------------------------|-------------------|------------------------|---------------------|
| **Ascoma**             | Globose to subglobose, rarely ovoid, irregularly rugged surface, bright white to yellowish beige | Subglobose to irregular, surface dry, elabrous, 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**              | Greyish 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 | Whittish to pale yellow | Whittish 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, (22.6–338.6–51.3) × (20.2–129.9–34.8) μm in diam. | Subglobose to ellipsoid, tapering toward base, 1–4 spored, 76–99 × 63–85 μm in diam. | Ovulate to broadly ellipsoid, 1–2 spored, (73–97) × (56–77) μm in diam. | Spindle to ovate, (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 (23–137–47–55) × (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 latusicollum* 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 clearly located apart from *T. borchii*, *T. flavidosporum* and *T. japonicum* (Figures 4 and 5). An analysis of the RPB2 DNA sequence also showed that GB20004 was 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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