RESEARCH ARTICLE

Taxonomic Re-evaluation of Korean *Gyromitra* Species Based on Morphological and Phylogenetical Studies

Sung-Eun Cho, Young-Nam Kwag, Sang-Kuk Han and Chang Sun Kim*
Forest Biodiversity Division, Korea National Arboretum, Pocheon 11186, Korea.

*Corresponding author: changsun84@korea.kr

ABSTRACT

Several species of *Gyromitra* are highly poisonous when consumed raw the presence of gyromitrin. In this study, *Gyromitra* specimens deposited in the Korea National Arboretum were re-examined and subjected to molecular sequence analysis. *G. gigas*, *G.* aff. *perlata*, and *G. tianshanensis* were identified based on their morphological features and molecular phylogenetic analysis for the first time in Korea. Furthermore, internal transcribed spacer and 28S rRNA sequences of *G. esculenta* and *G. infula* were analyzed to confirm their phylogeny.

Keywords: Discinaceae, false morels, *Gyromitra*, Pezizales, phylogeny

INTRODUCTION

Gyromitra is a genus of Ascomycota, and its species commonly referred to false morels, exhibit a mycorrhizal association. Some *Gyromitra* species, including *G. esculenta*, *G. fastigiata*, *G. gigas*, and *G. infula*, cause severe poisoning and even death humans the presence of toxic gyromitrins [1]. *Gyromitra* has a taxonomic history of transfers among various genera, including *Discina*, *Helvella*, and *Maublancomyces*. Traditional characters have been used to segregate species, including their ecological features and macro- and micro-scopic characteristics. [2]. Five subgenera have been recognized in *Gyromitra*: *Caroliniana*, *Discina*, *Gyromitra*, *Melaleucoides*, and *Pseudorhizina* [3]. Recently, the subgenus *Pseudoverpa* was described based on its unique morphoecological profile of the Cypriot collections [4]. In addition, internal transcribed spacer (ITS) and Large subunit (LSU) phylogenetic analyses showed that collections from Cyprus in a well-supported clade within *Gyromitra* distant from their closest neighbor by 26 positions at the LSU locus.

To date, two species of *Gyromitra* (*G. esculenta* and *G. infula*) have been recorded in Korea [5]. In this study, we analyzed the morphological characteristics and sequence data of three unrecorded *Gyromitra* species to confirm their taxonomic position.

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MATERIALS AND METHODS

Fungal materials and morphological observation

Twelve specimens of *Gyromitra* have been collected during mushroom field foray since 2009. Macro-morphological characteristics were examined using a dissecting microscope. For characterization, the paraphyses, asci, and ascospores were mounted in distilled water and 5% KOH, examined using an Olympus BX53 compound microscope (Olympus, MA, United States), and photographed with a Jenoptik ProgRes C14 Plus camera (Jenoptik Corporation, Jena, Germany). Microscopic parameters were measured and calculated using ProgRes Capture Pro v. 2.8.8 software (Jenoptik Corporation). The dried specimens were deposited in the Korea National Arboretum (KH).

DNA isolation, ITS sequencing, and phylogenetic analysis

Genomic DNA was extracted from 12 specimens using the DNeasy Plant Mini DNA Extraction Kit (Qiagen Inc., Valencia, CA, USA), following the manufacturer's instructions, and used for PCR amplification. ITS 1 and 2, along with 5.8S rDNA were amplified using the primers ITS1 and ITS4 [6]. The 28S nuclear ribosomal LSU was amplified using the primers LR0R and LR6 [2]. The sequences generated in this study were assembled using Chromas v. 2.6.6, and then edited using BioEdit v. 7.2.5 [7] and MEGA v. 7.0.26 [8]. equences were assembled and deposited in GenBank. The ITS and LSU accession numbers are in Table 1. For phylogenetic analyses, the were aligned using MAFFT v. 7.475 [9] using the auto-option mode. Phylogenetic trees were constructed using RAxML in the CIPRES Science Gateway v.3.3 portal [10]. The relative robustness of the individual branches was estimated by bootstrapping with 1,000 replicates.

Table 1. *Gyromitra* specimens used in this study including locality, ITS and LSU GenBank accession numbers.

Species	Specimen no.	Coll. Date	Locality	ITS	28S
G. esculenta	KA19-0028	3-May-2019	Pyeongchang-gun, Korea	MZ567191	MZ573190
	KA21-0149	4-May-2021	Pyeongchang-gun, Korea	MZ567194	MZ573193
	KA21-0150	4-May-2021	Pyeongchang-gun, Korea	MZ567195	MZ573194
	KA21-0151	4-May-2021	Pyeongchang-gun, Korea	MZ567196	MZ573195
G. gigas	KA19-0027	3-May-2019	Pyeongchang-gun, Korea	MZ567190	MZ573189
	KA21-0152	4-May-2021	Pyeongchang-gun, Korea	MZ567197	MZ573196
	KA21-0153	4-May-2021	Pyeongchang-gun, Korea	MZ567198	MZ573197
G. infula	KM09-0298	20-Jul-2009	Hongcheon-gun, Korea	MZ567199	–
G. aff. *perlata*	KA15-0013	7-May2015	Pocheon-si, Korea	MZ567189	–
	KA20-0002	9-Mar-2020	Pocheon-si, Korea	MZ567192	MZ573191
	KA20-0006	30-Mar-2020	Pocheon-si, Korea	MZ567193	MZ573192
G. tianshanensis	KA13-1244	26-Sep-2013	Ulleung-gun, Korea	MZ567188	MZ573188

ITS, internal transcribed spacer region; 28s: 28s nuclear ribosomal LSU

RESULTS

Phylogenetic analysis

The RAxML tree showed that specimens of *G. gigas, G.* aff. *perlata,* and *G. tianshanensis* formed well-resolved monophyletic clades with high bootstrap values (100, 100, and 90, respectively). In addition, the phylogenetic positions of *G. infula* and *G. esculenta* collected from Korea were confirmed. In a previous report [2], *G. gigas* was considered a species complex comprising six different species. In this study, *G. tianshanensis* clustered with *G. xinjiangensis* and *G. infula,* but differed from the sister group. A search showed that three specimens of *G. perlata* from Korea were 100% identical to those from France (MG846993) and China (MG871303). In the ITS and LSU phylogenetic trees constructed, sequences from the Korean specimens clustered with *G. perlata*.

Taxonomy

***Gyromitra gigas* (Krombh.) Cooke, Mycogr., Vol. 1. Discom. London (No. 5): 191. 1878. (Figs. 1-3)**

Korean name: Keunmagwigombo-beoseot (큰마귀곰보버섯); derived from the species epithet '*gigas*' that means "giant" in Latin.

Description: Apothecia stipitate, irregularly lobed, inflated, 5–12 cm when fresh, 1.5–3.5 cm dia and 2–3 cm high when dry; hymenium dull yellowish brown, cinnamon, reddish brown, dark brown to blackish when dry, undulate-rugose, receptacle surface white, cream, grayish white, light brown to black, glabrous; stipe subcylindrical, enlarged at the base, typically fluted with broadly rounded ribs, white, light brown to black, glabrous to subpubescent, internally hollow, 2–3 × 0.3–1.3 cm. Excipulum of textura intricate, 40–55 μm thick, hyphae hyaline, 35 μm dia. Asci (n = 20) cylindrical, tapering at the base, 8-spored, 270–330 × 17–21 μm, ave. 290–320 × 18.5–20 μm. Paraphyses cylindrical, septate, hyaline, 7–11 μm long. Ascospores (n = 20) ellipsoidal to subfusoid, hyaline, smooth, biguttulate, non-apiculate, irregularly biseriate when young, uniseriate at maturity, 26–32 × 11.5–14 μm, ave. 28–30 × 12–13.5 μm.

Material examined: Korea, Pyeongchang-gun, 2021, leg. C. S. Kim, KA21-0153.

Notes: *Gyromitra gigas* regarded as a species complex morphologically similar taxa. Recently, this species was separated into six taxa *G. gigas* (Krombh.) Cooke, *G. montana* Harmaja, *G. korfii* (Raitv.) Harmaja, *G. ticiniana* Littini, *G. pseudogigas* X.C. Wang & W.Y. Zhuang, and *G. khanspurensis* Jabeen & Khalid, on phylogenetic analysis [2]. Species within the *G. gigas* species complex are characterized by stipitate ascomata are saddle-shaped to irregularly lobed or cerebriform and wrinkled, and yellow-brown to brown reddish brown, ribbed to sulcate, white to yellow-brown stipe, ellipsoid to fusiform ascospores that are roughened to finely reticulate and uniguttulate or triguttulate with an inconspicuous to distinctive apiculus that is up to 4 μm long [2]. In addition, *G. gigas* has been reported only Europe and Asia [2]. In 2020, the *G. gigas* species complex was divided into six species (*G. gigas*, *G. montana*, *G. korfii*, *G. ticiniana*, *G. pseudogigas*, *G. khanspurensis*) based on phylogenetic analyses and morphology [2]. In this study, the Korean specimens formerly identified as *G. gigas* formed a monophyletic clade with other sequences of *G. gigas* sensu stricto.

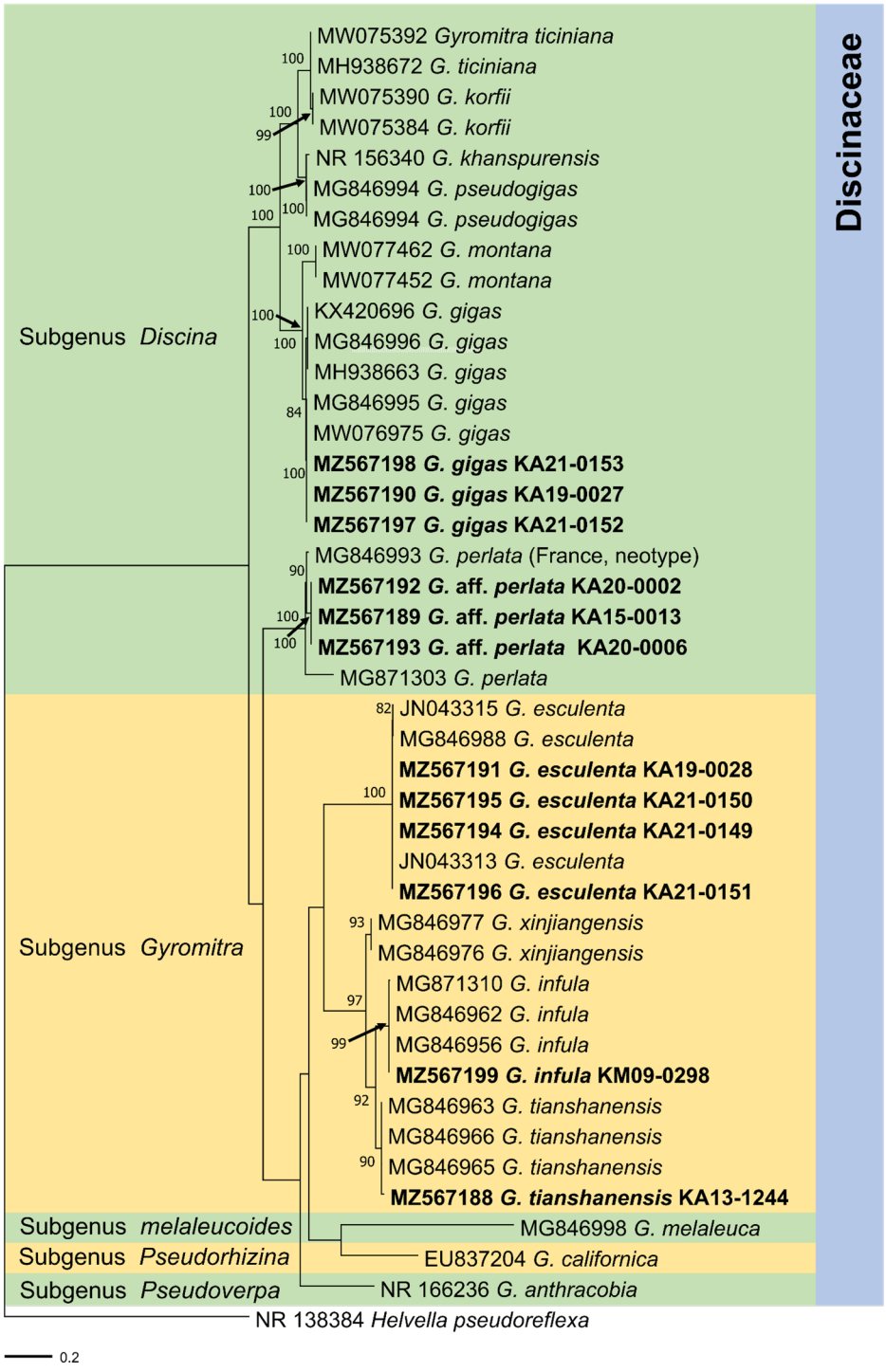

Fig. 1. Phylogenetic tree resulting from the RAxML analysis of ITS regions of the genus *Gyromitra*. Bootstrap values higher than 60% are shown in the branches. Bar, 0.2 Substitutions per nucleotide position.

***Gyromitra* aff. *perlata* (Fr.) Harmaja, Karstenia 9: 11. 1969. (Figs. 1, 2, and 4)**

Korean name: Neolbeunmagwigombo-beoseot (넓은마귀곰보버섯); derived from the species epithet '*perlata*'. Latin meaning perlatus (wide)

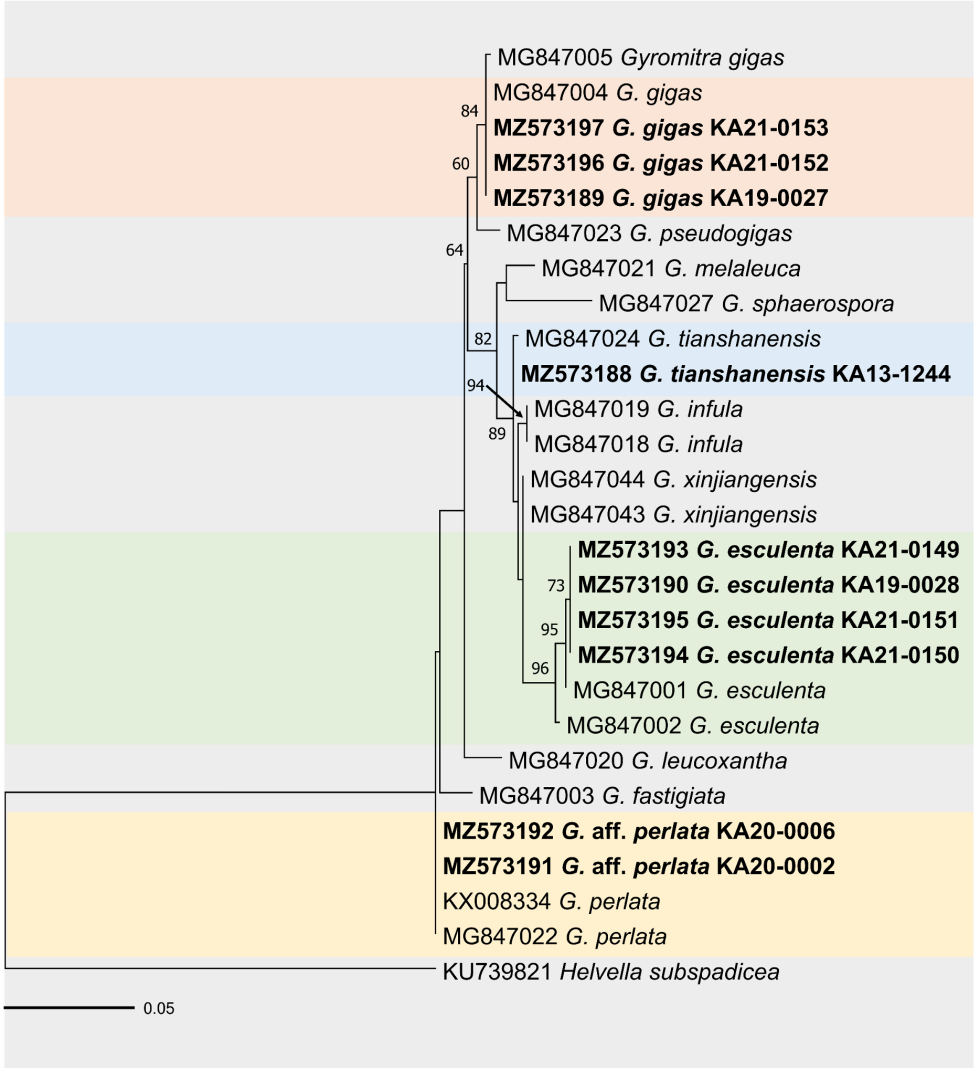

Fig. 2. Phylogenetic tree resulting from the RAxML analysis of LSU regions of the genus *Gyromitra*. Bootstrap values higher than 60% are shown in the branches. Bar, 0.2 Substitutions per nucleotide position.

Description: Apothecia cup-shaped, up to 7 cm high when fresh, smooth, wavy to wrinkled, or bumpy. Upper surface brown to reddish brown, darkening to dark brown or blackish. Under surface whitish to pale grayish brown or yellowish. Asci (n = 20) subcylindrical, tapering at the base, 8-spored, 160–210 × 12.5–15 μm, ave. 180–200 × 13–14.5 μm. Paraphyses filiform, septate, orangish to orange-brown, 6–9 μ m wide at apex. Ascospores (n = 20) fusoid, hyaline, smooth, apiculi pointed, 28–45 × 11–16 μm, ave. 32–42 × 13–15 μm.

Material examined: Korea, Pocheon-si, 2015, leg. S. K. Han, KA15-0013.

Notes: Based on the neotype specimen of *G. perlata* from France (MG846993), ascospores of this species were 40–46 × 13–17 μm. However, the ascospores of Korean *G. perlata* were 22.5–27 × 11–13 μm. Therefore, the size range of ascospores are quite different between French and Korean specimens . In a

previous report [11], *G. perlata* was classified as a species complex. However, the Korean specimens of *G. perlata* cluster with the neotype of *G. perlata* from France (MG846993) with strong support. Therefore, this species identified as *G.* aff. *perlata*.

***Gyromitra tianshanensis* X.C. Wang & W.Y. Zhuang, Mycologia 111(1): 72. 2018. (Figs. 1, 2 and 5)**

Korean name: Cheonsanmagwigombo-beoseot (천산마귀곰보버섯); derived from the species epithet '*tianshanensis*'. Mandarin tianshan (celestial mountains).

Description: Apothecia stipitate, cerebriform, irregularly lobed, convex, margin appressed and fused

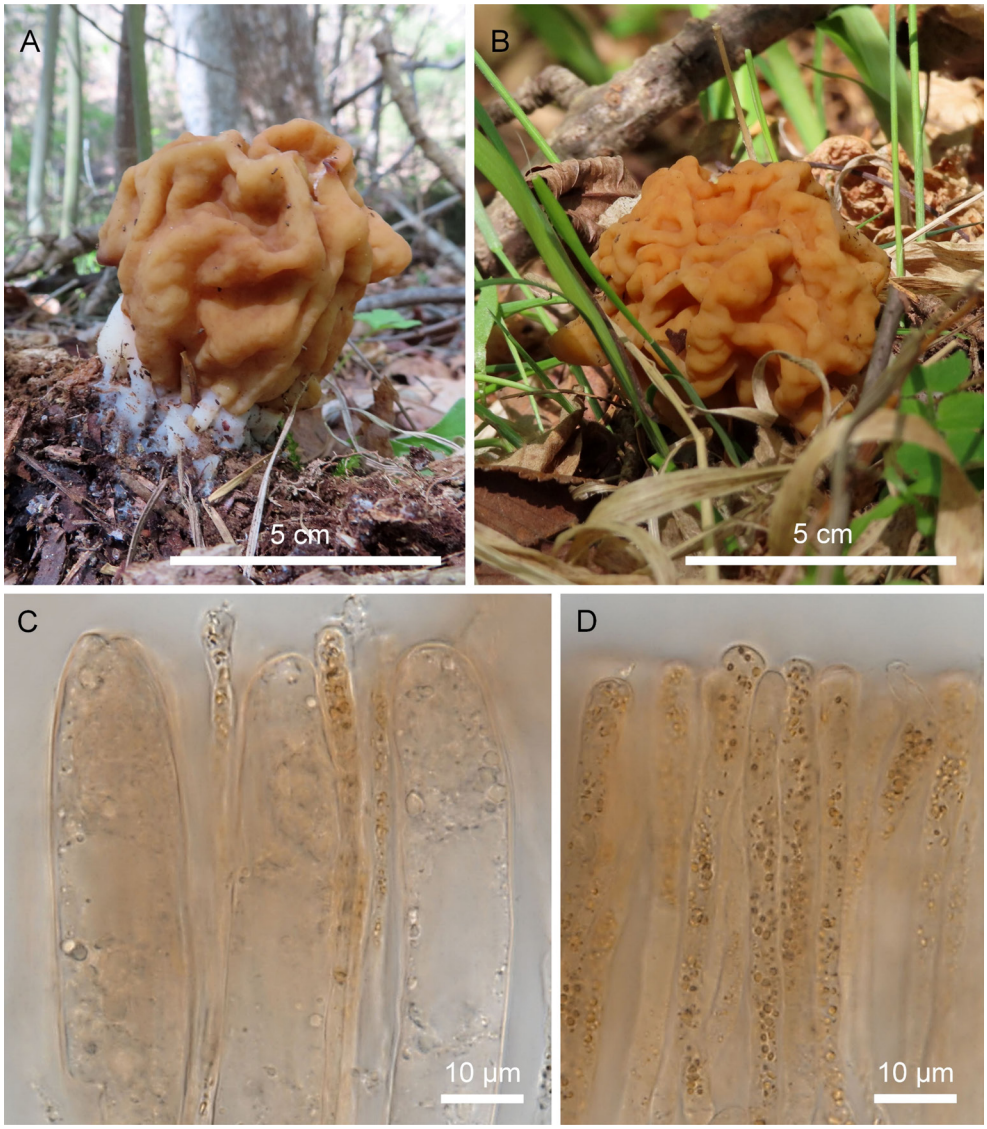

Fig. 3. Macro- and micro-scopic characteristics of *Gyromitra gigas* (KA19-0027). A, B: Mature apothecia; C: Immature asci; D: Paraphyses. Scal bars: A, B = 5 cm; C, D = 10 μm.

to the stipe, up to 4–6 cm high when fresh, 1.5–3.5 cm diameter and 2–3 cm high when dry; hymenium dull yellowish brown, cinnamon, reddish brown, dark brown to blackish when dry, undulate-rugose, receptacle surface white, cream, grayish white, light brown to black, glabrous; stipe subcylindrical, enlarged at base, typically fluted with broadly rounded ribs, white, light brown to black, glabrous to subpubescent, internally hollow, 2–3 × 0.3–1.3 cm. Excipulum of textura intricate, 100–220 μm thick, hyphae hyaline, 2–4 μm diameter. Asci (n = 20) subcylindrical, tapering at the base, 8-spored, 160–210 × 12–15 μm, ave. 170–200 × 13–14.5 μm. Paraphyses filiform, septate, hyaline, 7–9 μm wide at apex and 4.5–6 μm below. Ascospores (n = 20) narrowly ellipsoidal to fusoid, hyaline, smooth, biguttulate, non-apiculate, irregularly

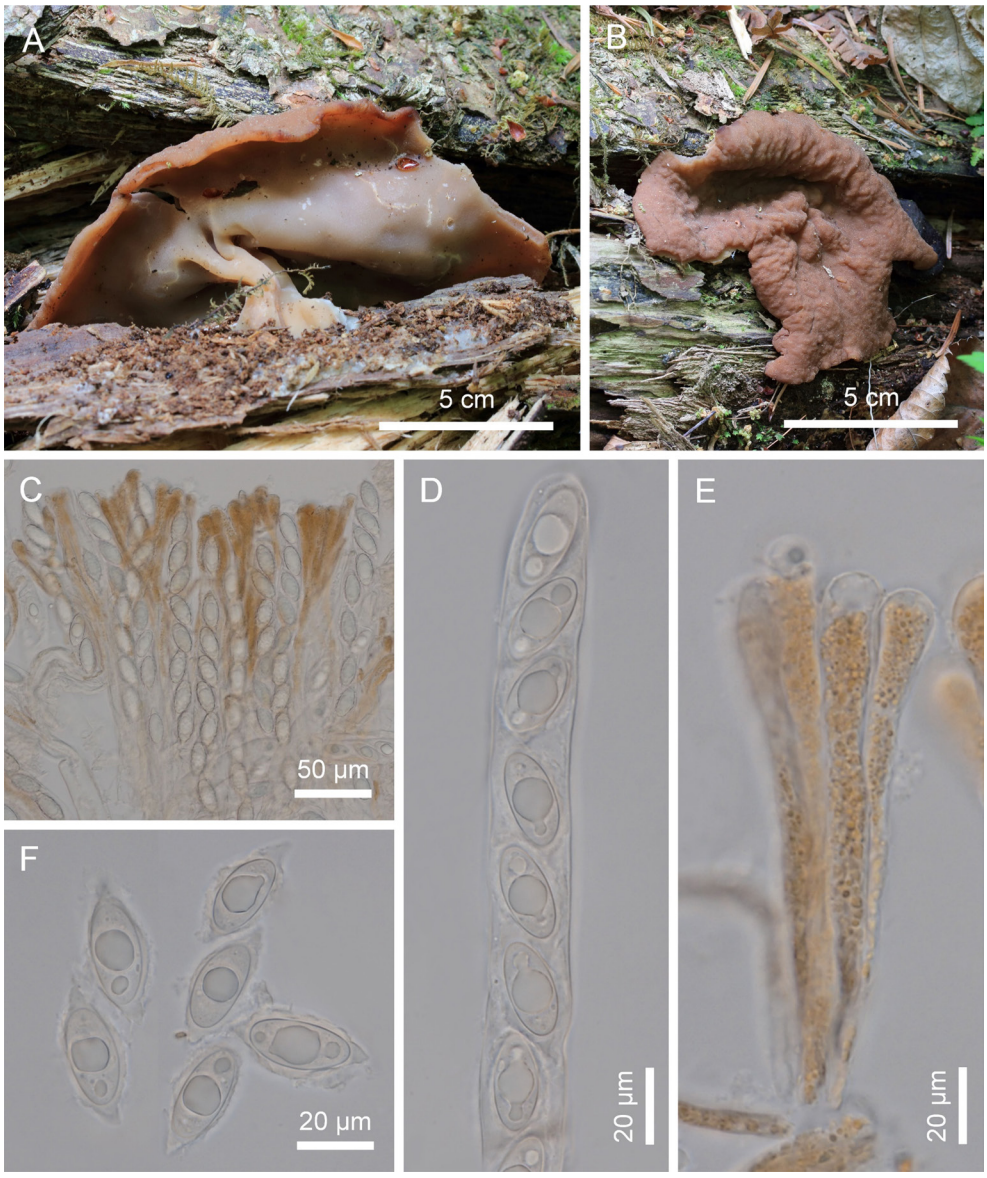

Fig. 4. Macro- and micro-scopic characteristics of *Gyromitra* aff. *perlata* (KA15-0013). A, B: Mature apothecia; C: Asci and paraphyses; D: Ascus; E: Tips of paraphyses; F: Ascospores.

biseriate when young, uniseriate at maturity, 17–21.5 × 6–9.5 µm, ave. 18–21 × 7–9 µm.

Material examined: Korea, Ulleung-gun, 26 Sep. 2013, leg. S. K. Han, KA13-1244.

Notes: *Gyromitra tianshanensis* clusters with *G. infula* and *G. xinjiangensis* but in morphological and molecular sequence. *G. tianshanensis* was introduced as a new species in Xinjiang, China and was collected rotten wood at 1700 m [3]. This species was collected from the rotten wood of *Picea* species

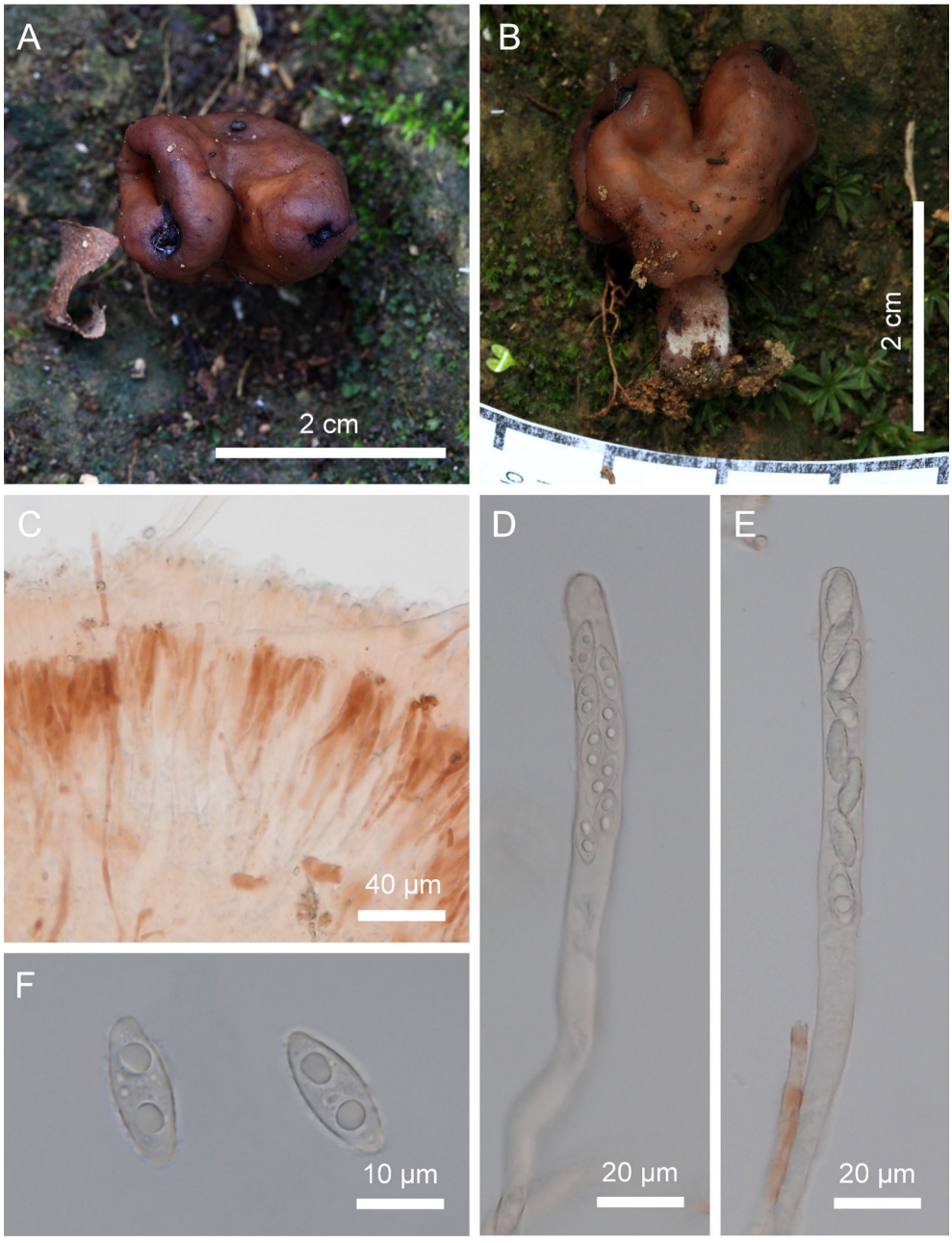

Fig. 5. Macro- and micro-scopic characteristics of *Gyromitra tianshanensis* (KA13-1244). A, B: Mature apothecia; C: Paraphyses; D, E: Asci; F: Ascospores.

at 1,900 m Ulleungdo Island, Korea. In China, *Gyromitra* species are usually associated with coniferous trees or grow in coniferous-deciduous mixed forests with *Picea*, *Pinus*, and *Betula* at altitudes of 800-4000 m [3]. Therefore, *Gyromitra* species can be related to altitude and geographical position, especially *G. tianshanensis*. The Korean collection was the first record outside its type locality.

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