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A new species of *Barssia* (Ascomycota, Helvellaceae) from Turkey

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Abstract: A new species of the genus *Barssia* was identified from Turkey using both morphological and phylogenetic analysis of the ribosomal internal transcribed spacer region (ITS rDNA) and nuclear ribosomal large subunit (LSU rDNA) gene sequences. Macroscopic and microscopic features of the fresh samples were photographed and a diagnostic key of the genus *Barssia* was built considering characteristics of known species.

Key words: Hypogeous fungi, phylogeny, taxonomy

1. Introduction

The genus *Barssia* Gilkey, which belongs to the family Helvellaceae, is currently represented by only five species in the world. All species of the genus *Barssia* were found at a latitude of 30° in the northern hemisphere until today (Figure 1). The first species of *Barssia* was collected by Barss in Oregon, USA, under *Rhamnus purshiana* DC. and named as *Barssia oregonensis* Gilkey (Gilkey, 1925). This was followed by *B. peyronelii* (Mattir.) Agnello & Kaounas (described as *Stephensia peyronelii* Mattir.) found under *Larix decidua* Mill. in Piedmont, Italy (Mattirolo, 1936; Agnello and Kaounas, 2017) and *B. yezomontana* (Kobayasi) Trappe (originally named as *Phymatomyces yezo-montanus* Kobayasi) found under *Abies mayriana* Miyabe & Kudô and *Picea jezoensis* Carrière in Yezo, Japan (Kobayasi, 1937; Gilkey, 1961). Later, *B. maroccana* G.Moreno, Manjón, Carlavilla & P.Alvarado was identified in a mixed forest of *Cedrus atlantica* G.Manetti and *Quercus ilex* L. in Morocco (Crous et al., 2014) and *B. hellenica* Kaounas, Agnello, P.Alvarado & Slavova was found under *Abies cephalonica* Loudon in Greece (Kaounas et al., 2015) (Figure 1). Recently, a new record for the genus *Barssia*, "*B. hellenica*", was given in Gaziantep (Turkey) by Uzun et al. (2018). According to the relevant literature, it can be said that *Barssia* is associated with specific coniferous tree hosts and makes mycorrhiza with them when published studies are taken into account.

In 2016, some specimens of *Barssia* were found in *Cedrus libani* A.Rich. forests of Turkey. The mushrooms were found incidentally while digging at the tree base with the anchor. Dogs or special equipment were not used while looking for fungal specimens and fungi were found 3–5 cm deep in the soil when they were being screened in the forest. The macro- and microscopy, ecology, and molecular data of these Turkish *Barssia* samples did not match those of any other species in the existing literature on *Barssia*. For this reason, we decided to formally describe these *Barssia* samples and propose a new species to accommodate them.

2. Materials and methods

2.1. Morphological studies

Barssia samples were collected from a plantation of *C. libani* forest from Kadirli district, Osmaniye, Turkey. Collected samples were photographed in the field, and morphological and ecological features such as color, smell, and vegetation type were recorded. Then samples were brought to the laboratory and dried in a dehydrator to prevent decay.

The *Munsell Book of Color* (Munsell, 1976) was used as the reference for macroscopic colors reported. Microscopic characters were measured with a Leica DM 3000 compound microscope. The samples were mounted in distilled water, Melzer's reagent (for amyloidity reaction), 2% KOH, Congo red (for staining cell walls of different elements), or cotton blue (to check spore ornamentation). Images were obtained using a Leica CF 450 camera coupled with the light microscope mentioned above. At least 20 mature ascospores were measured from each specimen, with Q

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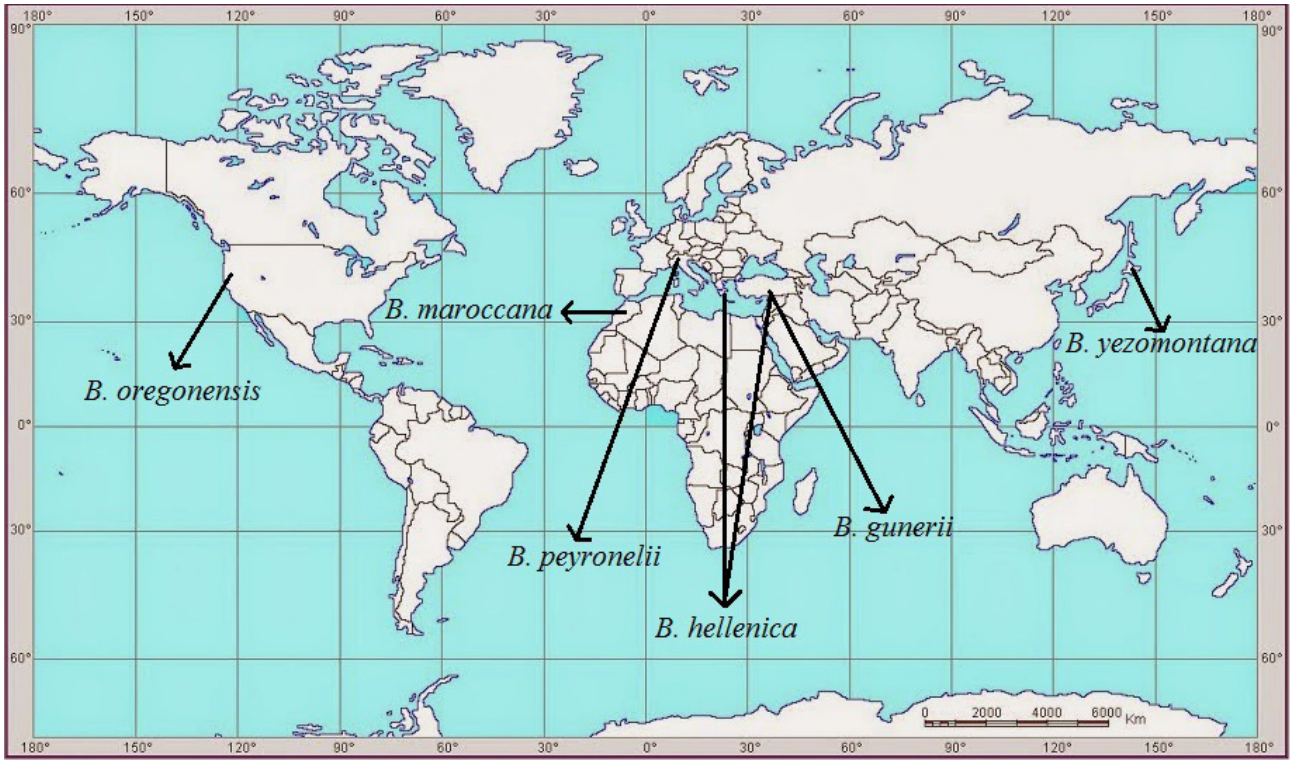

Figure 1. World distribution of *Barssia* species.

values (length divided by width) determined from water mounts from dried specimens.

All samples have been deposited in the Mushroom Application and Research Center of the Fungarium of Selçuk University, Konya, Turkey.

2.2. DNA extraction, PCR amplification, sequencing, and phylogenetic analysis

Total genomic DNA was isolated from dried samples with slight modifications (increasing the concentration (100 mg/mL and 10 mg/mL) and the volume (10 µL) of RNase A and proteinase K, respectively) to the protocol of the Eurx GeneMatrix Plant & Fungi DNA Purification Kit. PCR amplification of ITS rDNA and LSU rDNA was performed using ITS1F–ITS4 (White et al., 1990) and LROR–LR5 (Vilgalys and Hester, 1990) primers, respectively. PCR conditions were set as follows: 94 °C for 5 min, followed by 30 cycles of 30 s at 94 °C, 60 s at 52 °C for ITS rDNA, 55 °C for LSU rDNA, and 90 s at 72 °C and final extension 10 min at 72 °C. PCR amplifications were verified by electrophoresis on a 1.5% agarose gel and then DNA sequencing of successful amplifications was performed using the BigDye Terminator v3.1 Sequencing Kit, again with ITS1F–ITS4 (for ITS rDNA) and LROR–LR5 (for LSU rDNA) primers. An ABI 3730XL Sanger Sequencer (Applied Biosystems, Foster City, CA, USA) was used for running of sequencing reactions. Raw sequence chromatograms were edited and aligned using Sequencher version 5.4.5 (Gene Codes, Ann Arbor, MI, USA). The sequences obtained from this study were deposited in GenBank as accessions MF619953–MF619957 for LSU rDNA and MF619952–MF619955 for ITS rDNA.

Phylogenetic trees were obtained with maximum likelihood (ML) analysis by using *Helvella albella* and *Underwoodia singeri* as the outgroup for the ITS rDNA and LSU rDNA region in MEGA6 software, respectively. Support values (ML bootstrap percentages/Bayesian posterior probabilities) are given on the branches. The highest log likelihood of the trees for ITS rDNA and LSU rDNA were –2527.91 and –2478.58, respectively. The analysis involved 26 nucleotide sequences for LSU rDNA and 13 nucleotide sequences for ITS rDNA. All positions containing gaps and missing data were eliminated. There were a total of 503 positions for LSU rDNA and 487 for ITS rDNA in the final dataset.

3. Results

3.1. Taxonomy

Barssia gunerii H.H. Doğan, F. Bozok & H. Taşkın **sp. nov.**
Figures 2–4
MycoBank no.: MB 823062
Diagnosis: Differs from other *Barssia* spp. by its small (21.35 × 18.22 µm) subglobose to ovoid ascospores and the habitat under *C. libani*.

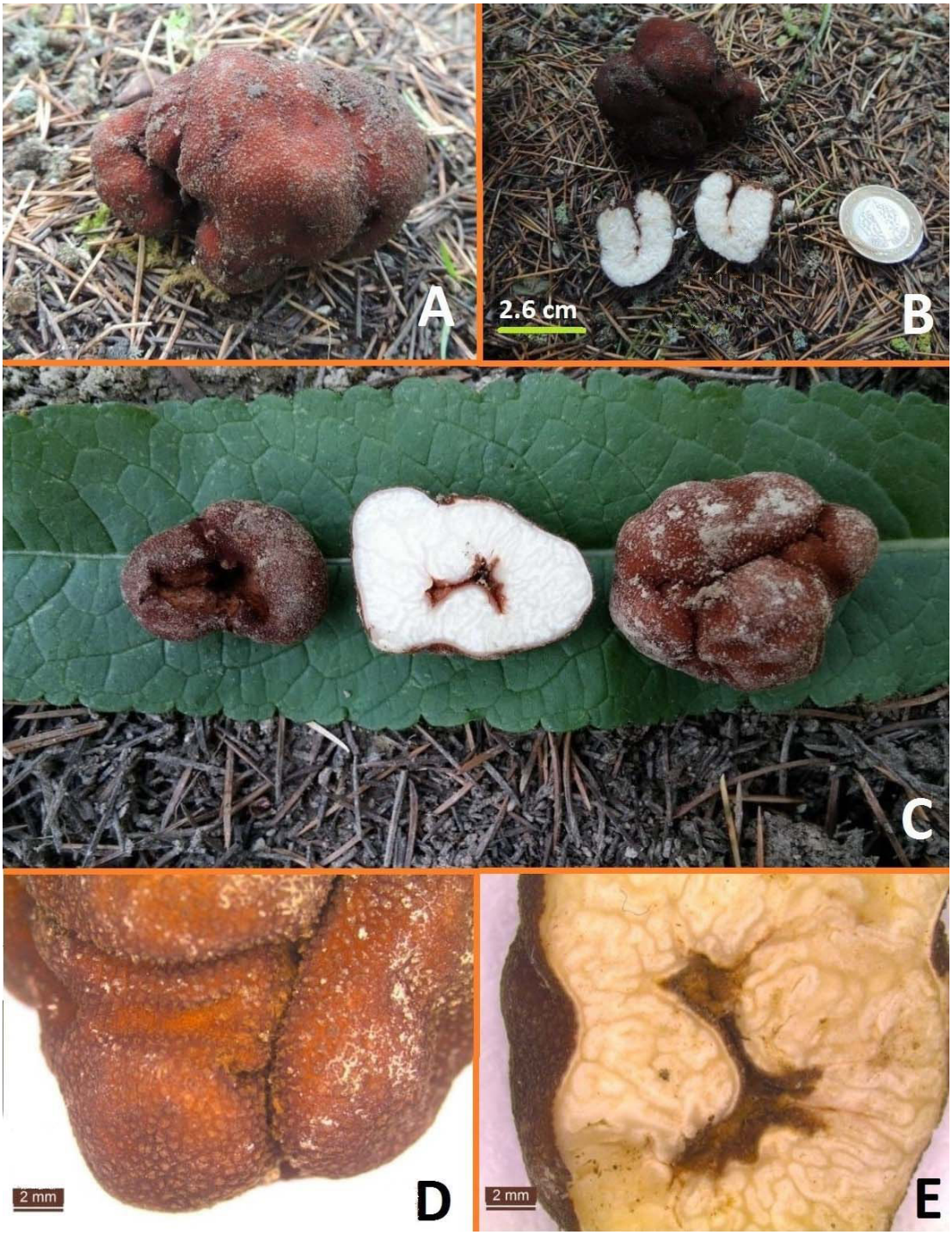

Figure 2. *Barssia gunerii*. A–E: Morphological views.

Type: TURKEY; Osmaniye Province, Kadirli, Uzunyazı plateau, Elmacık district (37°42′170″N, 36°12′135″E), in humus soil under *C. libani*, 1314 m, 7 Jun 2016, *det*. H.H. Doğan (holotype, HHD17617, isotype KONFUNGARIUM 5288).

Etymology: *gunerii* from Şaban Güneri, the person who first collected the specimens.

Hypogeous ascomata 0.9–3.3 × 0.7–2.5 cm in diam., irregularly globose or subglobose, spherical to subspherical, showing mostly an irregular shape, more

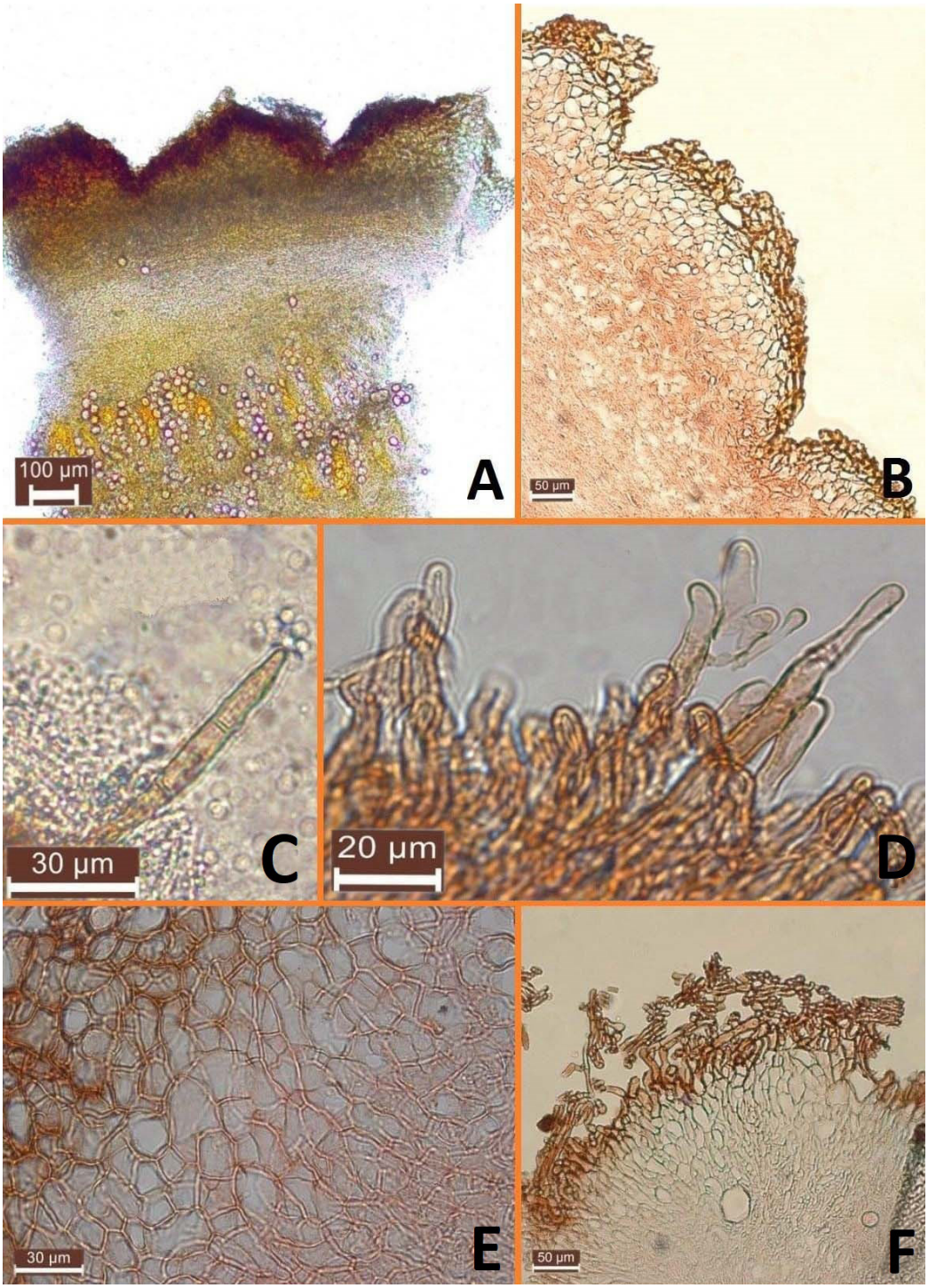

Figure 3. *Barssia gunerii*, microscopic view. A–B: Peridium, C–D: peridial hairs, E–F: gleba.

or less lobed, usually with an irregular apical depression, sometimes folded inside, red (5R 4/6), reddish orange (10R 6/6) to brownish red (10R 4/69) or blackish brown (5YR 2/2). Surface covered by irregular polygonal warts 4–6-angled, 0.450–0.609 × 0.114–0.293 mm diam., densely packed together when young and more isolated with age, sometimes like a network (Figures 2A–2E).

Peridium 190–550 µm, composed of a pseudoparenchymatous hyphal structure with polygonal cells (4–6-angled) measuring 15.0–27.5 × 7.5–18.0 µm,

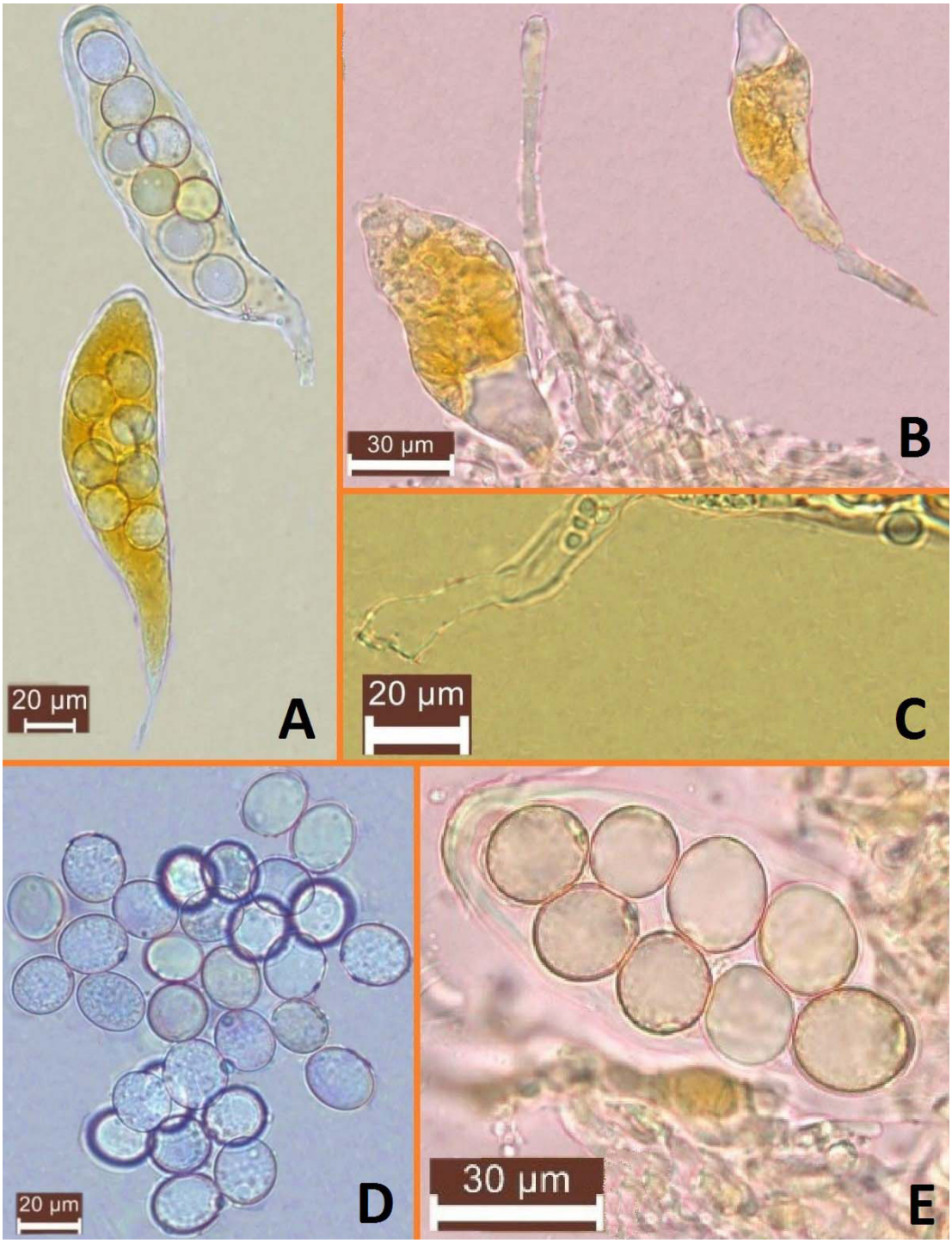

Figure 4. *Barssia gunerii*, microscopic view. A: Asci, B: paraphyses, C: pleurorynchous, D–E: ascospores.

red to brownish and thick walled (2.5–4.5 µm) in the outer layer, colorless and with thinner walls in the internal layer (Figures 3A and 3B). Yellowish to dark brown hairs are rare on the external surface, simple or branched, 2-4-septate, thick-walled, emerging from the terminal cells of the peridium, measuring up to 82–64 × 6–7.5 µm (Figures 3C and 3D).

Gleba whitish or yellowish, lubricous, generally compact or frequently presenting irregularly sinuous, labyrinth–like veins, with a prosenchymatic structure of

interwoven hyphae 3.6–5.6 µm in diam. (Figures 3E and 3F).

Asci mostly pear-shaped, broadly ellipsoid, or irregularly clavate with a protruding hump at the apex, inamyloid, 8-spored, 157–202 × 35.5–45.5 µm (Figures 4A and 4B), with a pleurorynchous base (Figure 4C). Paraphyses cylindrical, hyaline, uniseptate, longer than the asci, 5–9 µm wide (Figure 4B). Ascospores subglobose to ovoid, surface smooth, hyaline, (18–)19.5–23.5(–24.5) × (14.5–)17.2–20.5(–21.5) µm (21.35 × 18.22 µm on average), Q = 1.85–2.17 (Qm= 1.15) (Figures 4D and 4E).

Habitat: Hypogeous ascomata are found growing solitary or in clusters, under *Cedrus libani*, 1300 m a.s.l.

For molecular studies, ITS rDNA and LSU rDNA gene sequences obtained from 19 taxa and 34 samples selected from GenBank were used to compare our new species with the other *Barssia* species and show its place in the phylogenetic tree (Table; Figures 5 and 6). Four new

Table. Taxa and GenBank accession numbers for specimens used in the present study

			GenBank accession numbers	
Taxon	Location	Voucher no.	ITS	LSU
Underwoodia singeri	Argentina	JT26159	-	JQ925717
Underwoodia cf. *singeri*	Chile	MES161	-	JQ925718
Helvella silvicola	USA	N.S.Weber6219	-	JX993087
Helvella compressa	USA	UC1999259	-	KC122804
Helvella atra	USA	UC1999253	-	KC122802
Helvella dryophila	USA	UC1999238	-	KC122772
Helvella vespertina	USA	UC1999194	-	KC122778
Helvella crispa	Not available	FH-DSH97-050	-	AY789399
Helvella maculata	USA	UC1999255	-	KC122797
Helvella lacunosa	USA	UC1999199	-	KC122796
Helvella macropus	USA	MES198	-	KC122774
Balsamia platyspora	Norway	O-F245324	KP149497	-
Balsamia platyspora	Norway	O-F245320	KP149498	-
Balsamia vulgaris	Italy	Amer2482	-	KM243651
Balsamia vulgaris	Italy	Amer2403	-	KM243653
Balsamia vulgaris	USA	Not available	KM115881	-
Balsamia magnata	Not available	JMT13020		U42683
Balsamia nigrens	USA	Trappe19921		EU669425
Balsamia nigrans	USA	OSC146631	KU170039	-
Balsamia cf. *setchellii*	USA	MES84	-	JQ925657
Balsamia cf. *setchellii*	USA	SRC868	-	JQ925659
Barssia cf. *oregonensis*	USA	OSC100014	-	AY544652
Barssia oregonensis	USA	OSC:100014	-	NG027601
Barssia oregonensis	USA	OSC, RF 533	-	U42684
Barssia oregonensis	USA	JLF2161	KF983489	-
Barssia hellenica	Greece	MCVE28664	KT350942	KT350939
Barssia hellenica	Greece	MCVE28663	KT350941, NR138012	KT350940
Barssia maroccana	Morocco	AH44099	-	KM243654
Barssia maroccana	Morocco	AH39117	-	KM243655
Barssia maroccana	Morocco	AH39116	JN048885	-
Barssia maroccana	Morocco	AH44099	KM243648	-

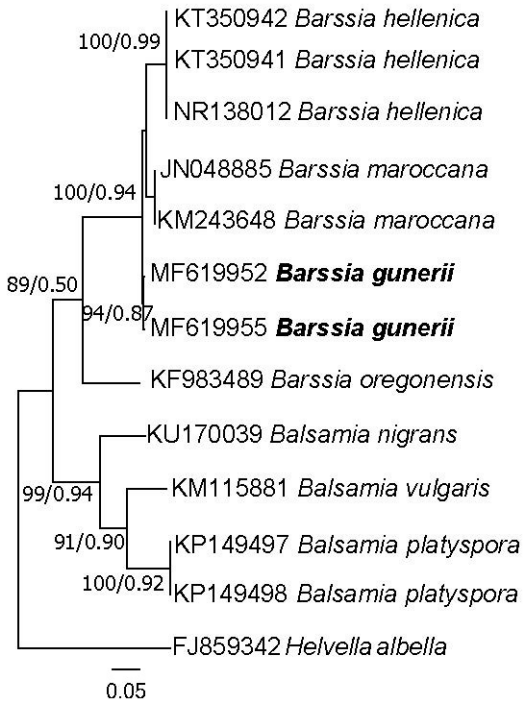

Figure 5. Maximum likelihood phylogenetic analyses of ITS rDNA sequences of *Barssia gunerii* obtained from this study and related species selected from GenBank.

sequence accessions (two ITS rDNA, two LSU rDNA) were added to GenBank with this study.

As a result of the GenBank database research, 20 sequences of the genus *Barssia* were found and most of these sequences were obtained from ITS and LSU rDNA gene regions. We compared *B. gunerii*, identified as a new species in this study, with the *Barssia* samples obtained from GenBank and found that the ITS rDNA and LSU rDNA sequences of *B. gunerii* showed similarity with *B. maroccana*, *B. hellenica*, and *B. oregonensis* at 96% and 99%, 95% and 99%, and 82% and 94%, respectively.

These results suggested that, while *B. gunerii* was closer to *B. maroccana* in terms of both ITS and LSU rDNA sequences (Figures 5 and 6), it is very easy to distinguish it by spore shapes, size, and habitats for both species.

4. Discussion

Macroscopic and microscopic characters are often very similar among known species of *Barssia*, but they can be easily discriminated by their spore size, shape, and host as shown in the synoptic key provided below.

1. Ascospores globose, ovoid
2. Ascospores ovoid
3. Ascospores bigger than 24 µm, under *Abies* .... *B. hellenica*

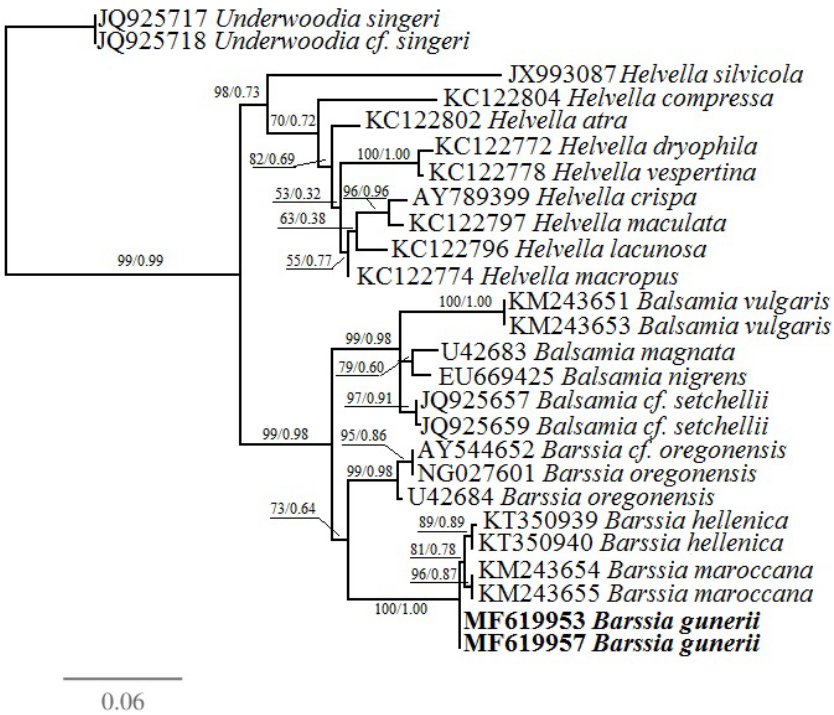

Figure 6. Maximum likelihood phylogenetic analyses of LSU rDNA sequences of *Barssia gunerii* obtained from this study and related species selected from GenBank.

3*. Ascospores subglobose to ovoid, smaller than 24 µm, under *Cedrus* ..... *B. gunerii*
2.*Ascospores globose, under *Abies myriana*, *Picea jezoensis* ..... *B. yezomontana*
1*. Ascospores elliptic
2. Ascospores bigger than 18 µm
3. Ascospores up to 26 µm, under *P. mensiesii* .... *B. oregonensis*
3*. Ascospores up to 36 µm, under *C. atlantica* .... *B. maroccana*
2*. Ascospores smaller than 18 µm ..... *B. peyronelii*

Barssia gunerii can easily be distinguished by its subglobose to ovoid and smaller spores (21.35 × 18.22 µm on average) than *B. hellenica* having ovoid with large oil droplet and larger spores (23.5 × 18.1 µm on average). Two other species, *B. oregonensis* and *B. maroccana*, have ellipsoid and definitely larger spores (24.0–32.0 × 12.0–17.0 µm in *B. oregonensis* and 29.0–36.0 × 16.0–22.0 µm in *B. maroccana*). In addition, *B. gunerii* was collected under *C. libani* while *B. maroccana* was found in mixed forest of *C. atlantica* and *Q. ilex*, *B. oregonensis* was associated with *Pseudotsuga menziesii*, and *B. hellenica* was found with *Abies cephalonica*. A new finding was made for *B. hellenica* in *A. cilicica* (Ant. & Kotschy) Carr. subsp. *cilicica* (Ant. & Kotschy) Carr. by Uzun et al. (2018).

Although morphologically *B. gunerii* looked similar to *B. yezomontana* because of its ascospore size (19–23 µm in *B. yezomontana*), it can be easily discriminated because of its smaller (157–202 × 35.5–45.5 µm) clavate or broadly ellipsoid asci, which are cylindrical bigger in *B. yezomontana* (200–220 × 40–50 µm). In addition, *B. yezomontana* has a different ecology (mixed forest of *A. mayriana* and *Picea jezoensis*). Lastly, *Barssia peyronelii* has a different ascospore shape (elliptic), ascospore size (14–18 µm), and ecology (*Larix decidua*).

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