Auricularia (wood ear mushroom) genus: a contribution to classification and new species records for Iran and world

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Abstract. *Auricularia* is one of the most widely distributed macrofungi, regarded as an edible medical mushroom, and is the third largest cultivated mushroom in the world due to its high nutrition and bioactivity properties. This research specifies the taxonomic status of this genus in Iran for the first time. After sample collection during 2017–2018, 19 samples were collected from Hyrcanian forests in Iran. Phylogenetic analyses and morphological examinations of 19 samples were carried out. Morphological studies identified five species *Auricularia auricula-judae*, *A. cornea*, *A. heimuer*, *A. iranica* sp. nov., and *A. mesenterica*. Subsequently, one representative sample from each group (species) was sequenced. Based on *rpb2* and ITS sequences, phylogenetic analyses were performed using Bayesian Inference analysis. The identification of five species of *Auricularia* was done according to molecular and morphological examinations. As expected from morphological characters, as well as ITS and *rpb2* regions sequences support, one new species, *Auricularia iranica* in the *A. mesenterica* complex, is described. The two known species, *A. auricula-judae* and *A. heimuer* belonging to the *A. auricula-judae* complex and *A. cornea* belonging to the *A. cornea* complex, are defined. An identification key for the accepted *Auricularia* species of Iran is proposed.

Key words: Hyrcanian Forests, Iranica, new species, phylogeny, taxonomic status

Introduction

Iran has a rich and diverse flora due to its geographical (south of the Caspian Sea) position in the subtropics and climatic differences from the east to the west of the country, which is covered with dense Hyrcanian forests that create good conditions for a high diversity of basidiomycete fungi. Despite the paramount importance of edible and medicinal mushrooms in the world, unfortunately, not only in the cultivation, but also in the taxonomy of these genera, including *Auricularia*, no research has been done. Until the last decade, almost all of the descriptions of *Auricularia* species were based on the macroscopic properties of basidiocarps including infrastructure, texture and color, size, and shape. However, these characters vary with the specimen age, moisture availability, nutritional condition, exposure to light and other environmental factors (Lowy 1951; Kobayashi 1981). Thus, this study was conducted to clarify the status of this genus and its possible species in Iran according to the current morphological concepts (Lowy 1951; Kobayashi 1981), along with the phylogenetic analyses of combined *rpb2* and ITS sequence data to determine the species.

Although *Auricularia* is recognized from another genera in the family *Auriculariacae*, it is difficult to identify species according to the macromorphological attributes including hair length, size, color, hyphae internal stratification and absence or presence of a medulla (Lowy 1952; Kobayasi 1981). Since the previous features were confusing to delimit the species of this genus, ITS and *rpb2* regions are as useful to establish species boundaries (Looney et al. 2013; Wang et al. 2013). Recently, molecular studies provided strong evidence for the genus taxonomy (Montoya-Alvarez et al. 2011; Looney et al. 2013; Wu et al. 2014). The most important aim of this study is to clarify the species diveriosity of *Auricularia* in Iran based on combined macromorphological features, molecular data and phylogenetic analyses.

Currently, it is estimated that there may be approximately 2–3 million species of fungi, of which 155,000 species have been described to date (Niskanen et al. 2023). Only 5,000 of these species were described in 2020 and 2021 (Index Fungorum 2021). The development of molecular genetics in the 20th century made it possible to determine the relationship between different types and revealed several misidentifications. However,

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many taxonomic groups still need to be investigated. Until the year 2013, research had shown that about 10 to 15 species had been described and recognized in this genus (Lowy 1952; Kobayasi 1981; Kirk et al. 2008; Looney et al. 2013), while according to Wu et al. (2021), 277 samples from 35 countries in Oceania, Africa, North and South America, Europe, and Asia were collected. Eventually, based on morphological studies and multi-gene phylogenetic analyses, 37 species belonging to five morphological groups: *Auricularia mesenterica*, *A. fuscosuccinea*, *A. delicate*, *A. cornea*, and *A. auricula-judae* were identified. An additional species named *A. cerrina* was introduced in 2022 (Kout & Wu 2022).

The most known species of this genus (except the species belonging to *Auricularia mesenterica* complex) are used as edible and medicinal species worldwide: e.g., *A. heimuer* and *A. cornea* in China, Indonesia, Malaysia, Philippines Thailand, and Vietnam (Peng et al. 2000; Chang & Lee 2004; Duc 2005; Tapingkae 2005; Dai & Yang 2008; Reyes et al. 2009; Irawati et al. 2012; Razak et al. 2013; Wu et al. 2019); *A. auricula-a judge* in Chile, China, Holland, Kenya, and Thailand (Onyango et al. 2011; Bao et al. 2016); *A. delicata* in China, India, Malwai, Mexico, Peru; and *A. fuscosuccinea* in Brazil, Mexico, United Republic of Tanzania (Bao et al. 2016).

Material and methods

Sample collection and morphological characterization

Regular pre-forecasted program according to the weather of the sampling areas was conducted to collect fruiting bodies of *Auricularia* on different hosts from different spots of the Hyrcanian forests in Iran during the rainy seasons of 2017–2018 (Fig. 1). During the sampling, the fruiting bodies of *Auricularia* were photographed, and the primary descriptions of species were done based on Lowy (1951, 1952), Kornerup and Wanscher (1978), and Petersen (1996). Also, the coordinates of the sample point were recorded and then, the specimens were sealed in paper bags containing 1,4-dichlorobenzene as a preservative and insectiside until laboratory work was performed. At room temperature, the fresh fruit body was sealed and dried in Ziplock plastic bags containing dehydrated silica gel as a desiccant to control moisture. All collected specimens were kept in the herbarium agriculture faculty of Tarbiat Modares University (TMU), Tehran, Iran, and specimen Fatima 1392 (IRAN 18459F) was deposited in the Fungus Collection of the Iranian Research Institute of Plant Protection (IRIPP), Tehran, Iran. To describe the microscopic features, first, the piece of basidiomata was soaked in distilled water for 20 minutes. The soaked fruit was cut into very thin cross-sections (as thin as possible) using a sharp sterile surgical blade. The sections were immersed in the diluted 5% potassium hydroxide (KOH) dye solution Cotton Blue (CB) and remained for 5 minutes. The thinnest sections were selected and placed on
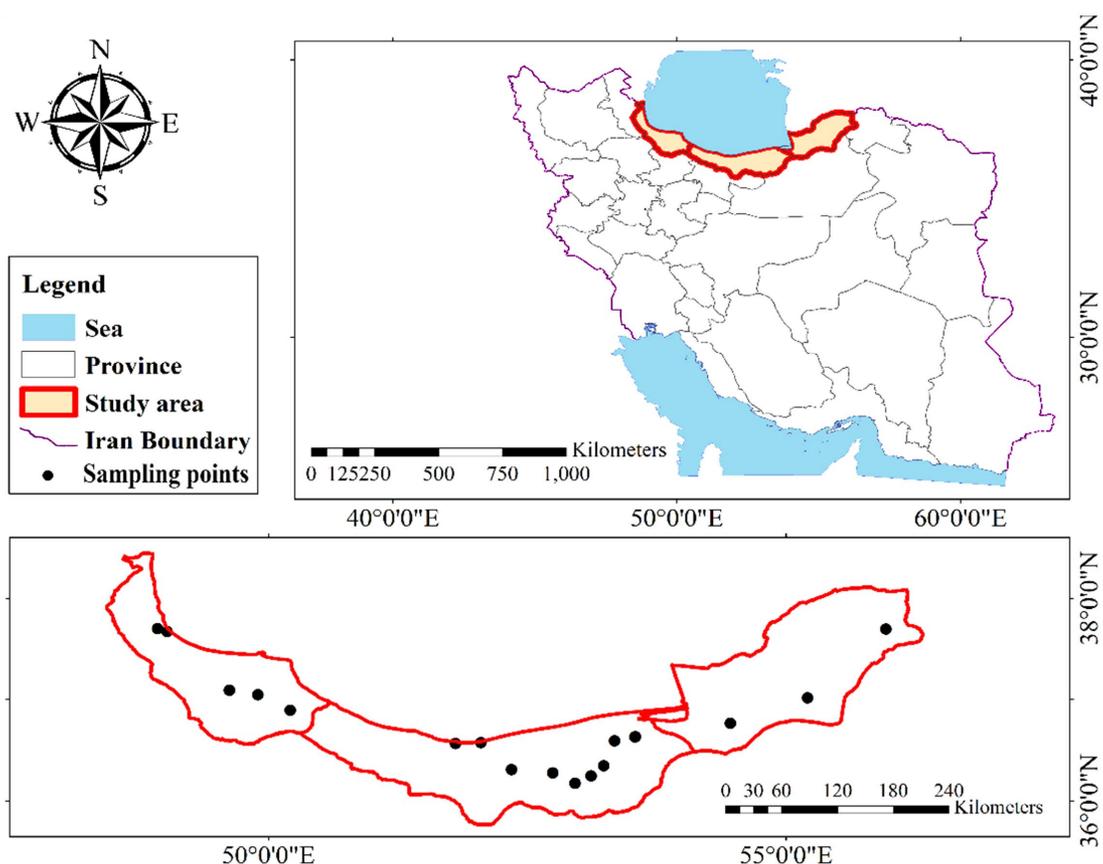

Figure 1. A schematic map of the sample collection areas of the present study.

lame and covered with a lamella. According to the target part, magnifications of 4, 10, 20, 50, and 100× standard optical microscopes with Differential interference contrast (DIC) (Olympus BX51, dic nomarski, Japan) were used. For example, 4× and 10× magnification were used for the general view of the cross-section and its constituent parts, abhymenial hair, and 100× magnification for basidiospores. Since there were variations in the size of spores and abhymenial hair, 5% of the data from each end of the range were removed and defined in parentheses. The following abbreviations were used: Q = (L/W) the spore shape quotient, Qm (mean of Q values ± SD), W = mean spore width (of 20 spores), L = mean spore length (of 20 spores), CB = Cotton Blue, and KOH = 5% potassium hydroxide.

DNA extraction, amplification, and sequencing

Genomic DNA was extracted (Safaie et al. 2005) from one representative speciemen of each group (species). DNA quality was measured with a spectrophotometer and diluted to 50 ng/µL in deionized water. ITS regions were amplified with primers ITS4 and ITS5 (White et al. 1990) and the *rpb2* with primers bRPB2-6F and bRPB2-7.1R (Binder & Hibbett 2003). PCR conditions were as follows: for ITS, initial denaturation at 95°C for 3 min, followed by 35 cycles at 94°C for 1 min, followed by 34 cycles at 55°C for 1.5 min, 72°C for 1.5 min, followed by 35 cycles at 72°C for 1.5 min and a final extension of 72°C for 5 min; and *rpb2*, initial denaturation at 95°C for 3 min, followed by 35 cycles at 94°C for 1 min, followed by 35 cycles at 57°C for 1.5 min and followed by 35 cycles at 72°C for 1.5 min and a final extension of 72°C for 5 min. PCR products were purified and sequenced in Biomagic Company, China, with the same primers and manually adjusted with Chromas software (www.technelysium.com.au/chromas.html). Newly generated sequences were submitted to GenBank and shown in Table 1.

Phylogenetic analyses

The Basic Local Alignment Search Tool (BLAST) (https://blast.ncbi. nlm.nih.gov/Blast.cgi) was used to compare the new ITS and *rpb2* sequences with other sequences accessible in the NCBI database. The datasets of combined sequences of ITS and *rpb2* sequences were separately aligned using the Q-INS-i algorithm of an online version of MAFFT v.7.205 (https://mafft.cbrc.jp/alignment/server/) (Katoh & Standley 2013). The Gblocks program (version 0.91b) with all three less stringent parameters (Allow smaller final blocks, Allow gap positions within the final blocks, and Allow gap positions within the final blocks) was utilized for editing the alignments. The best model for combined sequences of ITS and *rpb2* was chosen using PAUP/MrModeltest.2 (Nylander 2004). Bayesian analyses were carried out using MrBayes 3.1.2 (Ronquist & Huelsenbeck 2003) with the general time-reversible model, including a gamma distribution for rates across sites and a proportion of invariant sites (GTR + I + G) model for ITS-*rpb2* with three million generations. Dendroscope V.3.2.8 was used for the visualization of

output files prepared by phylogenetic programs (Huson & Scornavacca 2012). Branches that received Bayesian posterior probabilities (BPPs) greater than or equal to 0.9 were considered as significantly supported. To define the phylogenetic relations within the *Auriculariaceae*, a dataset composed of concatenated ITS + *rpb2* sequences was used. Another dataset composed of concatenated ITS + *rpb2* sequences was used in a final alignment comprising 100 different specimens to define phylogenetic relations within *Auricularia* species. *Elmerina efibulata* (Voucher Yuan4525) and *Exidia candida* (Voucher LE38198) were taken as the outgroup to root trees because they are closer to *Auricularia* than other genera of *Auriculariales* in the phylogeny (Malysheva & Spirin 2017).

Results

The ITS dataset included 49 *Auricularia* sequences with seven newly generated sequences and the *rpb2* dataset included 44 sequences with seven newly generated *rpb2* sequences. The final ITS alignment after noise removal contained 520 characters of the original 580 characters) and *rpb2* alignment contained 950 characters of the original 1,050 characters. The phylogenetic tree represented 32 species in the analyses of *rpb2* + ITS data with high Bayesian support (Fig. 2). Our seven representative sequences were grouped into 5 distinct species (Fig. 2).

The phylogenetic analyses provided a total of 32 species in three clades. Although clade C showed two separate subclades, it covered all of the species of *Auricularia mesenterica* complex. Clade B included the species of three complex species: *A. cornea* complex, *A. delicata* complex and *A. fuscusuccinea* complex. Clade 1 covered all species of the *A. auricula-judae* complex. The Bayesian Inference tree formed three main monophyletic lineages. Two taxa in our study (Fatima 1392 and Ayda 1399) were grouped with *A. mesenterica* complex, but they showed a separate clade with high support (BPP = 1.00). The analyses confirmed the morphological studies. All 32 species formed a monophyletic lineage with high support. In the present study, *A. auricularia-judae* (SAKH 61312751) grouped with the other two same species (*Auricularia auricula-judae* voucher Dai13210 and *Auricularia auricula-judae* voucher MT7).

Taxonomy

Auricularia auricula-judae (Bull.) Quél, Enchiridion Fungorum in Europa media et praesertim in Gallia Vigentium: 207. 1886.   (Fig. 3)

MycoBank MB 102281

Macroscopic description. Basidiocarps: gelatinous when fresh, reddish brown, after drying become yellowish brown or pink, usually grow solitary or caespitose. Pileus up to 8 cm, 1–2 mm thick when fresh, 0.5 mm thick when dry; the upper surface pilose, the surface of the hymenophore usually smooth and without folds, rarely with very small folds, turning black or cinnamon after drying. Medulla absent.

Table 1. Names, countries, voucher numbers and corresponding GenBank accession numbers of the taxa. Newly generated sequences are marked in bold.

| Species name	Country	Sample number	Accession numbers	
			ITS	*rpb2*
Auricularia africana	Uganda	Ryvarden 44929, holotype	MH213350	MZ740061
Auricularia americana	China	Cui 11509	KT152094	KT152127
Auricularia americana	China	Cui 11657	KT152095	KT152128
Auricularia angiospermarum	USA	TJV-93-12-SP	KT152096	KT15212
Auricularia angiospermarum	USA	Cui 12360, holotype	KT152097	KT15213
Auricularia australiana	Australia	HN 3213	MZ647504	–
Auricularia camposii	Brazil	URM 76905, holotype	MH213351	MH213427
Auricularia asiatica	Indonesia	OM 13932	MZ618931	MZ74004
Auricularia conferta	Australia	Dai 18825, holotype	MZ647500	MZ740048
Auricularia auricula-judae	France	Dai 13210	KM396769	KP729312
Auricularia auricula-judae	Czech Republic	MT 7	KM396771	KP729314
Auricularia auricula-judae	Iran	SAKH 61312751	**OQ799647**	**OQ808799**
Auricularia delicata	Cameroon	P 14, epitype	MH213364	–
Auricularia fibrillifera	China	Cui 6704	KP765613	MH213435
Auricularia fibrillifera	China	Dai 13598A	KP765615	KX022084
Auricularia lateralis	China	Dai 16417	MH213369	MH213440
Auricularia heimuer	China	Dai 13765, holotype	KM396793	KP729317
Auricularia heimuer	China	Dai 2291	KM396785	KP729315
Auricularia heimuer	Iran	Jahedi 1987	**OQ793046**	**OQ808802**
Auricularia fuscosuccinea	USA	OM 17909	KX022029	KX02209
Auricularia fuscosuccinea	Brazil	AG 1548	KX022028	KX022089
Auricularia cerrina	Czech Republic	HK 677	OM747871	–
Auricularia mesenterica	Iran	AS 1925	**OQ845732**	**OQ849589**
Auricularia mesenterica	Switzerland	Miettinen 12680	KP729286	KP72932
Auricularia mesenterica	Estonia	Kytovuori89333	KP729284	KP729321
Auricularia mesenterica	France	LYBR5353	KM396801	KP729318
Auricularia mesenterica	Uzbekistan	YG 037	MZ618939	MZ740056
Auricularia iranica sp. nov.	Iran	Fatima 1392, holotype	**OQ791312**	**OQ808800**
Auricularia iranica sp. nov.	Iran	Ayda 1399	**OQ793042**	**OQ808803**
Auricularia minutissima	China	Dai 14881, holotype	KT152104	KT152137
Auricularia minutissima	China	Dai 15455	KX022030	KX02209
Auricularia novozealandica	New Zealand	PDD 83897, holotype	KX02203	–
Auricularia nigricans	Costa Rica	Ahti 36234	KM396802	–
Auricularia nigricans	USA	TJY-93-242	KM396803	–
Auricularia cornea	China	Dai 15336	KX022014	KX022074
Auricularia cornea	China	Wu 07	MH213354	MH21343
Auricularia cornea	Iran	AJ01	**OQ073696**	**OQ107064**
Auricularia cornea	Iran	SJ 990224	**OQ793043**	**OQ808801**
Auricularia orientalis	China	Dai 14875, holotype	KP729270	KP72931
Auricularia orientalis	China	Dai 1831	KP729271	KP729311
Auricularia pilosa	Ethiopia	LWZ 201904217, holotype	MZ647506	–
Auricularia pusio	Australia	AK 547	MH213374	–
Auricularia sinodelicata	China	Cui 8596	MH213376	MH213444
Auricularia sinodelicata	China	Dai 13758	MH213378	MH213445
Auricularia subglabra	Brazil	Dai 17403	MH213382	MH213448
Auricularia scissa	Dominican Republic	Ahti 49388	KM396805	KP72932
Auricularia srilankensis	Sri Lanka	Dai 19575	MZ647502	MZ740058
Auricularia submesenterica	China	Dai 15450, holotype	MH213386	MH213449
Auricularia submesenterica	China	Dai 15451	MZ618942	MZ740059
Auricularia thailandica	Thailand	MFLU 130396	KR336690	–
Auricularia tibetica	China	Dai 15604	MH213388	MH213453
Auricularia tibetica	China	Cui 12267, holotype	KT152106	KT152139
Auricularia tremellosa	Brazil	Dai 17415	MH213390	MH213455
Auricularia villosula	China	Dai 13450	KM396812	KP729327
Auricularia villosula	China	Dai 13453	KM396813	KP729328
Auricularia brasiliana	Brazil	CRSL 886	KP729274	MZ740063
Elmerina efibulata	China	Yuan 4525	MZ618945	MZ740063
Exidia candida	Canada	LE 38198	KY801871	–
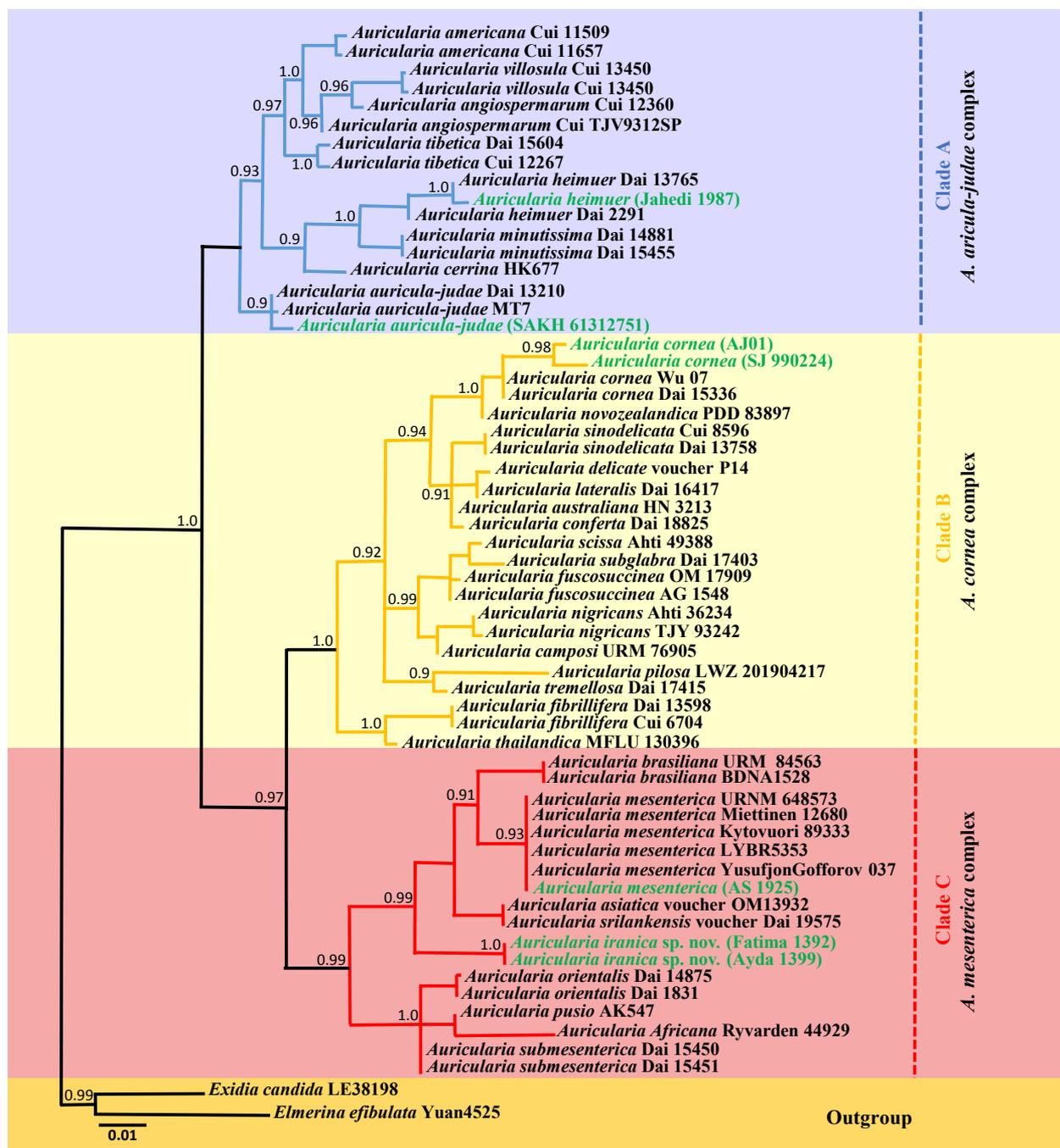
Figure 2. Bayesian phylogenetic tree reconstructed using to the sequences of *rpb2* and ITS regions according to the GTR + G + I evolutionary model. Branches are labeled with Bayesian Posterior Probabilities higher or equal to 0.90, respectively. Specimens sequenced during present study are shown in green font.

Microscopic description. Zona pilosa containing hyaline hairs with acute or obtuse apical tips, tufted, (41–)93–129(–145) × (3–)4.2–7(–7.5) µm, [110.04 ± 0.04 × 4.98 ± 0.14, Q = 20–21.2, Qm = 20.65 ± 0.1, n = 20]. Hymenium 60–80 µm wide, with elongated clavate basidia and elongated clubs with three transverse walls. Basidia 32–58 µm length. Cystidioles clavate with three transverse septa, (62.2–)63–86(–87.75) × 4–5(–5.7) µm, [75.33 ± 0.42 × 4.45 ± 0.44, Q = 15.33–16.82, Qm = 16 ± 0.11, n = 20]. Basidiospores hyaline, allantoid, thin-walled, usually with guttles, (13.3–)13.9–19(–20.2) × (3.7–)4–5.5(–6) µm, [16.53 ± 0.06 × 4.78 ± 0.44, Q = 3.13–3.54, Qm = 3.33 ± 0.07, n = 20].

Distribution. Europe.

Notes. In terms of appearance, it is similar to *Auricularia fuscisuccinea*, but in the cross-section (the transverse section of the basidiocarp), they are different, so that the *Auricularia auricula-judae* does not have a medulla. Lowy (1952) and Kobayasi (1981) described this species as *A. auricula*, while Swann and Taylor (1993) described it as *A. auricula-judae*. But in general, the name *A. auricula-judae* is generally accepted. Also can be mistaken by *A. heimuer* and *A. americana* while the only character that could be distinguished is its larger basidia 40–67 × 3–6.5 µm in *A. heimuer*, 55–71 × 4–5 µm in *A. americana* and basidiospores 11–13 × 4–5 µm in *A. heimuer*, 14–16.5 × 4.5–5.4 µm in *A. americana* (Wu et al. 2021).
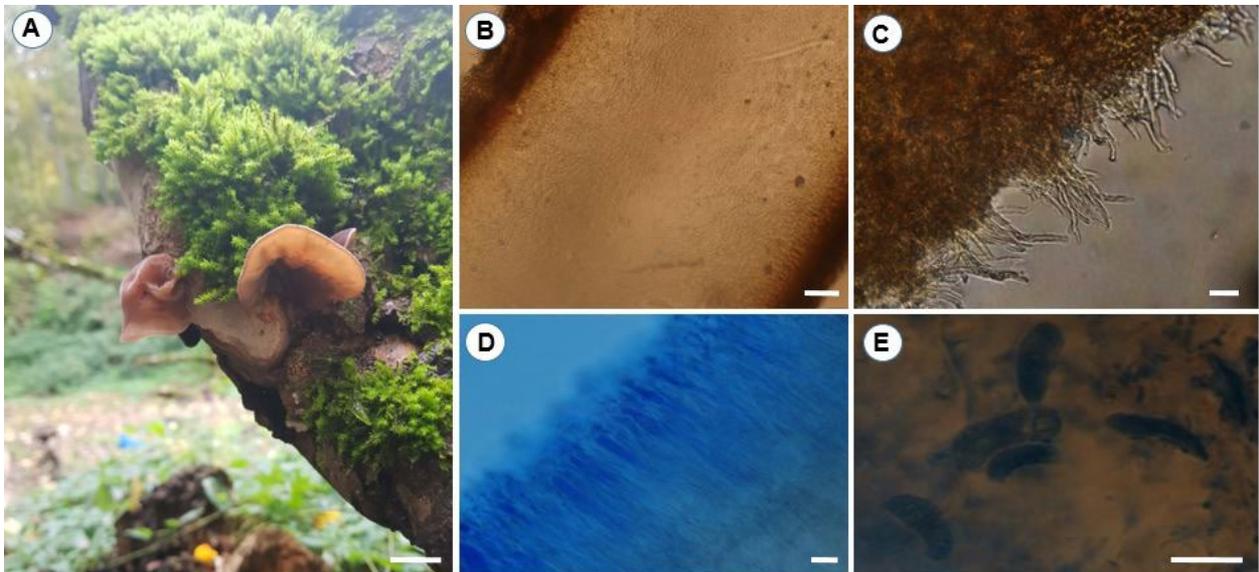
Figure 3. Macroscopic and microscopic characteristics of *Auricularia auricula-judae*. A – basidiocarp in the field; B – cross-section view; C – abhymenial hairs; D – basidia and basidiol; E – basidiospore. Scales: A = 3 cm; B = 100 μm; C = 20 μm; D, E = 10 μm.

Specimen examined. IRAN. Golestan Province, Glidagh–Kamerli, Marawa Tepeh, Hyrcanian Forests, 37°41′45″N, 55°58′01″E, 1150 m, on *Aluns glutinosa*, 8 Oct. 2018, A. Jahedi s.n. (SAKH 61312751), GenBank: ITS OQ799647, *rpb2* OQ808799.

Auricularia cornea Ehrenberg, Horae Physicae Berolinenses: 91. 1820. (Fig. 4)

Macroscopic description. Basidiocarp: solitary or collectively, up to 15 cm, on the branches or trunks of dead or decaying trees or rarely alive. It is shaped like an ear to almost circular, margin lobed, with a soft to hard jelly texture, the thickness of the fruit is 1.5–2.2 mm when fresh, and 1–0.5 mm when dry.

Microscopic description. Zona pilosa: no central lumen, rounded tip. Individual hairs are distinguishable, and tend to cluster at the apex, dimensions: (80–)150–400(–445) × (5.2–)5.5–8(–7.9) μm, [268.43 ± 0.02 × 6.12 ± 0.34, Q = 41.03–46.55, Qm = 43.8 ± 0.32, n = 20]. Zona compacta: compact and dense, with 70–80 μm width. Zona subcompacta superioris: width of 20–30 μm. Zona laxa superioris: with a width of 40–50 μm and hyphae with a diameter of 5–6 μm, sudden fusion with the medulla. Zona laxa inferioris: with a width of 30–40 μm and hyphae diameter 4–6 μm. Zona subcompacta inferioris: 70–80 μm wide and hyphae diameter 4–5 μm. Medulla: pale brown with a thickness of 230–300 μm, internal hyphae branched, hyaline, diameter 2 to 4.5 μm. Hymenium 57–95 μm. Cylindrical basidium with dimensions: (78–)81–83(–85) × (2.7–)3–6(–7.9) μm, [80.43 ± 0.32 × 4.3 ± 0.14, Q = 17.23–18.04, Qm = 17.65 ± 0.08, n = 20]. Sausage-shaped hyaline basidiospores usually with up to three large guttles, dimensions: (12.8–)13–15.5(–16.4)
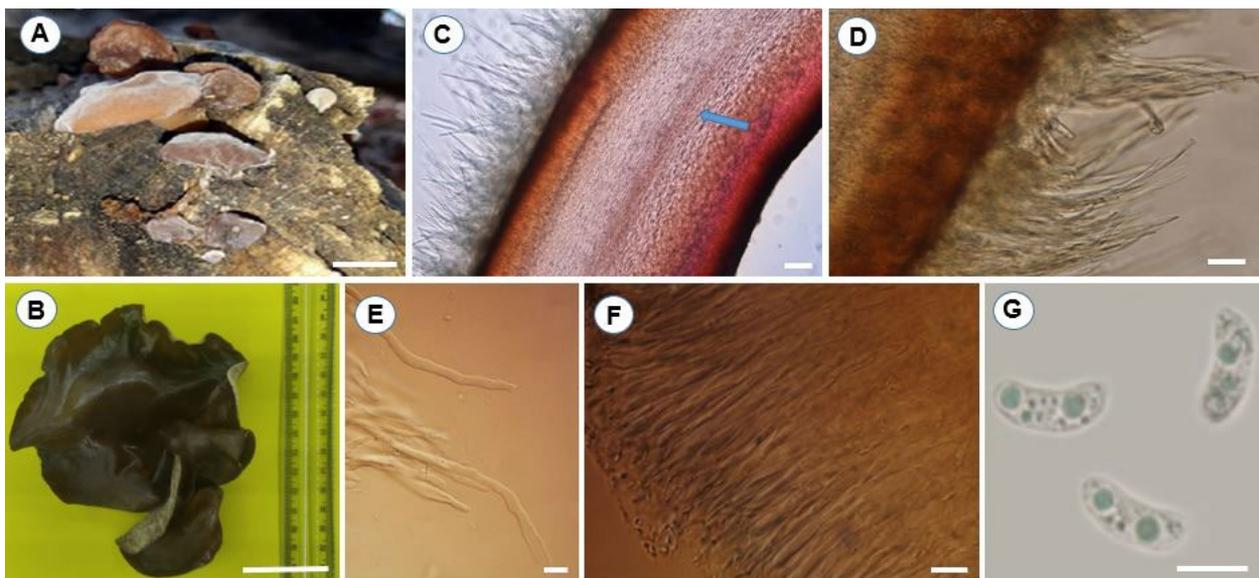

Figure 4. Macroscopic and microscopic characteristics of *Auricularia cornea*. A – basidiocarps in the field; B – cultivated basidiocarp; C – cross-section view, the medulla is shown by the arrow; D, E – abhymenial hairs; F – basidia and basidiols; G – basidiospores. Scales: A, B = 5 cm; C = 100 μm; D = 50 μm; E–G = 10 μm.

× (3.9–)4.5–6.1(–6.3) μm, [14.18 ± 0.36 × 5.22 ± 0.14, Q = 2.70–2.73, Qm = 2.68 ± 0.3, n = 20].

Distribution. Africa, America, Asia, Europe.

Notes. First reported by Patouillard and Olivier (1907) as *A. polytricha*, a name utilized in almost all Asia, Pacific areas, and China until 2015 (Teng 1939; Lowy 1952; Tai 1979; Kobayasi 1981; Ying et al. 1987; Imazeki et al. 1988; Park 1991; Musngi et al. 2005; Mao 2009; Huang et al. 2010; Li et al. 2015). However, *A. nigricans* is a synonym of *A. polytricha*. This is the first report in Iran (Fig. 4).

Specimen examined. IRAN. Mazandaran Province, Prim City, Sari, Hyrcanian Forests, 36°20′54″N, 53°14′24″E, 548 m, on *Carpinus betulus* (Hornbeam), 11 November 2018, A. Jahedi (AJ01), GenBank: ITS OQ073696, *rpb2* OQ107064.

Auricularia heimuer F. Wu, B.K. Cui and Y.C. Dai, Phytotaxa 186: 248. 2014.   (Fig. 5)

Macroscopic description. Basidiocarp: group, or solitary, up to 10 cm, usually substipitate, the thickness is 1–1.6 mm, gelatinous when fresh, grayish brown to brown as if it is sitting on the black colored when dry.

Microscopic description. piloza surface pilose, often with folds; Hymenophore surface smooth, rarely with shallow folds, medulla generally in the cross-section middle (a central band of parallel hyphae), crystals absent. Zona pilosa: Abhymenial hairs hyaline, thick-walled with a narrow lumen and swollen base, apical tips obtuse or acute, single or sparse clustered, dimensioned: (38–)55–155(–170) × (3–)4–6(–7.5) μm, [105.58 ± 0.16 × 5.1 ± 0.32, Q = 20.5–21.45, Qm = 20.96 ± 0.11, n = 20]. Basidiole without cystidiole and cylindrical basidium with three transverse septate, and dimensions: (25–)40–70(–80) × (4–)3–7(–8) μm, [51.08 ± 0.16 × 5.12 ± 0.32,

Q = 10.07–10.45, Qm = 10.26 ± 0.05, n = 20]. Basidiospores allantoid, thin-walled, hyaline, usually with two large guttles, dimensions: (7–)10–14 × (3.3–)3.5–4.6 μm, [11.68 ± 0.26 × 4.02 ± 0.32, Q = 2.67–2.95, Qm = 2.78 ± 0.31, n = 20] (Fig. 5).

Distribution. Asia.

Note. In terms of morphology, *A. villosula* had been misidentified as *A. heimuer* until 2014 (Wu et al. 2014). While the first one is distinguished from the latter by having larger basidiospores (13–15.5 × 5–6.1 μm). *A. heimuer* usually grows mostly on *Quercus* (Wu et al. 2014), but in the present study it is found on *Carpinus betulus*. Until some years ago, Asian *A. heimuer* was considered the same species as the European *A. auricula-judae* (Mao 1998; Dai et al. 2012).

Specimen examined. IRAN. Province, Larg Valley, Behshahr, Hyrcanian Forests, 36°37′59″N, 53°32′39″E, 793 m, on *Carpinus betulus* (Hornbeam), 21 April 2017, A. Jahedi (Jahedi 1987), GenBank: ITS OQ793046, *rpb2* OQ808802.

Auricularia iranica A. Jahedi, sp. nov.   (Fig. 6)

MycoBank MB 853065

Index Fungorum number: IF900448; Facesoffungi number: FoF 14161

Diagnosis: Differs from *Auricularia mesenterica* in having longer abhymenial hairs (1,030–2,200 μm, thiner zona laxa (30–60 μm), thicker zona subcompacta superioris (150–185 μm), thicker zona compacta (600–680 μm), lower hymenium (80–90 μm), longer and plump-shaped basidiospore (15–20 × 6–9 μm), presence of crystals, and lack of cyctidioles.

Type: Iran, Hyrcanian forests, savadkouh–lajim, Mazandaran province, 36°14′52″N, 53°07′01″E, on fallen dead wood of *Ulmus minor*, 7 June 2018, A. Jahedi, (Fatima 1392 – holotype), GenBank: ITS OQ791312, *rpb2* OQ808800.

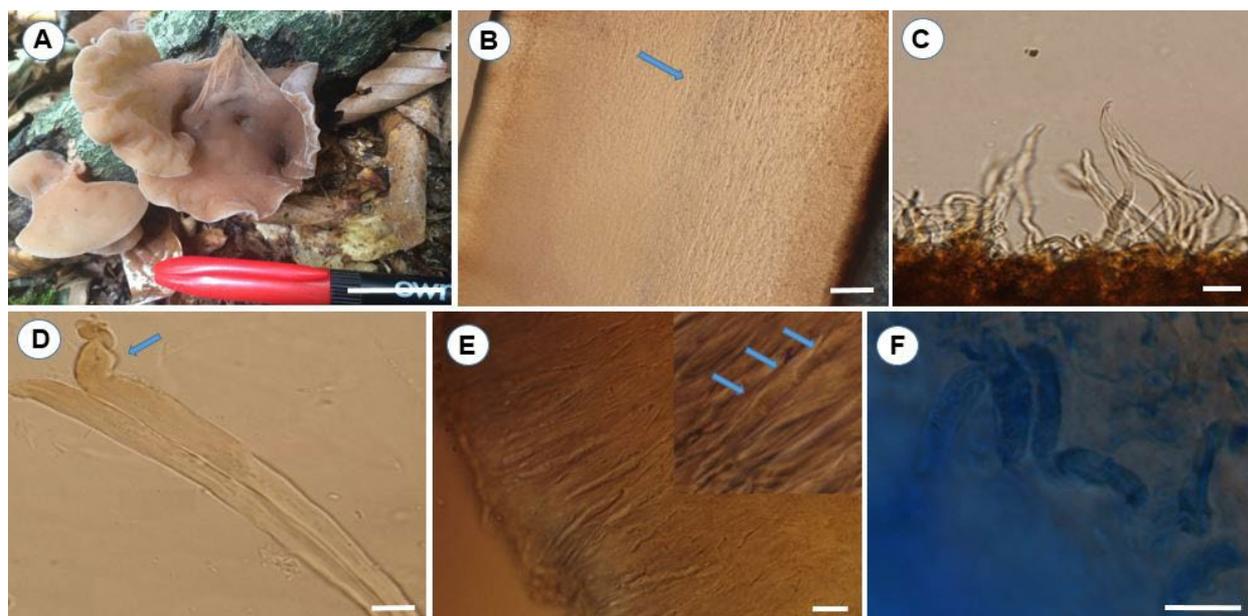**Etymology**. Refers to the distribution in Iran.

Figure 5. Macroscopic and microscopic characteristics of *Auricularia heimuer*. A – basidiocarps in the field; B – cross-section view, the medulla is shown by the arrow; C – abhymenial hairs; D – close up of abhymenial hairs, swollen base shown by arrow; E – close-up of hymenial layer, basidium with three transverse septa shown by arrow; F – basidiospores. Scales: A = 3 cm; B = 100 μm; C = 20 μm; D–F = 10 μm.
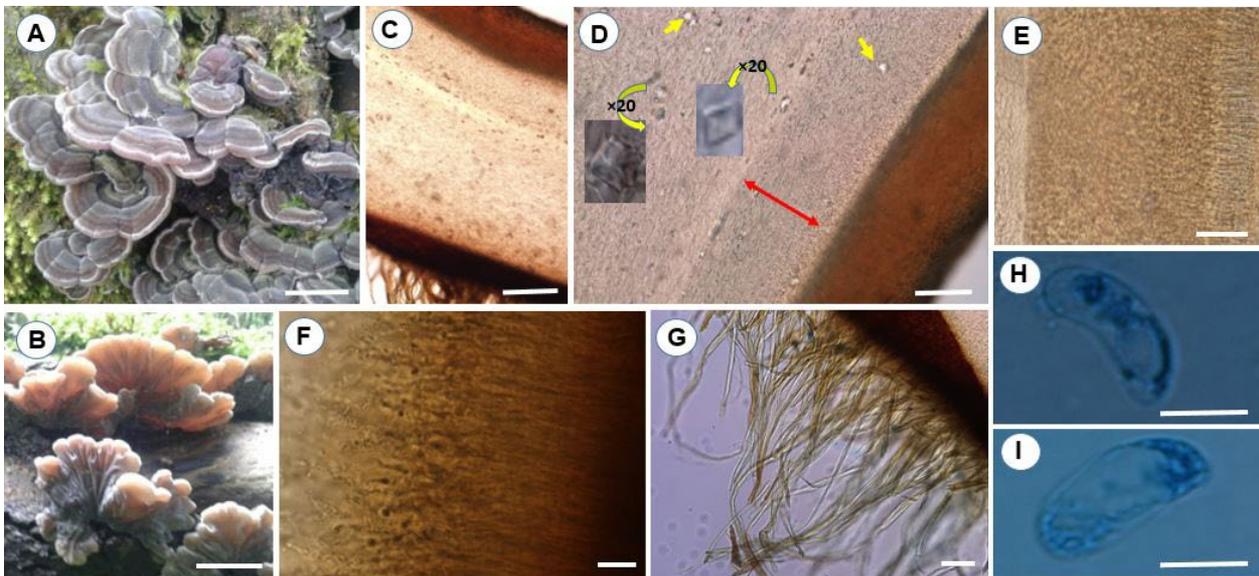
Figure 6. Macroscopic and microscopic characteristics of *Auricularia iranica* (Fatima 1392, holotype). A, B – basidiocarps in the field; C, D – cross-section view, a abnormal zona subcompacta inferior indicated with red arrow, two forms of crystals is shown by yellow arrow; E – close up of hymenium and a abnormal zona subcompacta inferior; F – basidia and basidiols; G – abhymenial hairs; H – basidium; I, J – basidiospores. Scales: A, B = 3 cm; C = 200 μm; D, E = 50 μm; G = 100 μm; H, I = 10 μm.

Description. Basidiocarp: fresh basidiocarps are gelatinous, streaks alternately with light brownish gray, similar to a rainbow, projecting up to 2–5(–6.5) cm, usually lobed, with many folds in the form of concentric veins, upper surface hispid, thick and fleshy texture with 2–3.5 mm thick, 0.2–1 mm thick when dry. Zona pilosa: dense, thick and longer abhymenial hair in dimensions: (900–)1,030–2,200(–2,600) × (1.7–)2–4.4(–5) μm, [1,650 ± 0.06 × 3.2 ± 0.52, Q = 490–510, Qm = 500 ± 0.42, n = 20]. Zona compacta: compact and dense, with a width of 30–60 μm. Zona subcompacta superiors: with a width of 150–185 μm. Intermedia laxa zona: with a width of 600–680 μm. Zona subcompacta inferiors: with a width of 170–190 μm. Hymenium: with a thickness of 80–90 μm and very compact, basidiospores allantoin, hyaline, thick-walled, plump, smooth, usually with fat parts and with dimensions: (14.6–)15–20(–21.2) × 6.5–9(–9.7) μm, [15.7 ± 0.61 × 6.2 ± 0.09, Q = 2.4–2.5, Qm = 2.58 ± 0.07, n = 20]. There is no medulla. Crystal present, cystidioles absent. Basidium clavite with three transverse septate and dimensions: 45–67(–70) × (3.3–)4.3–6(–6.8) μm, [55.5 ± .34 × 5.02 ± 0.09, Q = 11–11.1, Qm = 11.02 ± 0.08, n = 20].

Distribution. Only known from Iran.

Notes. Macro-morphologically, the closest species in *A. mesenterica* complex can be *A. mesenterica*, but differs simply from latter due to abnormal zona subcompacta inferioris and with crystals, but no cyctidioles vs. with cyctidioles, but no crystals (Fig. 6).

Specimen examined. IRAN. Gilan Province, Rudsar, Hyrcanian Forests, 36°53′45″N, 50°12′30″E, 620 m, on fallen dead wood of *Ulmus minor*, 17 July 2018, A. Jahedi (Ayda 1399), GenBank: ITS OQ793042, *rpb2* OQ808803.

Auricularia mesenterica (Dicks.) Pers., Mycologia Europaea 1: 97. 1822.   (Fig. 7)

MycoBank MB 178140

Macroscopic description. Basidiocarp: fresh basidiocarps are hard gelatinous (rubber state), dark brown to dark lilac, width of 2–7(–8) cm, usually lobed, dense upper surface, with many folds in the form of concentric veins, upper surface hispid, a smooth lower surface, 2–3 mm thick, 0.2–0.5 mm thick when dry.

Microscopic description. Zona pilosa: with hyaline to light yellow abhymenial hairs with a slightly swollen base, thick wall, with a narrow duct, in dimensions: (840–)940–2,050 × (1.8–)2–4(–4.1) μm, [1,500 ± 0.06 × 3 ± 0.52, Q = 500, Qm = 249.58 ± 0.32, n = 20]. Zona compacta; compact and dense, with a width of 32–46 μm. Zona subcompacta superiors: with a width of 140–160 μm. Intermedia laxa zona: with a width of 320–580 μm. Zona subcompacta inferiors: with a width of 138–152 μm. Hymenium: with a thickness of 68–120 μm and very compact, basidia with a length of 32–58 μm. There is no medulla. Crystals are absent, but cystidioles are present. Basidium cystidiole and clavate with three transverse septate and dimensions: (45–)47–67(–70) × (3.3–)4.3–5.8(–6.8) μm, [58 ± 34 × 5.03 ± 0.09, Q = 11.1–11.74, Qm = 11.38 ± 0.08, n = 20]. Basidiospores allantoin, hyaline, thick-walled, smooth, usually with fat parts and with dimensions: (12.6–)13–16.3(–17) × (3–)4.5–5.7(–9) μm, [14.5 ± 0.61 × 5 ± 0.09, Q = 2.65–2.9, Qm = 2.8 ± 0.04, n = 20].

Distribution. *Auricularia mesenteric* is a common species in tropical, subtropical and even temperate forests where it grows on different types of wood. This species was described from Europe, America, and Asia (Teng 1996; Montoya-Alvarez et al. 2011; Looney et al. 2013).
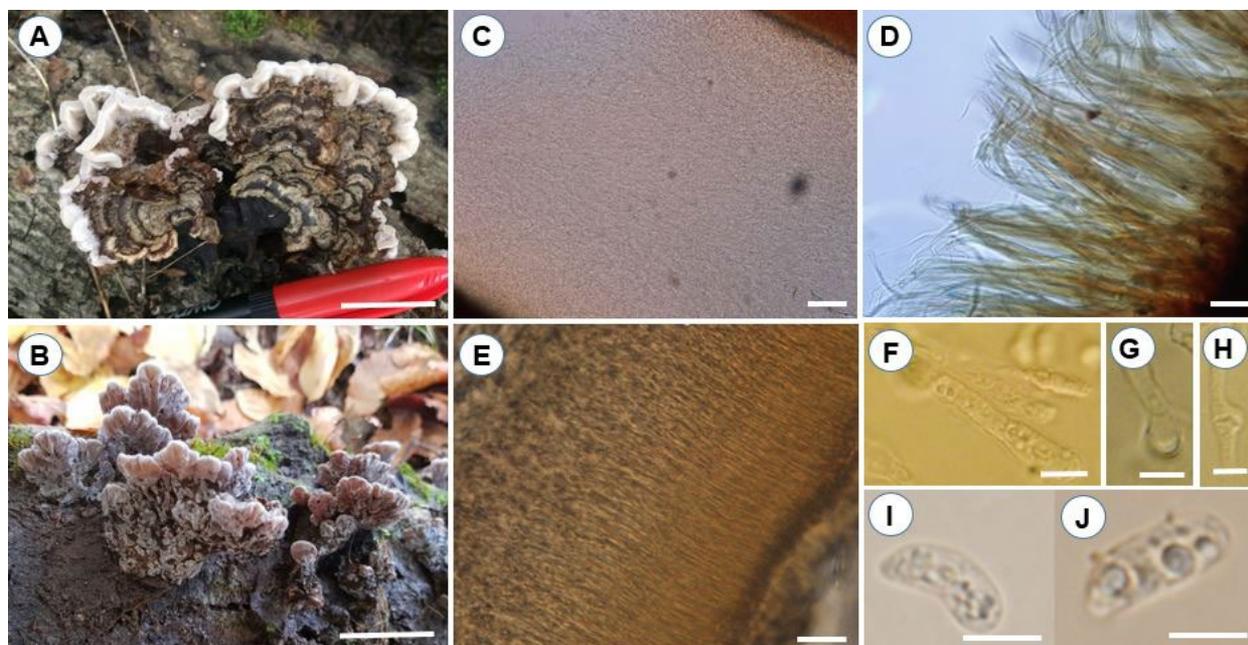
Figure 7. Macroscopic and microscopic characteristics of *Auricularia mesenterica*. A, B – basidiocarps in the field; C – cross-section view; D – abhymenial hairs; E – basidia and basidiols; F – basidium; G – cystidioles; H – clamp connection; I, J – basidiospores. Scales: A, B = 2 cm; C, D = 100 μm; E–J = 10 μm.

Notes. *Auricularia mesenterica* is a complex including eight species *A. asiatica*, *A. brasiliana*, *A. orientalis*, *A. africana*, *A. submesenterica*, *A. pusio*, *A. srilankensis*, and *A. mesenterica* (Berkeley 1881; Wu et al. 2015, 2021; Bandara et al. 2017). *A. mesenterica* s. str. had the largest population among the samples and is the only species of the *Auricularia* genus that was found in almost all forests (Fig. 7).

Specimen examined. IRAN. Mazandaran Province, Sisangan Park, Nowshahr, Hyrcanian forests, 36°34′06″N, 51°48′21″E, 65 m, on fallen dead *Acer nigrum* (Maple), 10 July 2018, A. Jahedi (AS 1925), GenBank: ITS OQ845732, *rpb2* OQ849589. Iran. Mazandaran Province, Larag Valley, Behshahr, Hyrcanian forests, 36°37′57″N, 53°32′27″E, 772 m, on fallen dead *Acer nigrum* (Maple), 21 April 2017, A. Jahedi (Jahedi 1987).

Discussion

This present study briefly discusses specific characteristics of this genus. Although some main characteristics of the *Auricularia*, such as the medulla, are used for species identification. In *Auricularia*, this trait sometimes is not able to be distinguished as in all of the species in *A. auricula-judae* complex, for previous research has claimed it has no medulla (Lowy 1952). Recently, species have been derived from this species complex both with and without the medulla (Wu et al. 2021; Kout & Wu 2022). An important point to consider in the morphological identification of this genus is the color of the basidiocarps, which can be misleading for intraspecific color variation, ranging from light brown to blackish (Mau et al. 1998; Lin et al. 2013; Wu et al. 2014a; Bandara et al. 2017a).

The first molecular study of this genus has been used since 1999–2002 to describe *Auricularia* species and has

intensified in the last few years (Looney et al. 2013; Wu et al. 2014; Bandara 2015; Wu et al. 2021; Kout & Wu 2022). Some researchers have utilized ITS rDNA (Weiß & Oberwinkler 2001; Montoya-Alvarez et al. 2011; De Leon et al. 2013; Bandara et al. 2020), while others have employed *rpb2* and ITS sequences to reconstruct the phylogeny of *Auricularia* (Bandara et al. 2017). For studies on the diversity of *A. mesenterica*, ITS, *rpb2*, and nLSU sequences have been used (Wu et al. 2015). However, there remain ambiguities regarding certain species, such as *A. cornea*, *A. nigricans*, and *A. polytricha*, within the *A. cornea* complex, and researchers often express differing opinions on the genes they chose. Multigenous phylogenetic studies (Wu et al. 2021), including ITS, *rpb1*, *rpb2*, nLSU, and SSU could be useful in separating *Auricularia* species.

However, in phylogenetic studies, several methods and approaches are commonly used. To clarify, the status of the five representative species based on morphology in this study, the ITS and *rpb2* sequences from all of the *Auricularia* species, were used in the phylogenetic analyses. Likewise, ITS rDNA and *rpb2* sequences separate these five species, placing them in five distinct clades.

Auricularia mesenterica was the only species reported from all three provinces (Gilan, Golestan, Mazanderan) on *Fagus orientalis* and some unknown hosts (Hallenberg 1979; Ershad 1995). *Auricularia auricula-judae* is the second species of this genus. As with the previous information from the reported field studies, it has been recorded with the name *Hironeola auricula-judae* (=*Auricularia auricula* (Soleimani 1976)) on *Fagus* sp. and some unknown hosts in Iran. It should be mentioned, that they merely reported that it existed, while no reliable, verified document is available. According to the results, the host range of the studied species is limited to six hardwoods: *Aluns glutinosa*, *Ulmus minor*, *Acer* sp., *Quercus castaneifolia*,

Carpinus betulus, and *Fagus orientalis*. The most of samples belong to *A. mesenterica* complex which was found on all six hosts, followed by *Auricularia auricula-judae* in *Aluns glutinosa* and *Quercus castaneifolia*, *A. cornea* on *Aluns glutinosa*, *Fagus orientalis*, and *A. heimuer* on *Carpinus betulus*, respectively. This study showed that this genus has the potential for host diversity. This is even though in previous studies, *A. auricula–judae* was only reported from *Sambucus nigra* and *Acer negunda* (Lowy 1952; Lassoe & Petersen 2019), but this study showed wider host range. The second issue is that the multiplicity of hosts in the *A. mesenterica* species complex led to the multiplicity of samples collected. This could be one of the reasons, but it cannot be generalized to the entire population. Typically, there are several hosts for a species, although only a few may be found in a forest. This shows that the species *A. mesenterica* is more compatible with different hosts, unlike other species. To find the answer to this question, more studies should be done in this field. According to the contents mentioned; as expected, *Auricularia iranica* as a new species is introducing various other species derivative from *A. mesenterica* complex; *A. mesenterica* (Lowy 1952), *A. orientalis*, *A. brasiliana* (Wu et al. 2015), *A. submesenterica*, *A. puiso*, *A. africana* (Wu et al. 2021), *A. asiatica* (Bandara et al. 2017) and *A. srilankanesis* (Wu et al. 2021).

The phylogenetic tree reveals *Auricularia iranica* as a monophyletic clade in the *A. mesenterica* species clade with 1.0 BI support. To be a new species, combined ITS and *rpb2* regions sequences are sufficient to support the same as in previous research (Jülich 1984; Wu et al. 2015, 2021). This character provide a significant confirmation, simultaneously with biogeographical area, because of the partial overlapping of morphological attributes in some, especially the closest species (Wu et al. 2015).

Auricularia iranica belongs to the *A. mesenterica* complex and is determined by streaks alternately with a light brownish-gray upper surface, similar to a rainbow. Macro-morphologically, the closest species in *A. mesenterica* complex can be *A. mesenterica*, but differs from later (Lowy 1952) due to longer abhymenial hairs (1,030–2,200 μm vs. 1,000–2,000 μm). Zona laxa, zona subcompacta superioris, zona compacta, zona subcompacta inferioris and Hymenium (30–60 μm vs. 80–90 μm, 150–185 μm vs. 130–150 μm, 600–680 μm vs. 575–600 μm, 170–190 μm vs. 165–180 μm and 80–90 μm vs. 115–125 μm), longer and plump basidiospore (15–20

× 6–9 μm vs. 15–18 × 5–6 μm) and with crystals, but no cyctidioles vs. with cyctidioles, but no crystals (Lowy et al. 1952; Wu et al. 2021). In the phylogeny, *Auricularia iranica* is distantly related to *A. mesenterica* and forms a single lineage with high support (Fig. 1). Compared to other species of the family, it differs from *A. africana* (Ryvarden 44929) and *A. asiatica* (Dai 16149) in having longer abhymenial hairs (1,030–2,200 μm vs. 300–500 μm, 800–1,200 μm), longer basidiospore (15–20 μm vs. 12–14 μm, 11.2–12.3 μm); from *A. brasiliana* (AN-MA 42) and *A. orientalis* (Dai 14875) in having crystals and longer abhymenial hairs (1,050–2,100 μm vs. 1,000–1,500 μm, 900–1,500 μm), longer basidiospore (14–20 μm vs. 11.5–12 μm, 12.5–14.2 μm); from *A. submesenterica*; *A. srilankensis*; *A. pusio* (AK 174); (Dai 15450) in having longer abhymenial hairs (1,050–2,100 μm vs. 400–800 μm, 600–1,000 μm), longer basidiospore (15–20 μm vs. 12–14 μm, 12–14.7 μm, 12.5–14.8 μm), respectively (Table 2).

Since *A. auricula-judae* (Pilát 1957) may be confused with very similar species, such as *A. heimur*, because previous mycologists expected only *Auricularia* species to be limited to a few species described by previous researchers. This is most likely because at that time there were fewer studies and usually only included morphological traits (Lowy 1951, 1952; Kobayashi 1981; Parmasto & Parmasto 1987; Wong & Wells 1987; Kirk et al. 2008). In addition, some dried specimens appear similar and it is improbable that anyone who runs a well-known *A. auricula-judae* is a plenty temperate species and it is currently the just species that can be mistaken for *A. heimuer*, *A. americana*, *A. cerrina A. angiospermarum* and *A. minutissima*, because of the morphological and spreading common area. *Auricularia auricula-judae* is mainly reported from *Sambucus nigra* and *Aluns glutinosa* (Lassoe & Petersen 2019), but it inhabits some species of woody plants (Wu et al. 2021). Before phylogenetic analyses were used for taxonomy, it was considered a widespread species (Lowy 1952). Based on our results, another important cultivated species of this genus, which is mistakenly used as *A. auricula-judae*, is *A. heimuer*, in this study, it was distinguished from *A. auricula-judae* by having a distinct medulla and forming a separate clade with strong support in the phylogeny. However, phylogenetic analyses showed that it was a species complex (Wu et al. 2015). Although *Auricularia auricula-judae* was limited to elderberry in the past (Jülich 1984), the number of its hosts is

Table 2. A comparison of the species in the *Auricularia mesenterica* complex (new species introduced in this study is in bold).

Species Name	Hairs (μm)	Basidia (μm)	Basidiospores (μm)	Upper Surface	Crystals	Cystidioles
A. africana	300–500 × 1–2	50–80 × 4.5–6	12–14 × 4.9–5.4	Tomentose	Present	Absent
A. asiatica	800–1200 × 1.5–3	40–52 × 3–6	11.2–12.3 × 4.5–5.2	Villose	Present	Absent
A. brasiliana	1000–1500 × 2–3.5	30–47 × 3–5	11.5–12 × 4.5	Tomentose	Absent	Absent
A. iranica	**1030–2200 × 2–4.4**	**45–67 × 4.3–6**	**15–20 × 6.5–9**	**Hispid**	**Present**	**Absent**
A. mesenterica	940–2000 × 2–4	48–68 × 4–6.5	14–17 × 4.7–5.2	Hispid	Absent	Present
A. orientalis	900–1500 × 1.5–2.5	50–72 × 3.5–6	12.5–14.2 × 5–6	Villose	Absent	Present
A. pusio	400–800 × 1.5–2	50–72 × 5–7.5	12–14 × 5–6	Tomentose	Present	Absent
A. srilankensis	400–800 × 1.5–3	50–70 × 5–7	12–14.7 × 4.5–6	Villose	Present	Absent
A. submesenterica	600–1000 × 2–2.5	37–50 × 4–6.5	12.5–14.8 × 5.5–6	Hispid	Present	Absent

increasing day by day (Kout & Wu 2022). Similarly, the abhymenial hair length has a vital determining position (Wu et al. 2015). Several studies show abhymenial hairs of *A. auricula-judae* up to 150 µm (Kobayasi 1981; Montoya-Alvarez et al. 2011; Wu et al. 2015). However, in different samples with different lengths, it is even more than 150 (in European samples) and less than 150 in the samples of the present study.

The present study was conducted for the first time to clarify the status of *Auricularia* in Iran. After sample collection during 2017–2018, 19 samples were collected from mushroom spots. Eventually, based on molecular (ITS and *rpb2* sequences) and morphological studies, five species (*Auricularia mesenterica*, *A. iranica*, *A. auricularia-judae*, *A. cornea*, and *A. heimuer*) were identified. The interesting point of this genus is that it may have specific host and growth preferences.

The previous research on the determination of *Auricularia* species had just relied on the morphological features in Iran. These attributes may vary with age, moisture, light exposure, nutritional condition, etc., leading to misidentifications. This study used morphological-molecular data to improve upon classifications and filter out previous mistakes of *Auricularia* species. Fruiting bodies collected during 2017–2018 were examined and sequenced for ITS and *rpb2* loci. Three of the five described species; *A. cornea* on *Aluns glutinosa*, *Fagus orientalis*, *Auricularia auricula-judae* on *Aluns glutinosa* and *Quercus castaneifolia*, and *A. heimuer* on *Carpinus betulus*, rare reported as new records from Iran. A combined *rpb2* and ITS phylogenetic tree showing the five *Auricularia* species placement found in Iran is provided.

The strong point of this study is that the taxonomic status of *Auricularia* (Wood ear mushroom) as the third edible-medicinal mushroom in the world was done for the first time using morpho-molecular methods leading to the introduction of four of the most important species of this genus in Iran and one new species for the world.

Conclusion

This research specifies the taxonomic status of genus *Auricularia* in Iran for the first time. After comprehensive sampling from Hyrcanian forests in Iran, the identification of five species of *Auricularia* was done according to morphological and molecular examinations. Based on *rpb2* and ITS sequences, phylogenetic analyses were performed using Bayesian Inference analysis methods. As expected from morphological characters, as well as ITS and *rpb2* region sequences, our results support one new species, *Auricularia iranica* in the *A. mesenterica* complex. The two known species, *A. auricula-judae* and *A. heimuer*, belonging to the *A. auricula-judae* complex and *A. cornea* belonging to the *A. cornea* complex, are defined.

Key to *Auricularia* species in Iran

1 Resupinate to effused-reflexed basidiomata, the upper surface usually with distinct and concentric zones, up to 7 cm, abhymenial hair > 1000 µm. 2

Basidiomata gelatinous when fresh, basidiocarps longer than 8 cm, abhymenial hair < 1000 µm. 3

2(1) Basidiocarp 2–3 mm thick, basidiospores 13–17 × 4.5–6 µm, Abhymenial hair 940–2000 µm (< 2050 µm) . . . . *A. mesenterica*

Basidiocarp 2–3.5 mm thick, basidiospores 15–20 × 6–9 µm, Abhymenial hair 1030–2200 µm . . . *A. iranica*

3(1) No medulla. . . . . *A. auricula-judae*

With medulla 4

4(3) Abhymenial hair 55–155 µm (< 155 µm), basidiocarp project up to 10 cm . . . . *A. heimuer*

Abhymenial hair 150–400 µm (> 150 µm), basidiocarp project up to 15 cm . . . . *A. cornea*

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