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Gyromitra persicula sp. nov. (Discinaceae, Pezizales), a novel discoid *Gyromitra* from Northwestern North America

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ABSTRACT

Gyromitra persicula (Discinaceae, Pezizales), a new species from Washington state, USA, is described. Like its sister species *G. leucoxantha*, *G. persicula* has reticulate ascospores with blunt apiculi that form concave depressions resembling a fishtail. *Gyromitra persicula* is differentiated from *G. leucoxantha* by its smaller size, peach to buff coloration, and nrDNA ITS. Further collections are required to better understand the distribution and abundance of this discoid *Gyromitra*, which is currently only known from a small geographic area of temperate rainforest.

Key words: false morel, gyromitrin, one new taxon, systematics, Washington

INTRODUCTION

Discinaceae is a morphologically diverse family of mushrooms ranging from discoid or saucer-shaped to stipitate with an apical hymenophore, as well as hypogeous, truffle-like forms (O'Donnell *et al.* 1997). Taxa with discoid ascomata have traditionally been placed in the genus *Discina* (Fries) Fries (1849: 348) and those with stipitate ascomata in the genera *Gyromitra* Fries (1849: 346), *Neogyromitra* Imai (1932: 174), or *Pseudorhizina* Jaczewski (1913: 414). Harmaja (1969) challenged the generic delimitation based on gross morphology and lumped these taxa together under *Gyromitra*, arguing that their microscopic characteristics represented a continuum—a taxonomic framework we follow here. Discinaceae species are difficult to separate with morphological analysis alone; contemporary studies utilize molecular phylogenetics in an integrative approach to resolve long-standing questions in Discinaceae taxonomy, tease apart species complexes, and describe new taxa (Miller *et al.* 2020, 2022a). As part of ongoing studies concerning the gyromitrin mycotoxin in Discinaceae (Dirks *et al.* 2023), Dr. Michael W. Beug contributed collections from Washington, USA, belonging to an unknown but locally abundant species. In *Ascomycete Fungi of North America*, Beug called them “mini peach pig’s ears” due to the pinkish buff color of the hymenium (Beug *et al.* 2014: 145). While comparable to *Gyromitra leucoxantha* (Bresadola) Harmaja (1969: 11) in microscopic traits, their coloration and consistently small size suggested they were an undescribed species. Here, we confirm this suspicion and describe the mini peach pig’s ears as new to science.

MATERIALS AND METHODS

Specimens were collected by Dr. Michael W. Beug from Gifford Pinchot National Forest and Mt. Baker-Snoqualmie National Forest in Washington, USA, between the years 2005 and 2022. Ascomata were dried at 35 °C in a food dehydrator and later deposited at the University of Michigan fungarium (MICH). Micromorphological features were studied by shaving off thin sections from dried ascomata and rehydrating the tissue in 5 % KOH stained with phloxine B. Sections were examined with an LW Scientific i4 Infinity compound microscope (Lawrenceville, GA) at $\times 400$ and $\times 1000$ magnification. Photographs and measurements were taken with an AmScope MU500 microscope camera (AmScope, Irvine, CA). When present, 25 mature, freely liberated ascospores, 25 paraphyses, and 10 asci were measured. Sizes are presented as the range consisting of the mean \pm one standard deviation. The mean length (L_m), mean width (W_m), length-width ratio (Q), and mean length-width ratio (Q_m) are also reported. Ascospore measurements include the perispore and apiculi consistent with Miller *et al.* (2022a). Images were processed with Adobe Illustrator 2024 and Adobe Photoshop 2024 (Adobe Systems Inc., Mountain View, California).

DNA extractions and PCR amplifications of the nuclear rDNA internal transcribed spacer region ITS1-5.8S-ITS2 (ITS barcode) and the D1–D2 domains of nuclear 28S large subunit rDNA (LSU) were carried out according to Dirks *et al.* (2023). PCR products were purified with ExoSAP-IT (Applied Biosystems, Waltham, Massachusetts) and submitted to Azenta Life Sciences for Sanger sequencing (Azenta Life Sciences, South Plainfield, New Jersey). Forward and reverse Sanger sequences were assembled with Geneious Prime (Dotmatrix, Boston, Massachusetts) and submitted to GenBank (PP051281–PP051297) (Table 1). ITS and LSU sequences were aligned separately with MUSCLE as implemented in SeaView 5.0.5 (Edgar 2004; Gouy *et al.* 2021). Ambiguous regions of the alignments were trimmed with trimAl using the “automated1” option (Capella-Gutiérrez *et al.* 2009) and concatenated with catfasta2phym1.1.0 (github.com/nylander/catfasta2phym1). ModelFinder was used to determine the optimal model of sequence evolution according to the BIC (Kalyaanamoorthy *et al.* 2017). Maximum likelihood phylogenetic analyses of the individual and partitioned concatenated alignments were conducted with 1000 ultrafast and SH-aLRT bootstrap replicates using IQ-TREE 1.6.12 (Guindon *et al.* 2010; Hoang *et al.* 2018; Nguyen *et al.* 2015). The most likely tree was visualized in FigTree 1.4.4 (github.com/rambaut/figtree) and further edited in Adobe Illustrator 2024.

TABLE 1. Taxa used in the molecular phylogenetic analysis with their corresponding fungarium accession number, voucher or collection number, location, GenBank accession numbers, and source.

Species	Fungarium	ID	Location	ITS	LSU	Reference
<i>Gyromitra leucoxantha</i>	MICH352087	ACD0326	USA-NY	ON693641	ON693593	Dirks <i>et al.</i> 2023
<i>Gyromitra leucoxantha</i>	ILLS00121418	MP 2018-136	Andorra	MW078428	-	Miller <i>et al.</i> 2020
<i>Gyromitra leucoxantha</i>	MICH25407	NSW4536	USA-MI	ON693659	ON693613	Dirks <i>et al.</i> 2023
<i>Gyromitra leucoxantha</i>	HMAS279665	NV2017.06.10	Spain	MG846991	MG847020	Wang & Zhuang 2019
<i>Gyromitra leucoxantha</i> HOLOTYPE	S-F11771	NA	Italy	OP265175	-	Miller <i>et al.</i> 2022b
<i>Gyromitra melaleucooides</i>	MICH352039	ACD0308	USA-OR	ON693638	ON693590	Dirks <i>et al.</i> 2023
<i>Gyromitra melaleucooides</i>	NA	iNat48465814	USA-WA	OQ701114	-	Bouchillon <i>et al.</i> unpublished
<i>Gyromitra melaleucooides</i>	MICH1455	NSW4520	USA-CO	ON693658	-	Dirks <i>et al.</i> 2023
<i>Gyromitra persicula</i>	MICH346505	ACD0508	USA-WA	PP051290	PP051281	This study
<i>Gyromitra persicula</i>	MICH346493	ACD0515	USA-WA	PP051291	PP051282	This study
<i>Gyromitra persicula</i>	MICH346500	ACD0521	USA-WA	PP051292	PP051283	This study
<i>Gyromitra persicula</i>	MICH346515	ACD0524	USA-WA	-	PP051284	This study
<i>Gyromitra persicula</i>	MICH346519	ACD0525	USA-WA	PP051293	PP051285	This study
<i>Gyromitra persicula</i>	MICH346499	ACD0526	USA-WA	PP051294	PP051286	This study
<i>Gyromitra persicula</i>	MICH346523	ACD0547	USA-WA	PP051295	PP051287	This study
<i>Gyromitra persicula</i> HOLOTYPE	MICH346504	ACD0548	USA-WA	PP051296	PP051288	This study
<i>Gyromitra persicula</i>	MICH346513	ACD0552	USA-WA	PP051297	PP051289	This study
<i>Gyromitra persicula</i>	FLAS-F-61956	04mwb050917	USA-WA	MN653042	-	Healy unpublished
<i>Gyromitra</i> sp.	NA	420526MF0212	China	ON554780	ON527645	Wang <i>et al.</i> 2023

RESULTS

The ITS was > 99.7 % identical (no more than two bp differences) across *Gyromitra persicula* specimens, excluding MICH346519 (Table 1). *Gyromitra persicula* MICH346519 had 7–10 bp differences in the ITS (98.9–99.0 % identical to other specimens) and one bp difference in the LSU, which was otherwise identical across all specimens. All *G. persicula* specimens were collected in Washington. For comparison, specimens of *G. leucoxantha* came from different continents (Table 1), and their ITS had no more than three bp differences (> 99.3 % identical). Between *G. persicula* and *G. leucoxantha*, the ITS was 95–97 % identical, except for *G. persicula* MICH346519 and *G. leucoxantha* MICH25407, which exhibited 97.8 % similarity. The individual ITS and LSU phylograms were congruent, thus we present just the phylogram inferred from the concatenated dataset (Fig. 1). The concatenated alignment contained 19 sequences with 1799 nucleotide sites, 334 of which were parsimony-informative. The best-fit substitution models were HKY+F+G4 for ITS and TNe+I for LSU. The most likely tree strongly supports the delimitation of *Gyromitra leucoxantha* and *Gyromitra persicula* as distinct species.

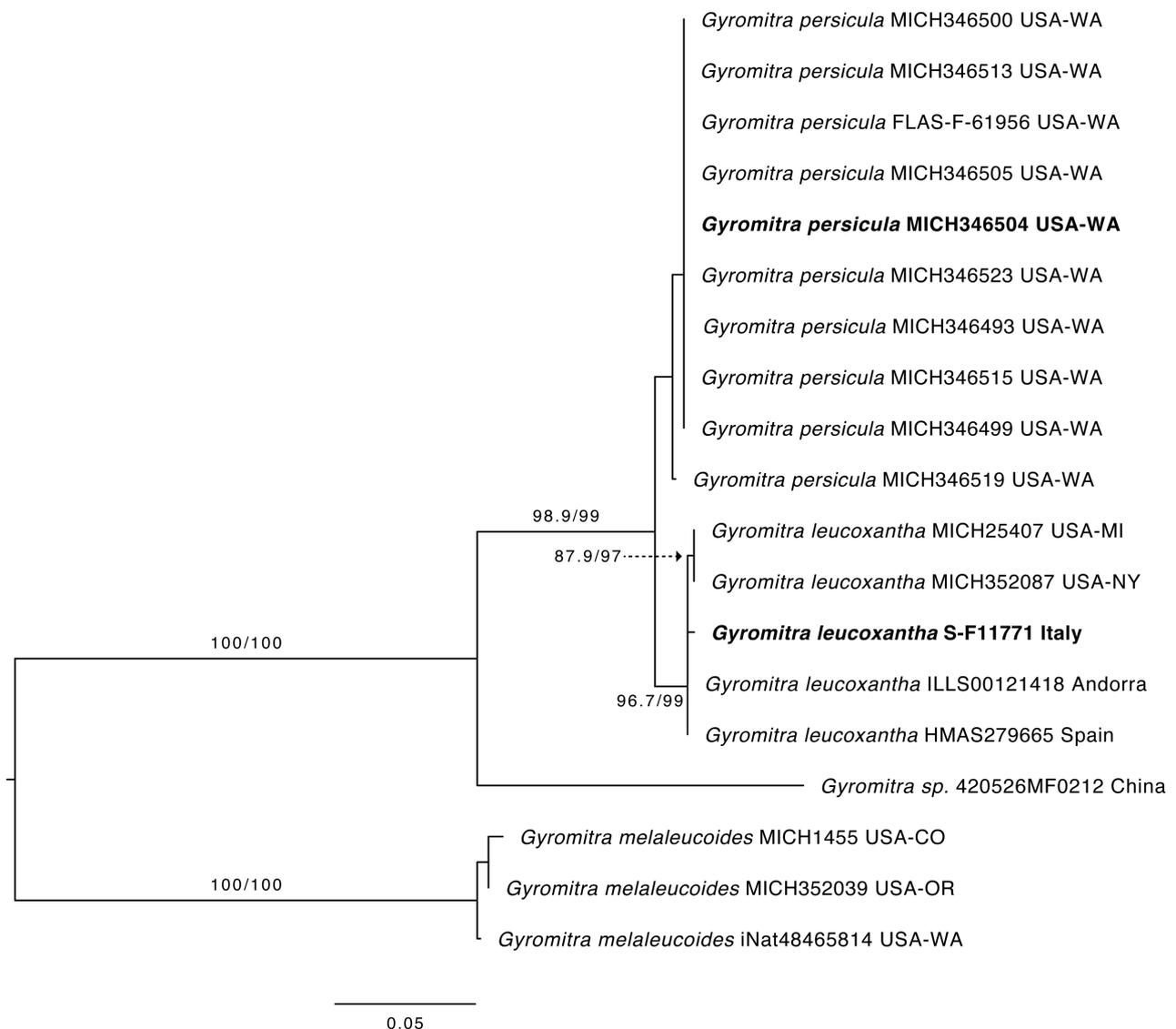


FIGURE 1. Phylogram of *Gyromitra persicula* and related taxa obtained from IQ-TREE maximum likelihood phylogenetic analysis of a concatenated ITS and LSU dataset. The tree is rooted with *Gyromitra melaleuroides* (Seaver) Pfister (1980: 615). The numbers above the branches are SH-aLRT and UFBoot support values. Only SH-aLRT values $\geq 80\%$ and UFBoot values $\geq 95\%$ are shown. Sequences from holotype specimens are in **bold**. The bar indicates the number of nucleotide substitutions per site.

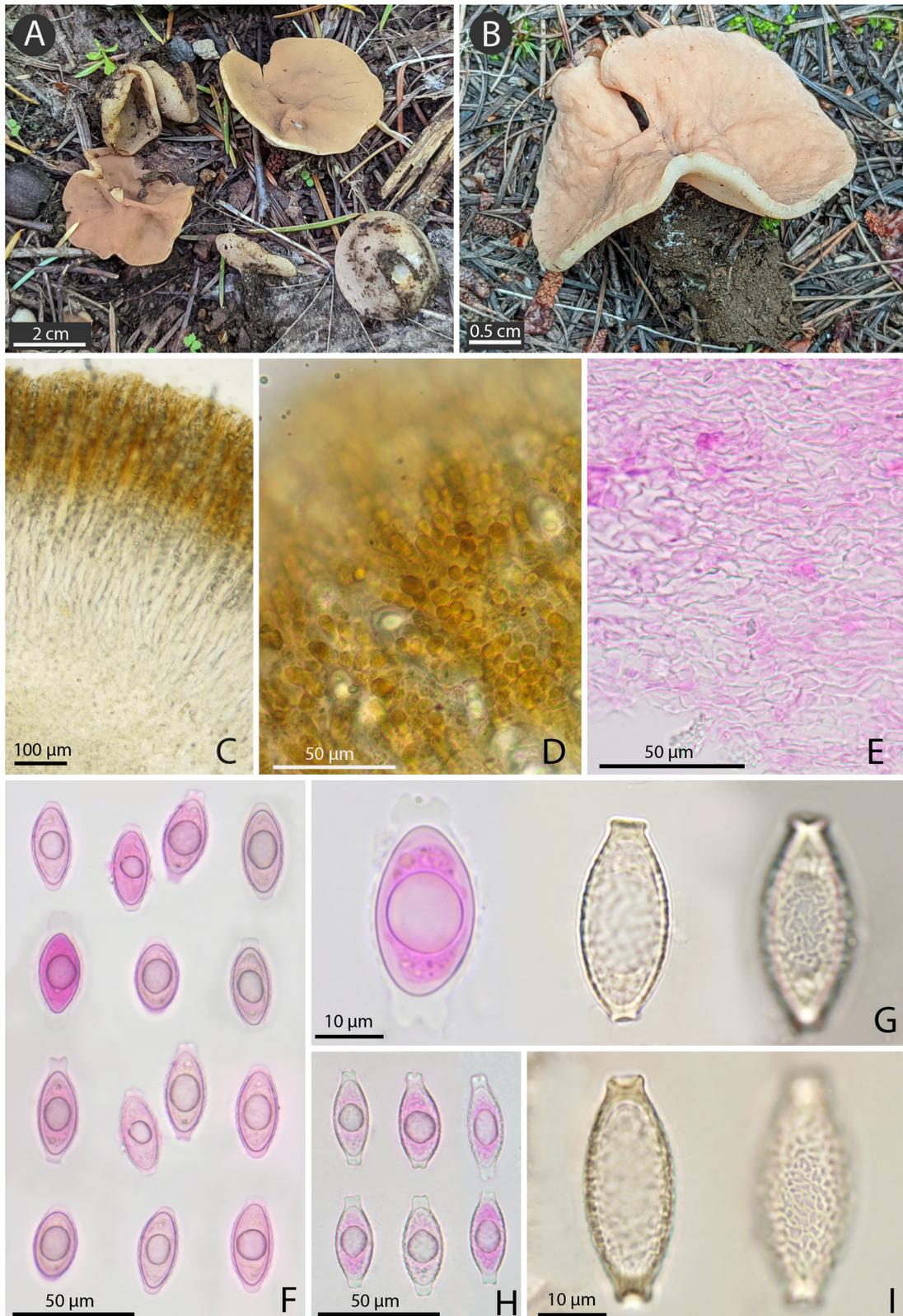


FIGURE 2. Ascomata and microscopic features of *Gyromitra persicula* (A–G) and ascospores of *Gyromitra leucoxantha* (H–I). A. Ascomata of the holotype collection (MICH346504). B. Peach-colored ascoma (MICH346523). C. Hymenium and excipulum (MICH346493). D. Asci and paraphyses with brown contents in KOH (MICH346493). E. Medullary excipulum of *textura epidermoidea* (MICH346493). F. Ascospores at various stages of maturity; note the blunt apiculi with a concave depression, large central guttule, and minute ornamentation (MICH346519). G. Close up of ascospores showing the pronounced “fishtail” apiculi and fine reticulation (MICH346519). H–I. Ascospores of *Gyromitra leucoxantha* (MICH25407); similar to those of *G. persicula* but with a more pronounced reticulation. Mounting medium: 5% KOH (C–D), 5% KOH stained with phloxine B (E–F, leftmost ascospore in G, H), Melzer’s reagent (two rightmost ascospores in G, I). Scale bars: 2 cm (A), 0.5 cm (B), 100 µm (C), 50 µm (D–F, H), 10 µm (G, I).

TAXONOMY

Gyromitra persicula Dirks & Beug, *sp. nov.* (Fig. 2)
MycoBank MB852786

Holotype:—USA. Washington: Klickitat Co., Mount Adams Horse Camp, 46.0515 -121.5357, growing from the ground, 30 May 2022, *leg.* Michael W. Beug, ACD0548, iNaturalist #119742009 (HOLOTYPE MICH346504; ISOTYPE ILLS00122612). Sequences: PP051296 (ITS), PP051288 (LSU).

Diagnosis:—*Gyromitra persicula* and *G. leucoxantha* are readily distinguished from other Discinaceae species by the scooped out “fishtail” apiculi of the mature ascospores. These two closely related taxa are separable by their size and coloration: *G. persicula* is small (up to 4.5 μm wide) and has a pinkish buff color, whereas *G. leucoxantha* is larger (up to 10 μm wide) and has an egg yolk yellow to yellow ochre color.

Description:—Ascomata consisting of a discoid hymenophore and pseudostipe, width 1.5–4.5 cm; hymenium buff to peach, smooth, becoming wrinkled; sterile underside off-white, drying tan, minutely pubescent; pseudostipe if present 1 \times 1 cm. Medullary excipulum a *textura epidermoidea*. Paraphyses cylindric, apices subclavate, apical width 6.3–8.5 μm ($W_m = 7.4 \mu\text{m}$), thin-walled, septate, unbranched, contents golden-brown in KOH. Asci 380–450 $\mu\text{m} \times$ 22–25 μm , cylindric, thin-walled, hyaline, eight-spored. Ascospores 36–44 $\mu\text{m} \times$ 16–19 ($L_m = 40 \mu\text{m}$, $W_m = 17 \mu\text{m}$, $Q = 2.1\text{--}2.5$, $Q_m = 2.3$), ellipsoid, with a large central guttule and a varying number of smaller polar guttules, hyaline in KOH, inamyloid. Perispore present, expanded up to 2 μm in KOH, less noticeable in water, appearing minutely roughened due to a fine reticulation when mature (best observed in water or Melzer’s reagent), forming blunt apiculi with concave depressions enlarged in KOH, 3.2–4.9 $\mu\text{m} \times$ 6.7–8.9 ($L_m = 4.0 \mu\text{m}$, $W_m = 7.8 \mu\text{m}$).

Etymology:—From the Latin *persica* meaning peach along with the diminutive suffix *-ula*, referring to the peach coloration and small size, hence the common name “mini peach pig’s ears”.

Habitat and distribution:—Growing from soil in association with conifers in Washington, 250–1300 m elevation.

Additional specimens examined:—USA. Washington: King Co., Middle Fork Snoqualmie Natural Area, 274 m, 47.4922 -121.6411, in a mixed conifer forest, 3 June 2011, *leg.* Michael W. Beug (MWB), ACD0515 (MICH346493); Klickitat Co., Gotchen Creek Trail, 46.0880 -121.4929, growing from the ground in a mixed conifer forest, 9 June 2011, *leg.* MWB, ACD0526 (MICH346499) (immature); *ibid.*, 46.1069 -121.5075, in a mixed *P. menziesii* forest, 21 June 2011, *leg.* MWB, ACD0521 (MICH346500) (immature); Klickitat Co., Mount Adams Horse Camp, 46.0499 -121.5327, 8 May 2022, *leg.* MWB, ACD0508, Mushroom Observer #491382 (MICH346505) (immature); Klickitat Co., Trout Lake, 46.0093 -121.5553, growing from the ground in a mixed conifer forest, 1 May 2005, *leg.* MWB, ACD0524 (MICH346515); *ibid.*, 46.0212 -121.5295, on soil, 30 May 2022, *leg.* MWB, ACD0552, iNaturalist #119741787 (MICH346513) (immature); Pierce Co., Mt. Rainier National Park, 46.7586 -121.5569, growing from the ground in an old growth forest, 17 May 2014, *leg.* MWB, ACD0525 (MICH346519); Skamania Co., Trout Lake, 45.9688 -121.6573, growing from the ground, 13 June 2022, *leg.* MWB, ACD0547, iNaturalist #121904369 (MICH346523).

Notes:—While separable by macromorphology, other distinguishing features of *Gyromitra persicula* and *G. leucoxantha* may be the degree of ascospore reticulation and prominence of the scooped-out apiculi in water. Whereas *G. persicula* shows a notable but somewhat faint ascospore reticulation, McKnight (1969) described the ascospores of *G. leucoxantha* as “very distinctly and coarsely reticulate”. We also observed a somewhat more pronounced reticulation in the one specimen of *G. leucoxantha* with mature ascospores that we studied (Fig. 2). The ascospores of both taxa differ in appearance according to the mounting medium (McKnight 1968). In water, the scooped-out apiculi of *G. persicula* could be overlooked but are prominent in KOH due to the expansion of the perispore, resulting in an overall increase in size of 10–15 %. In contrast, the scooped-out apiculi of *G. leucoxantha* are more obvious in water and are less likely to be overlooked. Discinaceae species mature over an extended period and are often inadvertently collected in an immature state when their ascospores have not yet attained their full size, ornamentation, and apiculi. More observations of ascospores from fresh deposits (*i.e.*, living ascospores) are required to delineate these finer microscopic details and to establish a uniform comparison across the discoid *Gyromitra* (Karakehian *et al.* 2021).

DISCUSSION

We describe *Gyromitra persicula* as new to science based on phylogenetic and morphological analyses. A sufficient barcode gap exists between *G. persicula* and *G. leucoxantha* for reliable identification with ITS; however, the ITS divergence exhibited by *G. persicula* MICH346519 suggests further collections are needed to document the full genetic diversity of *G. persicula*. More collections are also required to better understand the geographic range of the mini peach pig's ears mushroom, which so far is limited to temperate rainforests in Washington state. There is clearly undocumented diversity within the *G. leucoxantha* group, as indicated by the closely related and potentially novel species *Gyromitra* sp. 420526MF0212 sequenced by Wang *et al.* (2023). *Gyromitra persicula* should not be consumed because, like its sister species *G. leucoxantha*, it produces gyromitrin (Dirks *et al.* 2023, unpublished data).

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