

Rugosporella, a new genus to accommodate the North American species *Peziza atrovinosa* (Pezizaceae) and its predicted ectomycorrhizal status

Donald H. PFISTER
Benjamin LEMMOND
Rosanne HEALY
Katherine L. LOBUGLIO
Gregory BONITO
Matthew E. SMITH

Ascomycete.org, 16 (3) : 0–0

Reçu le 00/00/2024

Mise en ligne le 00/06/2024

 10.25664/ART-0000

Abstract: The genus *Rugosporella* is proposed to accommodate *Peziza atrovinosa* in the modern classification scheme of the *Pezizaceae*. We used DNA sequences of ITS, LSU and RPB2 to resolve the phylogenetic placement of this taxon. The species occupies a distinct position within a large and diverse clade of hypogeous and epigeous taxa, all of which are either known to be or presumed to be ectomycorrhizal. Although no DNA sequences of this species have been identified from ectomycorrhizal roots, we provide isotopic data supporting its ectomycorrhizal lifestyle. *Peziza atrovinosa* is distinctive in its moderately large vinaceous brown apothecia with thick flesh and the relatively small ascospores that are ornamented with a high reticulum and that become yellow-brown at maturity. This species is found across eastern North America.

Keywords: Ascomycota, ascospore pigmentation, isotopic analysis, molecular phylogeny, *Pezizales*.



Introduction

The operculate discomycete *Peziza atrovinosa* Cooke is commonly collected on soil in mixed woods in late summer and autumn in eastern North America. This species is moderately large (3–5 cm) and is distinctive in its relatively small ascospores, which have a high, coarse reticulum that at maturity becomes yellow-brown. This species has most often been confused with *Legaliana badia* (Pers.) Van Vooren (formerly *Peziza badia* Pers.). In *L. badia* the incomplete reticulum is finer and is not pigmented (VAN VOOREN, 2020).

Molecular phylogenetic studies have revealed relationships among *Pezizaceae*, both hypogeous and epigeous, that have led to major revision of the generic circumscriptions (HANSEN *et al.*, 2001; HANSEN *et al.*, 2005; HANSEN & PFISTER, 2006). The large, heterogeneous genus *Peziza* has been broken into several genera, some recognized by earlier workers. Other genera have been described anew as clades have been identified through sequence analyses (see PFISTER, 2015; VAN VOOREN, 2020). There remain described species named in the genus *Peziza* that have not been yet placed in modern genera. One such species is *Peziza atrovinosa*.

Peziza atrovinosa was described from material provided to M. C. Cooke by William R. Gerard, a druggist in Poughkeepsie, New York. From our present study we know that the species is broadly distributed across eastern North America. It is frequently collected on soil on trail verges and banks. Its moderate size makes it a target for general collectors. Although distinctive in size, color and notable spore characteristics, the species has not been without controversy. In the European literature it was suggested that *P. atrovinosa* and *P. ostracoderma* Korf were synonyms, the older name being *P. atrovinosa*. This confusion was discussed and clarified by HENNEBERT & KORF (1975). These two species are quite different in both morphology and in habitat. Although both have ascospores with reticulations, those in *Peziza atrovinosa* are prominent and become pigmented at maturity. The finely reticulate ascospores of *P. ostracoderma* remain hyaline. *Peziza atrovinosa* is collected on soil in forests with no anamorph recorded whereas *P. ostracoderma* is generally found as both teleomorph and chromelosporium-like anamorph in greenhouses on steam-sterilized soil or where the substrate has been exposed to heat and is often noted in its anamorph form (HENNEBERT & KORF, 1975). These authors state that *P. atrovinosa* has not been reliably reported in Europe. Our findings agree with this statement.

Still other questions remain, including potential synonyms of this species. At least two species described from eastern North America have been suggested as synonyms of *P. atrovinosa* (PFISTER, 1978a,

1978b). *Peziza retiderma* Cooke has been variously characterized but, aside from the type collection, it has not been reliably recollected. It has been reported from Australia (RIFAI, 1968) and Madagascar (LE GAL, 1953), but these reports have been revised (MORAVEC & SPOONER, 1988). That revisionary work resulted in the Austral-Asian material being named as a different species, *Peziza rifaii* J. Moravec & Spooner. Collections from Madagascar have been re-examined in our study. *Peziza chlamydospora* Ellis has also been considered a synonym of *P. atrovinosa* (SEEVER, 1928; PFISTER, 1978a). It too is known reliably only from type and authentic collections from the 1880s. In both cases the type material of these species was collected in eastern North America in regions and habitats where *P. atrovinosa* has been documented.

In this contribution we place *P. atrovinosa* in a new genus and resolve some of the questions surrounding synonymy.

Materials and methods

Morphology studies

The isotype of *Peziza atrovinosa* was borrowed from the Cornell University Plant Pathology Fungarium (CUP). Collections studied morphologically are cited with depositories in the specimens examined section. These specimens were examined microscopically using free hand sections mounted in tap water, 3% KOH, and in Melzer's reagent. Examination was carried out on an Olympus BX-40, or a Zeiss Axio Imager A2 compound microscope. Images were captured by an Axiocam 305 camera using Zen Pro v3.1 software (Carl Zeiss, Oberkochen, Germany). To more fully record the intricate spore ornamentations, multiple images were stacked using Helicon Focus v8.0.4 Pro (Helicon Soft Ltd 2000, Kharkiv, Ukraine).

Molecular techniques and phylogenetic analyses

From fresh and fungarium collections, small tissue samples were selected for DNA extraction and subsequent DNA sequencing from the *Peziza atrovinosa* specimens (Table 1). These include seven specimens of *P. atrovinosa* from the Farlow Herbarium, Harvard University Herbaria (FH), along with seven specimens from the University of Florida Fungarium (FLAS-F). DNA extraction of the ascotal samples from fungarium material was performed using either the Qiagen DNeasy Plant Mini Kit (Qiagen, Germantown, Maryland) or a modified CTAB-based chloroform extraction method (GARDES & BRUNS, 1993). Rapid extractions were performed on freshly collected material that was stored in alkaline extraction buffer following the

methods of VANDEPOL *et al.* (2020). Tissues were ground in Microependorf 1.5 ml tubes using Kimble's Pellet Pestle (catalogue no.749521-1500) and sterile sand. Standard PCR amplification and Sanger sequencing was performed on most samples.

The genomes of three of the FLAS fungarium specimens were also sequenced on the Illumina Novaseq 6000 platform (Illumina, San Diego, CA, USA) at the Genomics Core at Michigan State University (East Lansing, MI, USA), as described below. For all other specimens, DNA dilutions of either 1:10 or 1:100 of DNA template:water were used for polymerase chain reaction (PCR) amplification. For Sanger sequencing of DNA extracts of both fungarium and fresh material, the following gene regions were amplified: (i) the nuclear ribosomal internal transcribed spacer region (ITS1-5.8s-ITS2, abbreviated as ITS) using ITS1F (GARDES & BRUNS, 1993) or ITS5 as the 5' primer and ITS4 (WHITE *et al.*, 1990) as the 3' primer; (ii) the nuclear ribosomal 28S Large Subunit (LSU) using primers NL1 and NL4 (O'DONNELL, 1993) or LROR and LR5 (HOPPLE & VILGALYS, 1994); (iii) the RNA polymerase II gene (RPB2) using primers RPB2-200036F (PFISTER *et al.*, 2008) and RPB2-7CR (LIU *et al.*, 1999) or newly designed RPB2 primers specific for *Peziza atrovinosa* (5', Patro_RPB2_1528F: CAGCTCCACAACACCCATTG and 3', Patro_RPB2_2467R: TGAG-GAATCCAT GGAACGTG). The primer design used an RPB2 target sequence was derived from a *P. atrovinosa* genome using the NCBI (National Center for Biotechnology Information) primer design program Primer BLAST (YE *et al.*, 2012).

PCR amplifications were carried out in a Bio-Rad C1000 Thermal Cycler (Applied Biosystems, Hercules, CA, USA). For PCR, 5 µl of 1/10 dilutions of the fungarium DNA extracts were used as templates in a reaction volume of 20 µl, and 1 µl of the rapid extraction of fresh material was added to a reaction volume of 24 µl. The Bio-Rad Iproof High-Fidelity PCR Master Mix (Applied Biosystems, Hercules, CA, USA, catalogue no. 1725310) was used for PCR amplification of the ITS and LSU regions with thermal cycling parameters as described in PFISTER *et al.* (2020). High-fidelity platinum Taq by Invitrogen (Waltham, MA, USA catalogue no.11304011) was used for PCR amplification of the RPB2 region. PCR cycling parameters were described previously in PFISTER *et al.* (2020).

The PCR products of all genes were subsequently prepared for PCR purification and Sanger sequencing using GeneWiz Inc. (Cambridge, MA, USA) sequencing facilities or Eurofins Genomics (Louisville, KY, USA). The forward and reverse sequences from each PCR product were edited with Geneious 10.2.3 or Geneious Prime 2024.0.3 (<https://www.geneious.com>). The newly generated DNA sequences were submitted to the NCBI GenBank database (<https://www.ncbi.nlm.nih.gov/>; ITS accession numbers in Figure 2, RPB2 and LSU accession numbers in Table 1).

Additional ITS, LSU, and RPB2 sequences were extracted from three *de novo* genome assemblies of *P. atrovinosa* that were included in an ongoing genome sequencing project of diverse *Pezizomycetes* (LEMMOND *et al.*, unpublished). Briefly, genomic DNA extracted from three *P. atrovinosa* specimens (FLAS-F-68805, FLAS-F-69722 and FLAS-F64202) was sequenced with 150bp paired-end sequencing on the Illumina Novaseq 6000 platform at the Genomics Core at Michigan State University. *De novo* assemblies of resulting reads were conducted with a custom bioinformatic pipeline modeled after the Automatic Assembly for the Fungi (AAFTF) pipeline (STAJICH & PALMER, 2012). Amino acid sequences of the RPB2 locus were identified in each assembly using the Universal Fungal Core Genome (UFCG) pipeline (KIM *et al.*, 2023) and the corresponding nucleotide sequences were extracted from genome assemblies using Exonerate v2.4.0 (SLATER & BIRNEY, 2005). ITS and LSU sequences were extracted from genome assemblies using the BARRNAP tool (SEEMAN, 2018).

The species delimitation of *P. atrovinosa* and its phylogenetic placement within the *Pezizaceae* was estimated by two separate analyses: a Maximum Likelihood (ML) analysis of aligned ITS DNA sequences and a ML analysis of a concatenated alignment of LSU and RPB2 DNA sequences. For the ITS dataset, we included repre-

sentative sequences from ascomata, mitotic spore mats, and ectomycorrhizal root tips from *Pezizaceae*, including sequences from collections for a core set of genera (*Cazia*, *Chromelosporiopsis*, *Chromelosporium*, *Legaliana*, *Mycoclelandia*, *Ruhlandiella*, *Tirmania*, and *Velenovskya*) and closely related sequences. To ensure that we included as many closely related taxa as possible we used BLAST to check several sequences from each of these genera, as well as our *P. atrovinosa* sequences, and also used the detailed ITS analyses of Healy *et al.* (2022). We made a special effort to include as many ITS DNA sequences from ectomycorrhizal root tip samples (ECM) as possible. Taxa are listed by their GenBank accession numbers in Figure 2. The ITS DNA sequences were first aligned with MAFFT, through the CIPRES Science Gateway (MILLER *et al.*, 2010) or with MUSCLE v5.1 (EDGAR, 2022) in Geneious Prime. Maximum Likelihood (ML) phylogenetic analysis, using the GTRGAMMA substitution model, default parameters, and 1000 bootstrap replicates, was performed using the online version of RAXML-HPC2 on XSEDE v8.2.12 (CIPRES, MILLER *et al.*, 2010; STAMATAKIS, 2014).

To assemble the LSU and RPB2 datasets, we downloaded all *Pezizaceae* sequences for which both the LSU and RPB2 regions were available, starting with the backbone dataset from HANSEN *et al.* (2005). To recover any additional relevant *Pezizaceae* sequences and sequences closely related to *P. atrovinosa*, we used BLAST with our LSU and RPB2 sequences against the NCBI GenBank database. The ITS and RPB2-LSU alignments are available through Open Science Framework (https://osf.io/arp89/?view_only=a1b027f6704345a8acffced6c4815f65).

Isotopic analysis

Isotopic and compositional properties of two *P. atrovinosa* samples (FLAS-F-64202 and FLAS-F-68216) were measured at the Light Stable Isotope Mass Spectrometry Lab at the University of Florida (Gainesville, FL, USA). For each sample, %C, %N, $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ were measured. All carbon isotopic results were expressed in standard delta notation relative to Vienna Pee Dee Belemnite (VPDB). All nitrogen isotopic results are expressed in standard delta notation relative to air.

An analysis of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of the *P. atrovinosa* samples was conducted to test the hypothesis of ECM trophism for this group. This analysis used existing isotopic data from 813 fungal specimens of known trophic modes from MAYOR *et al.* (2009) as a training dataset for a quadratic discriminant analysis (QDA) model (BIRKEBAK *et al.*, 2013). A QDA model was selected as an appropriate model given the unequal variances of the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values used in the training dataset. This QDA model was used to predict the trophic status (either ECM or saprobic) of the two *P. atrovinosa* samples. The QDA analysis was conducted in RStudio (2023.06.2+561) using R v4.3.1 (R CORE TEAM, 2021) and the *qda* function in the MASS package (VENABLES & RIPLEY, 2002). Data were visualized in R using the ggplot2 package (WICKHAM, 2016).

Results

Phylogenetic results

The LSU alignment included 69 taxa and 923 characters and the likelihood score for the best tree was -6756.606787. The RPB2 alignment included 74 taxa and 521 characters and the likelihood score for the best tree was -9678.481038. There were no supported incongruences between the two phylogenetic trees, so the two alignments were concatenated. The RPB2-LSU alignment had 74 taxa and 1444 characters and the likelihood score for the best tree was -16675.34586. The RPB2 locus was represented for each taxon, but the LSU was missing for the following five accessions: *Peziza ammophila*, one of the two *P. lobulata* accessions, *P. pseudoammophila*, *P. subviolacea*, and one of the two *Sarcosphaera coronaria* accessions. *Ascobolus crenulatus* (*Ascobolaceae*) served as the outgroup for the *Pezizaceae*. The best tree is shown in Fig. 1. *Peziza atrovinosa*

Table 1 – *Peziza atrovinosa* herbarium specimens and GenBank DNA sequences included in the molecular phylogenetic study

Current Name	Herbarium	Locality	LSU	RPB2
<i>Amylascus cineraceus</i>	FLAS-F-64534	Chile	OQ270081	OQ230425
<i>Amylascus hallingii</i>	NY1491200	Australia	NG_242434	OQ230422
<i>Ascobolus crenulatus</i>	AFTOL-ID_181	unknown	AY544678	DQ470893
<i>Daleomyces exogelatinosus</i>	C:KH-00-029	Denmark	AY500545	AY500501
<i>Daleomyces ligni</i>	CBS 146637, CPC-39110	France	MW883832	MW890073
<i>Daleomyces petersii</i>	TAAM:187584	UK: England	MN737816	MN816681
<i>Daleomyces phillipsii</i>	TAAM:199363	Estonia	MN737802	MN816674
<i>Elaiopezia boudieri</i>	MPU:JCD 954-75	France	MT273595	MT274700
<i>Elaiopezia obtusapiculata</i>	C:TL-6474	Denmark	AY500550	AY500490
<i>Elaiopezia polaripapulata</i>	C:KH-96-11	Denmark	AY500551	AY500515
<i>Geoscypha tenacella</i> (P. subviolacea)	TAAM:165082	Estonia	–	MN816685
<i>Geoscypha ampelina</i>	C:KH-00-011	Denmark	AF335127	AY500492
<i>Geoscypha violacea</i> (P. lobulata)	FH:KH-03-157	USA	AY500548	AY500495
<i>Geoscypha violacea</i> (P. lobulata)	GM 010520142	Italy	–	MN816678
<i>Hansenopezia decora</i>	CNF 2/10621	Croatia	NG_241909	MK673767
<i>Hansenopezia retrocurvata</i>	C:KS-94-182	Denmark	AF335159	AY500516
<i>Hydnoplicata whitei</i>	C, OSC:Trappe 17049	Australia	AF335168	AY500491
<i>Hydnotryopsis</i> sp.	C, OSC:Trappe 17231	USA	AF335116	AY500472
<i>Iodophanus carneus</i>	C:JHP_00-027	Denmark	AY500534	AY500506
<i>Iodophanus hyperboreus</i>	C:Gr-83-06	Greenland	AY500535	AY500458
<i>Iodowynnea auriformis</i>	FH:18510_PAN	India	AF335118	AY500473
<i>Legaliana badia</i>	LY:NV 2019.09.14	France	MT273593	MT274699
<i>Legaliana limnaea</i>	C:HFG_94-2	Denmark	AF335147	AY500518
<i>Malvipezia emileia</i>	GM 03091006	Italy	KU898062	KJ728720
<i>Malvipezia howsei</i>	C:KH-97-98	Denmark	AF335146	AY500493
<i>Pachyphlodes annagardnerae</i>	MIN:925696	USA: IA	KJ775836	OQ230428
<i>Pachyphlodes thysellii</i>	FLAS-F-66243	USA: MN	JN121369	OQ230436
<i>Paragalactinia michelii</i>	C:TL-5692	Denmark	AY500549	AY500494
<i>Paragalactinia succosa</i>	C:KH-97-139	Denmark	AF335167	AY500517
<i>Paragalactinia succosa</i>	C:KH-98-07	Denmark	AF335166	AY500487
<i>Peziza ammophila</i>	LIP:791126	France	–	KX271742
<i>Peziza arvernensis</i>	C:KH-98-12	Denmark	AF335131	AY500497
<i>Peziza azureoides</i>	MPU:JCD 856-75	France	MT273592	MT274698
<i>Peziza badiofusca</i>	C:KH-98-113	Sweden	AF335132	AY500475
<i>Peziza depressa</i>	C:KH-98-28	Denmark	AF335135	AY500474
<i>Peziza echinispora</i>	TURA:JukkaVauras_9110F	Finland	AF335138	AY500496
<i>Peziza ellipsospora</i>	C, OSC:Trappe13017	USA	AF335139	AY500482
<i>Peziza nordica</i>	FH:G01_15	Norway	KU898046	KU898054
<i>Peziza nordica</i>	FH:G07_14	Norway	KU898047	KU898055
<i>Peziza oliviae</i>	OSC:JLF2140	USA: OR	KU898051	KU898059
<i>Peziza oliviae</i>	OSC:JLF2538	USA: OR	KU898050	KU898058
<i>Peziza pseudoammophila</i>	LIP:791104	France	–	KX271743
<i>Peziza saniosa</i>	C:KH-97-137	Denmark	AF335160	AY500476
<i>Peziza varia</i>	C:KH-97-107	Denmark	AF335150	AY500498
<i>Peziza varia</i>	C:KH-97-54	Denmark	AF335134	AY500519
<i>Peziza varia</i>	C:KH-99-04	USA	AF335151	AY500499
<i>Peziza vesiculosa</i>	OSC:100126, AFTOL-ID_507	unknown	DQ470948	DQ470898
<i>Phaeopezia calongei</i>	LIP:0002247	Spain	OL984070	OL984066
<i>Phaeopezia calongei</i>	MA-F-33516	Spain	OL984069	OL984065
<i>Phylloscypha phyllogena</i>	C:KH-99-03	USA	AF335155	AY500480
<i>Phylloscypha kallioi</i>	TUR s.n. (type)	Finland	AF335156	AY500481

Table 1 – (continued)

Current Name	Herbarium	Locality	LSU	RPB2
<i>Plicaria carbonaria</i>	FH:DHP 9215	USA	AY500553	AY500479
<i>Plicaria leiocarpa</i>	AFTOL-ID_1345, CBS-144.92	unknown	DQ842029	DQ842038
<i>Plicaria trachycarpa</i>	C:KH-97-93	Denmark	AY500554	AY500478
<i>Plicariella flavovirens</i>	FLAS-F-61549	USA: MN	JN121375	OQ230429
<i>Purpureodiscus bananincola</i>	FH:V-Demoulin-5529	New Guinea	AF335133	AY500483
<i>Purpureodiscus subisabellinus</i>	C:RK 96-54	Norway	AF335163	AY500484
<i>Purpureodiscus subisabellinus</i>	Winterhoff 8844	Germany	AF335164	AY500485
<i>Rugosporella atrovinosa</i>	FH 00465069	USA: MA	PP663026	PP681916
<i>Rugosporella atrovinosa</i>	FH 00822838	USA: ME	PP663025	PP681915, PP823944
<i>Rugosporella atrovinosa</i>	FLAS-F-64202	USA: OH	MT350464	PP823944
<i>Rugosporella atrovinosa</i>	FLAS-F-68805	Canada: QC	OR134533	PP823942
<i>Rugosporella atrovinosa</i>	FLAS-F-69722	USA: KY	PP808677	PP823943
<i>Ruhlandiella berolinensis</i>	C:Mycoflora_of_Macaronesia 1230	Spain: Canary Islands	AF335175	AY500477
<i>Ruhlandiella patagonica</i>	FLAS-F-62145	Argentina	MG947618	MH156156
<i>Ruhlandiella patagonica</i>	FLAS-F-62148	Chile	MG947619	MH156157
<i>Sarcopeziza sicula</i>	MCVE 25877	Italy	MH704523	MH709116
<i>Sarcosphaera coronaria</i>	C:KS-94-19	Denmark	–	AY500523
<i>Sarcosphaera coronaria</i>	C:KS-94-24A	Denmark	AY500555	AY863001
<i>Terfezia bertae</i>	PA-2022a_AH_51463	Spain	ON009056	ON012516
<i>Terfezia claveryi</i>	FH, OSC:Trappe 3195	Kuwait	AY500558	AY500503
<i>Tirmania nivea</i>	C, OSC:Trappe 23190	Israel	AF335177	AY500525
<i>Tirmania pinoyi</i>	C, OSC:Trappe 13587	Saudi Arabia	AF335178	AY500502
<i>Velenovskya vacini</i>	TUR-A 209626	Italy	ON775566	ON758342

was placed with strong support within a clade of *Pezizaceae* that included *Velenovskya*, *Ruhlandiella*, *Hydnoplicata*, *Tirmania*, *Legaliana*, *Terfezia*, *Peziza ellipsospora* and *Galactinia*. *Peziza atrovinosa* was not supported as a member of any other genus in the RPB2-LSU analysis.

Phylogenetic analyses of the ITS rDNA sequence dataset using ML (Fig. 2) indicate that the *P. atrovinosa* specimens form a highly supported monophyletic clade that is unique among genera of the *Pezizaceae*. All of the ITS rDNA sequences from the *P. atrovinosa* specimens in this study were 98% to 100% similar to each other. Inclusion of ITS rDNA sequences from ECM roots in the phylogeny demonstrate that the mycorrhizal habit is common among many genera in the *Pezizaceae*, including species of *Cazia*, *Chromelosporiopsis*, *Legaliana*, *Mycoclelandia*, *Ruhlandiella*, *Terfezia*, *Tirmania*, and many taxa that are considered currently as “*Peziza*” but require further taxonomic reconsideration.

Isotopic analysis results

Isotopic analysis of two *P. atrovinosa* specimens (FLAS-F-64202 and FLAS-F-68216) predicted an ECM trophic status for the *P. atrovinosa* lineage (Fig. 3). Both samples of *P. atrovinosa* were predicted by the QDA model as belonging to the ECM group (Fig. 3). FLAS-F-64202 ($\delta^{15}\text{N} = 3.56$, $\delta^{13}\text{C} = -26.11$) was predicted as ECM with 0.95 posterior probability, and FLAS-F-68216 ($\delta^{15}\text{N} = 6.42$, $\delta^{13}\text{C} = -25.94$) was predicted as ECM with 0.99 posterior probability.

Taxonomy

Rugosporella Pfister, Healy & LoBuglio, *gen. nov.* – MB 854056

Description: Apothecia shallow cupulate, often in groups, hymenium ranging in color from tawny buff to dark brown to nearly black on drying, outer surface concolorous or lighter than the hymenium, scurfy. Flesh thick, composed of *textura intricata* with intermixed

globose cells. Outer excipulum with low pustules composed of parallel cells oriented perpendicularly to the outer surface. Ascospores broadly ellipsoid, under 15 μm long, with two guttules, surface ornamented with a regular or irregular reticulum the veins of which become brown at maturity. Asci blue in iodine at the apex without a ring and blue in the upper half. Paraphyses slightly swollen at the tip, containing dark amorphous material.

Etymology: From Latin *rugo* – wrinkled or folded; *spora* – spore; *-ella*, implying the diminutive. Also referring to the name used by Gerard, *Peziza rugospora*, when sending material to M. C. Cooke.

Type: *Peziza atrovinosa* Cooke.

Rugosporella atrovinosa (Cooke) Pfister, Healy & LoBuglio, *comb. nov.* – MB 854057 – Fig. 4, 5.

Basionym: *Peziza atrovinosa* W.R. Gerard ex Cooke, *Bull. Buffalo Acad. Sci.*, 2: 288 (1875).

Typification: Holotype: (K); Isotype: USA. Dutchess Co., Poughkeepsie, *leg.* W. R. Gerard, lectotype K (designated by RIFA, 1968), isoelectotype CUP-D-03703 (27-31).

Homotypic synonymies: *Plicaria atrovinosa* (Cooke) D.S. Hone, *Minn. Bot. Stud.*, 4 (2): 79. (1909); *Aleurina atrovinosa* (Cooke) Seaver, *North American cup-fungi (Operculates)*: 101 (1928); *Galactinia atrovinosa* (Cooke) Le Gal, *Bull. Soc. mycol. Fr.*, 78: 207. (1962).

Taxonomic synonymies: *Peziza retiderma* Cooke, *Mycographia*, 1 (4): 176 (1877); *Phaeopeziza retiderma* (Cooke) Sacc., *Syll. fung.*, 8: 472. (1889); *Aleurina retiderma* (Cooke) Sacc. & P. Syd., *Syll. fung.*, 16: 739 (1902); *Galactinia retiderma* (Cooke) Le Gal, *Discomycètes de*

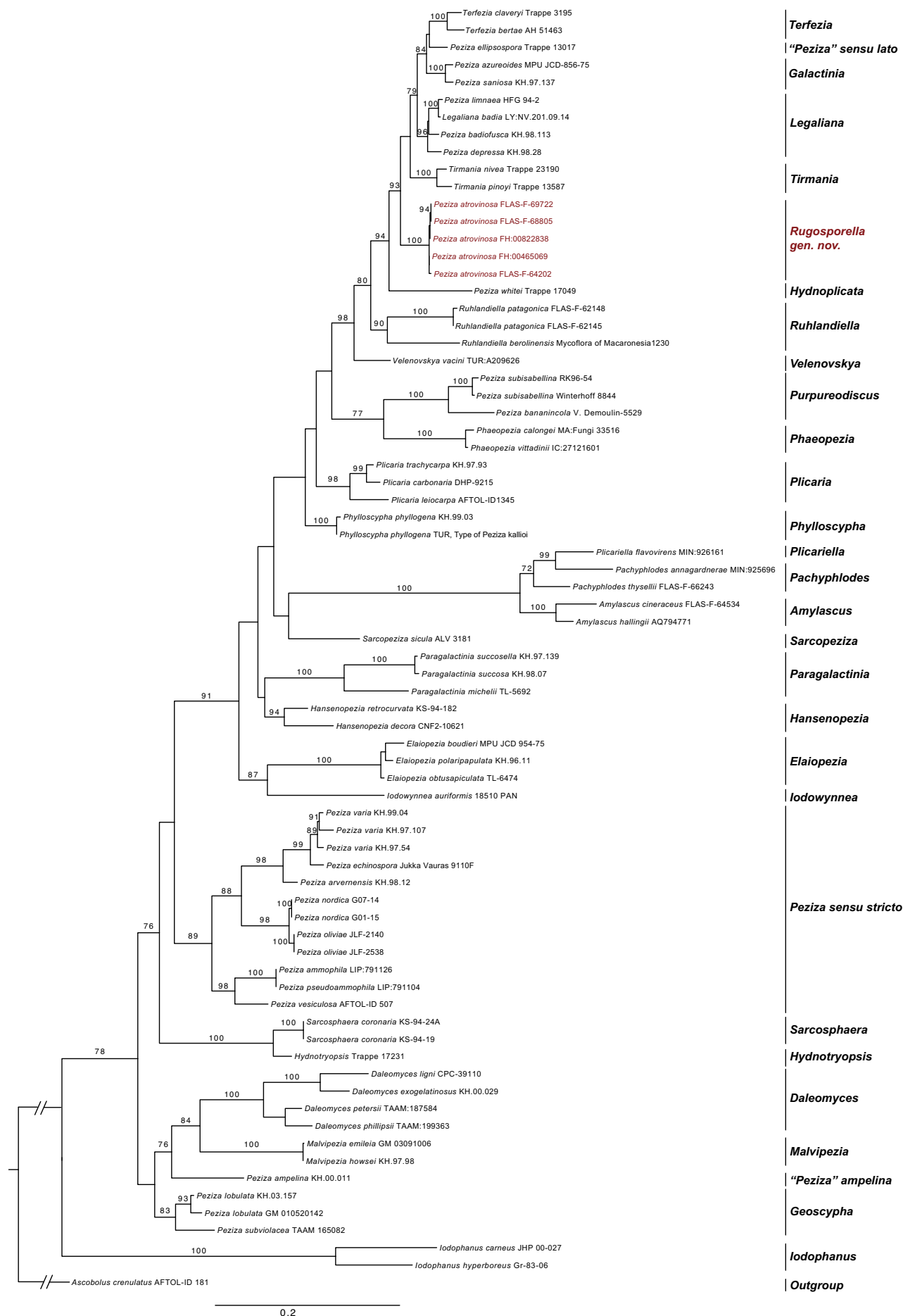


Figure 1 – Maximum Likelihood phylogeny based on concatenated LSU rDNA and RPB2 sequences from *Rugospora atrovinosa* (text in red) and other genera in the *Pezizaceae*. Fungarium or collector numbers follow species names. Bootstrap support ≥ 70 is shown on branches. *Ascobolus crenulatus* is the outgroup species.

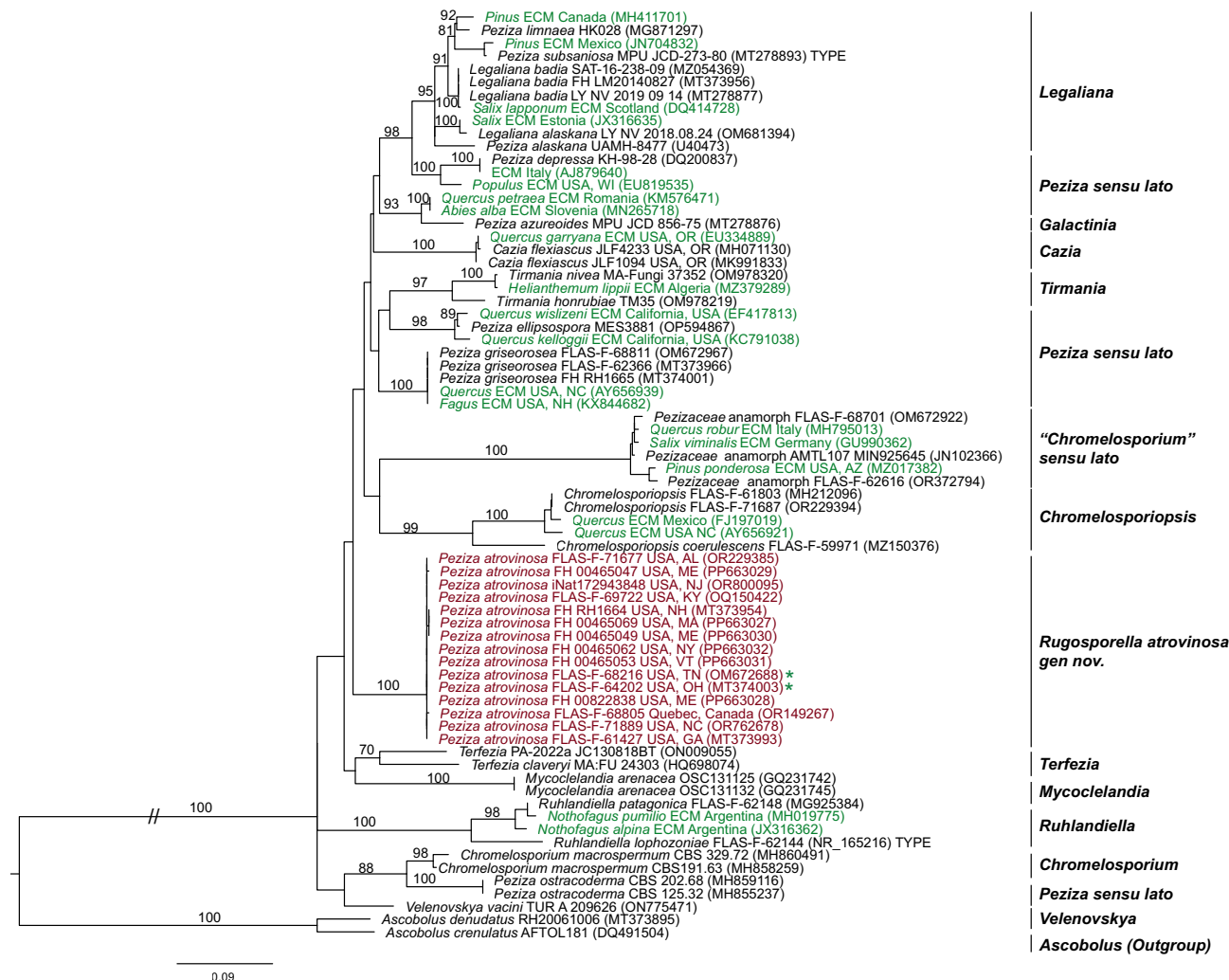


Figure 2 – Maximum Likelihood phylogeny based on ITS rDNA sequences from *Rugospora atrovinosa* fungarium specimens (text in red) and genera in the *Pezizaceae*. ITS DNA sequences from ectomycorrhizal tissue samples are indicated as “ECM” and are highlighted in green. The two *R. atrovinosa* specimens (FLAS-F-64202 and FLAS-F-68216) with predicted ECM trophic status based on isotopic analysis are indicated with a green asterisk. GenBank numbers associated with each taxon are in parentheses. Bootstrap support ≥ 70 is shown on branches. *Ascobotrya crenulatus* and *A. denudatus* are the outgroup species.

Madagascar: 57 (1953); *Peziza chlamydospora* Ellis & Everh., *Bull. Torrey Bot. Club*, 10: 98 (1883).

Macroscopic features: **Apothecia** gregarious to cespitose, medium sized 2.5–5 cm, sessile or with a very short stalk. **Disc** concave, smooth, pale brown or smoky, when dry, black to blackish brown, sometimes vinaceous. **Receptacle** cupulate to saucer-shaped, margin entire, sometimes contorted by mutual pressure, outer surface generally black when dry.

Microscopic features: **Excipulum** composed of large subglobose to pyriform cells, up to 100 μm diam., regularly interspersed with septate hyphae 5–7 μm in diam., up to 25 μm long. **Ectal excipulum** up to 160 μm thick; **medullary excipulum** about 250 μm thick. Toward the outer surface the cells become angular or compressed and are oriented in a somewhat parallel fashion, cells of the outer-most layer elongate to an outer layer of short cells which sometimes produce a pustulate appearance. **Subhymenium** of angular cells. **Hymenium** approximately 200–230 μm thick. **Asci** cylindrical, arising from croziers, apex blued in iodine solutions, of the “i” type of HANSEN *et al.* (2001)/ WT type of VAN VOOREN (2020), 200–260 $\times$ 10–12 μm , 8-spored. **Ascospores** uniseriate, biguttulate, ellipsoidal, at first hyaline and smooth-walled, but soon covered with yellow to brown ridges and warts which anastomose to form an irregular reticulum, in some cases an apiculus develops at each end of the spore. The markings reach a height of 2 μm , (13) 13.7–14.8

(15.6) $\times$ (7) 7.8–8.8 (9.2) μm including markings. **Paraphyses** rather stout, septate, unbranched, yellowish, 3–4 μm diam below, apex enlarged to 7 μm .

Habitat and phenology: Occurring on soil in mixed forests, often on trail verges, from July through September, in the northern range, and earlier toward the south.

Trophic status: Isotopic analysis strongly suggests this species is ectomycorrhizal.

Distribution: Present in northeastern North America, from Canada south to Alabama, USA and at least as far west as Kentucky, USA.

Specimens studied, including those sequenced: CANADA. Quebec, Chelsea, 45.549745 N -75.840063 E +97 m. WGS84, growing on the ground, mixed forest (Beech, Oak, Maple, Eastern Hemlock), *leg.* Igor Khomenko (2021-998), 15.VII.2021 (FLAS-F-68805). USA. Alabama. Cullman Co., Cullman, County Rd 177, Crane Hill, 33.969733 N -87.124675 E +8 m, on soil, woods of *Quercus*, *Pinus*, *Fagus*, privet, *Vitis*, *leg.* Cassie Pugh, *det.* Rosanne Healy, 23.IV.2023 (FLAS-F-71677). Connecticut. New Haven Co., New Haven, *leg.* R. Thaxter (sn), *det.* D. H. Pfister, 1888-1889 (FH00465067); New Haven Co., New Haven, on ground, *leg.* R. Thaxter (accession no 929),

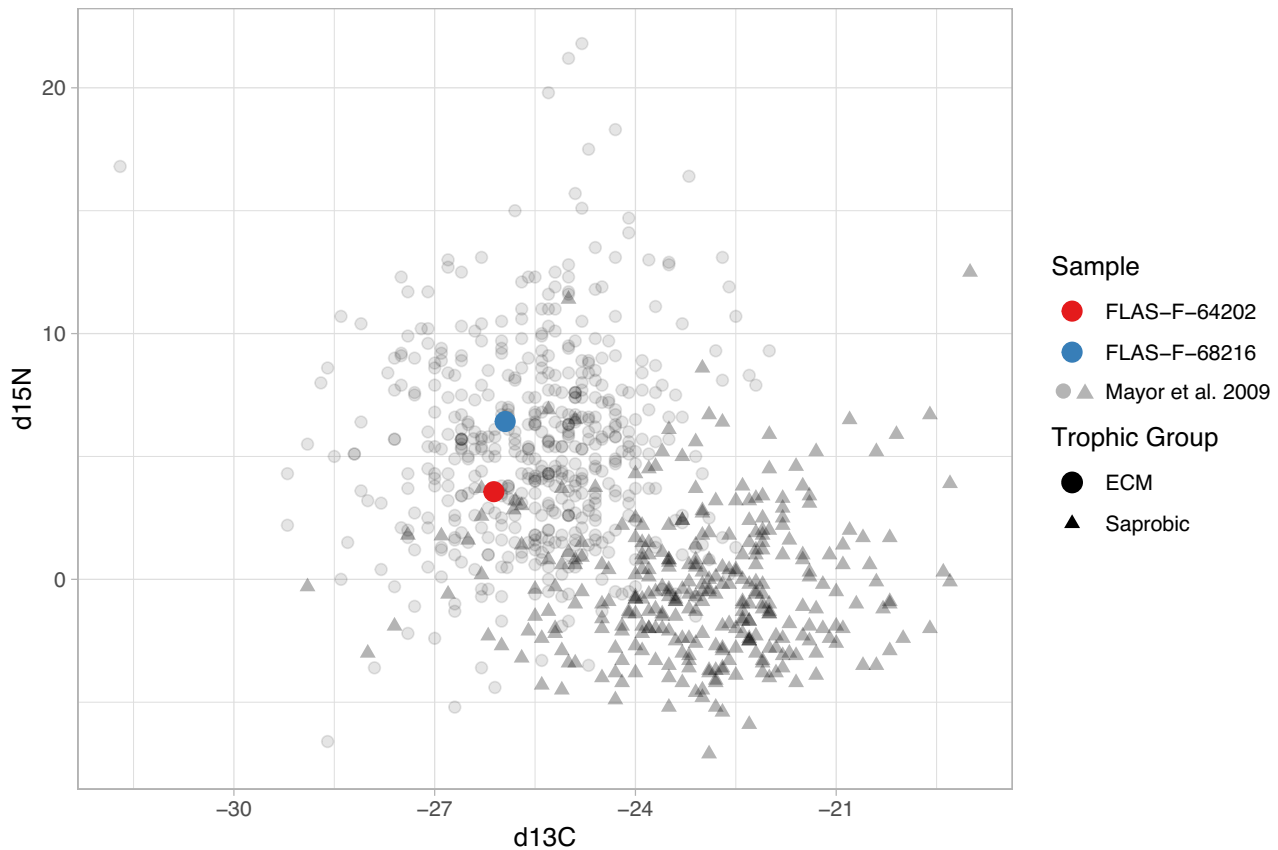


Figure 3 – Plot of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values. Grey background points represent data for known ECM (circles) and saprobic (triangles) taxa from MAYOR *et al.* (2009) used to train the quadratic discriminant analysis (QDA) model. Red and blue circles represent samples of *Rugosporrella atrovinosa* predicted as ECM by the QDA model.

det. D. H. Pfister, VIII. 1890 (FH00465068); Tolland Co., Storrs, near Feltton River, on soil, *leg.* D. H. Pfister (sn), 25.VII.1979 (FH00465060). Georgia. Pickens Co., Burnt Mountain Preserve, Burnt Mountain Champion Creek Trail, mixed woods, in moss along trail, *leg.* Rosanne Healy, 25.VIII.2020 (FLAS-F-67101); Greene Co., Eatonton, Rock Hawk/Oconee Archery Range in Oconee Wildlife Management Area, 33.339 N -83.162 E, *leg.* Curtis Peyer, 20.V.2020 (FLAS-F-68169). Kentucky. Laurel Co., Daniel Boone National Forest, Sheltowee Trace Trail, Van Hook Falls, 37.000188 N -84.286292 W, 1050 ft (320 m) a.s.l., on soil, *leg.* Judson Van Wyk, 21.IX.2022 (FLAS-F-69722), immature; Whitley Co., Daniel Boone National Forest, Dog Slaughter Falls trail, 36.903522 N -84.282789 E, 1270 ft (387 m) a.s.l., on soil, *leg.* Gregory Bonito, 20.IX.2022 (FLAS-F-69666). Maine. Aroostook Co., on soil in hardwoods, *leg.* M. E. Bigelow (Barr 1719), *det.* D. H. Pfister, 15.VII.1956 (FH00823575); *ibid.*, 4.VIII.1956 (FH00823575); Aroostook Co., near Madawaska Lake, on soil at edge of trail, *leg.* M. E. Bigelow (Barr 1730), *det.* D. H. Pfister, 18.VIII.1956 (FH00823547); Aroostook Co., near Madawaska Lake, on soil of trail, *leg.* H. E. Bigelow (4037/Barr 1718), *det.* D. H. Pfister, 15.VIII.1956 (FH00823629); Aroostook Co., near Madawaska Lake, on soil of trail, *leg.* H. E. Bigelow (Barr 1810), *det.* D. H. Pfister, 3.IX.1956 (FH00823505); Penobscot Co., Orono, County Rd., *leg.* D. H. Pfister, 13.VIII.1983 (FH00465047); Penobscot Co., East Millinocket, off Rt. 157, on soil, *leg.* H. E. & M. E. Bigelow (Barr 3693), *det.* D. H. Pfister, 26.VIII.1962, 11.VIII.1983 (FH00823633); Penobscot Co., soil, *leg.* H. E. & M. E. Bigelow (Barr 3746), *det.* D. H. Pfister, 21.X.1962 (FH00823657); Penobscot Co., Orono, on soil on trail, *leg.* D. H. Pfister (FH00465049); York Co., Kittery Point, *leg.* R. Thaxter, *det.* D. H. Pfister, 1911 (FH00465048); York Co., Kittery Point, *leg.* R. Thaxter, s.d. (FH00465054); Washington Co., Beddington, Lead Mountain, *leg.* M. E. Smith, *det.* D. H. Pfister, 5.VIII.2009 (FH00822838). Massachusetts. Berkshire Co., Windsor, Wahconash Falls, *leg.* H. E. and M. E. Bigelow (Barr 2590), *det.* D. H. Pfister, 22.VII.1959 (FH 00465078); *ibid.*, (FH00465079); Essex Co.,

Rockport, woods near Evans Field, close to train station, beech forest, on soil, *leg.* Michaela Schull, *det.* D. H. Pfister, 27.VI.2010 (FH00302477); Essex Co., Cape Ann, Dogtown, on soil in white pine and beech forest, *leg.* Lawrence Millman, *det.* D. H. Pfister, 2 IX 2004 (FH 00465052); Franklin Co., Conway State Forest, on soil, *leg.* H. E. Bigelow and M. E. Bigelow, *det.* D. H. Pfister, 2.IX.1965 (FH00465051), *ibid.* (Barr 3010), 23.VII.1961 (FH00465074); *ibid.* (Barr 2767) (FH00465075); Franklin Co., Conway State Forest, *leg.* H. E. & M. E. Bigelow (Barr 3012), *det.* D. H. Pfister, 23.VII.1961 (FH 00465083), *ibid.* (FH 00465070); Franklin Co., Conway State Forest, *leg.* H. E. Bigelow (Barr 2768), *det.* D. H. Pfister, 9.VIII.1960 (FH00465071); Franklin Co., Conway, *leg.* H. E. & M. E. Bigelow (Barr 3171), *det.* D. H. Pfister, 29.VII.1961 (FH00465082); Franklin Co., Warwich, Mt. Grace State Forest, *leg.* H. E. and M. E. Bigelow (Barr 2604), *det.* D. H. Pfister, 28.VII.1959 (FH00465080); Franklin Co., Mt. Toby, *leg.* H. E. and M. E. Bigelow (Barr 2531), *det.* D. H. Pfister, 4.X.1958 (FH00465081); Franklin Co., Colrain-Heath, *leg.* H. E. & M. E. Bigelow, F. Witham (Barr 2432), *det.* D. H. Pfister, 12.VII.1958 (FH00465073); Franklin Co., Leverett, *leg.* H. E. & M. E. Bigelow (Barr 2492), *det.* D. H. Pfister, 6.VII.1958 (FH 00465077); Hampshire Co., Amherst, Lover's Lane, on soil side of trail, *leg.* H. E. Bigelow (Barr 2430), *det.* D. H. Pfister, 9.VIII.1958 (FH00465076); Hampshire Co., Vicinity Amherst, on soil in woods, *leg.* H. E. Bigelow, M. E. Bigelow, F. Witham (Barr 2404), *det.* D. H. Pfister, 4.VIII.1958 (FH00465072); Middlesex Co., Carlisle, woody debris, *leg.* L. Millman, 7.VIII.2006 (FH00465069); Middlesex Co., Lincoln, around Walden Pond, mixed woods, trail verge, *leg.* Lawrence Millman, *det.* D. H. Pfister, 18.VIII.2008 (FH00822876). New Hampshire. Carroll Co., Pequasket, on Piper Trail, D. H. Linder and R. Singer, *det.* W. L. White, 25 – 26.VII.1941 (FH00465059); Carroll Co., Albany, World Fellowship Center, sandy soil, *leg.* Lawrence Millman, 21.IX.2014 (FH00822872); Carroll Co., Intervale, *leg.* R. Thaxter, VII.1907 (FH00465055); Intervale, VIII.1901, *det.* D. H. Pfister, VIII.1901 (FH00465057); Carroll Co., Sawyer Rock Picnic Area, White Mountain



Figure 4 – Field collection images showing the range of color variation of *Rugospora atrovinosa* apothecia. A. FLAS-F-67101 B-C. FLAS-F-71677. B shows top view. C shows underside with pustulate receptacle. Photos by Cassie Pugh. D. FLAS-F-68805 photo by Igor Khomenko. E. FLAS-F-68169 photo by Curtis Peyer. Bars: A, D, E = 2 cm; B, C = 1 cm.

National Forest, leg. H. E. & M. E. Bigelow (Barr 4042), det. D. H. Pfister, 10.VIII.1963 (FH00823658); Coös Co., Shelbourne, leg. W. G. Farlow, (FH00465066). New York. Dutchess Co., Poughkeepsie, leg. W.R. Gerard, isotype of *Peziza atrovinosa* (CUP-3703); Hamilton Co., Raquette Lake, Brown Tract Road, scattered in sandy soil in road bank, leg. T. Baroni (4614), det. D. H. Pfister, 19.VIII.1984 (FH00465061); *ibid.*, Baroni (4614) (FH00465062), *ibid.*, Baroni (4612) (FH00465064). North Carolina. Macon Co., Highlands Biological Station, 35.03 N, 83.12 W, leg. J. Kimbrough, as *P. retiderma*, 13.VIII.1982 (FLAS-F-52955). Ohio. Hocking Co., Old Man's Cave State Park, one soil in mixed woods, leg. W. B. & E. G. Cooke (46536), det. D. H. Pfister, 15.X.1972 (FH00465063); Hocking Co., Tar Hollow State Park and Forest, 39.3899 N 82.7535 W, on soil, mixed pine-hardwood forest, leg. Django Grootmyers (FLAS-F-64202). Pennsylvania. Chester Co., West Chester, 04.VIII.1885 [possible type of *P. chlamydospora*]; Huntingdon Co., Alan Seeger State Monument, leg. J. Kimbrough, det. R. Korf,

7.VII.1982 (FLAS-F-52966); Philadelphia Co., Philadelphia, leg. W. C. Stevenson, IX.1883. [possible type of *P. chlamydospora*]; Pike Co., approx. 6 mi. E of Green tree near Promise Land State Park, on soil with mosses, leg. G. Benny, 5.VIII.1986, det. J. Kimbrough (as *P. retiderma*) (FLAS-F-55016). Tennessee. Blount Co., Great Smoky Mts. National Park, Parsons Branch Rd. turnaround, Cades Cove, leg. L. Ryvarden, det. D. H. Pfister (Tenn 25), VIII.1977 (FH00465063); Blount Co., Great Smoky Mts. National Park, Schoolhouse Gap Trail, 35.636233 N -83.736143 E, 1810 ft (552 m) a.s.l., on soil in mixed forest of *Carpinus*, *Betula*, *Tilia*, *Pinus*, *Quercus*, and *Tsuga*, leg. R. Healy 16.VIII.2021 (FLAS-F-68216); Carter Co., Burbank, leg. R. Thaxter, det. D. H. Pfister, VIII-IX.1887 (FH00464046); Cocke Co., Cosby Campground, 35.75543 N, -83.20894 E, on soil under *Acer*, *Liriodendron*, *Cornus*, leg. Gregory Bonito, Judson Van Wyk, 12.VIII.2021 (FLAS-F-68274); Sevier Co., near Whaley-Big Greenbrier Cemetery, 35.7 N -83.38 E, 1870 ft (570 m) a.s.l., on soil, leg. Sarah Prentice 14.VIII.2021 (FLAS-F-68478). Ver-

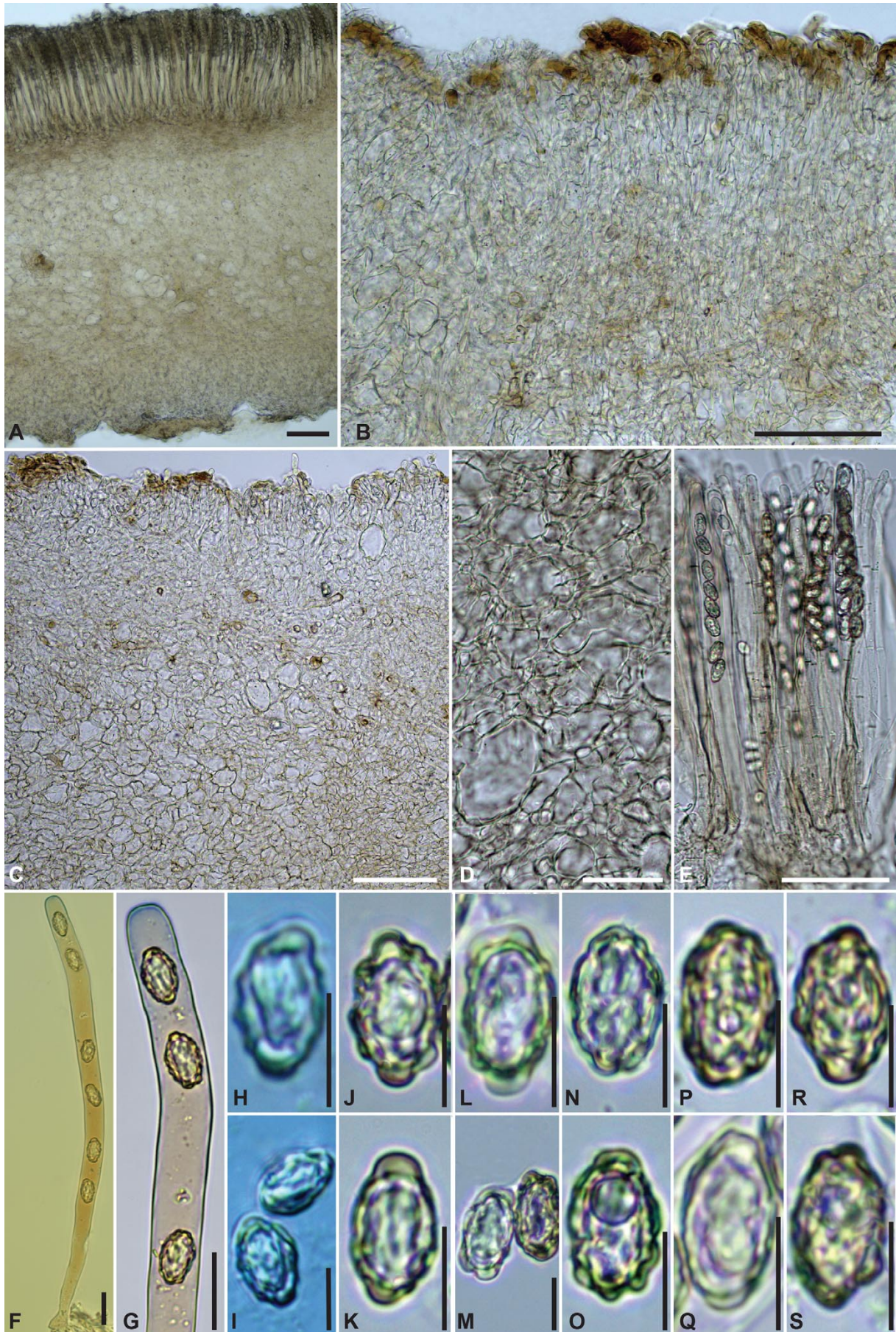


Figure 5 – Microscopy images of *Rugospora atrovinosa*. A. Through section of apothecium showing excipulum and hymenium (FLAS-F-68478). B. Ectal excipulum (FLAS-F-64202). C. Ectal and medullary excipulum (FLAS-F-64202). D. Higher magnification of medullary excipular cells (FLAS-F-68169). E. Asci and paraphyses (FLAS-F-68216). F, G. Ascus showing amyloid reaction in Melzer's solution (FLAS-F-64202). H, I. Differential interference contrast of ascospores (FLAS-F-68805). J, K. Ascospores (FLAS-F-69666). L, M (FLAS-F-68169). N. (FLAS-F-64202) O. (FLAS-F-69666). P, Q, R, S. Ascospores of isotype for *Peziza atrovinosa* (CUP-D-3703). Bars: A, B, C = 100 µm. D, E = 50 µm. F, G = 20 µm. L-S = 10 µm.

mont. Chittenden Co., Burlington, Indian Hill, *leg.* D. H. Pfister, 30.VIII.2000 (FH00465053); Lamoille Co., Stowe, Covered Bridge Rd, *leg.* H. E. & M. E. Bigelow (Barr 4386), *det.* D. H. Pfister, 23.VII.1964 (FH00823610); Lamoille Co., Ranch Brook, near Mt. Mansfield, *leg.* H. E. & M. E. Bigelow (Barr 4403), *det.* D. H. Pfister, 27.VII.1964 (FH 00823540); Rutland Co., Paulet, Haystack Mt., *leg.* C. W. Dodge (1864), 22.IX.1922 (FH00465080). West Virginia. Braxton Co., on US19, 3-4 mi S of Sutton, *leg.* J. Kimbrough, 12.VIII.1982 (FLAS-F-52961).

Discussion

A note on the authorship of *P. atrovinosa*. In the original publication COOKE (1875) lists the author of *P. atrovinosa* as "Cooke." It is also said to be from New Jersey, presumably a mix up with Ellis whose collections are also listed in Cooke's paper. *Peziza griseorosea* W.R. Gerard that precedes *P. atrovinosa* in Cooke's list is attributed to Gerard and gives the location as New York. COOKE (1877) corrected the locality later in this series to indicate New York as the locality and Gerard as the collector. SACCARDO (1889) lists the authorities as Gerard & Cooke, and he gives the location as "ad terram in Britannia." COOKE (1876) illustrated *P. atrovinosa* (Fig. 6) in his *Mycographia* and there he indicated "Gerard" as the author. We know from fungarium annotations that Gerard sent material to Cooke under the name *P. rugospora*, a name that had previously been used by Sowerby for a different species.

Rugospora atrovinosa has been confused with *Legaliana badia*. It has been shown through molecular phylogenetic analysis that *R. atrovinosa* is placed outside *Legaliana* in an independent, supported clade. *Rugospora atrovinosa* is situated within a larger clade that includes *Legaliana* and several other species in genera that are both epigeous and hypogeous (Fig. 1). No environmental or ectomycorrhizal root tip sequences have been recorded for *R. atrovinosa*. However, this larger clade includes species that are documented to be mycorrhizal, and we have provided isotopic evidence which suggests that *R. atrovinosa* is ectomycorrhizal as well.

Judging by sequence BLASTn searches there are several collections of *R. atrovinosa* that are misidentified on GenBank as *Phylloscypha phyllogena* (Cooke) Van Vooren. The spore ornamentation of *P. phyllogena* differs from both *Rugospora atrovinosa* and *Legaliana badia* in that it is made up of isolated warts. Additionally, *P. phyllogena* is a spring fruiting fungus that has violet tints at the base of the receptacle.

One of the species that has been considered along with *Rugospora atrovinosa* because of the highly marked, pigmented ascospores is *Plicariella vacini* Velen., now *Velenovskya vacini* (Velen.) Albanese, Boragine, M. Carbone & P. Alvarado, based on a molecular phylogenetic study (ALBANESE *et al.*, 2022). MORAVEC & SPOONER (1988) discuss this species, provide a description and compare it to *R. atrovinosa*. The two differ in morphology and in habitat. The yellow-brownish ascospores of *V. vacini* are ornamented with coarse "thick wing-like obtuse to pyramidal warts" (ALBANESE *et al.*, 2022) rather than a reticulum. Also, *V. vacini* occurs on burned areas, whereas *R. atrovinosa* is found directly on forest soil. Finally, our study shows that *V. vacini* is distantly related to our new genus.

Three names deserve further discussion. Two are represented by specimens collected within the known range of *Rugospora atrovinosa*. Citing authentic material in the Farlow Herbarium, PFISTER (1978a) considered *Peziza chlamydospora* Ellis & Everh. to be a synonym of *R. atrovinosa*. A restudy of that material confirms this synonymy. The type specimen was collected in eastern Pennsylvania, USA. The other species synonymized here is *P. retiderma*. The type collection of *Peziza retiderma* was collected in South Portland, Maine, USA by Charles Bowen Fuller, the longtime curator of the defunct Portland Society of Natural History. COOKE'S (1877) illustration is provided here as Fig. 7. The type specimen was studied by MORAVEC & SPOONER (1988). The name often has been used interchangeably with *P. atrovinosa* in North America. Our proposed synonym reflects our view that these two taxa fall within the range of variation of

R. atrovinosa and also occur in the same geographical area. No collections, other than the type specimen, have been authoritatively identified as *P. retiderma* in North America. A third name deserves mention. *Aleurina stipitata* Cash is an illegitimate name; it is a later homonym of *A. stipitata* Rodway, a species described from Tasmania. Cash's specimens were collected on soil in Panama. PFISTER (1979) studied the type specimen and pointed out its similarity to *P. atrovinosa* and *P. retiderma*. Resolution of its identity awaits study of additional material from Central America.

There are reports of *Peziza atrovinosa* from Mexico and Central America (DENISON, 1963; MEDEL *et al.*, 2013; SANCHEZ *et al.*, 2005). At least some of these are on burned areas and are being re-examined; the habitat suggests that they may belong to the genus *Velenovskya*. LE GAL (1953) named collections from Madagascar as *P. retiderma*. A re-examination of these Malagasy collections shows that the ascospore ornamentation differs from *R. atrovinosa* and thus, from *P. retiderma* as we have interpreted it. Specimens studied are: Madagascar. Mandraka, forêt orientales, 2 et 30 mars 1940, as cited by Le Gal, CUP-K-3992, CUP-K-3993, as *Galactinia retiderma* (Cooke) Le Gal. There are currently no sequences available for the Madagascar collections. *Peziza retiderma* was considered the type of the illegitimate genus *Aleurina* (Sacc.) Sacc. & P. Syd. (1902). ECKBLAD (1968) considered this to be the case, but the earlier *Aleurina* Massee is now recognized as the accepted genus and circumscribes a group of ectomycorrhizal taxa known primarily from the Southern Hemisphere (ZHUANG & KORF, 1986). The type is *Aleurina tasmanica* Massee.

Acknowledgments

We join in celebrating Trond Schumacher recognizing his contributions to the understanding of the systematics and ecology of the *Pezizomycetes*.

We thank Kevin Childs and the MSU RTSF Genomics Core for assistance with sequencing genomic DNA samples. We thank Jason Curtis and the staff at the University of Florida Stable Isotope Mass Spec Lab for processing isotope samples.

We warmly thank all of the collectors who directly contributed to this study or made their data available through iNaturalist: Timothy J Baroni, Django Grootmyers, Arthur Grupe, Sigrid Jakob, Larry Millman, Judson Van Wyk, and in particular, we thank Igor Khomenko, Curtis Peyer, and Cassie Pugh who provided images as well as collections. Alisha Millican coordinated efforts to send well-documented *Pezizales* collections to FLAS-F, for which we are very grateful. We are grateful for funding from the National Science Foundation (NSF DEB-1946445 to MES, GB, and RH), and NSF GRFP Fellowship (no. 2019277707, to BL). Collecting in the Great Smoky Mountains National Park was under permit GRSM-2021-Sci-2516 and these collections are on loan to FLAS-F. Permission for collecting in the Daniel Boone and Nantahala National Forests was granted in writing from the United States Forest Service.

We also thank the curatorial staff at the University of Florida (FLAS-F) and those at the Farlow Library and Herbarium of Cryptogamic Botany (FH). They are the keepers of biodiversity treasures.

Authors' contributions

DHP conceived the project, examined specimens and literature, and wrote the preliminary manuscript; BRL extracted the RPB2 sequences from low coverage genomes of FLAS-F *R. atrovinosa*, provided the isotopic data and analyses, reviewed the manuscript and obtained funding; RH performed sequencing, measured FLAS-F specimens, performed micrography, and analyses, reviewed the manuscript, prepared the figures and provided funding; KFL performed sequencing and analyses, prepared figures, and reviewed the manuscript; GB provided isotopic data and reviewed manuscript and obtained funding; MES reviewed the manuscript, gave advice and provided funding.

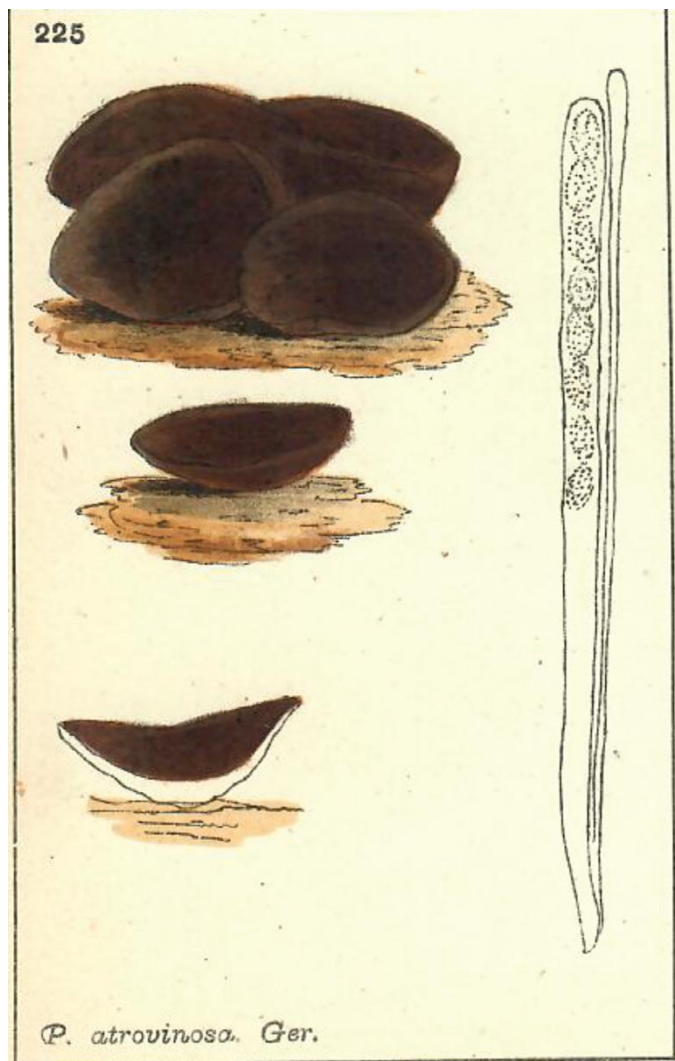


Figure 6 – Illustration of *Peziza atrovinosa* by M. C. Cooke (*Mycographia*, pl. 57, fig. 225. 1876)

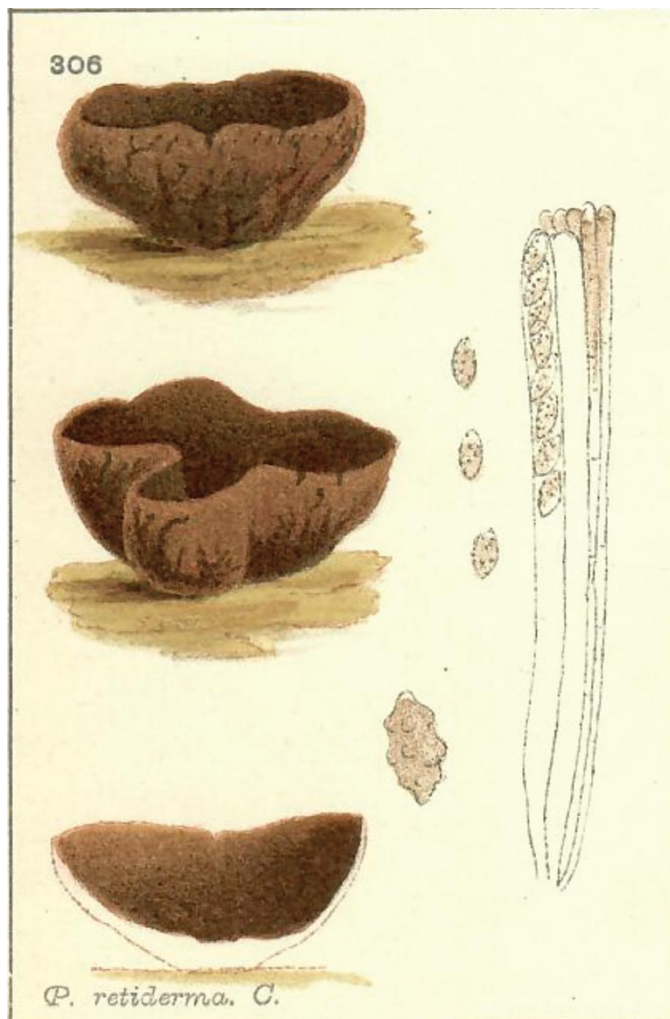


Figure 7 – Illustration of *Peziza retiderma* by M. C. Cooke (*Mycographia*, pl. 79, fig. 306. 1877)

References

- ALBANESE A., BORAGINE M., CARBONE M. & ALVARADO P. 2022. — *Velenovskya*, a new genus in the family *Pezizaceae* to accommodate *Plicariella vacini*. *Ascomycete.org*, 14: 109–115. doi: [10.25664/art-0353](https://doi.org/10.25664/art-0353)
- BIRKEBAK J.M., MAYOR J.R., MARTIN RYBERG K. & BRANDON MATHENY P. 2013. — A systematic, morphological and ecological overview of the *Clavariaceae* (*Agaricales*). *Mycologia*, 105 (4): 896–911. doi: [10.3852/12-070](https://doi.org/10.3852/12-070)
- COOKE M.C. 1875 [1874–1875]. — Synopsis of the Discomycetous fungi of the United States. *Bulletin of the Buffalo Academy of Science*, 2: 285–300.
- COOKE M.C. 1876. — *Mycographia, seu icones fungorum*. Vol. I. Discomycetes. Fasc. 2. London, Williams and Norgate.
- COOKE M.C. 1877. — Synopsis of the Discomycetous fungi of the United States (Part II). *Bulletin of the Buffalo Academy of Science*, 3: 21–32.
- COOKE M.C. 1877. — *Mycographia, seu icones fungorum*. Vol. I. Discomycetes. Fasc. 3. London, Williams and Norgate.
- DENISON W.C. 1963. — A preliminary study of the operculate cup-fungi of Costa Rica. *Revista de Biología Tropical*, 11(1): 99–129.
- ECKBLAD F.-E. 1968. — The genera of the operculate discomycetes. A re-evaluation of their taxonomy, phylogeny and nomenclature. *Nytt Magasin for Botanikk*, 15: 1–191.
- EDGAR R.C. 2022. — Muscle5: High-accuracy alignment ensembles enable unbiased assessments of sequence homology and phylogeny. *Nature Communications*, 13: 2968. doi: [10.1038/s41467-022-34630-w](https://doi.org/10.1038/s41467-022-34630-w)
- GARDES M. & BRUNS T.D. 1993. — ITS primers with enhanced specificity for basidiomycetes – application to the identification of mycorrhizae and rusts. *Molecular Ecology*, 2 (2): 113–118. doi: [10.1111/j.1365-294X.1993.tb00005.x](https://doi.org/10.1111/j.1365-294X.1993.tb00005.x)
- HANSEN K., LÆSSØE T. & PFISTER D.H. 2001. — Phylogenetics of the *Pezizaceae*, with an emphasis on *Peziza*. *Mycologia*, 93: 958–990. doi: [10.1080/00275514.2001.12063229](https://doi.org/10.1080/00275514.2001.12063229)
- HANSEN K., LOBUGLIO K.F. & PFISTER D.H. 2005. — Evolutionary relationships of the cup-fungus genus *Peziza* and *Pezizaceae* inferred from multiple nuclear genes: RPB2, beta-tubulin, and LSU rDNA. *Molecular Phylogenetics and Evolution*, 36: 1–23. doi: [10.1016/j.ympev.2005.03.010](https://doi.org/10.1016/j.ympev.2005.03.010)
- HANSEN K. & PFISTER D.H. 2006. — Systematics of the *Pezizomycetes* – the operculate discomycetes. *Mycologia*, 98: 1029–1040. doi: [10.3852/mycologia.98.6.1029](https://doi.org/10.3852/mycologia.98.6.1029)
- HEALY R.A., ARNOLD A.E., BONITO G., HUANG Y.-L., LEMMOND B., PFISTER D.H. & SMITH M.E. 2022. — Endophytism and endolichenism in the *Pezizomycetes*: the exception or the rule? *New Phytologist*, 233: 1974–1983. doi: [10.1111/nph.17886](https://doi.org/10.1111/nph.17886)
- HENNEBERT G.L. & KORF R.P. 1975. — The Peat Mould, *Chromelosporium ollare*, conidial state of *Peziza ostracoderma*, and its misapplied names, *Botrytis crystallina*, *Botrytis spectabilis*, *Ostracoderma epigaeum* and *Peziza atrovinosa*. *Mycologia*, 67: 214–240. doi: [10.1080/00275514.1975.12019745](https://doi.org/10.1080/00275514.1975.12019745)

- HOPPLE JR J.S. & VILGALYS R. 1994. — Phylogenetic relationships among coprinoid taxa and allies based on data from restriction site mapping of nuclear rDNA. *Mycologia*, 86: 96–107. doi: [10.1080/00275514.1994.12026378](https://doi.org/10.1080/00275514.1994.12026378)
- KIM D., GILCHRIST C.L.M., CHUN J. & STEINEGGER M. 2023. — UFCG: database of universal fungal core genes and pipeline for genome-wide phylogenetic analysis of fungi. *Nucleic Acids Research*, 51(D1): D777–D784. doi: [10.1093/nar/gkac894](https://doi.org/10.1093/nar/gkac894)
- LE GAL M. 1953. — *Les Discomycètes de Madagascar*. Prodrôme à une flore mycologique de Madagascar et dépendances. IV. Paris, Muséum national d'histoire naturelle, 465 pp.
- LIU Y.J., WHELEN S. & HALL B.D. 1999. — Phylogenetic relationships among ascomycetes: evidence from an RNA polymerase II subunit. *Molecular Biology and Evolution*, 16: 1799–1808. doi: [10.1093/oxfordjournals.molbev.a026092](https://doi.org/10.1093/oxfordjournals.molbev.a026092)
- MAYOR J.R., SHUUR E.A.G. & HENKEL T.W. 2009. — Elucidating the nutritional dynamics of fungi using stable isotopes. *Ecological Letters*, 112: 171–183. doi: [10.1111/j.1461-0248.2008.01265.x](https://doi.org/10.1111/j.1461-0248.2008.01265.x)
- MEDEL R., CASTILLO R., MARMOLEJO J. & BAEZA Y. 2013. — Analisis de la familia *Pezizaceae* (Pezizales: Ascomycota) en Mexico. *Revista Mexicana de Biodiversidad*, 84, supplement: S21–S38. doi: [10.7550/rmb.31741](https://doi.org/10.7550/rmb.31741)
- MILLER M.A., PFEIFFER W. & SCHWARTZ T. 2010. — Creating the CIPRES Science Gateway for inference of large phylogenetic trees. In: Institute of Electrical and Electronics Engineers. Proceedings of the Gateway Computing Environments Workshop (GCE), New Orleans, LA: 1–8. doi: [10.1109/GCE.2010.5676129](https://doi.org/10.1109/GCE.2010.5676129)
- MORAVEC J. & SPOONER B.M. 1988. — *Peziza vacinii* (Pezizales), with notes on taxonomy of related brown-spored species. *Transactions of the British Mycological Society*, 90: 43–48. doi: [10.1016/S0007-1536\(88\)80178-5](https://doi.org/10.1016/S0007-1536(88)80178-5)
- O'DONNELL K. 1993. — *Fusarium* and its near relatives. In: REYNOLDS D.R. & TAYLOR J.W. (eds.) *The Fungal Holomorph: Mitotic, Meiotic and Pleomorphic Speciation in Fungal Systematics*. Wallingford, CAB International: 225–233.
- PFISTER D.H. 1978a. — Type studies in the genus *Peziza*. II. Operculate discomycetes described by J. B. Ellis and co-authors. *Mycotaxon*, 7: 97–101.
- PFISTER D.H. 1978b. — Type studies in the genus *Peziza*. III. Operculate discomycetes collected by W. R. Gerard. *Mycotaxon*, 7: 209–213.
- PFISTER D.H. 1979. — Type studies in the genus *Peziza*. VIII-X. Species described by F. Massee, E. K. Cash, and F. J. Seaver. *Mycotaxon*, 9: 501–504.
- PFISTER D.H. 2015. — *Pezizaceae* taxonomy and classification: Boudier, Cooke and Korf. *Ascomycete.org*, 7(6): 239–244. doi: [10.25664/art-0145](https://doi.org/10.25664/art-0145)
- PFISTER D.H., SLATER C. & HANSEN K. 2008. — *Chorioactidaceae*: a new family in the *Pezizales* (Ascomycota) with four genera. *Mycological Research*, 112: 513–527. doi: [10.1016/j.mycres.2007.11.016](https://doi.org/10.1016/j.mycres.2007.11.016)
- PFISTER D.H., QUIJADA L. & LOBUGLIO K.F. 2020. — *Geodina* (Pezizomycetes: Wynneaceae) has a single widespread species in tropical America. *Fungal Systematics and Evolution*, 5: 131–138. doi: [10.3114/fuse.2020.05.08](https://doi.org/10.3114/fuse.2020.05.08)
- R CORE TEAM. 2021. — R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. <https://www.R-project.org/>
- RIFAI M.A. 1968. — The Australasian *Pezizales* in the herbarium of the Royal Botanic Gardens Kew. *Koninklijke Nederlandse Akademie van Wetenschappen. Afdeling Natuurkunde. Verhandelingen, Tweede reeks*, 57 (3): 1–295.
- SACCARDO P.A. 1889. — *Sylloge fungorum omnium hucusque cognitarum*. Vol. 8. Patavii, 1143 pp.
- SACCARDO P.A. & SYDOW P. 1902. — *Sylloge fungorum omnium hucusque cognitarum*. Vol. 16. Patavii, 1235 pp.
- SANCHEZ-JACOME M.D.R. & GUZMÁN-DAVALOS L. 2005. — New records of ascomycetes from Jalisco, Mexico. *Mycotaxon*, 92: 177–191.
- SEAEVER F.J. 1928. — *The North American cup-fungi (Operculates)*. New York. 284 pp.
- SEEMAN T. 2018. — Basic Rapid Ribosomal RNA Predictor (BARRNAP) (Version 0.9). <https://github.com/tseemann/barrnap>
- SLATER G.S.C. & BIRNEY E. 2005. — Automated generation of heuristics for biological sequence comparison. *BMC Bioinformatics*, 6: 31. doi: [10.1186/1471-2105-6-31](https://doi.org/10.1186/1471-2105-6-31)
- STAMATAKIS A. 2014. — RAXML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics*, 30: 1312–1313. doi: [10.1093/bioinformatics/btu033](https://doi.org/10.1093/bioinformatics/btu033)
- STAJICH J.E. & PALMER J.M. 2023. — Automatic Assembly For The Fungi (Version 0.5.0). <https://github.com/stajichlab/AAFTF>
- VANDEPOL N., LIBER J., DESIRÒ A., NA H., KENNEDY M., BARRY K., GRIGORIEV I.V., MILLER A.N., O'DONNELL K., STAJICH J. & BONITO G.M. 2020. — Resolving the *Mortierellaceae* phylogeny through synthesis of multi-gene phylogenetics and phylogenomics. *Fungal Diversity*, 104: 267–289. doi: [10.1007/s13225-020-00455-5](https://doi.org/10.1007/s13225-020-00455-5)
- VAN VOOREN N. 2020. — Reinstatement of old taxa and publication of new genera for naming some lineages of the *Pezizaceae* (Ascomycota). *Ascomycete.org*, 12 (4): 179–192. doi: [10.25664/art-0305](https://doi.org/10.25664/art-0305)
- VENABLES W.N. & RIPLEY B.D. 2002. — *Modern Applied Statistics with S*. Fourth edition. New York, Springer. <https://www.stats.ox.ac.uk/pub/MASS4/>
- WHITE T.J., BRUNS T., LEE S. & TAYLOR J.W. 1990. — Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: INNIS M.A., GELFAND D.H., SNINSKY J.J. & WHITE T.J. (eds.) *PCR protocols: a guide to methods and applications*. New York, Academic Press: 315–322. doi: [10.1016/B978-0-12-372180-8.50042-1](https://doi.org/10.1016/B978-0-12-372180-8.50042-1)
- WICKHAM H. 2016. — *ggplot2: Elegant Graphics for Data Analysis*. New York, Springer. <https://ggplot2.tidyverse.org>
- YE J., COULOURIS G., ZARETSKAYA I., CUTCUTACHE I., ROZEN S. & MADDEN T.L. 2012. — Primer-BLAST: a tool to design target-specific primers for polymerase chain reaction. *BMC Bioinformatics*, 13: 134. doi: [10.1186/1471-2105-13-134](https://doi.org/10.1186/1471-2105-13-134)
- ZHUANG W.-Y. & KORF R.P. 1986. — A monograph of the genus *Aleurina* Massee (= *Jafneadelphus* Rifai). *Mycotaxon*, 26: 361–400.



- 1: D. H. Pfister — Department of Organismic and Evolutionary Biology and Farlow Library and Herbarium of Cryptogamic Botany, Harvard University, 22 Divinity Ave., Cambridge, MA 02138, USA — dpfister@oeb.harvard.edu
- 2: B. Lemmond — Department of Plant Pathology, University of Florida, Fifield Hall, Gainesville, Florida 32611, USA
- 3: R. Healy — Department of Plant Pathology, University of Florida, Fifield Hall, Gainesville, Florida 32611, USA
- 4: K. F. LoBuglio — Department of Organismic and Evolutionary Biology and Farlow Library and Herbarium of Cryptogamic Botany, Harvard University, 22 Divinity Ave., Cambridge, MA 02138, USA
- 5: G. Bonito — Department of Plant, Soil and Microbial Sciences, Molecular Plant Science Building, 1066 Bogue Street, Michigan State University, East Lansing, MI 48824-6254, USA
- 6: M. E. Smith — Department of Plant Pathology, University of Florida, Fifield Hall, Gainesville, Florida 32611, USA