Paragalactinia pseudomichelii (*Pezizaceae*), a new species from eastern North America

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Abstract: Presentation and illustration of a new species of the genus *Paragalactinia*, *P. pseudomichelii*, collected in Canada and the USA, based on morphological and molecular characters. An updated key to the genus *Paragalactinia* is also provided.
Keywords: *Ascomycota*, *Pezizales*, 1 new species.

Résumé : présentation et illustration d'une nouvelle espèce du genre *Paragalactinia*, *P. pseudomichelii*, récoltée au Canada et aux États-Unis, à partir des caractères morphologiques et moléculaires. Une clé actualisée du genre *Paragalactinia* est également proposée.
Mots-clés : *Ascomycota*, *Pezizales*, 1 espèce nouvelle.

Introduction

During a field trip in Québec in September 2022, the first author had the opportunity to collect numerous specimens of a *Paragalactinia* species unknown to him but named as "*Peziza michelii*" by local mycologists. The second and third authors confirmed this species was quite frequent in Canada, from June to mid-October and not differentiated, microscopically, from *Paragalactinia michelii* (Boud.) Van Vooren. Phylogenetic analyses that included highly similar ITS sequences generated particularly from collections in the Appalachian Mountains by the M.E. Smith Lab (University of Florida) and G. Bonito Lab (Michigan State University) helped to delimit this undescribed species and revealed its distribution from as far north as Ontario (Canada) east to New Brunswick (Canada) south to Alabama (USA) and west to Minnesota and Iowa (USA). We present in detail this cup-fungus as a new species of temperate eastern North American woodlands. We also place this new *Paragalactinia* phylogenetically based on ITS and LSU rDNA sequences and show that additional taxa in this group are known from DNA sequences and still need to be described in the future.

Material and methods

Morphological studies

Fresh specimens of *Paragalactinia pseudomichelii* and *P. michelii* were studied using methods of "vital taxonomy" (Baral, 1992; Dougoud, 2024). Dried specimens of these species were studied after rehydrating pieces in tap water. For microscopic characters, squashed mounts of hand cut thin sections of fresh (or rehydrated) specimens were examined first in tap water. Melzer's reagent (MLZ) or Lugol's solution (IKI) were used for testing the amyloidity, and lactic acid cotton blue (LCB) for highlighting the spore ornamentation. All measurements were taken on fresh material mounted in tap water and are given on the basis of a number (n) of free ascospores in the mounts, ornamentation excluded. The following abbreviations are used: Me = arithmetic mean, Q = ratio between spore length and width, Qm = mean Q. Measurements obtained from rehydrated material are noted with the symbol †.

DNA extraction, amplification and sequencing

Total DNA was extracted from dry specimens employing a modified protocol based on Murray & Thompson (1980). PCR reactions (Mullis & Faloona, 1987) included 35 cycles with an annealing temperature of 54ºC in all cases. The primers ITS1F and ITS4 (White *et al.*, 1990; Gardes & Bruns, 1993) were used to amplify the ITS rDNA region, and LROR and LR5 (Vilgalys & Hester, 1990; Cubeta *et al.*, 1991) were used to amplify the 28S rDNA region (LSU). Successful PCR amplification was checked by gel electrophoresis, followed by illumination by UV light. Amplicons were sequenced with one or both PCR primers. Geneious Prime 2024.0.3 (www.geneious.com) or the BioEdit software v.7.2.5 (Hall, 1999) were used to remove messy ends of sequences, to form contigs of forward and reverse sequences, and to edit reading errors in chromatograms.

Phylogenetic analyses

We used the BLAST® search algorithm (Altschul *et al.*, 1990) to find the highly similar sequences from the International Nucleotide Sequence Database Collaboration (INSDC) public database (Arita *et al.*, 2021). Representative sequences that were ≥ 90% similarity were downloaded. Selected sequences were aligned using MUSCLE (Edgar, 2004) integrated in MEGA-X software (Kumar *et al.*, 2018) or Geneious Prime, with the default execution parameters, and then manually checked and corrected when obviously erroneous. The final dataset for the ITS analysis of *Paragalactinia* contains 105 nucleotide sequences and a total of 618 positions, 103 nucleotide sequences (including two sequences for the outgroup) and a total of 874 positions for the LSU analysis. The ITS RAxML analysis was performed on the Cyberinfrastructure for Phylogenetic Research Science Gateway (CIPRES) v.3.3 (Miller *et al.*, 2010) using the GTR model of evolution and 1000 bootstrap replications. The LSU analysis of maximum likelihood was performed in MEGA-X using a GTR evolutionary model and 500 bootstrap replications.

The phylograms were viewed in FigTree v.1.2.4 (Rambaut, 2009) and graphically edited with the online software iTOL v6 (https://itol.embl.de, Letunic & Bork, 2021) or Adobe Illustrator v. 15.1 (Adobe Systems Inc., San Jose, CA). The newly sequenced collections were deposited in GenBank (see Table 1).

Molecular results

Our phylogenetic analyses support the division of the genus *Paragalactinia* into two main clades: the first clade contains the species of the "*succosa* complex", *P. hypoleuca* (Boud. & Torrend) Van Vooren — a Mediterranean species —, and the hypogeous species *P. infossa* (Fogel & States) Van Vooren and *P. eriniae* (M.E. Sm.) M.E. Sm. & Van Vooren (Smith, 2014); the second clade contains *P. michelii*, *P. berthetiana* (Donadini) Van Vooren and a separate group corresponding to a previously unrecognized species that we describe here as *P. pseudomichelii*. The trees with the highest log likelihood for each locus are shown (Fig. 1-2): ITS = -2587.37 and LSU = -4920.39. The topology is the same in the ITS and LSU phylograms.

Table 1 – List of collections of *Paragalactinia* sequenced during this study

Species	Country	Voucher No.	GenBank Number	
			ITS	LSU
P. michelii	Canada, ON	IK 2020-234	PP789288	PP789301
P. michelii	Canada, ON	IK 2020-213	PP789287	PP789300
P. michelii	Canada, QC	HRL1708	–	PP789302
P. michelii	Canada, ON	FLAS-F-68824	OM672978	–
P. michelii	Switzerland	LY NV 2023.08.18	PP789286	PP789299
P. michelii	USA, MI	FLAS-F-72466	MT156478	–
P. pseudomichelii	Canada, QC	LY NV 2022.09.40	PP789289	PP789303
P. pseudomichelii	Canada, QC	HRL3696	PP789290	PP789304
P. pseudomichelii	Canada, QC	HRL0892	PP789291	PP789305
P. pseudomichelii	Canada, QC	HRL0215	–	PP789306
P. pseudomichelii	Canada, QC	FLAS-F-72467	MT156480	–
P. pseudomichelii	Canada, QC	FLAS-F-60037	PP789296	–
P. pseudomichelii	Canada, QC	FH JK13092607	MT373991	–
P. pseudomichelii	USA, MN	FLAS-F-61352	MT373999	–
P. pseudomichelii	USA, MN	FLAS-F-63923	MT373994	–
P. pseudomichelii	USA, TN	FLAS-F-68232	OM672694	–
P. pseudomichelii	USA, NC	FLAS-F-68348	OM672735	–
P. pseudomichelii	USA, AL	FLAS-F-68862	OR149273	–
P. pseudomichelii	USA, IA	FLAS-F-67060	OM672683	–
P. pseudomichelii	USA, KY	FLAS-F-69795	OQ150483	–
P. pseudomichelii	USA, NC	FLAS-F-69809	OQ150491	–
P. pseudomichelii	USA, NC	FLAS-F-69820	OQ150498	–
P. pseudomichelii	USA, TN	FLAS-F-69835	OQ150510	–
P. pseudomichelii	USA, TN	FLAS-F-69845	OQ150517	–
P. pseudomichelii	USA, AL	FLAS-F-70951	OR372830	–
P. pseudomichelii	USA, VT	FLAS-F-71109	OQ066230	–
P. pseudomichelii	USA, VT	FLAS-F-71111	OQ066233	–
P. pseudomichelii	USA, AL	FLAS-F-71676	OR229384	–
P. pseudomichelii	USA, MN	MIN RH1195	MT373997	–
P. cf. *succosa*	Canada, QC	LY NV 2022.09.39	PP789295	PP789310
P. cf. *succosa*	Canada, QC	LY NV 2022.09.57	PP789294	PP789309
P. cf. *succosa*	Canada, NB	NBM JK13092503	MT373951	–
P. cf. *succosa*	Canada, QC	FLAS-F-70799	OR372805	–
P. cf. *succosa*	USA, IA	FLAS-F-62625	MT373977	–
P. cf. *succosa*	USA, IA	FLAS-F-62626	MT373978	–
P. cf. *succosa*	USA, MN	FLAS-F-62646	MT373980	–
P. cf. *succosa*	USA, MN	FLAS-F-62652	MT373979	–
P. cf. *succosa*	USA, WV	FLAS-F-63906	MT373987	–
P. cf. *succosa*	USA, MN	FLAS-F-63925	MT374006	–
P. cf. *succosa*	USA, MN	FLAS-F-63927	MT374005	–
P. cf. *succosa*	USA, MN	FLAS-F-63930	MT374007	–
P. cf. *succosa*	USA, WV	FLAS-F-64205	MT374004	–
P. cf. *succosa*	USA, TN	FLAS-F-68567	OM672854	–
P. cf. *succosa*	USA, NC	FLAS-F-68674	OM672908	–
P. cf. *succosa*	USA, KY	FLAS-F-69759	OQ150458	–
P. cf. *succosa*	USA, KY	FLAS-F-69771	OQ150465	–
P. cf. *succosa*	USA, NC	FLAS-F-69821	OQ150499	–
P. cf. *succosa*	USA, AL	FLAS-F-70952	PP789298	–
P. cf. *succosa*	USA, GA	FLAS-F-72465	MT156450	–

Table 1 – (continued)

Species	Country	Voucher No.	GenBank Number	
			ITS	LSU
P. cf. *succosa*	USA, VA	FLAS-F-72468	MT156505	–
P. cf. *succosa*	Spain	MV220212-01	–	PP789311
P. cf. *succosella*	Malta	CS1383	PP789293	PP789308
P. cf. *succosella*	France	LY NV 2022.10.08	PP789292	PP789307

Our phylogenetic analyses included sequences of *Paragalactinia* species we collected in Canada, the USA and Europe. In addition to the delimitation of *P. pseudomichelii*, results of our analyses suggest that 1) *P. succosa* (Berk.) Van Vooren is a cryptic species complex represented by several different phylogenetic species that are present in Europe and in North America, as well as in Asia (Ashraf *et al.*, 2012); 2) North American collections of *P. michelii* show some ITS nucleotide variations and may represent additional distinct species, but more sequences including additional genetic loci from a larger sampling are required to evaluate this hypothesis.

Taxonomy

Paragalactinia pseudomichelii Van Vooren, Khomenko, Lebeuf & Healy, *sp. nov.* – MB 853996 – Plate 1–3.

Diagnosis: Differs from *Paragalactinia michelii* by its external color when young, whitish to pale greyish vs. yellow-ochre in *P. michelii*, and its occurrence in temperate eastern North America.

Holotype: coll. LY NV 2022.09.40.

Etymology: from Ancient Greek ψευδής (*pseudês*) meaning 'false' and *michelii* (named in honor to Pier Antonio Micheli), the epithet of the closest species.

Apothecia (10) 20–40 mm diam., fruiting singly or in clusters, sometimes reaching 50 mm diam., sessile, cupuliform; hymenium pink to violet in young specimens, quickly becoming dusty pink to beige, yellowish to ochre, purplish ochre in the end; external surface glabrous, first whitish to pale greyish, sometimes with pink to purplish tinges, becoming ochraceous beige. **Context** soft, exuding a whitish fluid when damaged, becoming yellowish when exposed to the air.

Excipulum entirely made of a *textura globulosa* of large hyaline cells 10–55 µm diam. with a yellowish intercellular pigment, mixed with some laticiferous hyphae 6–7 µm wide. **Asci** 260–300 × 13–14 µm, cylindrical, tapered at the base, with croziers, 8-spored, showing an amyloid reaction of WT type (based on typology of Van Vooren, 2020) in MLZ or IKI. **Paraphyses** cylindrical, few widened at the top, 5–6 µm diam., hyaline. **Ascospores** (14) 15–17.2 (18) × (6.5) 7.8–9.1 (9.6) µm, Me = 16.2 × 8.4 µm (n=123), Q = (1.6) 1.8–2.1 (2.3), Qm = 1.9, † (14.5) 15–16.6 (17.5) × (6.5) 7–8.5 (9) µm, † Me = 15.6 × 7.6 µm (n=60), † Q = (1.7) 1.9–2.2 (2.5), † Qm = 2.1, ellipsoid, hyaline, biguttulate, ornamented with cyanophilous warts; warts of irregular shape, angular or elongated (short ridges), curved or not, often protruding at the poles, up to 1 µm high.

Studied and sequenced collections: ***Paragalactinia pseudomichelii***. Canada. Québec, Saint-Étienne-des-Grès, Nature Park of La Gabelle, 46.445595° N 72.740517° W, 35 m a.s.l., on the ground, on naked soil or among wood debris, under various deciduous trees, *leg.* N. Van Vooren, 14 Sep. 2022, herb. LY NV 2022.09.40 (**holotype**). Québec, Saint-Narcisse, Parc de la rivière Batiscan, entrance to Barrage sector, 46.552003° N 72.416185° W, 86 m a.s.l., in a ditch by the roadside close to a deciduous forest (*Fagus grandifolia*, *Acer* sp., *Quercus rubra*, *Populus grandidentata*), on the ground, with calcareous gravel, *leg.* R. Lebeuf & A. Paul, 30 Jun. 2022, pers. herb. HRL3696. Québec, Sainte-Anne-de-Bellevue, Morgan Arboretum, approx. 45.431411° N 73.948483° W, 73 m a.s.l., on the ground, under *Quercus rubra* and *Carya ovata*, *leg.* R. Lebeuf & A. Paul, 13 Sep. 2011, pers. herb. HRL0892. Québec, Longueuil, approx. 45.549102° N, 73.461545° W, 54 m a.s.l., on soil, in an oak forest (*Quercus rubra*), *leg.* R. Lebeuf, 4 Jul. 2009, pers. herb. HRL0215. United States. Alabama, Cullman Co., 34.1957° N 86.862892° W, 290 m a.s.l., on edge of foot path in mixed woods (*Pinus*, *Magnolia*, *Liquidambar*, *Quercus*, *Juniperus*), *leg.* A. Millican, 13 May 2023, [iNaturalist #161498304], herb. FLAS-F-71673, immature. *Ibid*, [iNaturalist #161497760], herb. FLAS-F-71676. Iowa, Linn Co., Central City, Pinicon Ridge Park, 42.21135° N 91.54527° W, 270 m a.s.l., on soil in *Quercus* dominated woodland, *leg.* R. Heidt, 27 May 2020, rwh-cup-031, herb. FLAS-F-67060. Minnesota. Lake Co., about 20 miles N of Ely, on soil among moss by path in the Forest Service lease lot group on Snowbank Lake, 48.277743° N 91.418634° W, 480 m a.s.l., *leg.* K. Baumgarten & D. Mahoney, 10 Aug 2019, herb. FLAS-F-61352. ***Paragalactinia michelii***. Canada. Québec, Laval, boisé Sainte-Dorothée, 45.538178° N 73.8207° W, 64 m a.s.l., on the ground, in a deciduous forest, *leg.* R. Lebeuf & A. Paul, 1 Jul. 2014, pers. herb. HRL1708. Ontario, Ottawa, 45.408837° N 75.573625° W, 73 m a.s.l., on a mossy ground in deciduous forest (*Acer rubrum*, *A. ?saccharum*, *Fagus grandifolia*, *Populus grandidentata* and *P. tremuloides*), *leg.* I. Khomenko, 1 Jul. 2020, pers. herb. IK 2020-234 [iNaturalist #51770851]. Ontario, Ottawa, Pine Grove, 45.408828° N 75.573578° W, 93 m a.s.l., on mossy ground in deciduous forest, *leg.* I. Khomenko, 23 Jun. 2020, pers. herb. IK 2020-213 [iNaturalist #50694401]. Ontario, Ottawa, Greenbelt, 45.403057° N 75.587708° W, 81 m a.s.l., on the ground in a mostly broadleaf forest (*Acer rubrum*, *Populus grandidentata*, *Fagus grandifolia*, *Betula alleghaniensis*), *leg.* I. Khomenko, 8 Oct. 2021, ex IK 2021-1238, herb. FLAS-F-68824 [iNaturalist #97736497]. Switzerland. Fribourg, Gruyère, route du Petit Chalet Neuf, 46.57021° N 7.0098° E, 1170 m a.s.l., on soil, under *Picea abies*, *leg.* R. Dougoud & N. Van Vooren, 9 Aug. 2023, herb. LY NV 2023.08.18.

Sequenced and documented collections (not studied): ***Paragalactinia pseudomichelii***. Canada. New Brunswick, Fundy National Park, 45.54916° N 65.01929° W, 45 m a.s.l. on sandy soil by trail in coastal mixed forest dominated by *Picea rubens*, *Betula alleghaniensis*, and *B. cordifolia*, *leg.* J. Karakehian, 26 Sep. 2013, JK 13092607, FH. Ontario, Inverhuron Provincial Park, near Tiverton, on soil in mixed forest, *leg.* M.E. Smith, 10 Aug. 2014, MES-779, herb. FLAS-F-72467. Ontario, Havelock, Belmont Township 3 Line, 44.4570239° N 77.7999967° W, 193 m a.s.l., on soil, *leg.* J. Thornhill, 21 Aug. 2015, herb. FLAS-F-60037. United States. Alabama, Cullman Co., 1201 County Road 46, 34.001156° N 86.897965° W, 192 m a.s.l., on soil in mixed woods, *leg.* A. Millican, 8 Sep. 2022, herb. FLAS-F-70951. Alabama, Jefferson Co., Red Mountain Park, 33.447822° N 86.863367° W, 240 m a.s.l., on rocky soil among moss, *leg.* A. Millican, 16 Oct. 2021, herb. FLAS-F-68862. Kentucky, Pulaski Co., Daniel Boone National Forest, Whitaker Cemetery Rd. N of KY-80, 37.156794° N 84.296897° W, 271 m a.s.l., on loamy clay soil in hardwood forest near Rockcastle River (dominated by *Fagus*, *Betula*, with some *Quercus*, *Tilia*, *Acer*, and *Liriodendron*), *leg.* M.E. Smith, 19 Sep. 2022, herb. FLAS-F-69632. Ibid, herb. FLAS-F-69633. Kentucky, Whitley Co.,

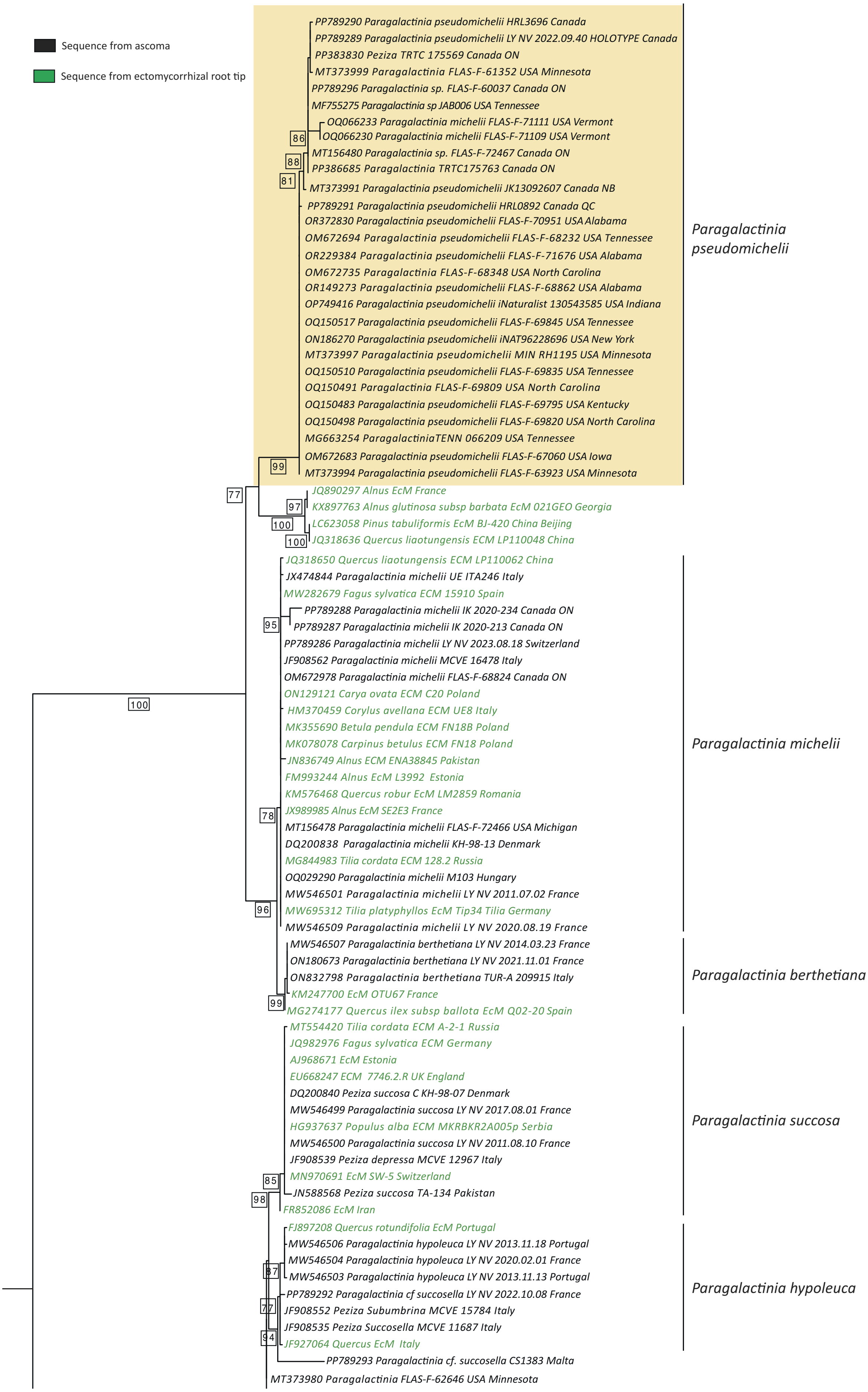

Fig. 1 – Phylogram of the genus *Paragalactinia* inferred from maximum likelihood (ML) analysis of the ITS region. The phylogram is mid-point-rooted and the nodes with ≥ 70% ML bootstrap support are annotated with their support values. Text in green indicates sequences from ectomycorrhizal root tips.

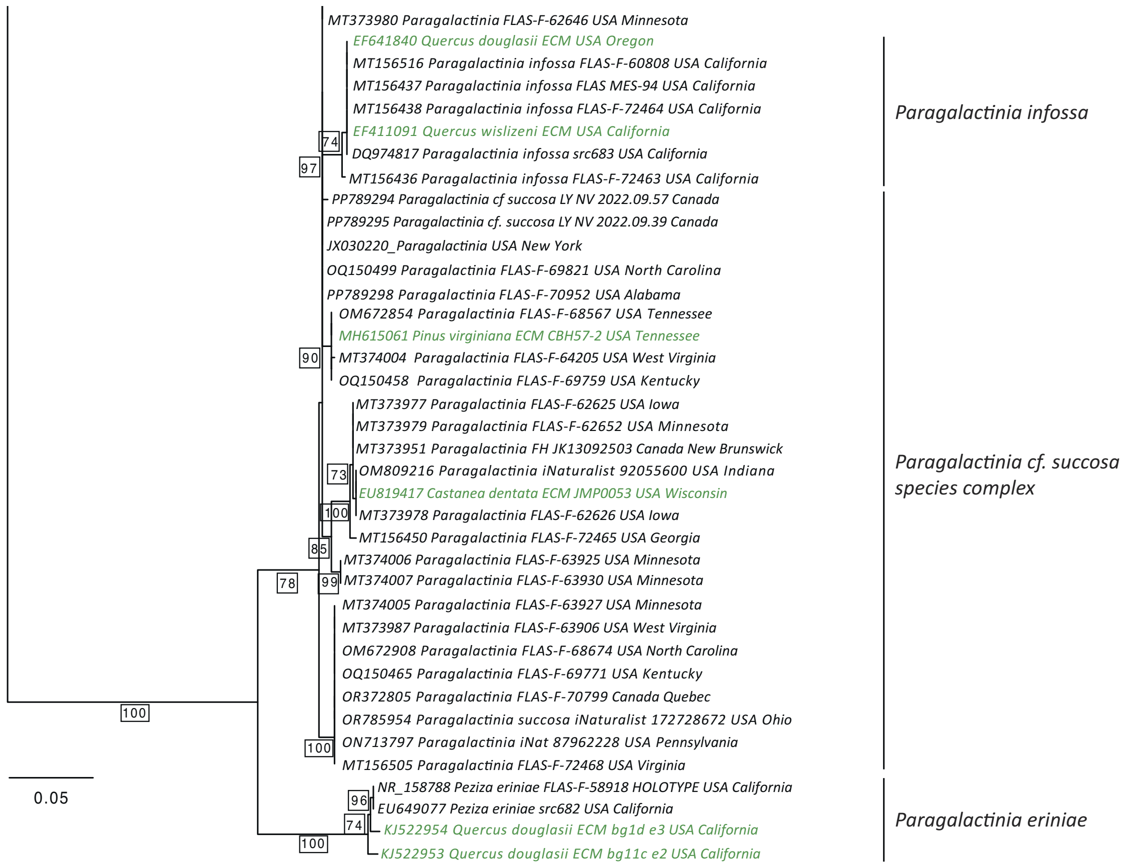

Fig. 1 – (continued)

Daniel Boone National Forest, Dog Slaughter Trail 414, upper trailhead, 36.848056° N 84.272778° W, 320 m a.s.l., on soil of creek bank, *leg.* J. Van Wyk, 23 Sep. 2022, herb. FLAS-F-69795. Minnesota, Rice Co., Nerstrand Big Woods State Park, on soil in hardwood forest dominated by *Quercus* and *Acer* spp., *leg.* A. Grupe, M. Jusino, N. Reynolds, M. Caiafa Sepulveda, 11 Aug. 2019, herb. FLAS-F-63923. Minnesota, Rice Co., Nerstrand Big Woods State Park, on soil in hardwood forest dominated by *Quercus* and *Acer* spp., *leg.* R. Healy, 12 Jul. 2010, herb. MIN RH1195. North Carolina, Appalachian Mountains, Great Smoky Mountains National Park, Winding Stair Gap, 35.121389° N 83.547778° W, 1121 m a.s.l., on soil in mixed forest, *leg.* M.E. Smith, 6 Sep. 2021, herb. FLAS-F-68422. North Carolina, Cherokee Co., Appalachian Mountains, Great Smoky Mountains National Park, Smokemont, Bradley Fork Trail, 35.563333° N 83.31° W, 674 m a.s.l., on soil bank by trail above river, *leg.* R. Healy, 24 Sep. 2022, herb. FLAS-F-69809. Ibid, 35.56° N 83.308889° W, 704 m a.s.l., 25 Sep. 2022, herb. FLAS-F-69820. South Carolina, Oconee Co., Appalachian Mountains, Nanatahala National Forest, Bullpen Road, Trail 81, 35.003056° N 83.054444° W, 875 m a.s.l., *leg.* R. Healy, 5 Sep. 2021, herb. FLAS-F-68348. Tennessee, Blount Co., Appalachian Mountains, Great Smoky Mountains National Park, Schoolhouse Gap Trail, 35.636111° N 83.736111° W, 552 m a.s.l., on soil in mixed forest of *Carpinus*, *Betula*, *Tilia*, *Pinus*, *Quercus*, and *Tsuga* spp., *leg.* R. Healy, 6 Aug. 2021, herb. FLAS-F-68232. Tennessee, Blount Co., Appalachian Mountains, Great Smoky Mountains National Park, Schoolhouse Gap Trail, 35.627778° N 83.726389° W, 485 m a.s.l., on soil in mixed forest (mainly with *Carpinus*, *Quercus*, *Tilia*, *Betula* and minor components of *Pinus* and *Tsuga*), *leg.* R. Swenie, 26 Sep. 2022, herb. FLAS-F-69835. *Ibid*. *leg.* R. Healy, herb. FLAS-F-69845. Vermont, Washington Co., Berlin, Irish Hill trail, along stream where it crosses the main trail, *leg.* B. Lemmond, 14 Aug. 2022, herb. FLAS-F-71109. Ibid, FLAS-F-71111.

Studied and documented collections (not sequenced): *Paragalactinia pseudomichelii*. Canada. Ontario, Lanark, 45.104042° N 76.341208° W, 183 m a.s.l., on soil, close to a log of *Abies balsamea*, in a mixed forest (*Thuja occidentalis, Abies balsamea*, *Ulmus*, *Acer saccharum*), *leg.* I. Khomenko, 6 Aug. 2023, IK 2023-2051, sample not kept [iNaturalist #177377601]. Ontario, Lanark, Rosetta Road, 45.10524° N 76.33899° W, 182 m a.s.l., on soil, in a mixed forest (mainly *Acer saccharum*, but also *Populus grandidentata*, *Quercus rubra*, *Thuja occidentalis*, *Abies balsamea* and *Picea glauca*), *leg.* I. Khomenko, 9 Jul. 2023, IK 2023-1975, sample not kept [iNaturalist #172034785]. Ontario, Ottawa, Greenbelt, 45.296328° N 75.841162° W, 113 m a.s.l., on the ground near a stump and logs, in a mixed forest, under *Thuja occidentalis* and *Picea glauca*, *leg.* I. Khomenko, 24 Sep. 2022, IK 2022-1749B, sample not kept [iNaturalist #136523718]. Ontario, Ottawa, Gloucester-Southgate, Orléans, 45.403417° N 75.58857° W, 81 m a.s.l., on the side of a path in a mixed forest mainly with *Acer rubrum*, but also *Pinus strobus* nearby, *leg.* I. Khomenko, 24 Jul. 2021, IK 2021-1019, sample not kept [iNaturalist #88451109].

Sequenced (not studied) collection: *Paragalactinia michelii.* USA. Michigan, Waterloo Recreational Area, along Oak Woods trail, northeast of Jackson, on soil, *leg.* N. Nuygen & M.E. Smith, 8 Jun. 2014, MES-752, herb. FLAS-F-72466.

Distribution: This new species is present in eastern temperate North American woodlands. Based on our collections, sequences available in GenBank and data found on the Internet (especially iNaturalist), it is found as far north as Ontario (Canada) east to New Brunswick (Canada) south to Alabama (USA) and west to Minnesota and Iowa (USA). It is probably frequent but confused with *P. michelii* or other species (see Discussion). To our knowledge, it is not present in Europe.

Fig. 2 – Phylogram of the genus *Paragalactinia* and some genera of *Pezizaceae* inferred from maximum likelihood (ML) analysis of the LSU region, rooted with two species of *Ascobolus* (*Ascobolaceae*). Nodes with ≥ 70% ML bootstrap support are annotated with their support values.

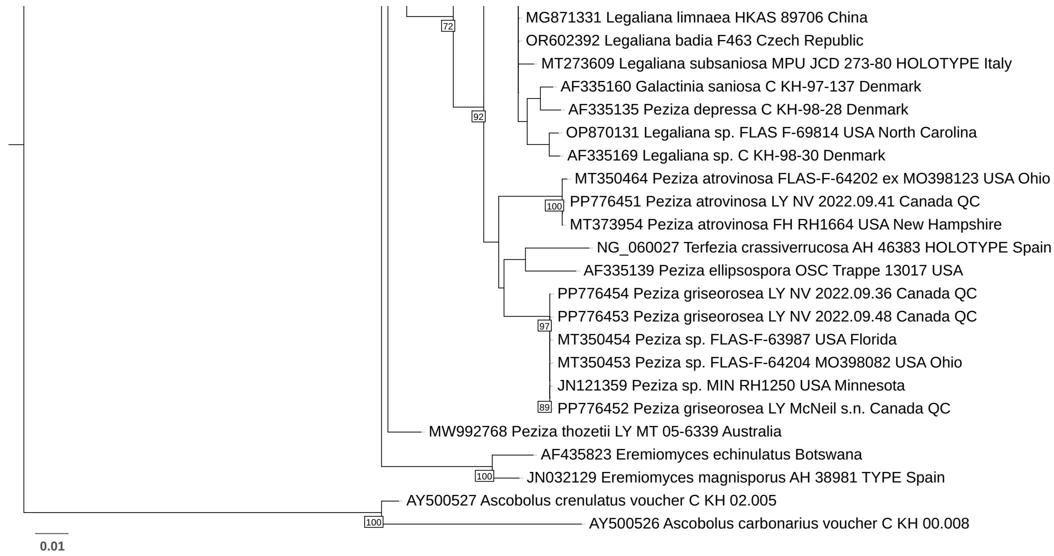

Fig. 2 – (continued)

Discussion

Paragalactinia pseudomichelii is described as a new North American species based on its morphological and molecular characters. This species is morphologically close to *P. michelii* but differs in the color of the external surface of the apothecia, whitish to pale greyish vs. yellow-ochre in *P. michelii* (Plate 4). The latter was described in detail by Van Vooren *et al.* (2022) and shows a certain variability, but it seems that the color of the external surface is rather constant. Both share some other characters such as a violet hymenium in most young specimens, presence of an exuded fluid when the context is damaged, and similar spore ornamentation. Older specimens may appear more reddish brown in *P. michelii*, but this feature is not apparent in *P. pseudomichelii*. Microscopically, the size and ornamentation of ascospores are very similar (Plate 5) and cannot be used to discriminate these two species. In this context, it is not surprising that they may be confused in the field, especially with old specimens, because these two species seem to overlap in Canada and the uppermost region of northeastern USA (Michigan). *Paragalactinia michelii* has not been documented further south than Michigan, while *P. pseudomichelii* appears to be relatively common on soil in eastern *Quercus*-dominated woodlands or mixed woodlands of North America. In contrast, *P. michelii* has rarely been collected in North America, mainly in Canada. In the North American literature devoted to discomycetes, *Paragalactinia michelii* is not cited by Seaver (1928), Abbott & Currah (1989) and Tylutki (1993). Pfister (1982: 17) applied the name *Peziza michelii* to what Seaver (1928) refers to as *Peziza brunneovinosa* Clem., in conformity with the type revision he made of Clements' collections (Pfister, 1978: "The species seems closest to *Peziza michelii* (Boud.) Dennis […]"). *P. brunneovinosa* was initially described in Bessey *et al.* (1896) from a collection made in Nebraska (USA). As the description reports "ascomata caespitose [...] hymenium deep brown-wine-color, beneath paler, or ochraceous", we think this cannot apply to *P. pseudomichelii* because this species does not form caespitose clusters and is much lighter and more yellowish in color.

Peziza griseorosea W.R. Gerard is quite variable based on our experience but may also be confused with *P. pseudomichelii*. This is because of its occurrence on soil in the same type of woodlands and similar geographic distribution, pink tinges on the hymenium, and its biguttulate ascospores that are similar in size, though the spore ornamentation is lower in *P. griseorosea*. Both have laticiferous hyphae in the excipulum and exude a whitish fluid when the context is damaged, but the color changes to yellowish in *P. pseudomichelii*, not in *P. griseorosea*. Phylogenetically *P. griseorosea* is distant from *Paragalactinia* (Fig. 2).

Peziza roseolilacina Clem. was reported from Nebraska (USA) as a small cup fungus, 10–15 mm diam., with a lilac hymenium, and uni- to biguttulate smooth ascospores measuring 17–18 × 10 µm (Clements, 1901). Unfortunately, the type material was not retrieved by Pfister (1978). It is not possible to confirm the spore ornamentation, a character that Clements incorrectly reported on several species based on type revisions done by Pfister (1978).

Galactinia viriditincta Clem. is another species described from Nebraska (Pound & Clements, 1896) with a milky context, but this fluid is reported as "grayish-green", the hymenium is brownish-olivaceous and the ascospores are uni- or biguttulate, 15 × 8 µm (15–17 × 7–8 µm after Pfister, 1978). Such characters point to the genus *Paragalactinia*, but to the "*succosa*" complex.

Heteroplegma caeruleum Clem. is another North American species described with a blue-colored fluid (Clements, 1903), and finely verrucose ascospores 18–20 × 8–9 µm, but its large apothecia, 50–80 mm diam., have an olive hymenium.

Recently, Beug *et al.* (2014: 59) illustrated *P. michelii* with a small picture, but this represents *P. pseudomichelii*, whilst the photograph illustrating *Peziza saccardoana* Cooke, on the same page, surely represents the true *P. michelii*. The latter is also cited in a key of the *Pezizaceae* of the Pacific Northwest (Coulter, 1988), but without evidence of its occurrence in this part of the USA (no picture, no collection recorded). Finally, *P. michelii* is described and illustrated in McNeil (2019: 100), but the collection presented might be *P. pseudomichelii* based on the photograph.

Paragalactinia is recognized as one of the ectomycorrhizal lineages within the *Pezizales* (Tedersoo *et al.*, 2010, as "*Galactinia*"). The trophic mode of *P. pseudomichelii* is uncertain since the species has not been included in stable isotope studies or been detected in ectomycorrhizal root tip studies. However, available evidence suggests that it is likely ectomycorrhizal because 1) it is nested within an ectomycorrhizal lineage, and 2) it fruits on soil in forests dominated by ectomycorrhizal trees. *Paragalatinia michelii* is known to be associated with *Betulaceae* (Tedersoo *et al.*, 2006; Tedersoo *et al.*, 2008; Ashraf *et al.*, 2012; Hanif & Khalid, 2023), *Juglandaceae* (*Carya*) (Wilgan & Leski, 2022), and *Malvaceae* (*Tilia*) (Dudka *et al.*, 2020), but may also form mycorrhizae with *Picea* (Tedersoo *et al.*, 2009). *Paragalactinia infossa* and *P. eriniae* are associated with *Fagaceae* (*Quercus*) (Morris *et al.*, 2008; Smith, 2014). Our phylogenetic analysis of root tip sequences downloaded from NCBI show that other species of *Paragalactinia* are also associated with the roots of *Fagaceae* (*Castanea*, *Fagus*, *Quercus*), *Juglandaceae* (*Carya*), and *Malvaceae* (*Tilia*)

Plate 1 – *Paragalactinia pseudomichelii* *in situ* – A: coll. LY NV 2022.09.40. B: coll. HRL0215. C: coll. HRL3696. D: coll. HRL0892. All photographs by R. Lebeuf, except A by N. Van Vooren.

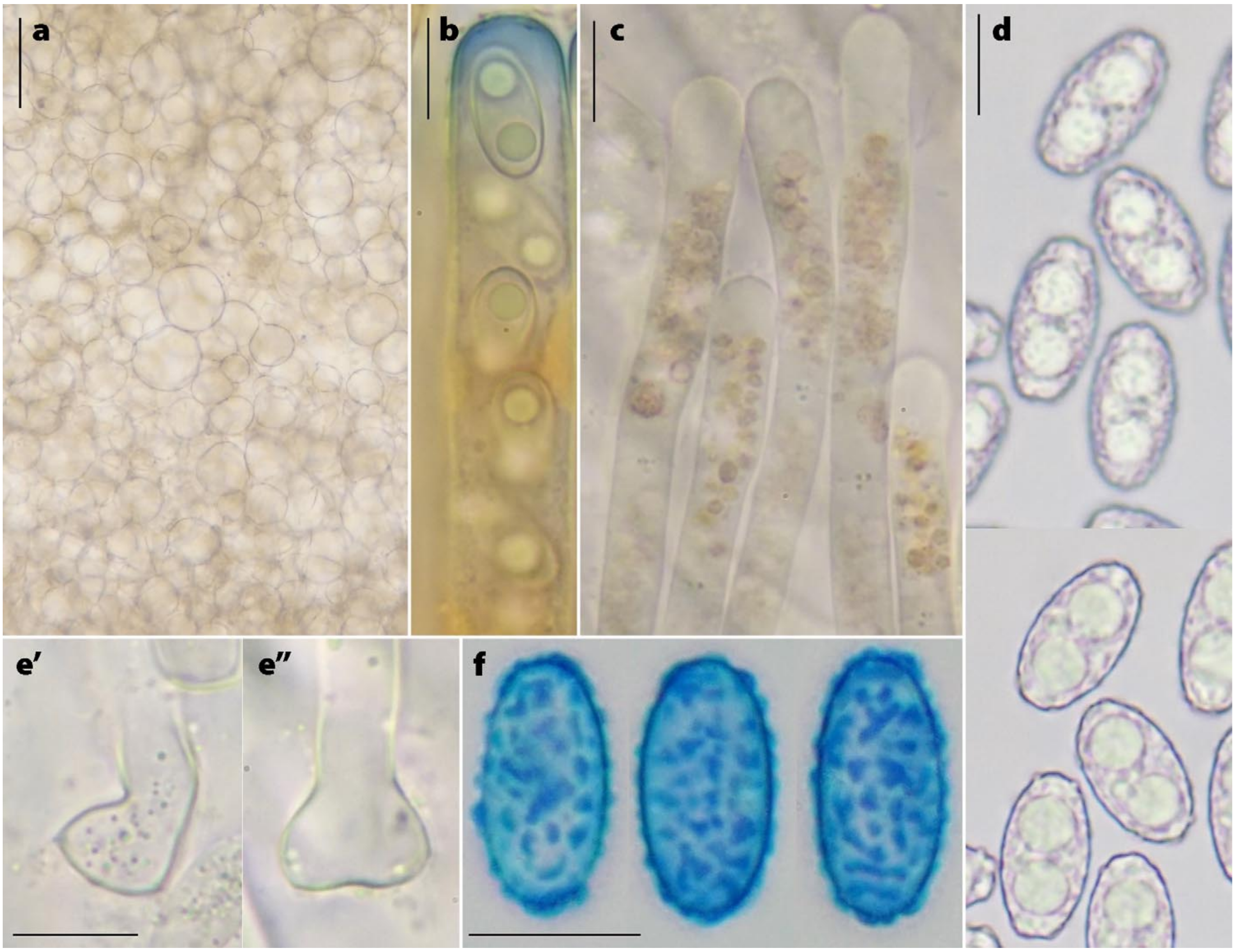

Plate 2 – *Paragalactinia pseudomichelii*. Microscopic characters (coll. IK 2023-2051) – a: Excipulum. b: Amyloid reaction of asci. c: Top of the paraphyses (coll. 2022-1749B). d: Ascospores. e'–e": Asci base. f: Detail of ascospore ornamentation. All photographs mounted in water, except b in Lugol's solution and f in Cotton blue. Scale bars = 10 µm, except a = 100 µm. Photos by I. Khomenko.

as well as *Betulaceae*. Although uncommon, some sequences of *Paragalactinia* spp. were detected from the roots of *Pinaceae* (*Pinus*) (Fig 1). We expect that future root tip studies or stable isotope studies in the eastern hardwoods of N.A. will show that *P. pseudomichelii* also forms ectomycorrhizae with diverse angiosperms and perhaps also with *Pinaceae*.

Updated key to *Paragalactinia*

This key is an updated version of the key published in Van Vooren (2022). It applies to a group of cup- or truffle-like fungi with a succulent context, i.e. exuding a fluid, becoming yellowish or greenish-yellow when exposed to the air, and, microscopically, with asci bluing in iodine solutions (amyloid reaction of WT type after Van Vooren, 2020), with or without croziers, containing eight uniseriate guttulate ascospores ornamented with warts. Information of distribution is given only if it seems localized to a specific area.

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Author's contributions

NVV was responsible for the conception of the study and has written the first version of the manuscript. All authors were responsible of their specimens sampling and morphological studies. NVV designed the plates 1 and 3 and IK designed the plates 2 and 4. RH managed the sequencing of samples, except those collected by NVV and RL. RH also registered the sequences in GenBank. The ML analysis on LSU was done by NVV, whilst the ITS analysis was conducted by RH. The successive versions of the manuscript were edited by all authors; they read and approved the final manuscript.

Worldwide key to *Paragalactinia*

1. Ascomata hypogeous [North America] 9
1. Ascomata epigeous.......... 2

2. Asci with croziers.......... 3
2. Asci without croziers.......... 6

3. Hymenium dark chestnut brown to blackish brown.......... ***P. benatii***
3. Hymenium first lilac to violet, becoming yellowish, pale brown to purplish brown 4

4. External surface yellow ochre when fresh 5
4. External surface whitish to pale greyish. Hymenium first lilac to violet, becoming yellowish to pale brown, often with purplish hues. Ascospores 15–17.2 × 7.8–9.1 µm, Qm = 1.9, ornamented with irregular warts, sometimes forming short ridges [North America] .... ***P. pseudomichelii***

5. Hymenium first violet, soon becoming purplish brown; external surface yellow ochre. Ascospores 15–17 × 7–9 µm, Qm = 1.9, ornamented with irregular, angular warts, often coalescent, and short ridges.......... ***P. michelii***
5. Hymenium vinaceous brown to dark violet, becoming purplish brown in the end. Ascospores 15–19 × 8–10 µm, Qm = 2.0–2.1, ornamented with small warts, more or less elongated, sometimes forming ridges [Mediterranean basin].......... ***P. berthetiana***

6. Ascospores mainly uniguttulate. Apothecia rather small, often < 20 mm diam. .... ***P. succosella***
6. Ascospores mainly biguttulate. Apothecia larger.......... 7

7. Ascospores narrowly ellipsoid, Qm ≥ 2.0 [Mediterranean basin].......... ***P. hypoleuca***
7. Ascospores with Qm < 2.0 8

8. Hymenium grey to grey-brown. Ascospores 16–20 × 8–12 µm, with irregular and coarse warts up to 2 µm high .... ***P. succosa*** [complex]
8. Hymenium fuliginous dark brown. Ascospores 15–19 × 8.5–10 µm, with smaller warts up to 1 µm high.......... ***P. infuscata***

9. Interior highly infolded; ascospores biseriate .... ***P. eriniae***
9. Interior less infolded with open spaces; ascospores uniseriate .... ***P. infossa***

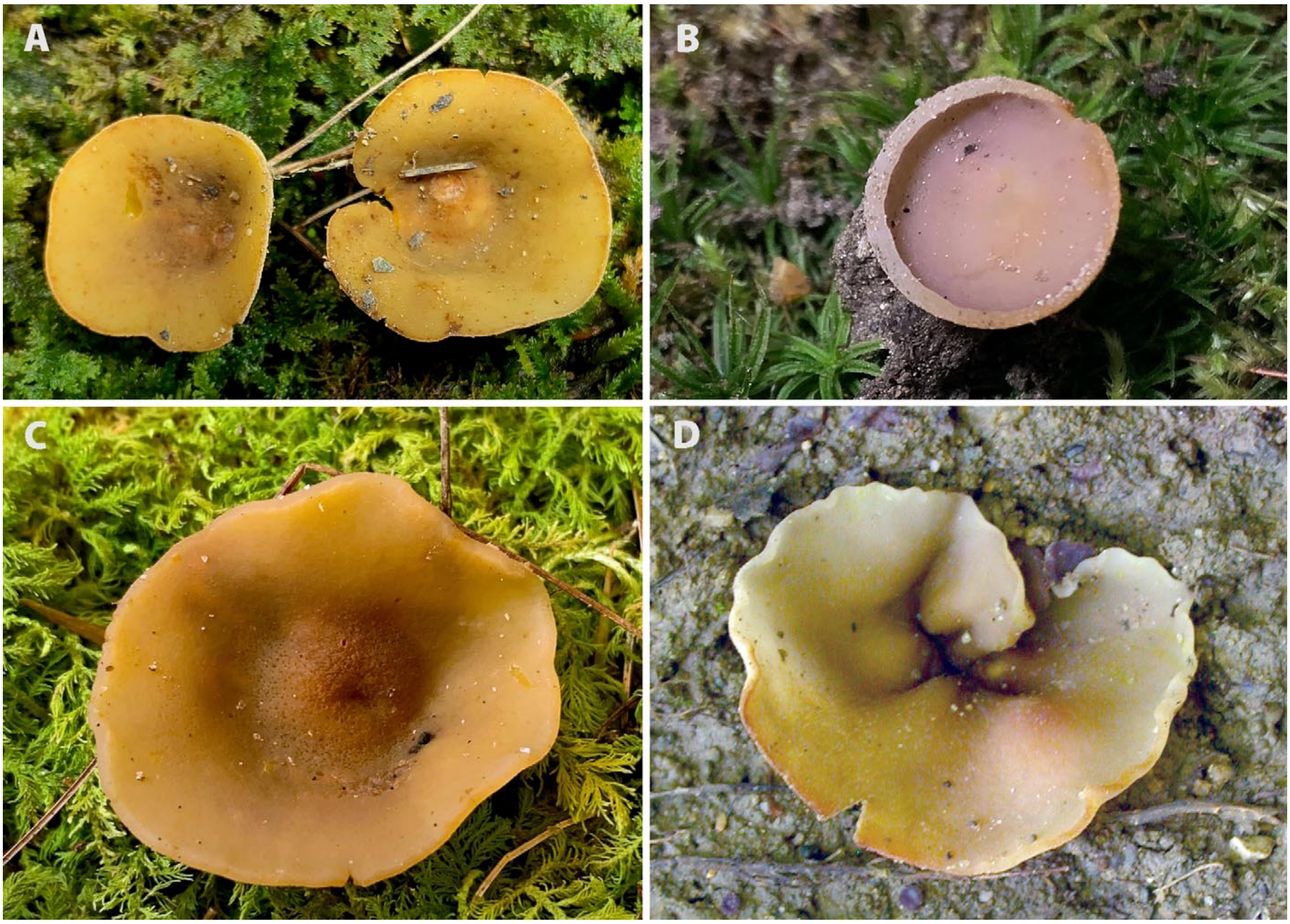

Plate 3 – *Paragalactinia pseudomichelii* – A: coll. FLAS-F-68244. B: coll. FLAS-F-69820. C: coll. FLAS-F-68348. D: coll. MIN RH1195. Photos A, C by M.E. Smith, B, D by R. Healy.

Plate 4 – *Paragalactinia michelii* *in situ* – A: coll. HRL1708. B: coll. FLAS-F-68824. Photo A by R. Lebeuf, B by I. Khomenko.

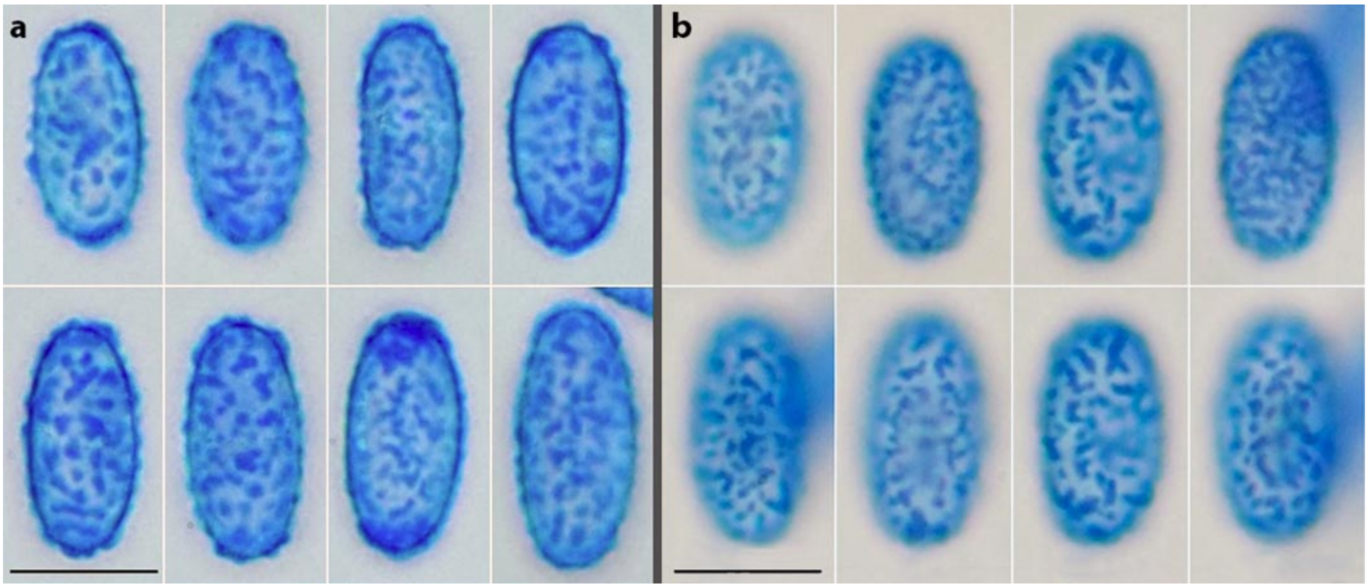

Plate 5 – Comparison of micrographs of ascospores mounted in Cotton blue of (a) *P. pseudomichelii* (IK 2023-2051) and (b) *P. michelii* (FLAS-F-68824). Scale bars = 10 µm. Photos by I. Khomenko.

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