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Pouzarella alissae, a new species from northwestern California, United States

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Abstract —*Pouzarella alissae* sp. nov. is known only from north coastal California. It is characterized by the evanescent silvery-white layer of fibrils and squamules initially covering the pileus and stipe surface, the dark greyish brown to dark brown innately fibrillose pileus and stipe, a pileipellis of entangled hyphae with cylindro-clavate terminal cells, a suprapellis with cytoplasmic pigments, a subpellis with encrusted pigments, heterogeneous lamellar edges, lageniform to rostrate-ventricose leptocystidia, cheilocystidia and pleurocystidia, abundant aborted basidia, and basidiospores averaging <11 µm in length.

Key words —*Basidiomycota*, *Entolomataceae*, phylogeny, taxonomy

Introduction

The name *Pouzarella* is applied to fungi in the *Entolomataceae* that possess stipitate–pileate basidiomata with the following features: basidiome typically mycenoid with a strigose stipe base, pileipellis disc composed of erect to semi-erect multicellular hyphae, tramal hyphae with encrusted pigments, nodulose heterodiametric 5–9-angled basidiospores, and the absence of clamp connections. Phylogenetically, these fungi form a well-supported clade (He et al. 2013), and *Pouzarella* Mazzer is applied as a generic concept (Mazzer 1976; Karstedt et al. 2007; Baroni et al. 2008, 2012; Horak 2008) or is treated as *Entoloma* subg. *Pouzarella* (Mazzer) Noordel. (Manimohan et al. 2006, Noordeloos 2004, He et al. 2013). We treat *Pouzarella* as a genus because all species form a monophyletic group with distinctive morphological characters.

Although *Pouzarella* species are distributed worldwide, they are encountered most frequently in mesic areas that receive >20 in. annual rainfall (Mazzer 1976). In North America, *Pouzarella* is common in the eastern United States

and Canada, but is rare in the west where it is restricted to a narrow coastal region extending from southern California to southern British Columbia (Largent 1994, Mazzer 1976).

Of the fifty-nine *Pouzarella* species reported worldwide in Index Fungorum, four have been described from western North America: *P. fernandae* (Romagn.) Largent and *P. fulvostrigosa* (Berk. & Broome) Mazzer from Washington, *P. araneosa* (Quél.) Mazzer from California, and *P. versatilis* (Gillet) Mazzer from California, Oregon, and Washington (Largent 1994, Mazzer 1976). In this report, we describe a third and new species from California, *P. alissae*.

Materials & methods

Macromorphological and micromorphological features

Techniques and equipment for collecting and describing basidiomata in the field and GPS coordinates have been described in Largent et al. (2011a,b), while techniques for color descriptions using Kornerup & Wanscher (1978) and factors determined from mathematical analyses in the descriptions are covered in Largent et al. (2013a,b). Dried specimens were examined microscopically with a research-grade trinocular Nikon Eclipse Ci-L compound microscope. Microphotographs were made using a Lumenera Infinity 2-5 CCD digital camera, and all microscopic measurements were obtained using Infinity Analyze software 6.5.0.

DNA sequences and phylogenetic analyses

Sequences of *P. alissae* were obtained from the mitochondrial small subunit ribosomal RNA (mtSSU), the nuclear large subunit ribosomal RNA (LSU), and second largest subunit of RNA polymerase II (RPB2) following protocols described in Largent et al. (2011b). Sequences were edited using Sequencher 4.2.2 (Gene Codes, USA) and aligned using MAFFT v. 7 (Katoh & Standley 2013). Thirty-eight sequences were generated for this phylogenetic analysis along with sequences obtained from GenBank including those for the majority of *Pouzarella* species and from species from other representative clades as defined in Baroni & Matheny (2011) (Table 1).

Table 1. Collections used in the phylogenetic analyses.
New sequences generated for this study are shown in bold.
Square brackets enclose GenBank names differing from those in Fig. 1.

Taxa	Collection identifier	GenBank accession numbers		
		mtSSU	LSU	RPB2
Alboleptonia angustospora [*Alboleptonia* aff. *sericella*]	MCA1978	GU384583	GU384609	GU384632
Alboleptonia sericella [*Entoloma sericellum*]	10	GQ289330	GQ289190	GQ289261
Catathelasma imperiale	11CA001A	**KR869913**	**KR869941**	KC816816
Claudopus minutoincanus	DLL9871	HQ731511	HQ731514	HQ731517
Claudopus parasiticus	10617 TJB	**KR233902**	**KR233872**	**KR233924**
Claudopus viscosus	DLL9788	HQ31513	HQ31516	HQ31518
Clitocella fallax [*Rhodocybe fallax*]	25668OKM	**KR869909**	AF261283	KC816937

Clitopilopsis hirneola	REH8490	GU384587	GU384611	KC816904
Clitopilus apalus	26394WAT	**KR869907**	**KR869936**	KC816906
Entocybe haastii [*Entocybe* sp.]	DLL10087	JQ793645	JQ793652	JQ793659
Entocybe trachyospora [*Rhodocybe trachyospora*]	5856 TJB	GU384605	GU384629	GU384658
Entoloma abortivum	5772 TJB	**KR233897**	**KR233869**	**KR233926**
E. albidoquadratum	i4	GQ289291	GQ289151	GQ289223
E. bloxamii	219	GQ289294	GQ289154	GQ289226
E. caespitosum	GDGM 27564	JQ993070	JQ410327	JQ993078
E. changchunense	HMJAU 3886	JQ993061	JQ993095	—
E. crassicystidiatum	GDGM 28821	JQ993058	JQ291567	JQ993083
	GDGM 27357	JQ993056	JQ291569	JQ993085
E. discoloratum	DLL10217	JQ793646	JQ793653	JQ793660
E. fragilum	MCA2415	KJ021690	KJ021700	KJ021694
E. furfuraceum	GDGM 28818	JQ993062	JQ993094	JQ993084
E. indoviolaceum	i13	GQ289312	GQ289172	GQ289243
E. myrmecophilum	231	GQ289314	GQ289174	GQ289245
E. omiense	GDGM 27563	JQ993067	JQ410330	JQ993079
E. pallidocarpum	GDGM 28828	JQ993074	JQ410331	JQ993080
E. praegracile	GDGM 29251	JQ993072	JQ320129	JQ993077
E. procerum	70	GQ289323	GQ289183	GQ289254
E. prunuloides	40	GQ289324	GQ289184	GQ289255
E. readiae	102	GQ289326	GQ289186	GQ289257
E. rhodopolium	8	GQ289327	GQ289187	GQ289258
E. rugosiviscosum	DLL9676	JQ793647	JQ793654	JQ793661
E. sericatum	28	GQ289329	GQ289189	GQ289260
E. sinuatum	50	GQ289333	GQ289193	GQ289264
E. subaraneosum	KA12-1534	—	KJ523137	—
	GDGM 28823	—	JQ291568	—
E. aff. *subsinuatum* [*E. sinuatum*]	5349 TJB	**KR233896**	**KR233868**	AY691891
E. tenuissimum	GDGM 28813	JQ993059	JQ993097	JQ993086
	GDGM 28814	JQ993060	JQ993096	JQ993087
E. undatum	18	GQ289342	GQ289202	GQ289270
E. valdeumbonatum	189	GQ289343	GQ289203	GQ289271
E. violaceotinctum	DLL10088	JQ793643	JQ793650	JQ793657
E. violaceovillosum	i2	GQ289345	GQ289205	GQ289273
E. yunnanense	GDGM 28815	JQ993057	JQ320128	—
Inocephalus hypipamee	DLL10071	JQ624604	JQ624609	JQ624616
I. "*lactifluus*"	7962 TJB	—	AF261304	—
I. plicatus	DLL9691	JQ624605	JQ624610	JQ624617
I. virescens	DLL9772	**KR233889**	**KR233859**	**KR233918**
Lepista nuda	11CA041	**KJ021692**	**KJ021705**	KJ136110
Leptonia ambigua	DLL9872	JQ756399	JQ756414	JQ756429
L. boardinghousensis	DLL10086	JQ756398	JQ756413	JQ756428
L. omphalinoides	DLL9673	JQ756400	JQ756416	JQ756431
L. odorifera	6534 TJB	KR233899	KR233870	KR233927
L. poliopus	DLL10209a	JQ756402	JQ756418	JQ756433

L. serrulata [*Entoloma serrulatum*]	163	GQ289332	GQ289192	GQ289263
L. "subdecurrentiba" [*L.* sp.]	MCA1486	GU384589	GU384623	GU384635
L. tjallingiorum [*Entoloma tjallingiorum*]	243	GQ289337	GQ289197	GQ289267
L. trichomata	11CA006	**KR233873**	**KR233873**	**KR233903**
L. umbraphila	DLL9793	JQ756409	JQ756424	JQ756440
Mycena aff. *pura*	11CA007	**KR869914**	**KR869942**	KC816995
Nolanea cetrata	DLL9531	KF738927	KF738942	KF771346
N. cf. conferenda [*Entoloma conferendum*]	6	GQ289300	GQ289160	GQ289231
N. hebes [Entoloma hebes]	46	GQ289310	GQ289170	GQ289241
N. sericea	DLL9527	**KR233882**	**KR233851**	**KR233913**
N. strictior var. *isabellina* [*E. strictius* var. *isabellinus*]	7710 TJB	GU384594	GU384618	GU384641
Panellus stipticus	11CA052	**KR869915**	**KR869943**	KC816996
Paraeccilia sericeonitida [*Entoloma sericeonitidum*]	7144 TJB	EF421098	AF261315	EF421016
Pouzarella albostrigosa	DLL9641	HQ876557	HQ876535	HQ876513
	DLL9663	HQ876558	HQ876536	HQ876514
P. alissae	DLL10490	**KT074359**	**KT074357**	**KT074361**
P. araneosa [*Entoloma araneosum*]	14	GQ289293	GQ289153	GQ289225
P. debilis	DLL9784	HQ876550	HQ876528	HQ876506
P. farinosa	DLL9900	HQ876537	HQ876515	HQ876494
	DLL9934	HQ876538	HQ876516	HQ876495
P. fusca	DLL9623	HQ876549	HQ876527	HQ876505
P. lageniformis	DLL9895	HQ876545	HQ876523	HQ876501
P. lasia	DLL9662	HQ876551	HQ876529	HQ876507
	DLL9670	HQ876552	HQ876530	HQ876508
	DLL9729	HQ876553	HQ876531	HQ876509
	DLL9807	HQ876555	HQ876533	HQ876511
	DLL9811	HQ876556	HQ876534	HQ876512
P. farinosa	DLL9900	HQ876537	HQ876515	HQ876494
	DLL9934	HQ876538	HQ876516	HQ876495
P. nodospora	5716 TJB	**KT074358**	**KT074356**	**KT074360**
P. pamiae	DLL9794	HQ876539	HQ876517	HQ876496
	DLL9808	HQ876540	HQ876518	HQ876497
	DLL9834	HQ876541	HQ876519	HQ876498
P. parvula	DLL9901	HQ876546	HQ876524	—
P. pilocystidiata	DLL9848	HQ876542	HQ876520	HQ876499
P. setiformis	DLL9809	HQ876547	HQ876525	HQ876503
	DLL9810	HQ876548	HQ876526	HQ876504
Rhodocybe roseiavellanea	8130 TJB	**KR869901**	**KR869930**	KC816982
R. spongiosa	MCA2129	GU384604	GU384628	GU384657
Rhodophana nitellina	7861 TJB	**KR869900**	**KR869929**	KC816959
Tricholoma flavovirens	11CA038	KJ021691	KJ021704	KC816997
Trichopilus porphyrophaeus [*Entoloma porphyrophaeum*]	113	GQ289322	GQ289182	GQ289253

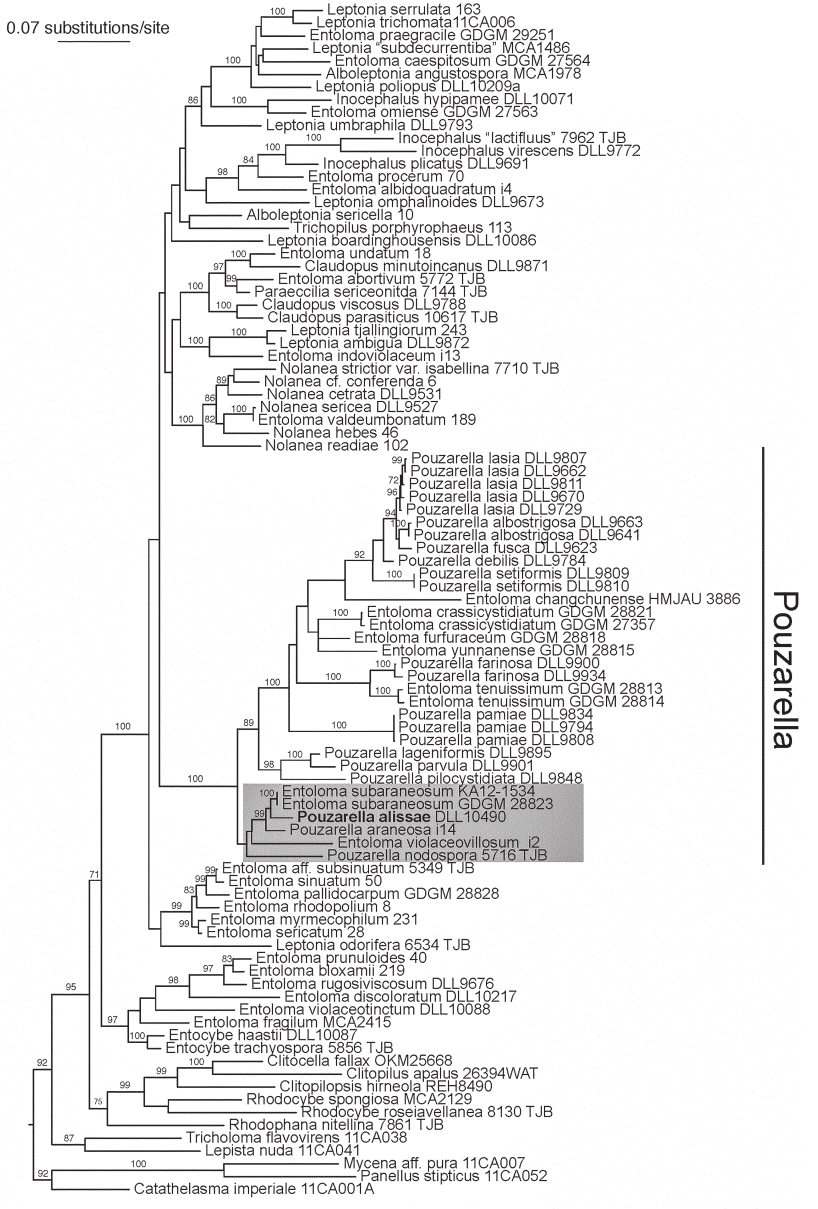

Figure 1. Maximum-likelihood topology, highlighting the sub-clade within *Pouzarella* containing the new species, *Pouzarella alissae*. Each sequence is labeled with the GenBank-listed isolate identifier. Branches with ≥70% support from 1000 rapid bootstraps are shown. *Catathelasma imperiale*, *Lepista nuda*, *Mycena* aff. *pura*, *Panellus stipticus*, and *Tricholoma flavovirens* were used as the outgroup.

After exclusion of the RPB2 introns and mtSSU hypervariable regions and manual optimization, alignment lengths were 592 bp (mtSSU), 1498 bp (LSU), and 1078 bp (RPB2). A Maximum Likelihood (ML) analysis based on a concatenated dataset with 1000 replicates and specifying a GTRGAMMA model using RAXML-HPC2 v. 8.1.11 (Stamatakis 2014) was carried out with a partitioned dataset for each RPB2 codon (three partitions) and separate partitions for mtSSU and LSU. One thousand bootstrap (BS) replicates were generated for assessment of branch supports.

Phylogeny

Monophyly of *Pouzarella* is well supported (BS = 100, Fig. 1), and *Pouzarella alissae* clusters with other representatives of *P.* sect. *Versatiles* (*P. araneosa* and *Entoloma subaraneosum*) with significant support (BS = 99, Fig. 1). These three species share similar features including a fibrillose pileus and stipe and thin-walled hymenial cystidia. Additionally, all three species have dark brown basidiomata, evanescent silvery-white coverings on stipe and pileus, appressed fibrillose pileipelli that become subglabrous, intracellular pigments in the pileus suprapellis, hyphae with encrusted pigments in the pileus subpellis and trama, and lageniform to rostrate-ventricose leptocystidioid cheilocystidia. They cluster within a larger clade with other members of *Pouzarella* sect. *Pouzarella* (*P. nodospora* (G.F. Atk.) Mazzer and *Entoloma violaceovillosum* Manim. & Noordel.), but without support (Fig. 1). Whether this lack of support for the morphologically based infrageneric classification is due to a lack of adequate taxon sampling or gene resolution will require further research.

Taxonomy

Pouzarella alissae Largent & Bergemann, **sp. nov**. Plates 1, 2

MycoBank MB 812300

Differs from *Pouzarella araneosa* by its dark brown pileus, shorter basidiospores, shorter cheilocystidia, and the presence of pleurocystidia.

Type — United States, California, Humboldt County, Prairie Creek Redwoods State Park, Intersection of Big Tree Trail and Prairie Creek Highway, in duff beneath *Acer circinatum* and *Polystichum munitum*, within 20 m of 41°22′33.15″N 124°00′51.66″W, 62 m elev., 22 November 2014, DL Largent 10490 (**Holotype**, HSC; GenBank KT074359 (mtSSU), KT074357 (LSU), KT074361 (RPB2)).

Etymology — named for Alissa Allen, who first discovered this species.

Basidiomata with the pileus and stipe initially covered with silvery-whitish evanescent layer of fibrils that forms squamules over the pileus surface, small pointed squamules on the pileus margin, and longitudinally arranged appressed fibrils on the stipe. When fresh, the stipe fibrils can be rubbed off revealing a dark brownish fibrillose layer on the stipe surface. In older basidiomata, this layer first disappears from the pileus before eventually disappearing on the

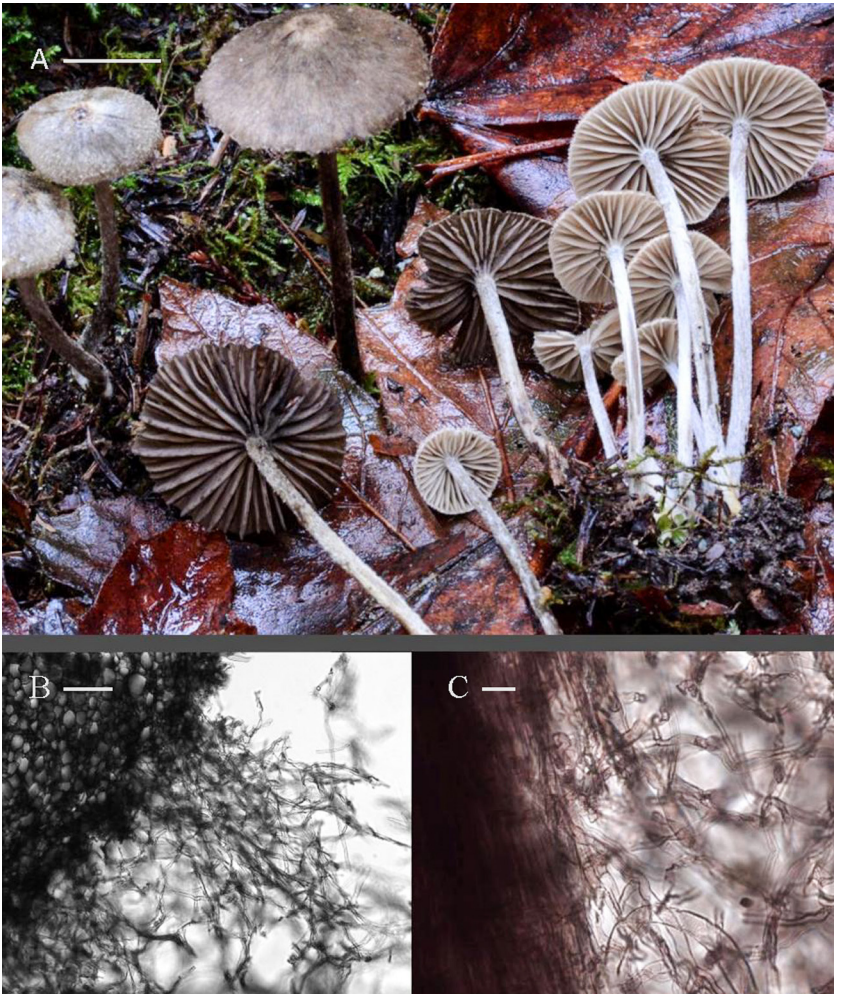

Plate 1. *Pouzarella alissae* (HSC DLL10490, holotype): A. basidiomata (Photo by Noah Segal); B. cross section through strigose stipe base; C. longitudinal section through strigose stipe base. Scale bars: A = 8 mm; B, C = 20 µm.

stipe. In silica gel-dried basidiomata, this layer is absent when sections of the basidiomata are viewed under the compound microscope.

Pileus 8–30 mm broad, 2–7 mm high, conic to campanulate, becoming convex to broadly convex, then plane, and finally uplifted; initially lacking an umbo or obscurely umbonate, broadly umbonate when mature; opaque (not

translucent and thus not translucent-striate), dull, scurfy or tomentulose on disc, innately fibrillose elsewhere; margin incurved then decurved and eventually plane; color overall dark brownish grey (4–5F2–3) fading to dark brown (5F3–4); flesh ≤1.5 mm above the stipe, pale greyish brown. Lamellae 6–12 mm long, 1.25–4 mm deep, initially adnexed and sigmoid and later narrowly to broadly adnate (at times with a small subdecurrent tooth), subdistant to distant, moderately broad, pale greyish-tan when young, maturing dark greyish-brown (5F2) with smoky greyish brown (5–7F5) spots and blotches, when fully mature with a pinkish cast from spores; edge fairly thick, at first concolorous and then developing whitish areas with spore maturation; lamellulae 3–4 between lamellae in two series [one always long (4–8 mm) and one always short (1–2.5 mm)]. Stipe 33–88 mm long, apex 1–2.5 mm diam. and base 1.25–4.5 mm diam., cylindrical, equal when young and enlarged at the base when mature, very finely pruinose at the apex and innately and appressed-fibrillose elsewhere, in very scattered places the fibrils separating and becoming matted in clumps; hairs or setae absent; basal area decidedly strigose up to 7–8 mm from substrate, brownish with reddish tones; basal tomentum also present, whitish (in some basidiomata developing orangish areas). Taste and Odor not distinctive. Spore deposit pinkish to dingy salmon-tan.

Basidiospores with 5–6 distinct angles in side and profile views, heterodiametric in side, profile, dorsal, and ventral views, 9–12 × 6–8 µm (m = 10.2 ± 0.8 × 7.2 ± 0.5 µm; E = 1.2–1.8; Q = 1.4 ± 0.1; n/1 = 40). Basidia cylindro-clavate, 34–47 × 8–13 µm (m = 41.2 ± 3.5 × 9.8 ± 1.5 µm; E = 3.2–6.0; Q = 4.3 ± 0.7; n/1 = 10), 4-sterigmate; aborted basidia abundant on the lamellar edge and face, wall thickening at first after which the entire contents become reddish brown in 10% NH_4OH or 3% KOH, cylindro-clavate, 21–39 × 4–11 µm (m = 29.9 ± 4.2 × 6.5 ± 1.6 µm; E = 3.1–8.3; Q = 4.8 ± 1.3; n/1 = 20). Lamellar edge heterogeneous. Cheilocystidia (leptocystidia) thin-walled, when present scattered (but often absent on some lamellae), colorless or staining reddish brown in alkali solutions, versiform (cylindro-clavate, clavate, obclavate), 34–62 × 10.5–16.4 µm or lageniform with a short to medium neck or rostrate-ventricose, 46–656 × 13–23.5 µm (including the neck or rostrum), rostrum typically short to medium (rarely longer), 3.1–7.0(–17.5) × 3.0–3.5 (–6.0) µm (n/1 = 4)). Pleurocystidia colorless, uncommon and very scattered, obclavate, acuminate-ventricose, lageniform, or rostrate-ventricose, 40–42.5 × 12–17 µm (including rostrum, which is 6.4–7.0 µm long and 2.7–7.0 µm diam.). Lamellar tramal hyphae subparallel, 125–564 × 9.2–42 µm (E = 9.2–19.1; n/1 = 10). Pileipellis composed of entangled hyphae, erect and resembling a trichodermium on the disc, semi-erect around the disc, becoming

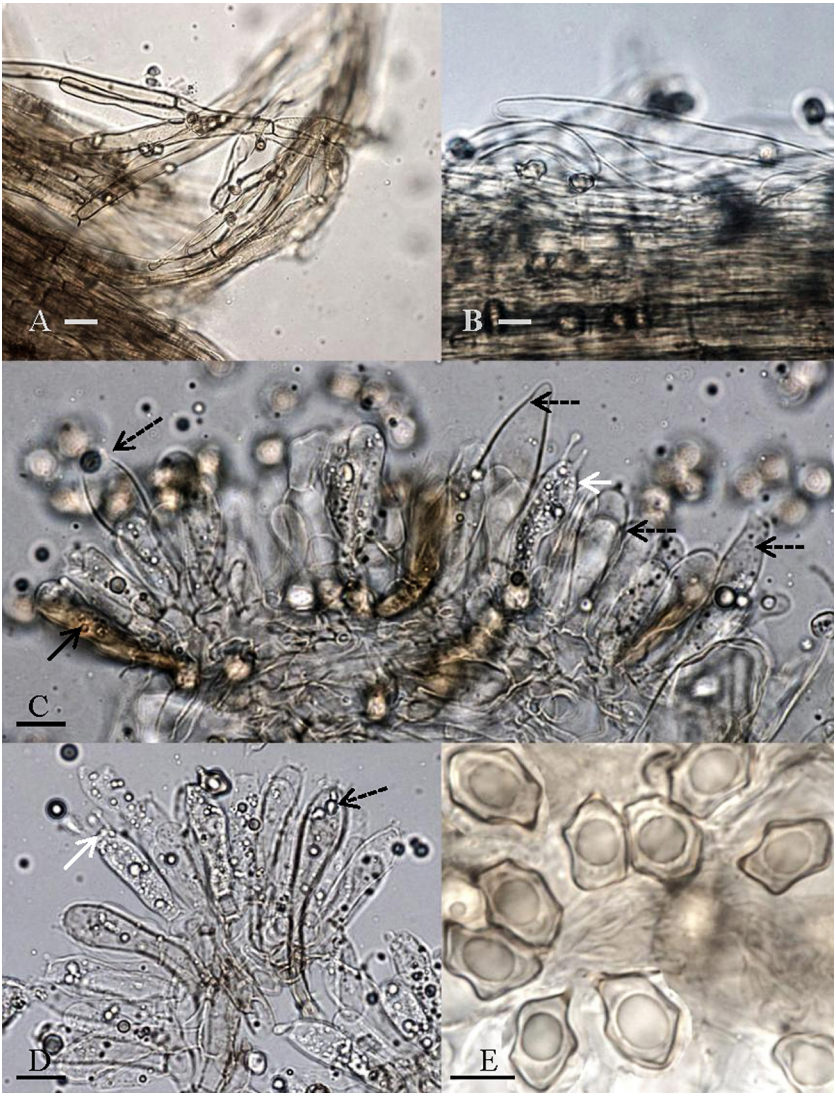

Plate 2. *Pouzarella alissae* (HSC DLL10490, holotype): A. pileipellis radial section from disc; B. stipitipellis with caulocystidium (longitudinal section from mid-stipe); C. basidium (white arrow), aborted basidium filled with dark pigment (black solid arrow), and cheilocystidia (dotted arrows); D. basidium (white arrow) vs. aborted basidium (dotted arrow); E. basidiospores. Scale bars: A, B = 12 µm; C = 15 µm; D = 10 µm; E = 7 µm.

prostrate towards the margin; PILEOCYSTIDIA (terminal cells) on or around the disc cylindro-clavate with a rounded apex 93–184 × 9–18 µm (*m* = 131.9 ± 31.1 × 12.6 ± 2.4 µm; E = 8.6–14.3; Q = 10.6 ± 2.1; n/1 = 11), towards and at the margin/edge longer and tapered, 166–218 × 14–17 µm. PILEAL TRAMAL HYPHAE subparallel and entangled, 49–310 × 3–30.5 µm (*m* = 152.2 ± 71.6 × 13.7 ± 9.4 µm; E = 4.9–46.3; Q = 15.9 ± 12.3; n/1 = 13). STIPITIPELLIS at the apex composed of hymenial elements (= pruinose), below the apex hyphae primarily prostrate, 2–4 cells deep but occasionally out-turned in some areas; caulocystidia (terminal cells) cylindro-clavate and similar in shape to the pileocystidia, 67–136 × 9–14 µm (m = 89.4 ± 24.4 × 11.2 ± 1.9 µm; E = 5.8–12.2; Q = 8.2 ± 2.5; n/1 = 6). STIPE TRAMAL HYPHAE nearly parallel, somewhat entangled, 9–67 µm diam. (n/1 = 7). OLEIFEROUS HYPHAE rare in the pileal trama. BRILLIANT GRANULES scattered to somewhat abundant in the basidia. LIPOID GLOBULES absent. PIGMENTS brownish, cytoplasmic and parietal on the apical 1–4 cells in the pileus and stipe suprapelli, encrusted as rings in the pileus and stipe subpelli, and decidedly encrusted on all tramal hyphae. Clamp connections absent.

ECOLOGY & DISTRIBUTION — Solitary, in clumps or loose clusters in wet duff under *Acer circinatum* Pursh and *Polystichum munitum* (Kaulf.) C. Presl in Prairie Creek Redwoods State Park.

COMMENTS — Morphologically, *P. alissae*, *P. araneosa*, *P. celata* Mazzer, *P. versatilis*, and *Entoloma subaraneosum* Xiao L. He & T.H. Li are placed in *Pouzarella* sect. *Versatiles* Mazzer because of their innately fibrillose pileus and stipe, thin-walled hymenial cystidia, and basidiospores that average <13 µm in length. *Pouzarella araneosa* and *E. subaraneosum* can be differentiated from *P. alissae* by their longer spores (9.5–15 µm for *P. araneosa*; 9.5–13 µm for *E. subaraneosum*), longer cheilocystidia (45–100 µm) with much longer necks, and lack of pleurocystidia (He et al. 2013; Mazzer 1976; Noordeloos 1992, 2004).

In North America, *P. celata,* described from Michigan, and *P. versatilis,* described from Europe (Noordeloos 1979, 1992, 2004; as *Entoloma versatile* (Gillet) M.M. Moser) and reported from western North America including California (Mazzer 1976) and Washington (Largent 1994), are similar in size and also produce dark brown colored basidiomata. Both species can be distinguished from *P. alissae* by their abundant and longer cheilocystidia and pleurocystidia and absence of silvery white evanescent fibrils and squamules on the pileus. *Pouzarella celata* also differs by its longer (11–14 µm) basidiospores and glabrous pileus surface that is streaked by innate silky grey fibrils while *P. versatilis* is distinguished by its abundant pleurocystidia, olivaceous green

tints or olivaceous brown pileus color (Noordeloos 2004, Largent 1994), and the metallic luster of the dried pileus (Mazzer 1976).

Pouzarella araneosa, reported from California under *Acer circinatum,* consisted of one basidiome with a missing stipe base (Largent 1994). The collection differs from *P. alissae* and *P. araneosa* by the absence of a silvery fibrillose layer, its medium dark brown (5–6F4) pileus, its translucent-striate pileal margin, and its broader and longer cheilocystidia; until more material is obtained, the diagnosis of the collection formerly reported as *P. araneosa* remains doubtful.

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