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Pouzarella (*Agaricales*, *Entolomataceae*) species from New South Wales (Barrington Tops National Park) and northeastern Queensland, Australia

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Abstract —Ten *Pouzarella* species are described from New South Wales and northern Queensland, Australia, of which eight (*P. albostrigosa*, *P. farinosa*, *P. fusca*, *P. lageniformis*, *P. pamiae*, *P. parvula*, *P. pilocystidiata* and *P. setiformis*) are new to science and two (*P. debilis* and *P. lasia*) are reported for the first time for Australia. Phylogenetic analyses of the mitochondrial small subunit rRNA (mtSSU), two variable domains (D1, D2) of the 25-28S large subunit rDNA (LSU), and a portion of the second largest subunit of the RNA polymerase (RPB2) gene support separation of these species into three clades. Morphological characters common to these clades are described, and a key to species based on the characters that are distinct between clades is included.

Key words — *Basidiomycota*, phylogeny, mtSSU, LSU, RPB2

Introduction

The genus *Pouzarella* Mazzer (*Entolomataceae, Agaricales*) was first monographed on a worldwide basis by Mazzer (1976). He proposed replacing *Pouzaromyces* Pilát with *Pouzarella* because the type species of *Pouzaromyces*, *Agaricus fumosellus* G. Winter, had no extant type material and the protologue description had been interpreted as probably referring to a coprinaceous species (Mazzer 1976, Noordeloos 1984, 1992; see Baroni et al. 2008 regarding nomenclature). The name *Pouzarella* has since been accepted by many researchers (Largent 1994, Karstedt et al. 2007, Baroni & Ortiz 2002, Baroni et al. 2008, Horak 2008).

Pouzarella combines the following features: typically mycenoid basidiomata (often resembling a smallish *Inocybe* (Fr.) Fr.); strongly fibrillose, squamulose, or hispid squamulose pilei; often radially strigose stipe bases; tramal hyphae with externally incrusting pigments; pileipellis and stipitipellis hyphae with parietal and/or externally incrusting pigments; a pileipellis (at least on the pileus center) with erect to semi-erect hyphae composed of chains of cells; nodulose-angular, heterodiametric basidiospores with 6–9 facets; and a hymenium frequently, but not always, with dark brown contents or aborted basidia.

Because there have been no *Pouzarella* cited in Australian publications on *Entolomataceae* Kotl. & Pouzar (Gates & Noordeloos 2007, Gates et al. 2009, Noordeloos & Gates 2009, May & Wood 1997, Grgurinovic 1997), we provide the first reports and descriptions of *Pouzarella* in Australia. Of the ten *Pouzarella* species covered, four are newly described from the Barrington Tops National Park in central New South Wales, while four new species and two new records are cited for the wet tropical rainforests of northeastern Queensland. We also present a phylogeny based on a supermatrix of 1985 characters from three partial gene regions: mitochondrial small subunit (mtSSU), 25–28S nuclear rDNA large subunit (LSU), and a portion of the gene encoding the second largest subunit of RNA polymerase gene (RPB2). The sequence analyses strongly support separation of the ten Australian taxa into three clades, each of which can be diagnosed by micromorphological characters.

Materials & methods

Macromorphological and micromorphological features

Specimens were collected during February–April in 2009 and 2010 from different sites within the Wet Tropics Bioregion in northeastern Queensland and in April 2010 in the Barrington Tops National Park of central New South Wales. Basidiomata collected in the field were stored in plastic containers for transport to the laboratory. Macroscopic features were described from recently collected fresh materials. Field GPS coordinates for each collection were taken using a Garmin GPSmap 60CSx. Colors were described subjectively and coded according to Kornerup & Wanscher (1978), with color plate designations noted in parentheses. Color plate abbreviations itemize page number, column, and row (e.g., 8D-F5-6 = p. 8, columns D–F, rows 5–6.)

Dried specimens were examined microscopically with a trinocular research-grade Nikon Labophot compound microscope fitted with bright field optics according to Largent (1994: 1–3); basidiospore measurements follow Baroni & Lodge (1998: 681). Digital microphotographs were made using a Nikon Coolpix 990 camera focused through the trinocular head of the compound microscope.

All microscopic measurements were obtained using a GTCO Corporation Graphic Digitizer, Model DP5A-111A, connected to a laptop computer. The software utilized, Measure Me 101 v 1.0, was modified for use on a contemporary computer from the BASIC program, Metrics5 (David Malloch, University of Toronto). The mathematical factors determined using this program include: arithmetic mean (x_m) of basidiospore

length and width ± standard deviation for n objects measured; quotient of basidiospore length by width (E) indicated as a range for n objects measured; mean of E values (Q) ± standard deviations. The sample size (n) equals the quotient (n = x/y) of the total number of microscopic structures measured (x) and the number of basidiomata studied (y).

All collections for New South Wales cited in 'Additional collections examined' are deposited in The Plant Pathology Herbarium, Orange Agricultural Institute (DAR); the Queensland collections were split with duplicates deposited in the Australian Tropical Herbarium (CNS) and The Queensland Herbarium (BRI). All holotype and isotype collections are deposited in the herbaria designated in the Latin descriptions using acronyms from Holmgren et al. (1990).

DNA sequences and phylogenetic analyses

Lyophilized basidiomata were pulverized (4 mps for 20 sec) with glass beads (FastPrep FP120 homogenizer, QBiogene, Carlsbad CA USA). DNA was extracted with 2 × cetyl trimethylammonium bromide (CTAB) buffer, purified with phenol-chloroform-isoamyl alcohol (25:24:1) bound to silica columns (GeneClean Turbo Columns, MP Biomedicals, Solon OH USA), and washed with 70% ethanol (Baumgartner et al. 2010). Portions of three loci were amplified using Polymerase Chain Reaction (PCR). PCR primers used were MS1 and MS2 for mtSSU (White et al. 1990); Ctb6 and Tw13 for nuclear rDNA LSU (variable domains D1 and D2; White et al. 1990); and for the RPB2 portion either pb2i6f and rpb2i7r (Co-David et al. 2009) or our *Pouzarella*-specific primers, rpb2-PouF (5' to 3' - GAA GGT CAA GCT TGT GGT C) and rpb2-PouR (5' to 3' - CAT GCT YGG ATG GAT TTC).

The mtSSU and LSU genes were amplified in 25 µL reactions containing 1× GoTaq Flexi Buffer (Promega, Madison WI USA), 2 mM $MgCl_2$, 0.2 mM dNTPs, 500 nM forward and reverse primers, 0.2 mg/mL bovine serum albumin, 0.2 M betaine, 0.025 U Taq polymerase, and 1–2 µL of diluted template DNA. RPB2 was optimally amplified according to the primer pair used. The rpb2-PouF and rpb2-PouR primer-based amplifications utilized 25 µL reactions using the above protocol, except that betaine was increased to 1 M. The Co-David et al. (2009) based RPB2 amplifications increased the concentrations of primers rpb2-i6f and rpb2-i7r to 1 µM and the betaine concentration to 1 M. PCR cycling conditions were as follows: mtSSU and LSU: 94°C for 1 min, followed by 40 cycles of 94°C for 1 min, 50°C for 1 min, and 72°C for 1 min, followed by a final extension of 72°C for 7 min. RPB2: EITHER the touchdown protocol using primers rpb2-i6f and rpb2-i7r (Co-David et al. 2009) OR [*Pouzarella* primers rpb2-PouF, rpb2-PouR] 95°C for 5 min, followed by 40 cycles of 95°C for 30 sec, 50°C or 52°C for 1 min 30 sec, and 72°C for 1 min, followed by a final extension of 72°C for 7 min. Unresolved sequences with overlapping chromatograms were resolved by sub-cloning PCR products with the TOPO TA cloning kit (Invitrogen, Carlsbad CA USA) according to Bergemann et al. (2009).

PCR products were cleaned using 1 µL of ExoSAP-IT (GE Healthcare, Pittsburgh, PA USA). Sequencing reactions were carried out in 10 µL reactions containing 1 µL of Applied Biosystems BigDye ver. 3.1 (Applied Biosystems, Carlsbad CA USA), 0.4 µm primer, 0.875 × Sequencing Buffer (Applied Biosystems, Carlsbad CA USA) and 1 µL PCR water. Cycling parameters followed: 39 cycles of 96°C for 10 sec, 50°C for 5 sec,

60°C for 4 min with an infinite hold at 15°C. Reactions were purified with 3 M NaOAc, 250 mM EDTA (pH 8) and ethanol by centrifugation at 2500 G. Reactions were washed once with 70% EtOH and centrifuged at 2500 G for 15 min. Precipitated products were stored at –20°C.

Forward and reverse sequences for each locus were generated on an Applied Biosystems 3130xl Genetic Analyzer at Middle Tennessee State University. Sequence contigs were edited in Sequencher 4.8 (GeneCodes Corp., Ann Arbor, MI USA) and manually aligned in SE-Al (Rambaut 2002). Prior to tree estimation, the model selection feature in TOPALi v. 2.5 (Milne et al. 2004) was used to test for the best-fit model of nucleotide substitution using three selection criteria (AIC1 = Akaike information criterion, AIC2 = second order correction and BIC = Bayesian information criterion). Each criterion selected general time-reversible (GTR) as the best fitting substitution model. All phylogenetic analyses were conducted on the combined dataset (mtSSU + LSU + RPB2) using *Lyophyllum decastes* (Fr.) Singer, *L. leucophaeatum* (P. Karst.) P. Karst., and *Calocybe carnea* (Bull.) Donk as outgroups and including two additional taxa (*Entoloma violaceovillosum* Manim. & Noordel. and *E. araneosum* (Quél.) M.M. Moser) known to have typical *Pouzarella* features (Co-David et al. 2009). Maximum likelihood (ML) analyses implemented in TOPALi v. 2.5 (Milne et al. 2004) used a heuristic search algorithm based on a rapid branch swapping operation (PhyML; Guindon & Gascuel 2003). The level of clade support was assessed using 100 bootstrap (BS) replicates. Bayesian inferences (BI) were implemented in MrBayes ver. 3.1.2 (Ronquist & Huelsenbeck 2003) using the GTR set to two runs of one million generations sampling every 100 generations. After convergence, Bayesian posterior probabilities (BPP) were determined after calculating a 50% majority rule consensus tree.

GenBank accession numbers for each sequence are listed at the end of the type description for each species along with the habitat data and GPS co-ordinates of the holotype collection. Sequences for all other collections are listed after the collection number in the 'Other collections examined' section. GenBank accession numbers for other species included in the phylogenetic analyses are as follows (mtSSU, LSU, and RPB2, respectively): *Entoloma violaceovillosum* (GQ289345, GQ289205, GQ289273), *E. araneosum* (GQ289293, GQ289153, GQ289225), *Calocybe carnea* (AF357097, AF223178, DQ367432), *Lyophyllum leucophaeatum* (AF357101, AF223202, DQ367434), and *L. decastes* (AF357136, AF042583, DQ367433).

Results

We obtained sequences for all three loci (mtSSU, LSU, RPB2) for 22 *Pouzarella* collections except that we omitted one RPB2 sequence (for *P. parvula* DL Largent 9901) after several amplification attempts failed to produce PCR amplicons that could be sequenced. The dataset comprised 686 (mtSSU), 661 (LSU), and 638 (RPB2) aligned characters assembled into a supermatrix consisting of 1985 characters. Of these, 122 mtSSU, 61 LSU, and 104 RPB2 characters were excluded at the 5' or 3' ends due to heterogeneity in the lengths of the sequences obtained for different samples.

We conducted ML and BI analyses on the combined dataset. As the two tree topologies were fully congruent, we present only the ML tree with corresponding

BPP values included for comparison. Most branches are strongly supported by BS values greater than 70% and BPP values greater than 0.95, although in some cases, high levels of BPP support (0.99–1.00) were found when BS values were less than 70%. The Australian collections formed three clades (Plate 21): Clade I formed a well-supported (BS >70, BPP = 1.00) group comprising five species (*Pouzarella lasia*, *P. albostrigosa, P. fusca, P. debilis, P. setiformis*). Clade II included two species (*P. pamiae, P. farinosa*) with high BPP (0.99) but low BS (<70%) support. Clade III formed a well-supported group (BS >70, BPP = 1.00) of three species (*P. pilocystidiata, P. parvula, P. lageniformis*). The clades can be distinguished using micromorphological features (cheilocystidia, aborted basidia, and pileocystidia; see Key). The Australian taxa are not resolved as monophyletic in relation to the two European taxa included (*E. araneosum* and *E. violaceovillosum*), though the grouping of the Australian clade III with these species received low BS (but high BPP) support.

Taxonomy

1. ***Pouzarella pilocystidiata*** Largent & Skye Moore, **sp. nov.** Plates 1–2
MycoBank MB 519567

Habitu Pouzarellae fulvostrigosae *similis, sed centro pilei hispido-squamuloso, atrofusco, cheilocystidiis versiformibus, incrustationibus externis in hyphis tramalibus distinctis valdis, caulocystidiis copiosis, pseudocystidiis nullis atque superficie pilei argentea differt.*

Type — Australia, New South Wales, central Hunter District, Barrington Tops National Park, end of Blue Gum Loop Track, 32°08′31.7″S 151°30′56.4″E, 366.4 m, 22 April 2010, DL Largent 9932 (**holotype** DAR), sequences: HQ876543 (mtSSU), HQ876521 (LSU), HQ876500 (RBP2).

Etymology — Derived from the Latin *pilus* (= hair) + *cystidium*, referring to the long pileocystidia.

Pileus 8–19 mm broad, 2–7 mm high, convex or conic-campanulate and suggestively umbonate becoming broadly convex and non-umbonate with expansion, densely hispid-squamulose on the disc, hispid-squamulose to squamulose near the disc, matted appressed squamulose to appressed fibrillose elsewhere, with age the marginal area becomes orange-white to orange-gray (5-6A-B2) as the trama is exposed through separation of the cuticle; squamules and fibrils dark brown (5-7E-F5-6) on dark blond to brown (5E-F4) background, dull, opaque and not hygrophanous; margin decurved at all times, not translucent, even. Trama less than 1 mm thick, dark brown. Taste farinaceous. Odor mild or somewhat pungent. Lamellae 3–8 mm long, 1–5 mm high, narrow when young, moderately broad, broad or ventricose when mature, at first light brownish-orange (6C4 camel) then orange-white to orange-gray or reddish-white to orangish-gray (5-7A-B2-3), with age developing brown, irregularly arranged spots, adnate to short decurrent or with a distinct decurrent tooth,

close then subdistant; margin whitish and ± fimbriate because of cystidia, becoming eroded with age, bruising brownish with handling. Lamellulae 3 (2 short, 1 medium to medium-long) between lamellae. Stipe 23–45 mm long, 0.5–2 mm broad, equal, dark brown (5-7E-F4-6), darkens upon handling to dark brownish-gray to grayish-brown (7F2-3), at first squamulose-hispid with erect squamulose tips at the apex, elsewhere entirely covered with entangled squamules and fibrils, with age, rain or handling the squamules and fibrils get plastered on the surface and thus, the stipe appears fibrillose; stipe base with orange cinnamon strigose hairs. Bruising reaction absent.

Basidiospores nodulose angular with 6–8 angles, 9.2–14.6(–15.1) × 5.4–8.7 (–9.7) µm (x_m = 11.6 ± 1.1 × 7.2 ± 0.7 µm; E = 1.22–2.08; Q = 1.63 ± 0.16; n = 118/4). Basidia 4-sterigmate, cylindro-clavate to clavate, moderately long to long, 32.5–50.1 × 6.3–12.7 µm (x_m = 40.8 ± 4.4 × 10.2 ± 1.6 µm; E = 2.90–6.27; Q = 4.12 ± 0.81; n = 34/4). Cheilocystidia abundant, colorless, thin-walled, versiform (clavate, acuminate, aciculate, ventricose-rostrate), most common near the pileal margin and on the lamellulae, 39.5–82.8 × 4.3–24.0 µm (x_m = 57.8 ± 11.4 × 13.5 ± 4.3 µm; E = 2.27–12.34; Q = 4.90 ± 2.45; n = 24/4). Pleurocystidia absent. Pseudocystidia absent. Aborted Basidia absent. Hyphae of lamellar trama very long, similar in size to *Inocephalus* (Noordel.) P.D. Orton, 164.1–748.0 × 3.8–26.8 µm. Pileipellis 45–400 µm deep, composed of entangled strands of thick-walled hyphae in chains of up to 10 cells, entirely erect and trichodermial when young, upon pileal expansion remaining so in the center, semi-erect near the disc and repent at the margin. pileocystidia cylindric, cylindro-clavate, narrowly obclavate, 28.1–171.3 × 6.8–20.1 µm (x_m = 79.7 ± 25.7 × 11.3 ± 3.6 µm; E = 3.56–10.81; Q = 7.16 ± 2.40; n = 19/3). Hyphae of the pileal trama similar in length and width to the lamellar trama. Stipitipellis loosely entangled layer of hyphae, 45–205 µm deep. Caulocystidia cylindric to cylindro-clavate 53.2–125.4 × 4.5–9.4 µm. Oleiferous hyphae absent. Lipoid bodies absent. Pigmentation with strong, external incrustations in the pileal, lamellar and stipe tramas; parietal as internal incrustations in the pileipellis and stipitipellis.

Ecology and distribution: Solitary or scattered in soil either amongst leaf litter or under a log in amongst rocks, subtropical and temperate gallery rainforests; central Hunter district, New South Wales.

Additional collections examined —**AUSTRALIA**. **New South Wales**, central Hunter District, Barrington Tops National Park, Williams Day Use Area, end of blue gum track, 32°09′03.0″S 151°31′28.2″E, 366.4 m, 8 April 2010, DL Largent 9848 (mtSSU HQ876542, LSU HQ876520, RPB2 HQ876499); Pool of Reflections track, 32°08′29.5″S 151°30′55.0″E, 366.4 m, 20 April 2010, DL Largent 9921; Lion's Rock Track, 32°09′10.2″S 151°3′38.6″E, 366.4 m, 26 April 2010, DL Largent 9949 (mtSSU HQ876544, LSU HQ876522, RPB2 HQ876501).

Comments— *Pouzarella pilocystidiata* is diagnosed by a pileus with a squamulose-hispid disc and elsewhere appressed squamulose to appressed

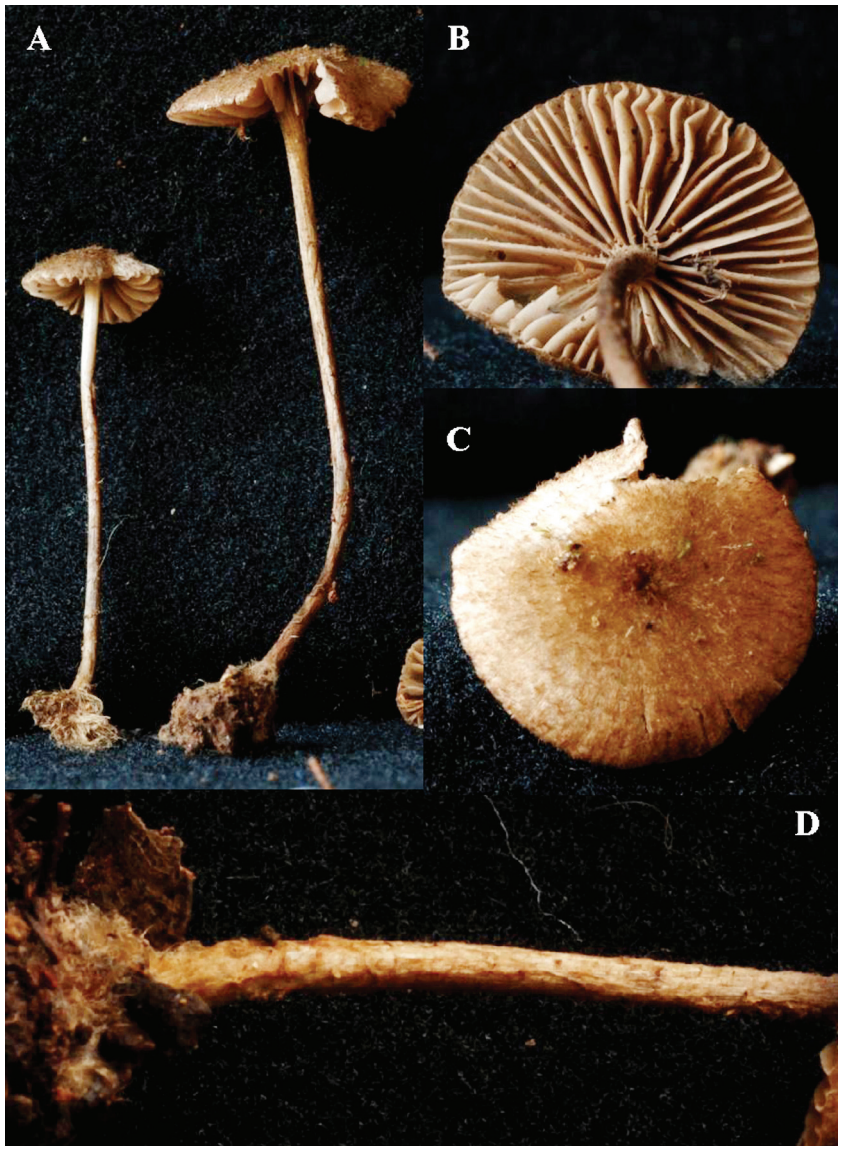

Plate 1 – *Pouzarella pilocystidiata*. A: Basidiomata (DLL 9932, holotype); B: Lamellae (DLL 9932); C: Pileus surface (DLL 9932); D: Strigose stipe base, stipe surface (DLL 9949).

fibrillose, a tomentulose to densely hirsute stipe apex, a dark brown stipe that bruises dark grayish-brown with a strigose base with light-colored to orange cinnamon hairs, a farinaceous taste, basidiospores generally smaller than 12.0

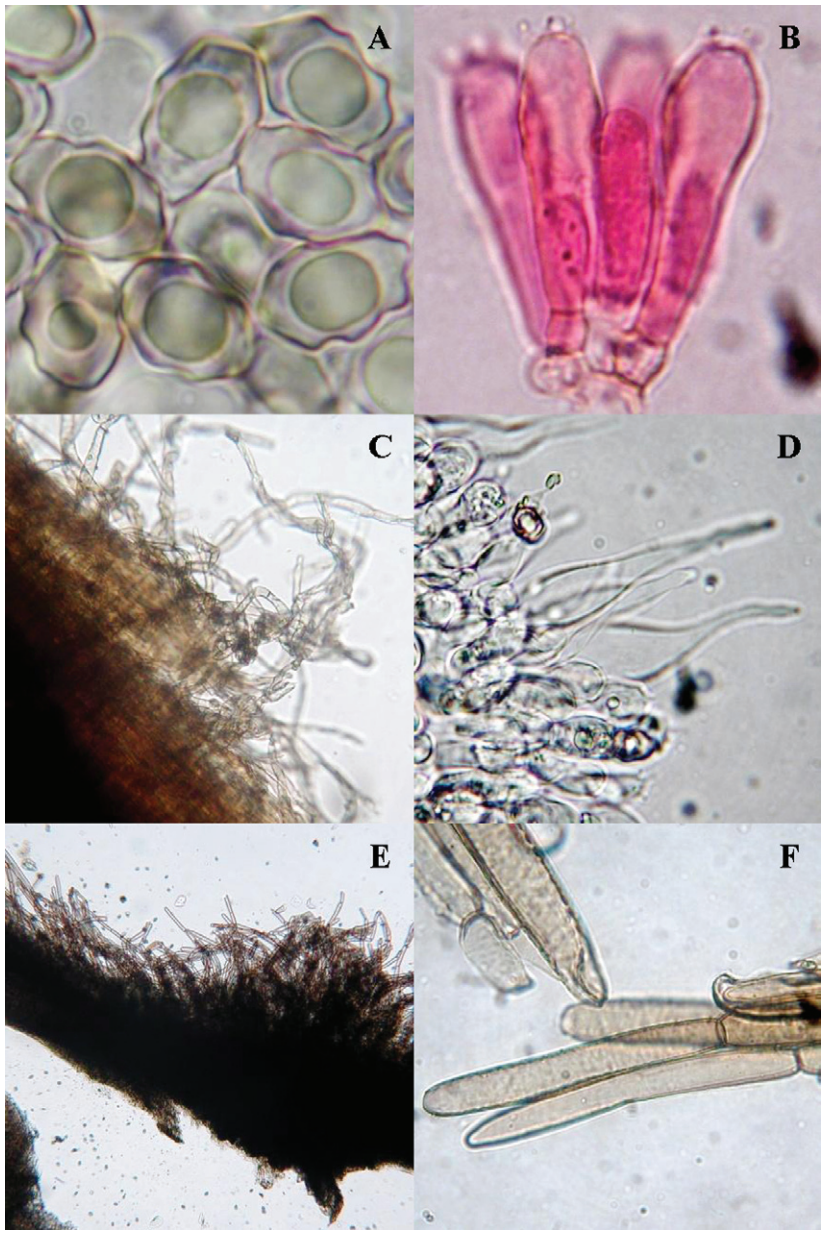

Plate 2 – *Pouzarella pilocystidiata*. A: Basidiospores (1000×) (DLL 9949); B: Basidia (400×) (DLL 9959); C: Stipitipellis (100×) (DLL 9949); D: Cheilocystidia (400×) (DLL 9932, holotype); E: Pileipellis on and near disc (100×) (DLL 9921); F: Pileocystidia (400×) (DLL 9949).

× 7.5 µm, basidia narrower than 11.0 µm, pileipellis and stipitipellis ≤400 µm thick, and ≤175 µm long cylindro-clavate pileocystidia and caulocystidia.

Pouzarella pilocystidiata morphologically resembles *P. fulvostrigosa* (Berk. & Broome) Mazzer, which is distinguished by a densely silvery fibrillose mouse gray pileus, lageniform cheilocystidia, minutely incrusted tramal hyphae, scattered pseudocystidia, and caulocystidia (Noordeloos 1979).

Australian taxa similar to *Pouzarella pilocystidiata*

Pouzarella pilocystidiata shares with *P. lageniformis* and *P. parvula* nodulose angular basidiospores (x_m <13.5 µm long), externally incrusted tramal hyphae, abundant, thin-walled, versiform (rostrate, ventricose, lageniform) cheilocystidia, long cylindro-clavate pileocystidia, and the absence of aborted basidia. Morphological features unique to *Pouzarella pilocystidiata* include the small hispid-squamulose dark brown/dark blond pileus, densely hirsute stipe surface, farinaceous taste, basidiospore size, rare acuminate pileocystidia, abundant cylindro-clavate caulocystidia, and lack of setiform pileocystidia. *Pouzarella lageniformis* is differentiated by a 11 × 2 mm hispid-squamulose orange-white/grayish orange pileus, entirely pruinose stipe surface, mild taste, slightly larger basidiospores, acuminate pileocystidia, and lack of setiform and aculeate thick-walled caulocystidia. *Pouzarella parvula* differs by its smaller matted squamulose pileus, adnexed lamellae, glabrous stipe, larger basidiospores, versiform (some rostrate ventricose) cheilocystidia, entangled pileipellis, and lack of aborted basidia and caulocystidia.

2. ***Pouzarella lageniformis*** Largent & Skye Moore, **sp. nov.** Plates 3–4

MycoBank MB 519568

A speciebus congenericis omnibus ceteris pileo juventute omnino hispido-squamuloso, superficie pilei omnino pallide griseo-brunnea squamulis aurantiaco-albis ornata, apice stipitis pruinoso, alibi stipite fasciculis fibrillarum rarenter praedito, basidiosporis 10.4–13.8 × 6.6–8.7 um, mediane 12.06 × 7.55 um, basidiis mediane 36.9 × 11.9 um, pileocystidiis clavatis acuminatis vel aculeatis, cheilocystidiis versiformibus aliquibus rostrato-ventricosis, caulocystidiis aculeatis differt.

Type — Australia, New South Wales, central Hunter District, Barrington Tops National Park, Williams Day Use Area, end of Blue Gum Track, 32°09′01.9″S 151°31′28.7″E, 365.5 m, 18 April 2010, DL Largent 9895 (**holotype** DAR), sequences: HQ876545 (mtSSU), HQ876523 (LSU), HQ876502 (RBP2).

Etymology — Derived from the Latin *lagena* (= flask) + *forma* (= shaped), referring to the flask-shaped or rostrate ventricose cheilocystidia.

Pileus 11 mm broad, 2 mm high, broadly convex, when young entirely erect hispid-squamulose with the squamules somewhat scattered, when mature remaining so on and near the center, semi-erect near the center, repent elsewhere, squamules orange-white (5-6A1-2), background light grayish-brown (6C-D3), overall grayish-orange (6B-C2), dull, dry, opaque, not hygrophanous,

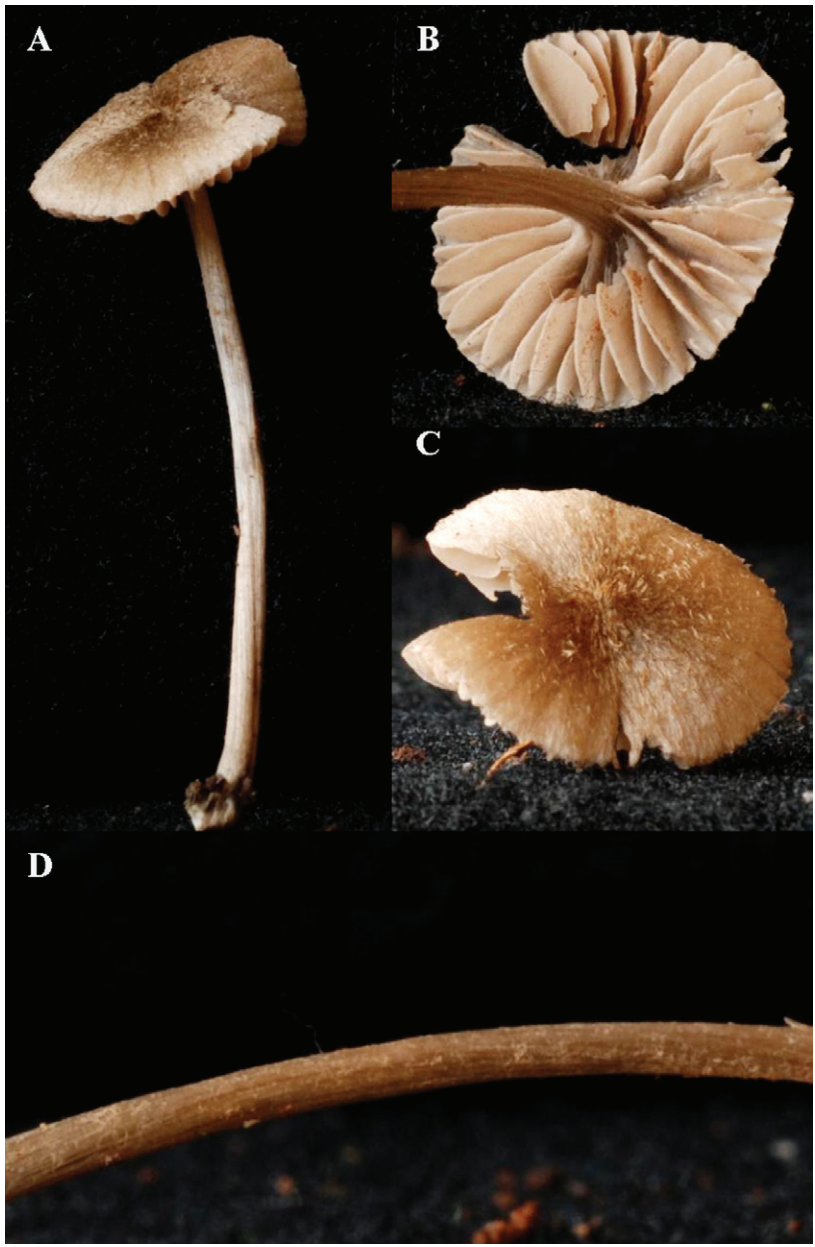

Plate 3 – *Pouzarella lageniformis* (DLL 9895, holotype)
A: Basidiomata; B: Lamellae; C: Pileus surface; D: Stipe surface.

striate with striae colored as the background. Margin decurved, fringed in places. Taste and Odor indistinct. Lamellae 5 mm long, 1.5 mm high, broad and ventricose, short-decurrent, orange-gray (5A2), light-colored when dried, subdistant, margin suggestively and minutely serrulate, whitish. Lamellulae 3 (2 short, 1 medium to medium long) between lamellae. Stipe 33 mm long, 1 mm broad at apex, 0.75 mm broad at base, slightly tapered, entirely pruinose with the pruinae scattered and orange-white to orange-gray on a grayish-brown (6D3) background, dull, hollow; stipe base densely pruinose. Bruising reaction absent.

Basidiospores angular-nodulose with 6–8 angles, heterodiametric in profile view, 6-angled and isodiametric in polar view, 10.4–13.8 × 6.6–8.7 µm (x_m = 12.1 ± 1.0 × 7.6 ± 0.6 µm; E = 1.38–1.87; Q = 1.6 ± 0.14 (heterodiametric); n = 29/1). Basidia 4-sterigmate, sterigmata 0.7–3.5 µm long, base of basidia 3.3–5.8 µm wide, relatively small, full of small droplets, 29.3–43.6 × 9.9–14.2 µm (x_m = 36.9 ± 3.3 × 11.9 ± 1.4 µm; E = 2.56–4.32; Q = 3.13 ± 0.48; n = 13/1). Cheilocystidia abundant, scattered, versiform (long ventricose-rostrate, acuminate), 47.5–133.6 × 8.7–28.8 µm (x_m = 85.6 ± 22.4 × 17.9 ± 6.0 µm;; E = 3.16–10.75; Q = 5.24 ± 2.28; n = 10/1). Pleurocystidia absent. Pseudocystidia absent. aborted basidia absent. Hyphae of lamellar trama relatively long 230.2–418.7 × 11.3–31.4 µm. Pileipellis 117–333 µm deep, composed of loosely entangled, long strands of hyphae with chains of 3–5 cells, individual cells relatively long, entirely erect to semi-erect and trichodermial in the center when young, upon pileal expansion remaining so in the center, becoming semi-erect near the center and repent elsewhere. Pileocystidia very long, clavate, aculeate, or acuminate, 105.4–192.7 × 10.7–20.2 µm. Hyphae of the pileal trama 194.6–451.3 × 9.4–17.1 µm. Stipitipellis 30–150 µm deep, composed of scattered strands of thick-walled hyphae. Caulocystidia aculeate, thick-walled, 20.5–144.4 × 5.0–9.2 µm. Pigmentation externally incrusted on the tramal hyphae of the pileus, stipe, lamellae, parietal with internal incrustations in the pileipellis and stipitipellis.

Ecology and distribution: Solitary amongst leaf litter, subtropical gallery rainforest; known only from type locality.

Comments— *Pouzarella lageniformis* is diagnosed by an entirely hispid squamulose young pileus with orange-white squamules on a light grayish brown background, stipe with a pruinose apex and rare fibrillose bundles elsewhere, basidiospores averaging 12.1 × 7.6 µm, basidia generally >11.0 µm long, clavate, acuminate, or aculeate pileocystidia, versiform (some rostrate ventricose) cheilocystidia, and aculeate caulocystidia.

See *P. pilocystidiata* for comparisons with Australian and other taxa related to *P. lageniformis*.

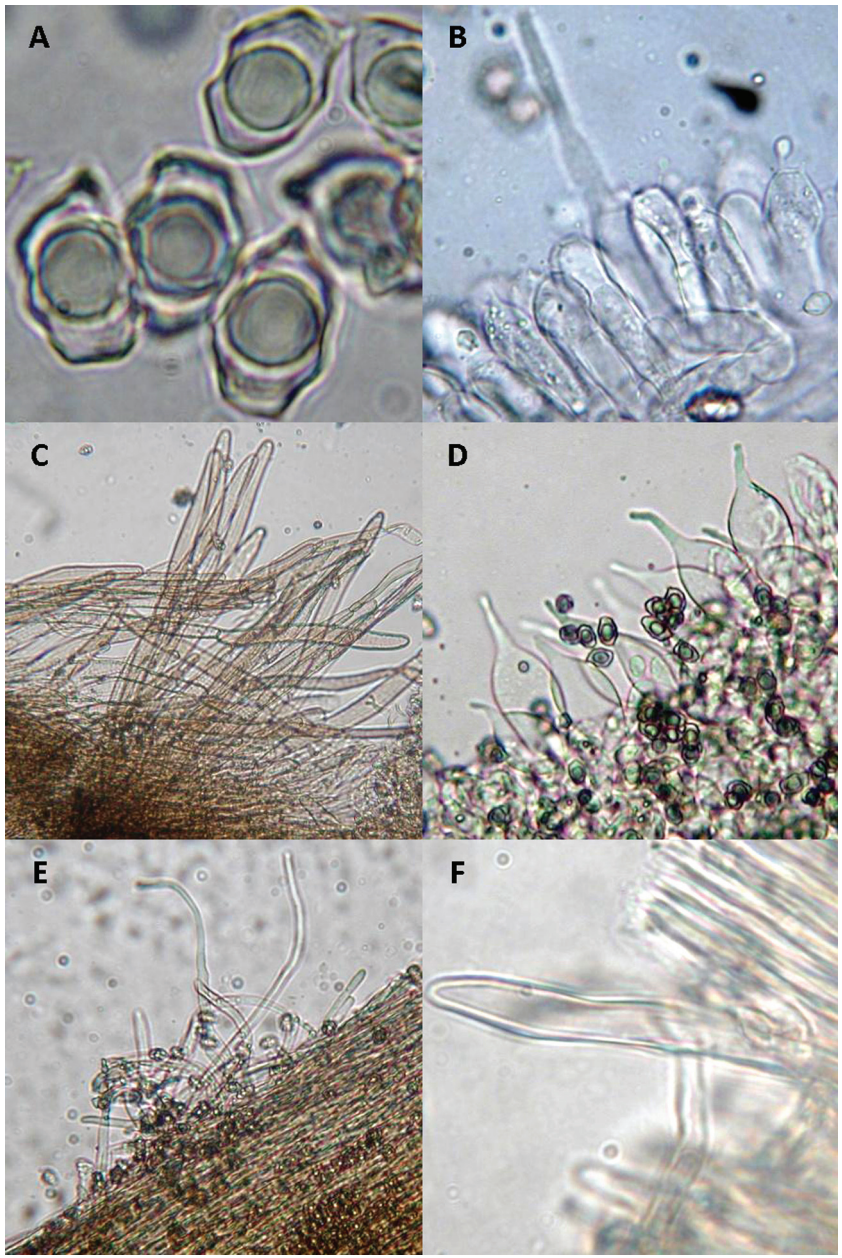

Plate 4 – *Pouzarella lageniformis* (DLL 9895, holotype). A: Basidiospores (1000×); B: Basidia and narrow rostrate-ventricose cheilocystidia (400×); C: Pileocystidia and pileipellis (180×); D: Cheilocystidia (100×); E: Stipitipellis (100×); F: Thick-walled caulocystidium (400×).

3. ***Pouzarella parvula*** Largent & Skye Moore, **sp. nov.** PLATES 5–6

MYCOBANK MB 519569

A speciebus congenericis omnibus ceteris pileo parvo, 6 mm lato, 2 mm alto, implexo, squamuloso, lamellis adnexis, stipite glabro, basidiosporis mediane 13.23 × 7.7 um, cheilocystidiis versiformibus aliquibus rostrato-ventricosis, pileipelle implexa, pseudocystidiis caulocystidiisque nullis differt.

TYPE — Australia, New South Wales, Central Hunter District, Barrington Tops National Park, Williams River Day Use Area, end of Blue Gum Track, 32°09′13.6″S 151°31′39.7″E, 355.1 m, 18 April 2010, DL Largent 9901(**holotype** DAR), sequences: HQ876546 (mtSSU), HQ876524 (LSU), HQ876543 (RBP2).

ETYMOLOGY — Derived from the Latin *parvulus* (= very little), referring to the very small pileus.

PILEUS 6 mm broad and 2 mm high, convex, entirely densely matted squamulose, overall orange-gray to grayish-orange (5B2-3) dull, even, dry, opaque and not hygrophanous; margin decurved, fringed, eroded and ± crenulate. TASTE AND ODOR indistinct. LAMELLAE 2 mm long, 1 mm deep, narrow, adnexed, orange-gray (5B2), distant; margin smooth but lighter in color; 1 lamellula between lamellae. STIPE 20 mm long, <1 mm broad, equal, dull, pruinose at the apex, glabrous elsewhere, orange-gray to grayish-orange (5B2), hollow and very fragile; stipe base not pruinose. BRUISING REACTION absent.

BASIDIOSPORES nodulose angular with 6–8(–9) angles, heterodiametric in profile view, 6-angled and isodiametric in polar view, 10.5–15.0 × 6.3–8.6 µm, (x_m = 13.2 ± 1.0 × 7.7 ± 0.6 µm; E = 1.35–2.04; Q = 1.72 ± 0.14; n = 29/1). BASIDIA 4-sterigmate, cylindro-clavate to clavate 32.5–40.4 × 10.8–14.8 µm, (x_m = 36.3 ± 2.6 × 12.8 ± 1.5 µm; E = 2.47–3.37; Q = 2.87 ± 0.29; n = 9/1). CHEILOCYSTIDIA solitary to scattered, versiform (aciculate, acuminate, obclavate, rostrate-ventricose), 36.0–88.6 × 14.8–21.3 µm. PLEUROCYSTIDIA absent. PSEUDOCYSTIDIA absent. ABORTED BASIDIA absent. PILEIPELLIS 100–200 µm deep, an entangled layer of hyphae composed of chains of thick walled cells, individual cells long and slender. PILEOCYSTIDIA cylindro-clavate, aciculate, aculeate to distinctly setiform 57.6–106.0 × 6.1–13.2 µm, (x_m = 74.8 ± 16.8 × 8.6 ± 2.2 µm; E = 7.01–16.00; Q = 9.04 ± 2.6; n = 11/1). STIPITIPELLIS a cutis from near the apex to the base of the stipe; apex overlooked because of stipe size. CAULOCYSTIDIA absent where studied. PIGMENTATION distinctly externally incrusted on the hyphae of the pileal stipe and lamellar trama; parietal and in the form of internal incrustations in the pileipellis.

ECOLOGY AND DISTRIBUTION: Solitary in soil amongst mosses on a bank cut in a wet sclerophyll subtropical gallery rainforest; known only from the type locality.

COMMENTS— *Pouzarella parvula* is differentiated by a small (6 × 2 mm) matted squamulose orange-gray pileus, adnexed lamellae, stipe pruinose at the apex and glabrous elsewhere, basidiospores averaging 13.2 × 7.7 µm, versiform

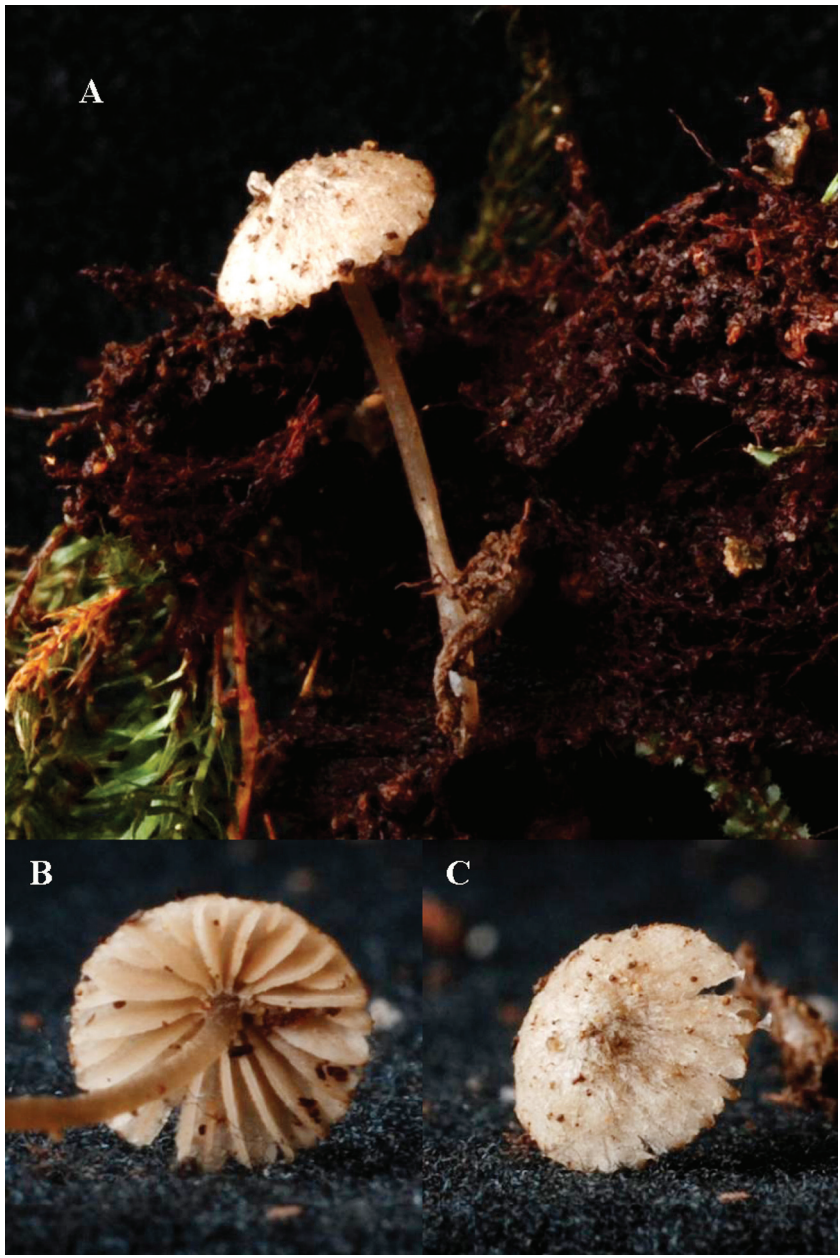

Plate 5 – *Pouzarella parvula* (DLL 9901, holotype). A: Field photo of basidiomata; B: Lamellae and lamellulae; C: Pileal surface and eroded pileal margin.

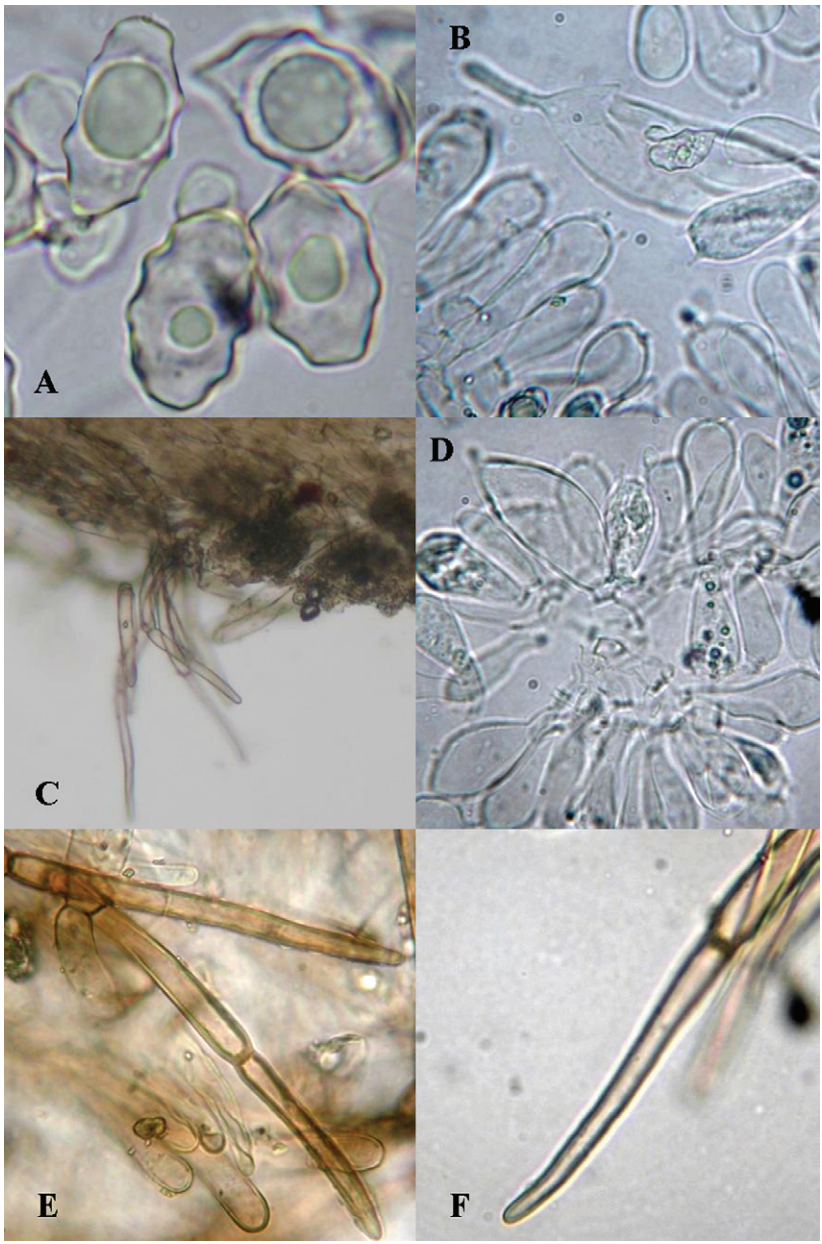

Plate 6 – *Pouzarella parvula* (DLL 9901, holotype). A: Basidiospores (1000×); B: Basidia and cheilocystidium (400×); C: Pileipellis near pileus margin (100×); D: Cheilocystidia (400×); E: Setiform pileocystidia (400×); F: Setiform pileocystidium (400×).

(some rostrate ventricose) cheilocystidia, entangled pileipellis with setiform pileocystidia, and lack of aborted basidia and caulocystidia.

Pouzarella minuta (E. Horak) E. Horak from New Zealand (Horak 2008), *Pouzaromyces napaliensis* Desjardin & T.J. Baroni from the Hawaiian Islands, and *Entoloma testaceostrigosum* Manim. & Noordel. from India have nearly the same size pileus as *P. parvula*. *Pouzarella minuta* differs by a smaller (2–4 × 1 mm) stipe densely covered with brownish or white fibrils and much larger basidiospores (16–20 × 10–12 µm) (Horak 1980, 2008). *Entoloma testaceostrigosum* and *Pouzaromyces napaliensis* both possess setiform pileocystidia and caulocystidia and lack cheilocystidia (Desjardin & Baroni 1991, Manimohan et al. 2006).

See *P. pilocystidiata* for comparisons with Australian species with a resemblance to *P. parvula*.

4. ***Pouzarella pamiae*** Largent, **sp. nov.** PLATES 7–8

MYCOBANK MB 519570

Habitu Pouzarellae squamifoliae *similis, sed coloribus basidiomatum fulvis, pileo latiore usque ad 3–8 mm, stipite minore, 12–25 × 0.5–0.75 mm atque contusis atro-fuscis differt.*

TYPE — AUSTRALIA, Queensland, Cook Region, Mossman Gorge National Park, 16°28′17.6″S 145°19′51.7″E, 84.4 m, 18 March 2010, DL Largent 9794 (**holotype** BRI, **isotype** CNS), sequences: HQ876539 (mtSSU), HQ876517 (LSU), HQ876496 (RBP2).

ETYMOLOGY —in honor of Pamela Largent, the collector and inspiration for the study of entolomatoid fungi in northeastern Queensland.

PILEUS 3–8 mm broad, 2–4.5 mm high, at first conic to conic-campanulate and densely erect squamulose-hispid over the entire surface expanding to convex and eventually convex-campanulate, remaining erect squamulose hispid on the center but becoming appressed squamulose from the margin first and then to near the center, at first entirely reddish-golden to brownish-orange (6-7C-D5-7) darkening to medium brown (6-7E-F5-6 teak brown to burnt umber) then dark brown (6-7E-F6-7 burnt umber to dark brown), with maturity remaining darker on the disc but lightening to yellowish-white to orangish-white (4-5A2-3), squamule tips lose color and separate showing the whitish-yellow background, not umbonate, opaque, dry and dull; margin decurved and fringed with squamule tips then some eroded as the tips collapse with age. TASTE indistinct. ODOR indistinct to pungent. LAMELLAE 3–4 mm long, 1.25–1.5 mm high, pale orange (6A3) then brownish (6E5 sunburn) with basidiospore maturity, narrow and then moderately broad, subdistant to nearly distant; no bruising reaction observed in the margin, fimbriate to serrulate and light-colored when lamellulae are mature. LAMELLULAE nearly non-existent with at most one lamellula between lamellae. STIPE 12–25 mm long, 0.5 mm broad at the apex, 0.75 mm broad at base, equal to slightly clavate, at first covered by a yellowish-white to orangish-white (4-6A2-3) dense layer of entangled

Plate 7 – *Pouzarella pamiae* (DLL 9794, holotype). A: Basidiomata, note densely hispid squamulose pileal surface and stipe base colored like pileus; B: Pileus with fringed pileal margin (left), lamellae (center) and young pileus surface (right).

fibrils, with maturity the fibrils darken to colors similar to those of the pileus, at times the consistency and color of the fibrils at the base are identical to those on the pileal surface, eventually the stipe surface becomes slightly roughened as the fibrils collapse onto the surface and then the stipe darkens to medium dark brown (6E6); the surface discolors to dark brown (near 6F6) when bruised; stipe base strigose or matted. Bruising reaction absent.

Basidiospores 7–9-angled, nodulose-angular, subisodiametric to heterodiametric in profile view, 5–6-angled and isodiametric in polar view, 10.0–15.9 (–17.3) × 7.1–11.2 µm (x_m = 13.07 ± 1.3 × 8.5 ± 0.8 µm; E = 1.18–1.85; Q = 1.53 ± 0.16; n = 82/2). Basidia 4-sterigmate, clavate, tapered, filled with a droplet, 31.8–48.3 × 11.5–15.3 µm (x_m = 42.3 ± 5.6 × 13.6 ± 1.2 µm; E = 2.17–3.78; Q = 3.11 ± 0.4; n = 11/1). Cheilocystidia abundant, broadly clavate to broadly acuminate, with a faint brownish cytoplasmic pigment, ± thick-walled, 47.1–77.6 × 10.1–26.2 µm and located as terminal cells arising from tramal hyphae with at least one subterminal cell inflated. Pleurocystidia absent. Pseudocystidia absent. aborted basidia inconspicuous or rare. Hyphae of the lamellar trama 79.3–240.3 × 5.9–15.3 µm. Pileipellis 111–551 µm deep, an entangled layer of long, rather slender hyphae with cells ± in chains, in young specimens entirely erect and trichodermoid, in older specimens trichodermoid only in the center, semi-erect towards the margin and repent at the margin; distinctly thick-walled in the apical 1–5 cells. Pileocystidia cylindric, cylindro-clavate, broadly clavate, or broadly obclavate, thick-walled and with parietal pigment, 26.0–78.7 × 6.6–13.0 µm (x_m = 46.8 × 9.8 µm; E = 2.87–7.52; Q = 4.8; n = 9/1). Hyphae of the pileal trama same size and pigmentation as lamellar trama. Stipitipellis similar to the pileipellis except with the addition of clusters of hymenial elements at the apex; Caulocystidia similar to the pileocystidia, 36.1–79.1 × 8.4–19.9 µm. Pigmentation externally incrusted in the trama, parietal and faintly externally incrusted in the pileipellis and stipitipellis.

Ecology and distribution: scattered in tan-colored soil beneath and protected by an overhanging boulder in a complex mesophyll vine forest on well-drained alluvium (Mossman Gorge National Park) or solitary in hard-packed soil (Danbulla National Park, Lake Euramoo).

Additional collections examined —**AUSTRALIA**. **Queensland**, Cook Region, Mossman Gorge National Park, 16°28′17.2″S 145°19′50.2″E, 86.6 m, 26 March 2010, DL Largent 9834 (mtSSU HQ876541, LSU HQ876519, RPB2 HQ876498); Danbulla National Park, Lake Euramoo Track, 17°09′42.3″S 145°37′45.6″E, 762.6 m, 21 March 2010, DL Largent 9808 (mtSSU HQ876540, LSU HQ876518, RPB2 HQ876497).

Comments— *Pouzarella pamiae* is diagnosed by its 3–8 mm broad densely squamulose-hispid pileus with brownish-orange squamules, long (12–25 mm) narrow stipe that bruises dark brown and has a base covered with squamules identical to those on the pileus, thick-walled acuminate to broadly clavate cheilocystidia, and basidiospores averaging 13.1 × 8.6 µm.

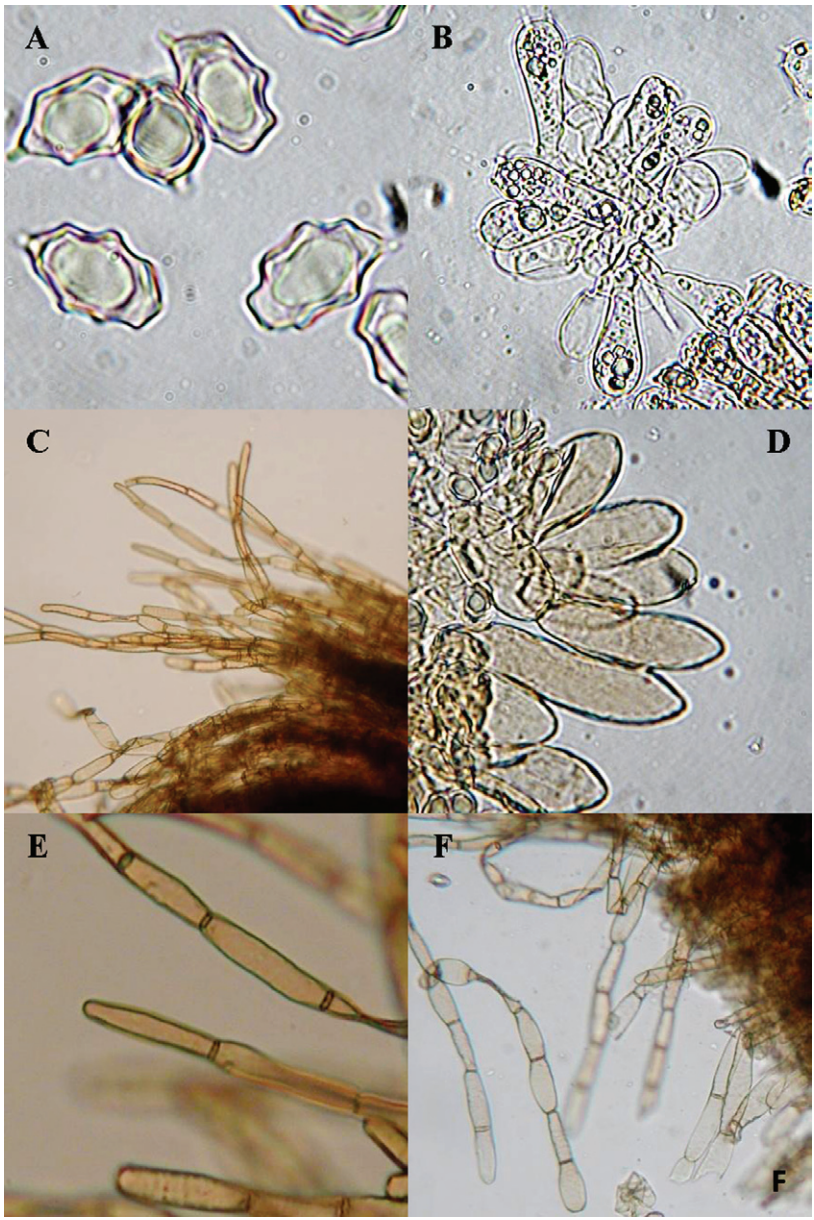

Plate 8 – *Pouzarella pamiae*. A: Basidiospores (1000×) (DLL 9808); B: Basidia (400×) (DLL 9794, holotype); C: Pileocystidia (100×) (DLL 9794); D: Thick-walled cheilocystidia with brownish pigment (400×) (DLL 9794); E: Cylindro-clavate pileocystidia (180×) (DLL 9794); F: Stipitipellis and chains of hyphae with terminal caulocystidia (100×) (DLL 9794).

Other species with a brownish-orange, densely squamulose-hispid pileus and stipe include *P. fulvolanata* (Berk. & Broome) Mazzer and *P. myoderma* (Berk. & Broome) T.J. Baroni (both from Ceylon), *P. squamifolia* (Murrill) Mazzer from Jamaica, *P. sepiaceobasalis* (E. Horak) T.J. Baroni from Argentina, and *P. ferreri* T.J. Baroni et al. from the Dominican Republic, Brazil, and Costa Rica.

Mazzer (1976) described *P. fulvolanata* with yellowish to tawny colors and basidiospores in the same size range (12–16 × 7–8 µm) as *P. pamiae*, but it can be differentiated by subpyriform cheilocystidia and larger pileus (13 mm broad) and stipe (32 mm × 2 mm). Noting their similar stature, coloration, and pileus and stipe surfaces, Mazzer (1976) differentiated *P. fulvolanata* and *P. squamifolia* based on distribution and stipe color: *P. squamifolia,* described from Jamaica, has a pallid stipe with ferruginous hairs whereas *P. fulvolanata,* described from Ceylon, has tawny hairs on the stipe.

The basidiospores, basidia, cheilocystidia, pileipellis, pileocystidia, stipitipellis and caulocystidia of *P. pamiae* are identical to those described for the Jamaican holotype of *P. squamifolia* (Mazzer 1976, Baroni et al. 2008). However, *P. squamifolia* is differentiated by ferruginous colors (tawny in *P. pamiae*), wider (10 mm broad) pileus, larger (40 × 1.5 mm) stipe, and the absence of a bruising reaction.

Pouzarella sepiaceobasalis is differentiated from *P. pamiae* by the longer basidiospores (15–19 µm) and longer stipe (60 mm) with a base that gradually becomes blue-black to black (Baroni et al. 2008).

Pouzarella ferreri is separated by a more reddish coloration, longer (≤60 mm) stipe, larger (~16.2 × 10.7 µm) basidiospores, globose to sphaeropedunculate cheilocystidia, and black staining reactions (Baroni et al. 2008).

On the basis of type studies, Pegler (1977, 1986) synonymised *A. fulvolanatus* Berk. & Broome under *A. myodermus* Berk. & Broome, for which he reported basidiospore measurements of 9–13(–15) × 5.5–7.0 µm (average 10.5 ± 1.0 × 6.5 ± 0.34 µm). He could not verify the presence of cheilocystidia, commenting, "Lamella-edge not revived." Based on further type studies, Horak (1980) synonymised both *A. myodermus* and *A. fulvolanatus* under *Agaricus lasius*, for which he reported basidiospores measuring 10.5–13.5 × 6–8 µm, cheilocystidia absent, and robust basidiomata with ≤20 mm broad pilei and 15–50 × 1–3 mm stipes. Therefore, *P. pamiae* is differentiated from both *Pouzaromyces myodermus* sensu Pegler (1977) and *Pouzaromyces lasius* sensu Horak (1980) by larger basidiospores, abundant clavate to acuminate cheilocystidia, and a smaller stature.

Australian taxa related to *Pouzarella pamiae*

The ML phylogeny weakly (BS <70) supports the clade including *P. pamiae* and *P. farinosa*. Both species possess ± thick-walled pigmented napiform to

clavate cheilocystidia and versiform pileocystidia. The two species are easily distinguished by basidiospore size (~15.5 × 10.0 µm in *P. farinosa*) and coloration (orange-gray squamules on a grayish-brown background in *P. farinosa*).

5. ***Pouzarella farinosa*** Largent & Skye Moore, **sp. nov.** PLATES 9–10

MYCOBANK MB 519571

Habitu Entolomate dysthali *(subg.* Pouzarellae*) similis, sed sapore farinaceo, squamulis pilei aurantiaco-griseis, cheilocystidiis latioribus, 21.1–51.1 × 16.7–30.9 um differt.*

TYPE — Australia, New South Wales, Cook Region, Central Hunter District, Barrington Tops National Park, Pool of Reflections Track, 32°08′15.8″S 151°30′38.2″E, 727.6 m, 22 April 2010, DL Largent 9934 (**holotype** DAR), sequences: HQ876538 (mtSSU), HQ876516 (LSU), HQ876495 (RBP2).

ETYMOLOGY — derived from the Latin *farinosus* (= mealy), referring to the mealy or farinaceous taste.

PILEUS 8–14 mm broad, 2–6 mm high, convex to broadly convex, in some with a slight, broad umbo, at first entirely squamulose-hispid with densely packed upright tufts of orange-gray (5B2) squamules on a grayish-brown (5E3) background, the overall color light grayish-brown (5C-D3), upon expansion remaining so in or near the center, becoming appressed squamulose and then appressed fibrillose towards the margin as the squamules get pressed onto the surface, dry, dull, opaque, not hygrophanous but becoming striate with age; margin decurved then slightly uplifted, striate with age, the striae grayish-brown (5D-E3). TASTE slightly to distinctly farinaceous. ODOR indistinct. LAMELLAE 3–6 mm long, 1–2 mm high, narrow, adnate with a distinct subdecurrent tooth, subdistant to distant, at first brownish-gray (5C3) then orange-white (6A2) with maturation of the basidiospores; margin whitish, smooth when very young eventually becoming minutely serrulate to fimbriate because of cystidia. STIPE 30–54 mm long, 1–1.25 mm broad, equal, fragile, dull, at first entirely orange-gray (5B2) and covered with dense visible tangled masses of fibrils and squamules that are more abundant towards the basal part of the stipe, with maturity and handling, the overall color grayish-brown (6C3-4) with areas darkening to dark brown (5F6) with age as the fibrils and squamules become appressed to the stipe surface. BRUISING REACTION absent.

BASIDIOSPORES 6–8-angled and nodulose angular in profile view, 6-angled in polar view, 12.0–19.8 × 7.9–11.9 µm (x_m = 15.5 ± 1.8 × 10.0 ± 0.84 µm; E = 1.2–1.8; Q = 1.56 ± 0.14 (heterodiametric); n = 63/2). BASIDIA 4-sterigmate, broadly clavate to clavate, tapered, 4-sterigmate with the sterigma long, slender and nearly needle-like with large droplets, 40.6–53.1 × 13.6–20.3 µm (x_m = 47.2 ± 3.5 × 16.3 ± 1.7 µm; E = 2.29–3.49; Q = 2.29 ± 0.34; n = 18/2). CHEILOCYSTIDIA abundant and forming a sterile layer mostly near the pileal margin, nearly globose, vesiculate, or nearly napiform, ± thick-walled, 25.1–51.7 × 16.7–30.9

Plate 9 – *Pouzarella farinosa.*
A: Basidiomata and stipe surface (DLL 9934, holotype); B: Pileus surface (DLL 9900).

µm, (x_m = 37.3 ± 8.2 × 23.0 ± 4.3 µm; E = 1.14–2.61; Q = 1.65 ± 0.40; n = 14/1). Pleurocystidia absent. Pseudocystidia absent. Aborted Basidia absent. Hyphae of the lamellar trama subparallel with relatively short cells, narrow and slender but more broad than those in the pileal trama, 4.4–87.4 × 3.6–10.7 µm. Pileipellis a densely entangled layer of hyphae in chains of 4–6 cells, ± palisade and erect in the center and near the center, semi-erect near the center and repent near the margin. Pileocystidia relatively short, clavate, broadly clavate or nearly napiform on or near the center, 21.4–54.1 × 8.6–20.4 µm (x_m = 42.4 ± 11.5 × 14.2 ± 4.3 µm; E = 1.35–4.87; Q = 3.24 ± 1.24; n = 9/1). Hyphae of the pileus trama similar to but more narrow than those in the lamellar trama. Stipitipellis 76–233 µm, similar to the pileipellis, composed of abundant clusters of hyphae in 5–6-celled chains. Caulocystidia clavate, cylindro-clavate, narrow to broadly aculeate, or even acuminate, longer than the pileocystidia, 32.4–76.6 × 9.1–20.6 µm, (x_m = 55.8 ± 14.4 × 14.3 ± 3.0 µm; E = 1.57–8.13; Q = 4.17 ± 1.74; n = 14/2). Oleiferous hyphae absent. Lipoid bodies absent in the trama; large globules present in the basidia. Pigmentation strongly incrusted on the pileal, lamellar and stipe trama; parietal and internally incrusted in the stipitipellis and pileipellis.

Ecology and distribution: Solitary to gregarious in soil, often amongst rocks and ferns in warm temperate and subtropical gallery rainforests, Central Hunter District, New South Wales, Australia.

Additional collections examined — **AUSTRALIA. New South Wales,** Cook Region, Barrington Tops National Park, Williams Day Use Area, end of Blue Gum Loop Track, 32°09′03.3″S 151°31′28.2″E, 366.9 m, 18 April 2010, DL Largent 9900 (mtSSU HQ876537, LSU HQ876515, RPB2 HQ876494).

Comments— *Pouzarella farinosa* is diagnosed by an entirely squamulose-hispid pileus covered by densely packed upright tufts of orange -gray squamules, basidiospores measuring 12.0–19.8 × 7.9–11.9 µm, nearly globose, vesiculate, or nearly napiform cheilocystidia, and the lack of aborted basidia.

The new species resembles *Entoloma dysthales* (Peck) Sacc. f. *dysthales* in basidiospore size, cheilocystidia, and overall stature and coloration. However, *E. dysthales* lacks the orange-gray colors of the *P. farinosa* squamules and possesses a mild (not farinaceous) taste and odor, abundant aborted basidia, and longer, narrower (21–82 × 8–21 µm) cheilocystidia. Our collections are similar to Gates 2265, the proposed holotype for a new species to be described in a forthcoming book on the entolomatoid fungi in Tasmania (Gates pers. comm.), but differs in the absence of aborted basidia, cheilocystidial shape (clavate to broadly clavate or obovoid in Gates 2265), and farinaceous taste.

See *P. pamiae* for comparisons with taxa closely related to *P. farinosa*.

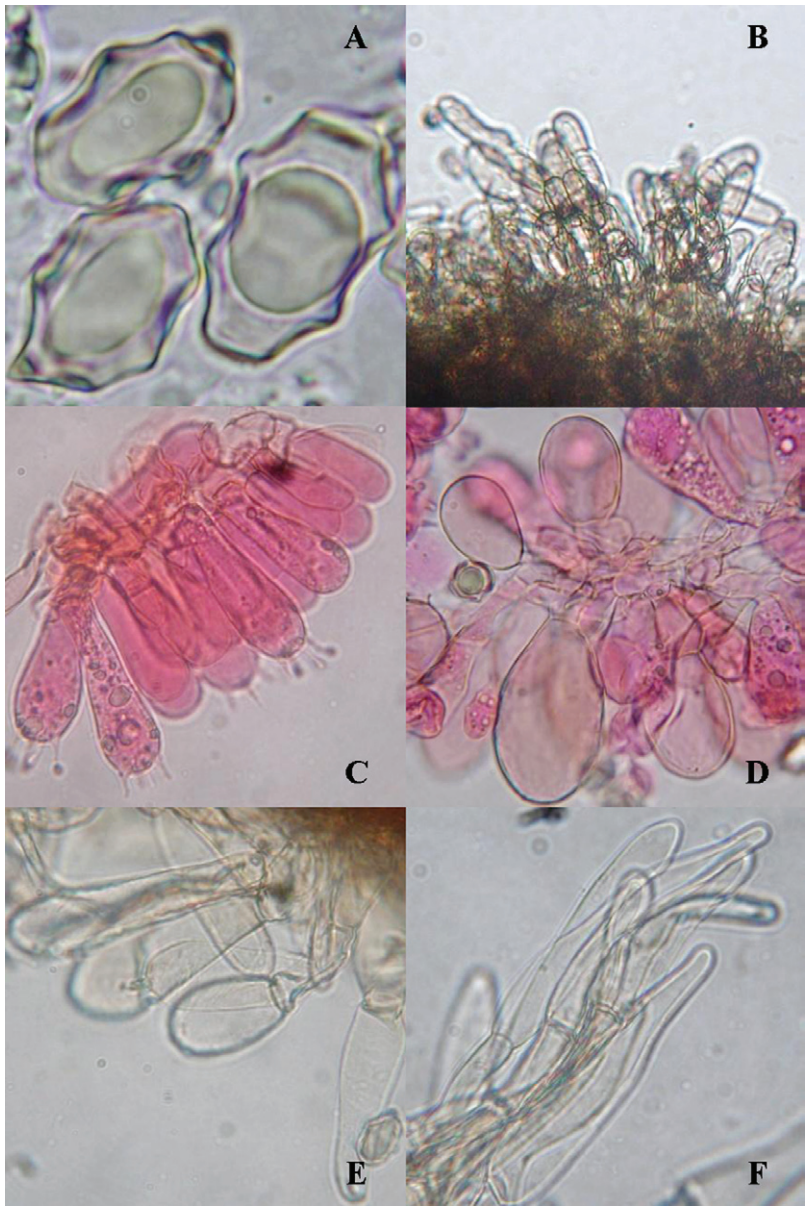

Plate 10 – *Pouzarella farinosa*. A: Basidiospores (1000×) (DLL 9900); B: Pileipellis (400×) (DLL 9934, holotype); C: Basidia (400×) (DLL 9900); D: Thick-walled cheilocystidia with brownish pigment (400×) (DLL 9934); E: Pileocystidia (400×) (DLL 9900); F: Acuminate caulocystidia (400×) (DLL 9934).

6. ***Pouzarella setiformis*** Largent & Abell-Davis, **sp. nov.** PLATES 11–12

MYCOBANK MB 519586

Habitu Pouzaromyceti transito *similis, sed pileo juventute hispido squamuloso, squamulis primo pallido-coloratis tum cito atro-griseo-brunneo, postremo aetate pallide vel mediocriter brunneolo-griseo decolorantibus, peileocystidiis caulocystidiisque multicellularibus setiformibus differt.*

TYPE — Australia, Queensland, Cook Region, Danbulla National Park, Lake Euramoo Track, 17°09′41.1″S 145°37′46.9″E, 714.5 m, 21 March 2010, DL Largent 9809 (**holotype** BRI, **isotype** CNS), sequences: HQ876547 (mtSSU), HQ876525 (LSU), HQ876503 (RBP2).

ETYMOLOGY — derived from the Latin *seta* (= bristle) + *forma* (= shape), for setiform pileocystidia and caulocystidia.

PILEUS 8.5–12.5 mm broad, 3–5.5 mm high, convex then broadly convex, scurfy with ± erect light colored (6C3) brownish-orange ± hispid squamules on the disc, tomentulose to tomentose elsewhere, overall dark grayish-brown (6-7E-F2-3) fading to light or medium brownish-gray (6D3) with age, dull, opaque, dry, even when young becoming ± sulcate and striate with age, not hygrophanous; margin decurved then plane, minutely hispid. TASTE and ODOR indistinct. LAMELLAE 3–5.5 mm long, 1–2 mm high, narrow to moderately broad, adnexed to adnate, subdistant to distant, dark brownish-gray to dark grayish-brown at first (7-8E-F2-3); margin smooth and concolorous. LAMELLULAE 1–3 (2 short, 1 medium long) between lamellae. STIPE 15–25 mm long, 0.75–1.0 mm broad, equal, stiff but hollow, fragile and breaks easily, with abundant stiff, light brownish-gray (6D3 or 7C3) ± tomentulose pointed hairs and squamules at the apex, pruinose to fibrillose dark brownish-gray (6-7E-F3); elsewhere, dull, suggestively strigose at the base. BRUISING REACTION absent.

BASIDIOSPORES nodulose-angular with 6–8 angles, heterodiametric in profile view, isodiametric and 6-angled in polar view, 8.2–13.9 × 4.8–7.9 µm (x_m = 10.7 ± 1.0 × 6.3 ± 0.7 µm; E = 1.4–2.5; Q = 1.73 ± 0.24 (heterodiametric); n = 56/2). BASIDIA 25.7–32.9 × 8.5–12.2 µm; E = 2.11–3.85; Q = 2.95. CHEILOCYSTIDIA absent. PSEUDOCYSTIDIA absent. ABORTED BASIDIA often with dark brown contents in 3% KOH, aborted sterigma and/or aborted basidiospores often present, 26.2–41.0 × 6.8–10.7 µm. Subhymenium composed of thin and short hyphae. HYPHAE OF LAMELLAR TRAMA long and broad, 58.3–253.7 × 3.1–26.6 µm. PILEIPELLIS a dense compactly entangled chain of 3–5 cells, 55–144 µm deep in the center and towards the margin, at the margin with tufts of hyphae, 93–150 µm deep. PILEOCYSTIDIA cylindro-clavate in the center to near the margin, 23.9–64.9 × 8.8–17.4 µm (x_m = 40.5 × 12.8 µm; E = 2.20–4.07; Q = 3.13; n = 9/1), at the margin thick-walled and setiform, 42.4–69.9 × 4.5–5.7 µm. STIPITIPELLIS at the apex with tufts of heavily incrusted hyphae, 74–190 µm deep, entangled hyphae between the tufts. CAULOCYSTIDIA aculeate to aciculate, clear and ± setiform when in abundant tufts, 47.0–121.0 ×

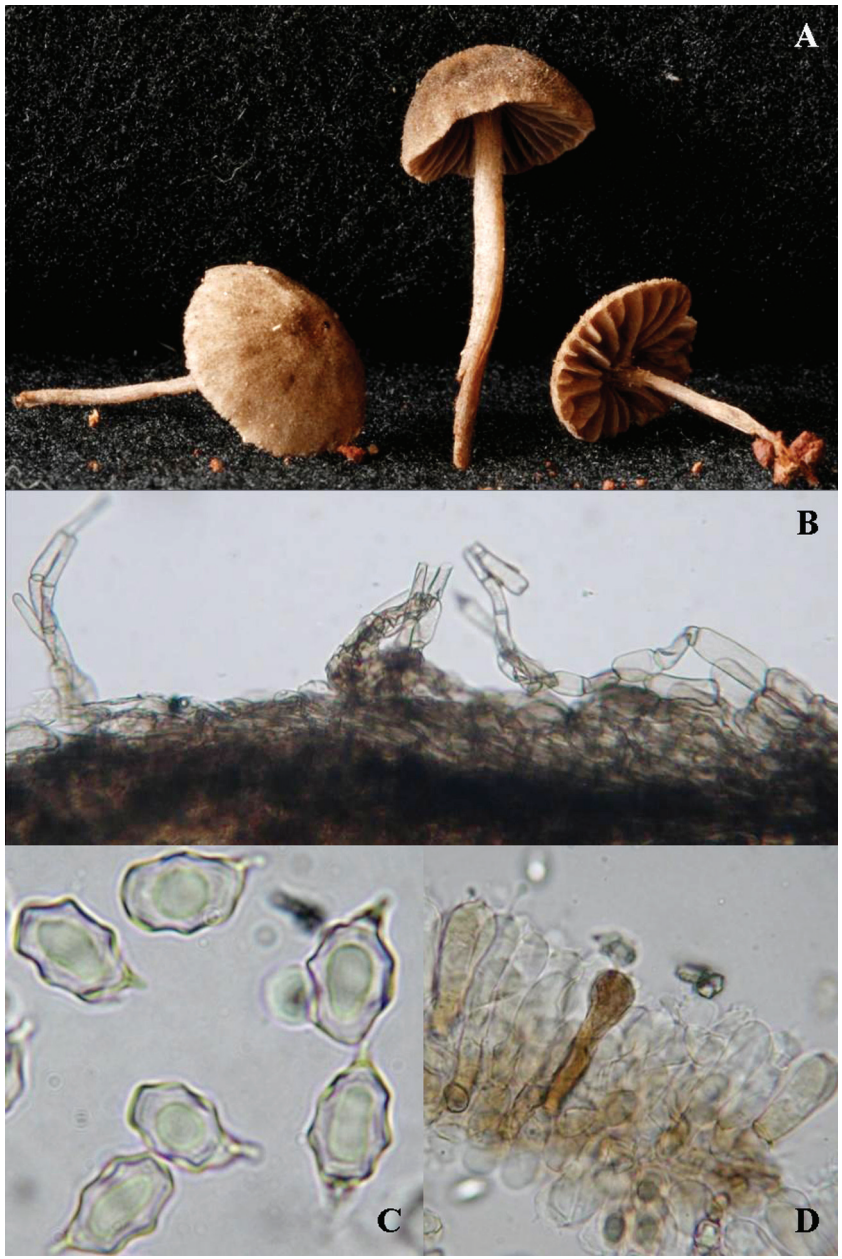

Plate 11 – *Pouzarella setiformis* (DLL 9809, holotype). A: Basidiomata;
B: Pileipellis (100×); C: Basidiospores (1000×); D: Basidia and pseudocystidia (400×).

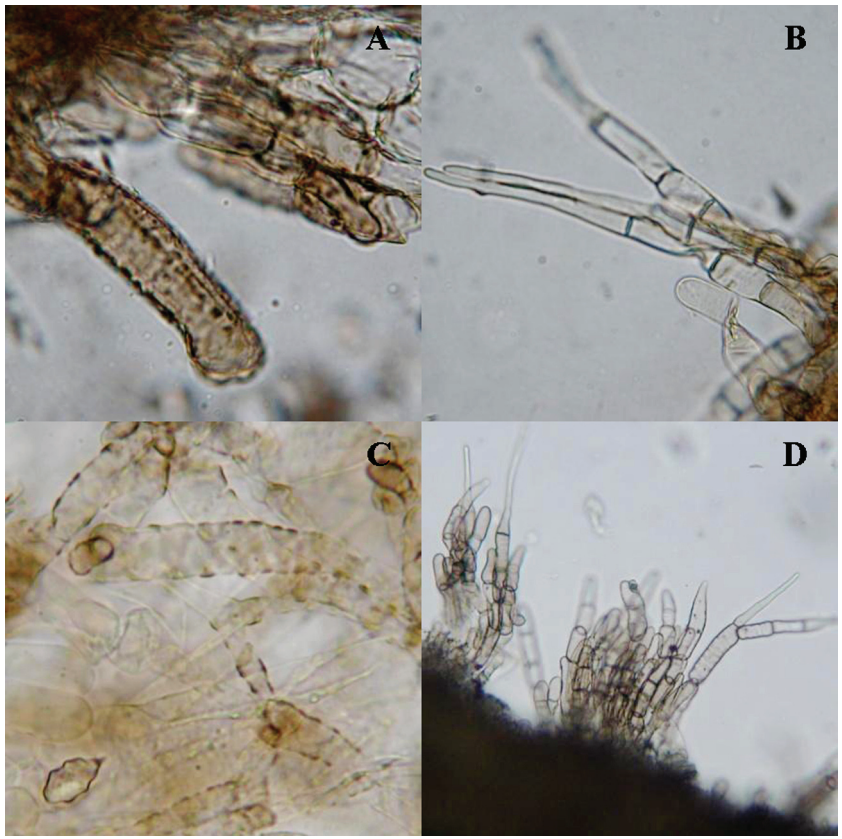

Plate 12 – *Pouzarella setiformis*. A: Pileocystidia with parietal pigmentation (400×) (DLL 9809, holotype); B: Multicellular setiform pileocystidia (400×) (DLL 9809); C: External incrustations on tramal hyphae of pileus (400×) (DLL 9810); D: Multicellular setiforma caulocystidia (100×) (DLL 9809).

4.8–7.9 µm; E = 8.44–17.50; Q = 10.89; clear and acuminate when not in tufts, 17.4–61.3 × 5.5–11.6 µm; E = 1.93–6.15; Q = 3.13. Pigmentation heavily incrusted on the outer walls of the tramal hyphae of the stipe, pileus and lamellae; parietal and heavily incrusted on inner and outer cell walls of the pileipellis and stipitipellis excluding the pileocystidia and caulocystidia; thick-walled, not incrusted, ± suggestively parietal but mostly clear in the setiform pileocystidia and caulocystidia.

Ecology and distribution: Solitary or scattered in hard packed soil in a complex mesophyll vine forest; northeastern Queensland.

Additional collections examined — **AUSTRALIA**. **Queensland**, Cook Region, Danbulla National Park, Lake Euramoo track, 17°09′41.6″S 145°37′40.7″E, 714.5 m, 21 March 2010, DL Largent 9810 (mtSSU HQ876548, LSU HQ876526, RPB2 HQ876504).

Comments— *Pouzarella setiformis* is diagnosed by a pileus that is hispid-squamulose on disc and elsewhere tomentulose to tomentose with hairs that are initially light colored but quickly become dark gray brown before fading to light or medium brownish-gray with age and a young even margin that becomes ± sulcate and striate with age, nodulose-angular, heterodiametric basidiospores averaging 10.7 × 6.3 µm, the absence of cheilocystidia, and the presence of aborted basidia, setiform pileocystidia and caulocystidia, and encrusted pileipellis and stipitipellis hyphae.

Pouzaromyces napaliensis from the Hawaiian Islands, *P. transitus* E. Horak from Papua New Guinea, and *Entoloma testaceostrigosum* from India have nodulose-angular heterodiametric basidiospores and setiform caulocystidia and lack cheilocystidia, features common to *P. setiformis*. *Pouzaromyces napaliensis* is distinguished by its slightly larger (11.2–15.4 × 7–9.6 µm) basidiospores, uniformly beige pileus and lamellae, smaller size (2–5 mm broad pileus, 3–4 mm long stipe), and weakly incrusted tramal hyphae (Desjardin & Baroni 1991). *Pouzaromyces transitus* differs in its black to soot-brown basidiomata and pileus hairs and single-celled setiform caulocystidia (Horak 1980). *Entoloma testaceostrigosum* is separated by its larger (12.5–17.0 × 7–10 µm) basidiospores and absence of encrusted pileipellis and stipitipellis hyphae (Manimohan et al. 2006).

Australian taxa related to *Pouzarella setiformis*

Pouzarella setiformis, P. albostrigosa, *P. fusca*, *P. lasia* and *P. debilis* form a monophyletic group that share a brown squamulose hispid pileus, stipe surface composed of a layer of entangled brownish fibrils, pileipellis composed of a chain of cells with clavate to broadly clavate pileocystidia, more or less stipitipellis with occasional entirely clavate to broadly cylindro-clavate caulocystidia, basidiospores averaging 10.0–12.0 µm long, aborted basidia that become reddish-brown in 3% KOH, and absence of cheilocystidia.

Within this group *P. setiformis* differs from *P. debilis*, *P. fusca*, *P. albostrigosa,* and *P. lasia* by a stipe surface with abundant stiff, ± tomentulose pointed hairs that is pruinose to fibrillose except for the apex squamules and a stipitipellis and pileipellis with scattered to abundant setiform caulocystidia and pileocystidia (most evident at the margin). The other taxa in this clade (*P. debilis*, *P. fusca*, *P. albostrigosa* and *P. lasia*) possess instead a stipe surface that is densely wooly or covered with abundant loose fibrils to nearly glabrous and non-setiform cystidia.

Pouzarella debilis, P. fusca, and *P. albostrigosa* share a convex to broadly convex pileus, a relatively shorter (typically ≤20 mm) stipe, aborted basidia typically imbedded in the hymenium, and caulocystidia, pileocystidia, and many subterminal cells with strong parietal pigmentation comprising coarse bands and obvious internal incrustations. *Pouzarella fusca* produces a young

pileus that is opaque and black to dark gray brown, basidiospores averaging 10.0 × 7.3 µm, and caulocystidia of 2 types (some thick-walled and pigmented). *Pouzarella albostrigosa* has light colored squamules when young, basidiospores averaging 11.5 × 7.7 µm, and caulocystidia of 2 types (some thick-walled and pigmented). *Pouzarella debilis* combines light-colored squamules when young, basidiospores averaging 10.4 × 6.3 µm, and thin-walled ± colorless caulocystidia, similar in shape to the pileocystidia.

Lastly, *P. lasia* produces a conic to campanulate pileus (at least when young), a stouter ≤50 mm long stipe, aborted basidia that distinctly project beyond the hymenium, caulocystidia and pileocystidia with obscure bands and minute internal incrustations, and absence of setiform pileocystidia and caulocystidia.

7. ***Pouzarella debilis*** (Corner & E. Horak) Largent & Abell-Davis, **comb. nov.**

MycoBank MB 519572 Plates 13–14

≡ *Pouzaromyces debilis* Corner & E. Horak, Beih. Nova Hedwigia 65: 42 (1980).

Pileus 5 mm broad, 4 mm high, convex, at first entirely opaque and hispid-squamulose with the squamules off-white (5A-B1-2) and erect in the center, suberect elsewhere, base color of the center grayish-brown (6F3-4) and elsewhere slightly lighter grayish-brown (6E-F3-4) when faded remaining so on the disc but becoming appressed squamulose and striate to the margin, elsewhere with the base color light grayish-brown (near 6C3-4) and the striae darker grayish-brown (6E3-4), dry, dull, not hygrophanous, even; margin decurved, minutely fringed and crenulate at the very edge of the margin. Taste and Odor unknown. Lamellae 7.5 mm long, uncinate with a short decurrent tooth, close to subdistant, narrow (1 mm deep); margin smooth and concolorous. Lamellulae very short, commonly 1, infrequently 3 (all short) between 2 lamellae. Stipe 15 mm long, 0.5 mm broad, equal, on the surface with loosely entangled fibrils or scattered pruina, obvious at the apex and base, inconspicuous elsewhere, surface fibrils similarly colored to those of the pileus, base color grayish-orange (6B-C3-4) and upon handling the base bruises a grayish-brown (6E3-4), hollow and fragile because of size. Bruising reaction absent.

Basidiospores nodulose-angular with 6–8 angles, heterodiametric in profile view, isodiametric and 6-angled in polar view, 8.7–12.0 × 5.0–7.4 µm, (x_m = 10.44 ± 0.8 × 6.3 ± 0.6 µm; E = 1.36–2.27; Q = 1.67 ± 0.22; n = 29/1). Basidia 4-sterigmate, clavate, tapered, 27.6–36.5 × 8.9–12.8 µm, (x_m = 31.8 ± 2.7 × 11.1 ± 1.2 µm; E = 2.33–3.49; Q = 2.88 ± 0; n = 10/1). Cheilocystidia absent. Pseudocystidia absent. Aborted basidia abundant on the gill edge and face, colored reddish-brown in 3% KOH, often with 1–2 sterigma and aborted basidiospores, slightly projected beyond the hymenium, 33.6–46.8 × 7.4–11.6 µm. Hyphae of lamellar trama subparallel, moderately long,

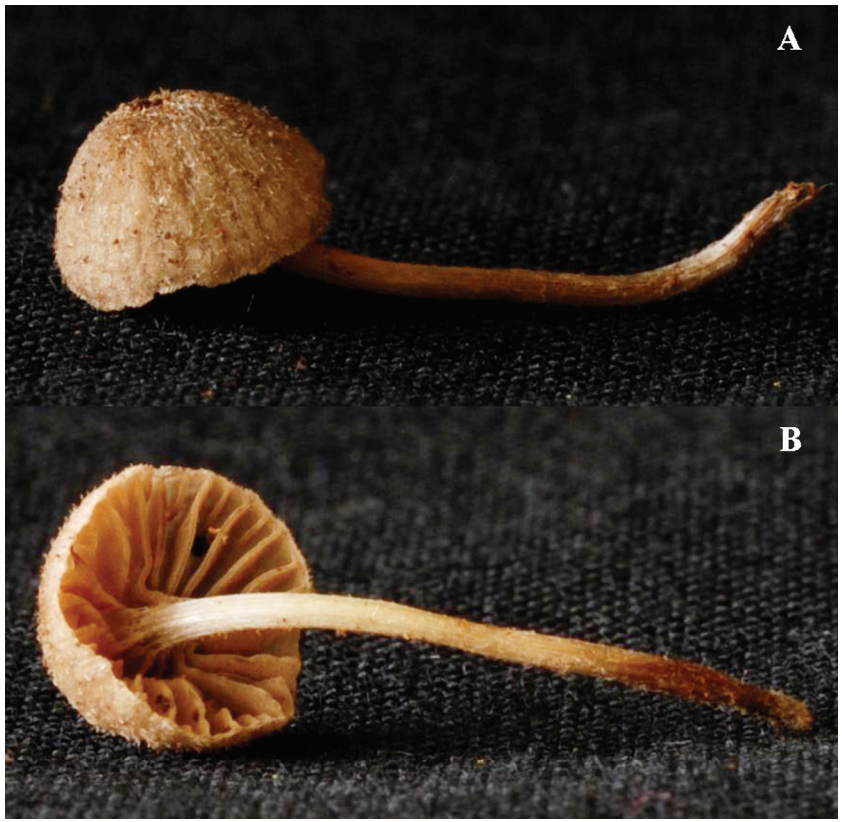

Plate 13 – *Pouzarella debilis* (DLL 9784). A–B: Basidiomata.

narrow to fairly wide, 184.9396.8 × 6.2–30.8 µm. Pileipellis with scattered, entangled hyphae composed of chains of 3–5 cells, subpellis without inflated cells, hyphae similar to pileal trama. Pileocystidia broadly cylindro-clavate 35.1–58.4 × 9.7–20.0 µm (E = 2.49–4.10; Q = 3.2). Stipitipellis with scattered areas of clustered hyphae similar to those of the pileipellis, 29–103 µm long, a cutis between the clusters. Caulocystidia similar to pileocystidia, 20.7–54.4 × 7.0–16.5 µm (x_m = 34.9 ± 10.6 × 11.6 ± 2.7 µm; E = 1.82–6.41; Q = 3.14 ± 1.31; n = 16/1). Pigmentation moderately externally incrusted in the trama of the pileus, stipe and lamellae; parietal in the form of minute to moderate internal incrustations in the hyphae of the stipitipellis and pileipellis.

Ecology and distribution: Solitary in dark crumbly soil on an open hillside in complex mesophyll vine forest of cloudy to moist highlands on granite; Mt. Hypipamee National Park. Previously reported from Papua New Guinea and the Solomon Islands.

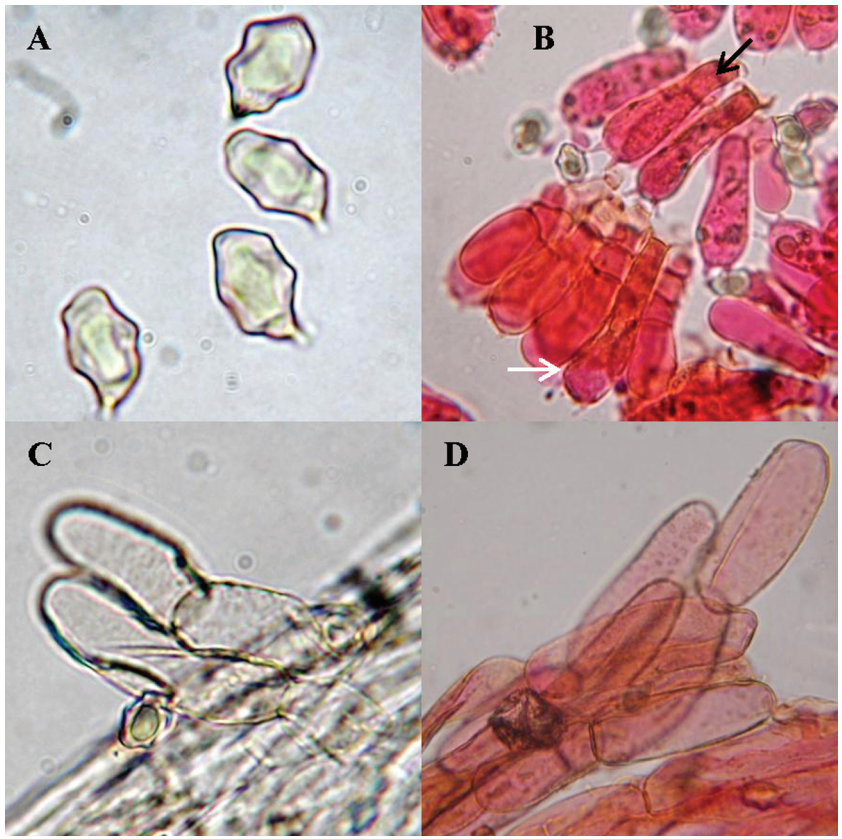

Plate 14 – *Pouzarella debilis* (DLL 9784). A: Basidiospores (1000×); B: Basidium (black arrow), pseudocystidium (white arrow) (400×); C: Caulocystidia (400×); D: Pileipellis hyphae with clavate to broadly cylindro-clavate pileocystidia (400×).

Collections examined — **AUSTRALIA. Queensland**, Cook Region Mt. Hypipamee National Park, Falls Loop Track, 17°25′36.9″S 145°29′07.9″E, 714.5 m, 16 March 2010, DL Largent 9784 (holotype BRI, isotype CNS; mtSSU HQ876550, LSU HQ876528, RPB2 HQ876506).

Comments— *Pouzarella debilis* is diagnosed by small fragile basidiomata (pileus 5 mm broad, stipe 15 × 0.5 mm), a hispid-squamulose pileus at first with off-white squamules that are erect on the disc and elsewhere suberect over a medium to dark grayish-brown base color and in age becoming striate from the margin inwards and fading to light grayish-brown, aborted basidia that project slightly beyond the hymenium, absence of cheilocystidia, caulocystidia resembling the pileocystidia, and basidiospores averaging 10.4 × 6.3 µm, (Q = 1.67). The macro- and micro-morphological features of the Australian

collection *P. debilis* are nearly identical with those described for *Pouzaromyces debilis* from Papua New Guinea and the Solomon Islands (Horak 1980).

Pouzarella domingensis T.J. Baroni from the Dominican Republic and *P. dunstervillei* (Dennis) Mazzer have moderate-sized basidiospores and lack cheilocystidia, features also noted for *P. debilis*. *Pouzarella domingensis* differs in the lack of light-colored squamules in young pilei, inflated subpellis hyphae, and larger basidiospores averaging 11.0 × 7.6 µm (Baroni et al. 2008), while *P. dunstervillei* (which also lacks light-colored squamules in young pilei) has a nearly glabrous pileus and stipe (Mazzer 1976). *Pouzarella pulverea* (Rea) Mazzer from Great Britain and the Netherlands, *Entoloma romagnesii* Noordel. from France, and *E. lomapadum* Manim. et al. from India also have similarly sized basidiospores, but all three lack light-colored squamules in the young pilei and possess thin walled cheilocystidia (Mazzer 1976, Manimohan et al. 1995, Noordeloos 1979, 2004).

See *P. setiformis* for comparisons with other Australian entolomatoid fungi similar to *P. debilis*.

8. ***Pouzarella fusca*** Largent & Abell-Davis, **sp. nov.** Plates 15–16

MycoBank MB 519573

Habitu Entolomate romagnesii *(subg.* Pouzarellae*) similis, sed pileo infuscate atro-brunneo, pigmento extrorsum in hyphis ad partes pileipellis stipitipellisque pertinentibus incrustato, pigmentatione perietali pileocystidiorum cauocystidiorumque, cheilocystidiis nullis differt.*

Type — Australia. Queensland, Cook Region, Dinden National Park, 17°02′14″S 145°36′4″E, 727.6 m, 24 February 2009, DL Largent 9623 (**holotype** BRI, **isotype** CNS), sequences: HQ876549 (mtSSU), HQ876527 (LSU), HQ876505) (RBP2).

Etymology — derived from Latin *fuscus* (= dark brown), referring to the dark blackish-brown coloration.

Pileus 3.5–10 mm broad, 1.5–3.0 mm high, consistently convex, minutely but entirely squamulose, erect in the center, imbricate squamulose elsewhere, becoming appressed squamulose at the very margin, black to a very dark gray brown (not matching any color to Kornerup & Wanscher (1978), fading to dark gray-brown (6F3) except a medium gray-brown (6E-F3) on the margin, dull, opaque, not hygrophanous, not striate; trama dark gray to black, less than 0.5 mm thick above the stipe; margin slightly incurved to decurved, entire. Taste mild. Odor somewhat pungent. Lamellae 2.5–3.5 mm long, 0.5–1.0 mm high, concolorous with the pileus, adnate, subdistant and narrow; margin smooth and concolorous. Lamellulae 3 (2 short, 1 medium) between lamellae. Stipe 8–17 mm long, 0.8–2.0 mm broad, equal, dark gray (6F1), minutely and entirely squamulose to covered with loose fibrils; basal tomentum scarce, strigose base present. Bruising reaction absent.

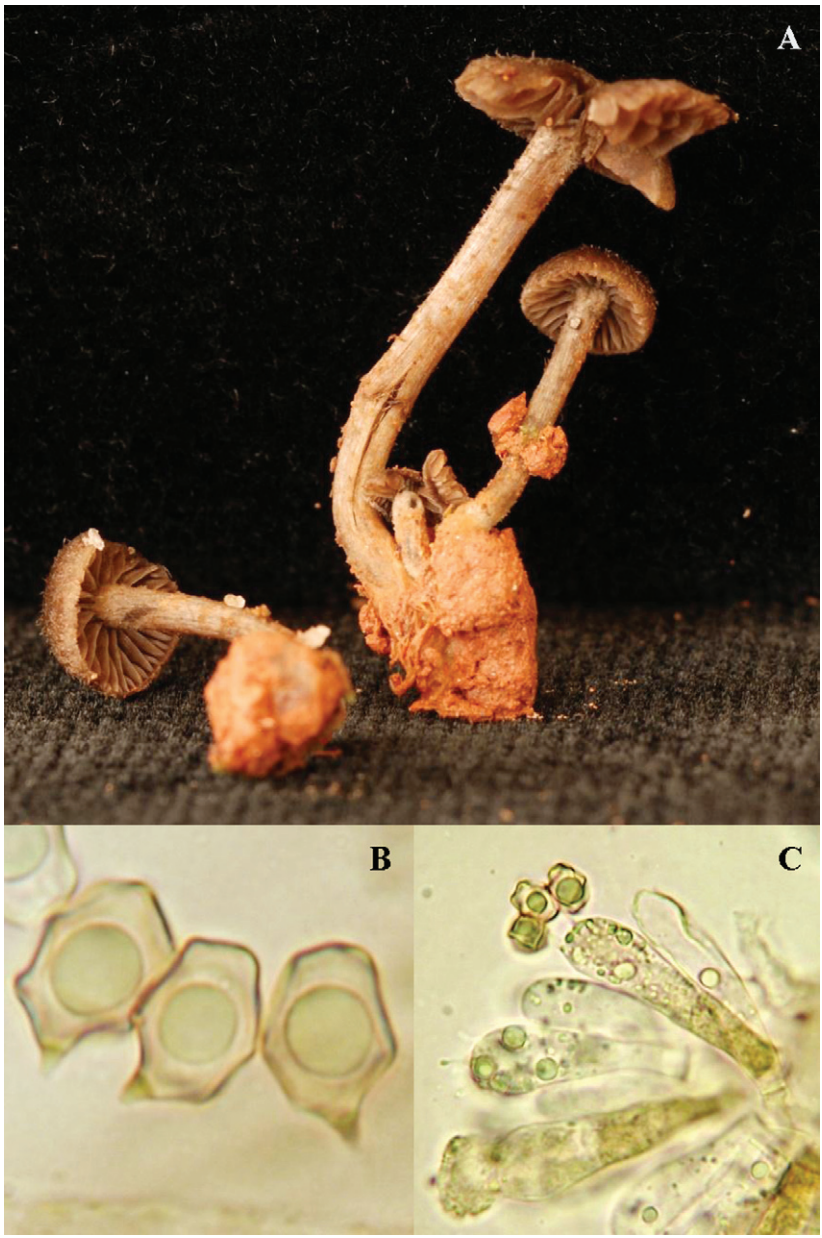

Plate 15 – *Pouzarella fusca* (DLL 9623, holotype).
A: Basidiomata; B: Basidiospores (1000×); C: Basidia with basidiospores (400×).

Basidiospores distinctly angular to slightly nodulose, in profile view typically 6-angled, often 7–8-angled, nearly or definitely heterodiametric, 8.6–11.5 × 6.1–8.7 µm (x_m = 10.0 ± 0.8 × 7.3 ± 6 µm; E = 1.22–1.54; Q = 1.38 ± 0.09 (heterodiametric); n = 28/1). Basidia 4-sterigmate, sterigma rather long, 4.2–5.5 µm; clavate, origin unusual for entolomatoid fungi, originating from a basal cell in the subhymenium, 33.9–47.2 × 8.9–12.6 µm (E = 2.8–4.7; Q = 3.83); base 3.0–6.5 µm. Pleurocystidia absent. Pseudocystidia absent. aborted basidia abundant, but scattered on the lamellae, clavate, often with aborted sterigma, not projecting beyond the hymenium and filled with opaque contents that stain reddish-brown in 3% KOH, 35.0–44.6 × 8.7–13 µm (x_m = 40.9 × 11.0 µm; E = 3.1–4.6; Q = 3.78; n = 7/1). Hyphae of the lamellar trama rather short, 29.6–179.0 × 4.7–11.0 µm. Pileipellis 240–400 µm deep, composed of chains of cells, erect or suberect, on or near the disc and towards the margin, becoming repent at the margin. Pileocystidia clavate to broadly cylindric, 36.5–118.9 × 5.2–21.4 µm (E = 2.6–6.1; Q = 5.04). Hyphae of the pileal trama rather short, 45.5–105.4 × 2.1–8.0 µm. Stipitipellis similar to the pileipellis and composed of solitary caulocystidia. Caulocystidia of two types; cylindro-clavate and the terminal cells comprised of a loosely entangled hyphae, 20.8–73.2 × 4.7–10.9 µm (E = 3.6–8.1; Q = 5.53), and clavate to broadly napiform and the terminal cells comprised of solitary hyphal walls and in clusters with the hyphae originating from a common base, 22.3–57.7 × 9.1–14.3 µm (E = 1.7–5.4; Q = 2.71). Hyphae of the stipe trama parallel to subparallel, 60.5–336.3 × 4.5–12.5 µm. Oleiferous hyphae rare in the trama of the stipe, pileus and lamellae. Lipoid bodies present in the basidia, absent in the aborted basidia and tramal hyphae of the pileus, lamellae and stipe. Pigmentation externally incrusting the hyphae of the basal portion of the pileipellis and stipitipellis and distinct and strong on the tramal hyphae of the lamellae, pileus and stipe; suggestively externally incrusting the outer hyphal and more obviously parietal on the inside the hyphal walls of the terminal 2–3 cells of the pileipellis and stipitipellis, including the pileocystidia and the caulocystidia.

Ecology and distribution: Scattered or connate in dark soil on high banks along road just above bridge at end of Davies Creek Road in a simple to complex notophyll vine forest, Dinden National Park.

Comments— *Pouzarella fusca* is diagnosed by the dark gray to dark blackish-brown to black coloration when young, a pileus that is initially squamulose on the disc and imbricate squamulose near the margin, only slightly nodulose

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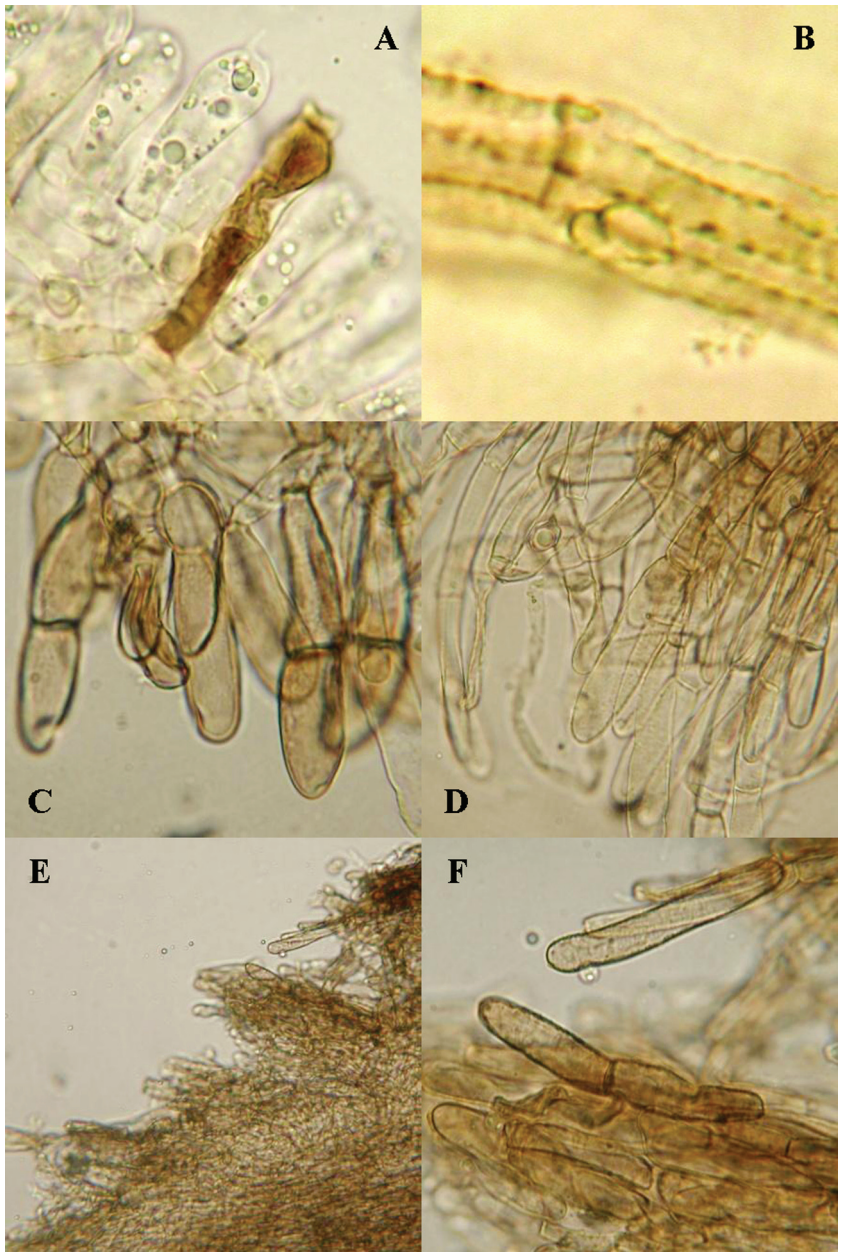
Plate 16 – *Pouzarella fusca* (DLL 9623, holotype). A: Pseudocystidium, origin from incrusted hyphae in subhymenium, basidium origin from non-incrusted hyphae (400×); B: Externally incrusted pigment on walls of lamellar trama (400×); C: bullet-shaped caulocystidia (400×);



D: cylindro-clavate caulocystidia (400×); E: Pileipellis near pileus center (100×); F: Cylindro-clavate to clavate pileocystidia (400×).

6–8 angled basidiospores averaging 10.0 × 7.3 µm, caulocystidia of two types (cylindro-clavate or napiform to clavate), and abundant aborted basidia.

The distinctive features of *P. fusca* are not found in any other *Pouzarella* species. *Entoloma romagnesii* described from France has similarly shaped and sized basidiospores but has a dark yellow-brown pileus and stipe, broadly ellipsoid to clavate cheilocystidia, and non-encrusted pileus and stipe hairs (Noordeloos 1979).

See *P. setiformis* for comparisons with other Australian entolomatoid fungi similar to *P. fusca*,.

9. ***Pouzarella albostrigosa*** Largent & Abell-Davis, **sp. nov.** PLATES 17–18

MYCOBANK MB 519587

Habitu Pouzarellae lasiae *similis, sed pileo convexo vel late convexo, base stipitis alba distincte strigosa, stipite minus robusto usque ad 22 mm longo, pseudocystidiis in hymenio inclusis, caulocystidiis pileocystidiisque pigmentatione parietali in parietibus internis e fasciis tumoribusque composita differt.*

TYPE — Australia, Queensland, Cook Region, Mt. Hypipamee National Park, Kauri Creek Track, 17°25′35.7″S 145°29′11.4″E, 964.7 m, 10 March 2009, DL Largent 9641 (**holotype** BRI, **isotype** CNS), sequences: HQ876557(mtSSU), HQ876535(LSU), HQ876513 (RBP2).

ETYMOLOGY — derived from the Latin *albus* (= white) + *strigosus* (= bristly), referring to the white stipe base.

PILEUS 3–10 mm broad, 0.8–2.5 mm high, convex to broadly convex, at first opaque, entirely hispid-squamulose with light colored squamules (between white and orange-white or reddish-white, 5-7A-B1-2) on a dark smoky brown to blackish-brown background (6-7F2-3), upon expanding and at maturity remaining hispid-squamulose on the disc, appressed squamulose to appressed fibrillose toward the margin and appressed fibrillose at the margin with an overall color of blackish-brown on the disc (close to but darker than 7F2) and slightly lighter elsewhere (close to but darker than 6-7F2-3) and becoming striate up to the center, eventually fading overall to dark grayish-brown (5F3) but remaining striate, dull, not hygrophanous and even; margin entire, decurved and opaque then striate. TASTE indistinct to suggestively farinaceous. ODOR indistinct. LAMELLAE 2.5–5.5 mm long, adnate to uncinate, subdistant to distant, narrow to moderately broad (1.0–1.8 mm high), dark gray to dark brownish-gray (7F2), and then appearing dark grayish-brown (5E-F4) and eventually light grayish-brown (5C-D3) after basidiospore maturation; margin smooth, somewhat lighter than the surface. STIPE 6–22 mm long, 0.8–1.0 mm broad at the apex, equal, covered with a dense layer of light colored fibrils or squamules on a dark brown (5-7E-F3-4) faintly fibrillose surface, solid at first, then hollow, basal tomentum absent to scarce; basal area pruinose just above white, distinctly strigose base. BRUISING REACTION stipe bruising dark

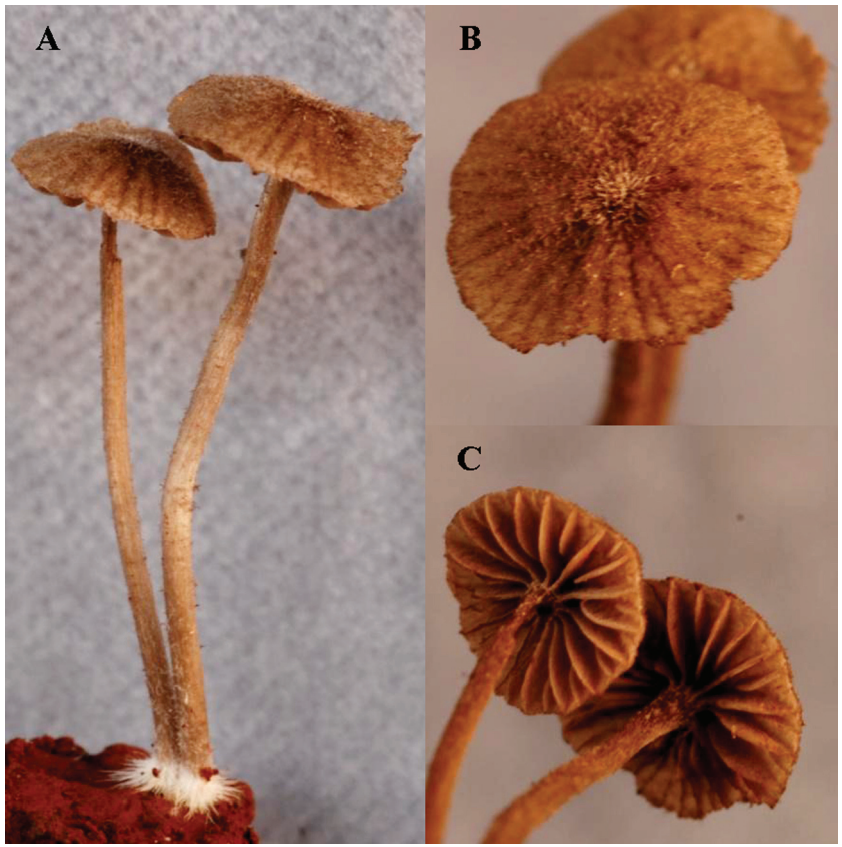


Plate 17 – *Pouzarella albostrigosa* (DLL 9641, holotype).
A: Basidiomata stature; B: Pileal surface; C: Lamellae and lamellulae.

gray brown when surface fibrils become appressed to the surface. Bruising reaction absent.

Basidiospores moderately nodulose-angular with 7–9 angles, heterodiametric in profile and side views, isodiametric and 6-angled in polar view, 9.1–15.6 × 6.7–9.2 µm (x_m = 11.5 ± 1.4 × 7.7 ± 0.6 µm; E = 1.27–2.16; Q = 1.49 ± 1.6; n = 57/3). Basidia 2–4 sterigmate, clavate and tapered, colorless in 3% KOH, 21.0–69.4 × 7.1–13.2 µm (x_m = 38.8 ± 14.0 × 9.7 ± 1.6 µm; E = 2.06–6.71; Q = 4.07 ± 1.50; n = 24/2). Pleurocystidia absent. Pseudocystidia absent. aborted basidia very abundant, nearly always with 2–4 sterigma and a few with aborted basidiospores, clavate and tapered, equal to but more often ⅓ to ¼ more narrow at the base than the basidia, embedded in the hymenium and not projecting beyond the basidia, often but not always, originating from the outer portions of the lamellar trama, dark reddish-brown in 3% KOH, 28.6–41.9 ×

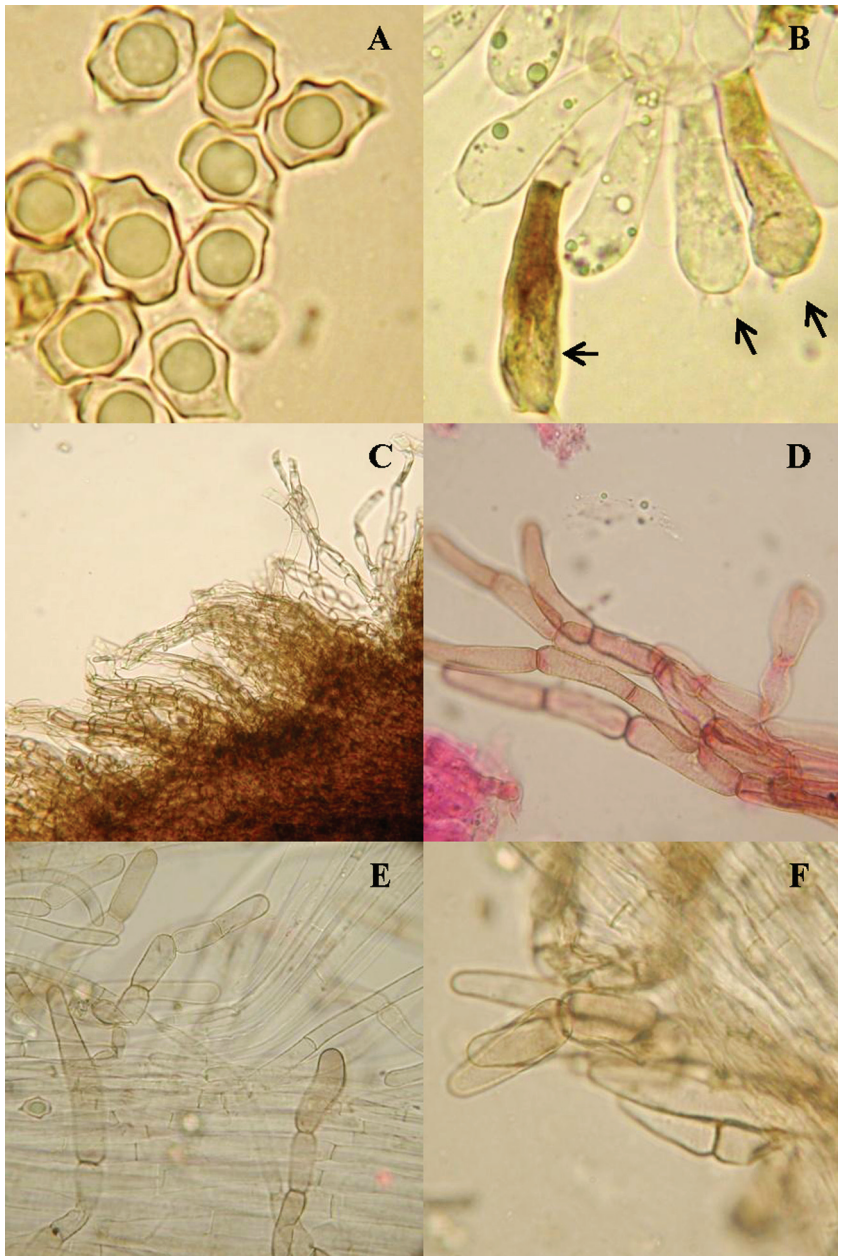


Plate 18 – *Pouzarella albostrigosa*. A: Basidiospores (1000×) (DLL 9641: holotype); B: Pseudocystidia (black arrows) (400×) (DLL 9641); C: Pileipellis in disc area (100×) (DLL 9663);

7.1–12.2 µm (E = 2.69–4.33; Q = 3.71 ± 0.48; n = 11/1). Lamellar tramal hyphae did not revive well and was not measured. Pileipellis up to 100–150 µm deep, composed of clusters (i.e. the squamules) of 5–10 laterally attached hyphae, each hypha with 5–10 cells; the clusters tapered with 5–10 hyphae laterally agglutinated at the base and 2–4 hyphae laterally agglutinated at the apex, erect on the disc, semi-erect towards the margin and nearly repent at the margin. Pileocystidia typically narrowly cylindro-clavate, at times broadly cylindro-clavate, rarely clavate, 22.5–63.6 × 5.7–21.6 µm (x_m = 41.2 ± 9.1 × 11.1 ± 3.8 µm; E = 1.42–8.40; Q = 4.20 ± 1.80; n = 36/3) Pileal tramal hyphae did not revive well and was not measured. Stipitipellis a cutis of pigmented hyphae between abundant scattered clusters of pigmented hyphae with clavate, broadly obclavate or broadly cylindro-clavate thick-walled; pigmented caulocystidia and scattered clusters of loosely entangled hyphae, 5–10 cells long, with cylindro-clavate to clavate, colorless caulocystidia. Caulocystidia 21.0–69.4 × 7.9–13.2 µm (n = 24/3). Stipe tramal hyphae parallel, 86–330 × 6–29 µm (n = 17/1). Oleiferous hyphae rare to nearly absent, reddish-brown in 3% KOH. Pigmentation distinct, coarsely and externally incrusting the tramal hyphae of the stipe and most probably the pileus and lamellae; parietal and with bands and bumps on the inner walls of the pileipellis, stipitipellis, pileocystidia and some caulocystidia, externally incrusting the outer walls of the stipitipellis; uniformly cytoplasmic in some pileocystidia and caulocystidia.

Ecology and distribution: Solitary in dark crumbly soil on hillside with water pipe above a trail or in hard packed soil within a complex mesophyll vine forest of high rainfall; northeastern Queensland.

Additional collections examined — **AUSTRALIA**. **Queensland**, Cook Region, Mt. Hypipamee National Park, 17°25′35.7″S 145°29′11.4″E, 964.7 m, 18 March 2009, DL Largent 9666; Danbulla National Park, Lake Euramoo Track, 17°09′42.3″S 145°37′45.6″E, 762.6 m, 18 March 2009, DL Largent 9663 (mtSSU HQ876558, LSU HQ876536, RPB2 HQ876514).

Comments— *Pouzarella albostrigosa* typically has a convex pileus that is hispid-squamulose on the disc and imbricate squamulose elsewhere and initially covered with white to orange-gray or reddish-gray tipped squamules that become dark brownish-gray with age and exposure, a ≤22 long stipe covered by light-colored layer of entangled fibrils overlying longitudinally appressed dark brown fibrils and with distinct white strigose fibrils at the base, nodulose-angular basidiospores averaging 11.5 × 7.7 µm, absence of cheilocystidia, and the presence of aborted basidia embedded in the hymenium, pileocystidia and caulocystidia with internal parietal pigmentation in the form of bands and

D: Pileocystidia (400×) (DLL 9641); E: Stipitipellis squash mount showing chains of cells and caulocystidia as terminal cells (400×) (DLL 9641); F: Stipitipellis periclinal section showing chains of cells with caulocystidia as terminal cells (400×) (DLL 9641).

bumps (some uniformly pigmented) and both thick-walled pigmented and thin-walled colorless caulocystidia.

See *Pouzarella setiformis* for comparisons with Australian taxa related to *P. albostrigosa*.

10. ***Pouzarella lasia*** (Berk. & Broome) Largent & Abell-Davis, **comb. nov.**

MycoBank MB 519574 Plates 19–20

≡ *Agaricus lasius* Berk. & Broome, J. Linn. Soc., Bot. London. 11: 539 (1871).

Pileus 4–10 mm broad, 2.5–7 mm high, conic to campanulate, at first hispid-squamulose on the disc and imbricate squamulose elsewhere, remaining so on the disc when mature but becoming appressed squamulose towards the margin and appressed fibrillose on the margin, the squamules entirely pallid to brownish-orange (5C3) at first, with age darkening from the base towards the tip, dark brownish-gray (5E-F3), eventually entirely dark brownish-gray (5-7F2-3) on the disc and a lighter brownish-gray elsewhere (5-7E2-3); marginal fibrils concolorous with nearby squamules dull, opaque and even at first, becoming striate when faded; margin decurved, entire. Taste and Odor indistinct. Lamellae 2–6 mm long, rarely adnexed to more commonly uncinate, subdistant, narrow to moderately broad (1–2 mm deep) when 2–6 mm long, when young entirely brownish-gray (5E-F3-4) and then appearing brownish-orange (5C3) with a lighter-colored margin with basidiospore maturation, margin smooth. lamellulae typically 3 between lamellae and in 2 tiers (2 short and 1 medium long). Stipe 11–40(–50) × 0.5–1.3 mm, equal, with a superficial, orange-gray (5B2-3) to brownish-orange (5C3) layer of entangled fibrils overlying longitudinally appressed, grayish-brown (5D-E3-4) fibrils, solid becoming hollow with age; basal tomentum scarce to absent; stipe base pallid and obscurely strigose. Bruising reaction observed in the stipe (7F2-3) but not the strigose base, darkening upwards.

Basidiospores nodulose-angular with 7–9 angles in profile and side views, heterodiametric, 8.9–14.5 × 5.1–8.7 µm (x_m = 11.5 ± 1.2 × 7.2 ± 0.6 µm; E = 1.29–1.99; Q = 1.61 ± 0.14; n = 99/4). Basidia 2–4 sterigmate, clavate but hardly tapered, colorless in 3% KOH, 34.6–42.7 × 7.7–11.8 (widest point) × 5.2–7.9(base) µm (E = 2–96–5.28; Q = 4.02). Pleurocystidia absent. Pseudocystidia absent. Aborted basidia abundant, nearly always with 2–4 sterigma and a few with aborted basidiospores, clavate and tapered to a narrow end, often embedded in the hymenium but a few projecting up to 17 µm beyond the basidia, often but not always, originating from the outer portions of the lamellar trama, dark reddish-brown in 3% KOH, 36.2–59.0 × 7.1–11.2(widest point) × 2.8–5.2(base) µm (E = 4.76–5.58; Q = 5.02). Lamellar tramal hyphae parallel to subparallel, 22.8–215.2 × 4.0–25.7 µm; narrow hyphae abundant, broad hyphae rare to uncommon; subhymenium indistinct.

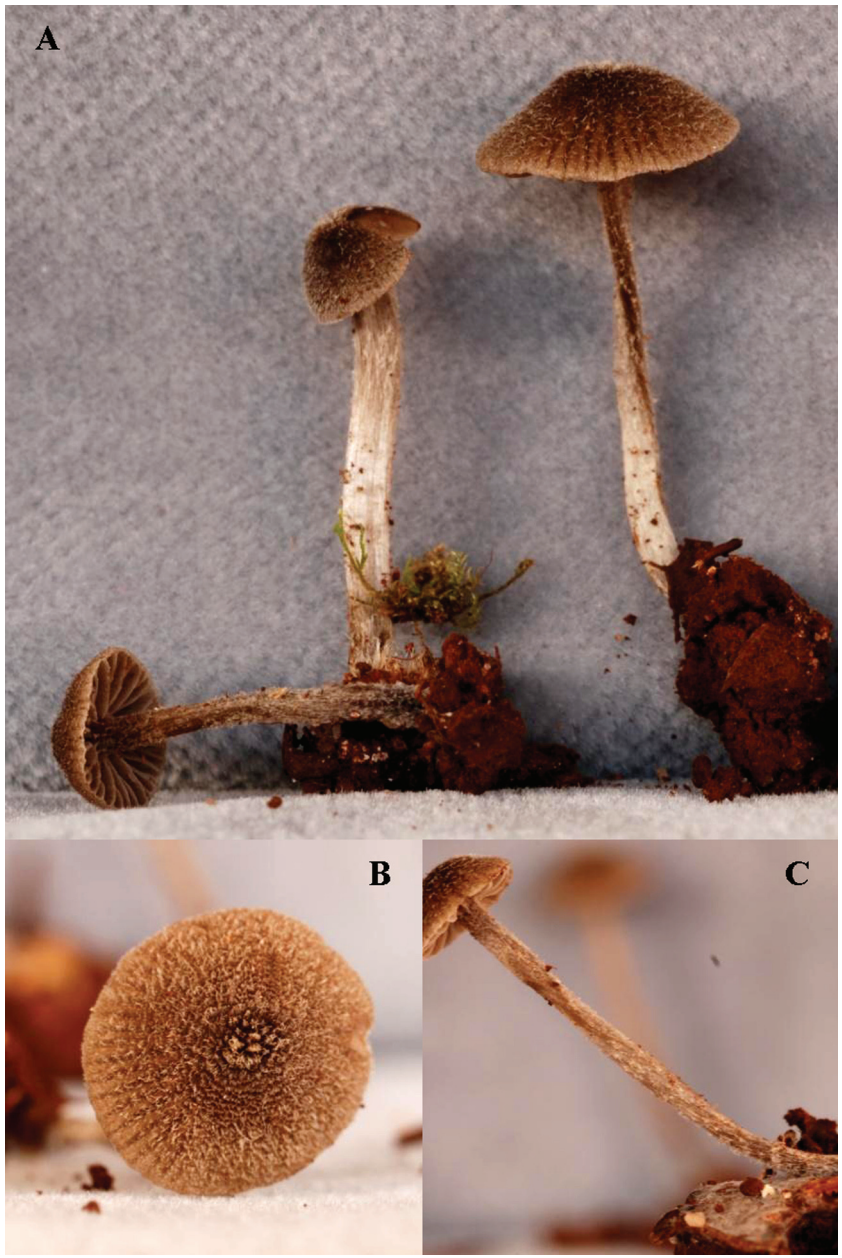


Plate 19 – *Pouzarella lasia* (DLL 9662).
A: Basidiomata stature; B: Pileus surface; C: stipe surface.

Pileal tramal hyphae 56.0–162.3 × 3.0–13.5 µm. Pileipellis up to 240 µm deep, composed of clusters (squamules) of 5–10 laterally attached hyphae, each hypha with 10–20 cells that have parietal pigmentation; the clusters tapered with 5–10 hyphae at the base and 2–4 hyphae at the apex, erect on the disc, semi-erect towards the margin and nearly repent at the margin. Pileocystidia typically narrowly to broadly cylindro-clavate, at times clavate to nearly napiform, 24.4–66.1 × 6.8–19.0 µm (x_m = 41.7 × 11.6 µm; E = 2.0–6.9; Q = 3.89; n = 13/1). Stipitipellis a cutis between abundant, densely scattered clusters of colorless 1–3 celled hyphae with clavate, broadly obclavate or broadly cylindro-clavate caulocystidia and scattered clusters of loosely entangled hyphae, 5–10 cells long, with cylindro-clavate to clavate, colorless caulocystidia. Caulocystidia 16.5–66.4 × 7.1–13.9 µm. Stipe tramal hyphae parallel, 101.4–355.5 × 5.4–13.4 µm. Oleiferous hyphae rare in the pileal and stipe trama, absent in the gill trama, reddish-brown in 3% KOH. Pigmentation coarsely externally incrusted on the tramal hyphae of the lamellae, pileus and stipe and in the basal cells of the pileipellis; obscurely internally parietal in the hyphae of the stipitipellis and in the outermost 1–3 cells of the hyphae of the pileipellis.

Ecology and distribution: Scattered to gregarious in hard packed soil in complex mesophyll vine forest in areas with high rainfall; Lake Euramoo, Danbulla National Park, Ceylon and Papua New Guinea.

Collections examined — **AUSTRALIA**. **Queensland**, Cook Region, Danbulla National Park, Lake Euramoo Track, 17°09′43.0″S 145°37′44.7″E, 763.2 m, 18 March 2009, DL Largent 9662 (mtSSU HQ876551, LSU HQ876529, RPB2 HQ876507); 19 March 2009, DL Largent 9670 (mtSSU HQ876552, LSU HQ876530, RPB2 HQ876508), DL Largent 9671; 21 March 2010, DL Largent 9807 (mtSSU HQ876555, LSU HQ876533, RPB2 HQ876511), DL Largent 9811 (mtSSU HQ876556, LSU HQ876534, RPB2 HQ876512), DL Largent 9812; 24 March 2010, DL Largent 9818, 9827; Cook Region, Danbulla National Park, Kauri Creek Track, 17°07′49.1″S 145°35′58.2″E, 725.1 m, 21 February 2010, DL Largent 9729 (mtSSU HQ876553, LSU HQ876531, RPB2 HQ876509); 13 March 2010, DL Largent 9778 (mtSSU HQ876554, LSU HQ876532, RPB2 HQ876510).

Comments— *Pouzarella lasia* is diagnosed by a typically conic to campanulate to parabolic pileus that is hispid-squamulose on the disc and imbricate squamulose elsewhere and covered when young with pallid to brownish-orange tipped squamules that become dark brownish-gray with age and exposure; a ≤50 mm long stipe covered by an orange-gray to brownish-orange layer of entangled fibrils overlying longitudinally appressed grayish-brown fibrils and with an obscurely strigose base, nodulose-angular basidiospores averaging 11.5 × 7.2 µm, absence of cheilocystidia, presence of aborted basidia that project beyond the hymenium, internally parietal pigmented pileocystidia and caulocystidia, ± thick-walled strongly encrusted basal pileipellis hyphae, and a nondistinctive taste and odor.

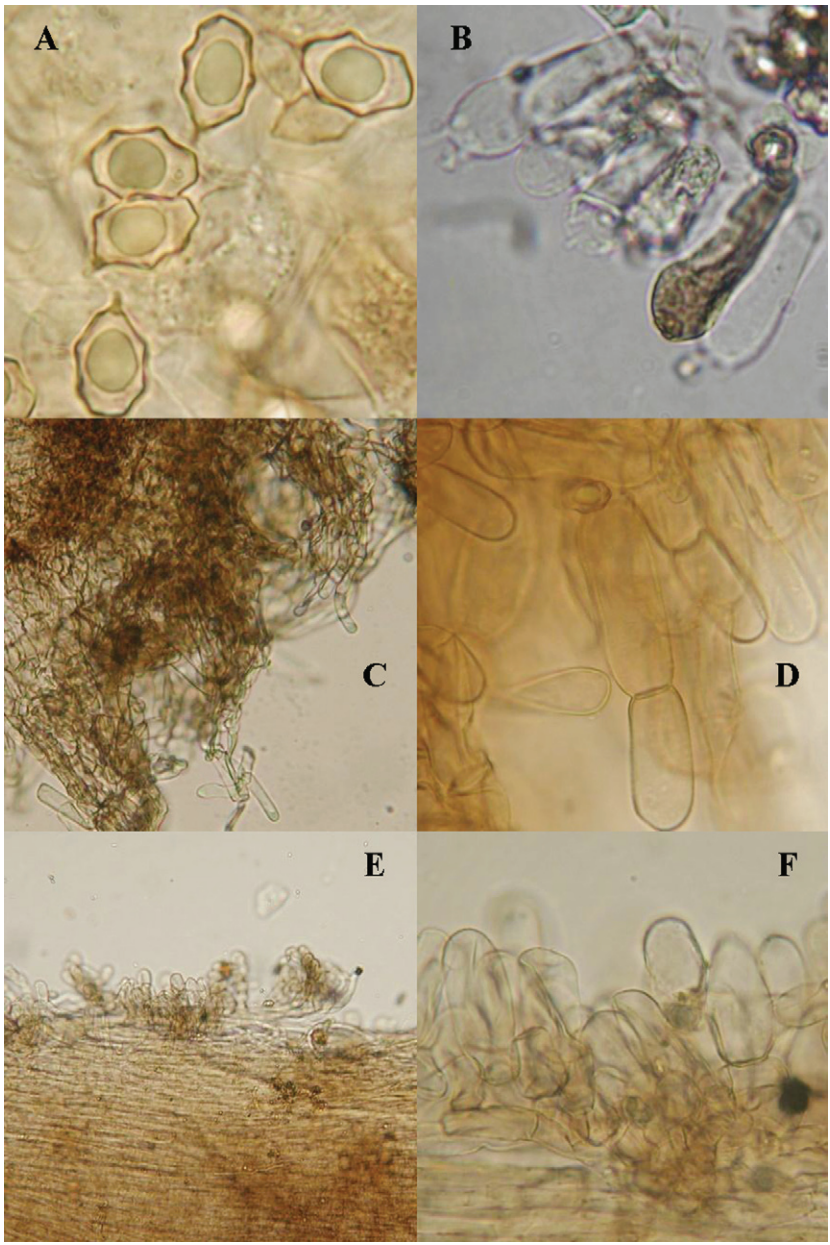


Plate 20 – *Pouzarella lasia* (DLL 9662). A: Basidiospores (1000×); B: Basidia (colorless) and pseudocystidia (dark) (400×); C: Pileipellis on disc (100×); D: Pileocystidia (400×); E: Stipitipellis (100×); F: Caulocystidia (400×).

In Australia, we observed 30–40 *P. lasia* basidiomata in the same habitat and locality twice over a 2–4 day period in mid-March 2009 and mid- to late-March 2010. Most pilei from basidiomata collected during the beginning days possessed light-colored squamules over the entire surface but the squamule base of the remaining basidiomata began to develop dark gray brown tints. Pileal surfaces from basidiomata collected at the end of the 2–4 day period had only dark gray brown squamules and were otherwise morphologically identical to *Pouzaromyces lasius* as described and illustrated by Horak (1980). With the exception of the light colored squamules, the basidioma features from the beginning of the time period also matched those of *Pouzaromyces lasius*. Horak apparently either overlooked the light colored squamules in young specimens or examined only mature specimens.

Horak (1980) and Pegler (1977) studied the holotype of *Agaricus lasius.* Referring to his type study in his *Pouzaromyces lasius* description, Horak noted basidiospores measuring 10.5–13.5 × 6–8 µm, cheilocystidia absent, and the terminal (1–3 cell thick) hairs on stipe [and pileus?] as thin-walled and remaining cells as thick-walled. For the holotype, Pegler (1977) reported basidiospores averaging 14 × 8.5 µm (11.5–15.5 × 8–9.5 µm) and a 4-µm thickness for the cell walls of the pileal hairs. It is rather perplexing to have two investigators study the same specimens and report such distinct differences.

Pouzarella lasia, P. domingensis from the Dominican Republic, *Pouzaromyces napaliensis* from the Hawaiian Islands, and *Entoloma testaceostrigosum* from the state of Kerala in India lack cheilocystidia and have similarly shaped and sized basidiospores. *Pouzarella domingensis* differs in the presence of inflated cells in the pileal subpellis (Baroni et al. 2008), while *Pouzaromyces napaliensis* has aciculate to setiform caulocystidia and pileocystidia, smaller basidiomata (2.5 mm broad pileus, 3–4 × <1 mm stipe), and a pale yellowish-brown pileus (Desjardin & Baroni 1991). *Entoloma testaceostrigosum* is separated by its larger (12.5–17.0 × 7–10 µm) basidiospores and absence of encrusted pileipellis and stipitipellis hyphae (Manimohan et al. 2006).

Pouzarella lasia was found fruiting abundantly in two locales. All these collections were made in a similarly complex mesophyll vine rainforest in Danbulla National Park in northeastern Queensland. Four collections —DL Largent 9662 (18 March 2009), 9670 (19 March 2009), 9807 and 9811 (21 March 2010)— came from populations in identical habitats on the Lake Euramoo Track, while two —DL Largent 9729 (21 February 2010), 9778 (13 March 2010)— were from the same population and identical habitat on the Kauri Track. We found no evidence of genetic isolation among populations separated by 4 km and the level of allelic variation within each locus is consistent with the variation expected in interbreeding populations with a large effective population size. Future studies of these and other populations

of *P. lasia* may provide insight into the extent of gene flow or genetic isolation among populations.

See *P. setiformis* for comparisons with Australian taxa related to *P. lasia*,.

Discussion

In the past decade, efforts to gain insight into the morphological features that unite mushroom-forming fungi with their sequestrate relatives (*Basidiomycota, Agaricales*) have pinpointed some unique characters that can form the basis of natural classifications (e.g. Moncalvo et al. 2004, Matheny et al. 2006). However, defining these boundaries for large and diverse families such the *Entolomataceae* with over 1500 mushroom-forming species and few sequestrate taxa (Largent 1994, Moncalvo et al. 2004, Co-David et al. 2009) can be challenging. The basis for the early classification of all members in the *Entolomataceae* is the pinkish basidiospore deposit and angular basidiospores observed in polar view, features that may form the basis of a natural classification based on the observed monophyletic support using DNA sequence data (Moncalvo et al. 2004, Matheny et al. 2006, Co-David et al. 2009).

Despite the support for the monophyly of the *Entolomataceae*, defining generic boundaries within the family may be challenging. For example, several of the proposed generic concepts based on morphological features have been questioned based on the lack of observed monophyletic support in phylogenetic analyses (Moncalvo et al. 2002, 2004, Co-David et al. 2009). Large genera that exemplify these problems include *Entoloma* (Fr.) P. Kumm., recovered as either polyphyletic (Moncalvo et al. 2004) or paraphyletic (Co-David et al. 2009). A similar lack of support has been observed for *Claudopus* Gillet, *Leptonia* (Fr.) P. Kumm., *Rhodocybe* Maire, and *Nolanea* (Fr.) P. Kumm. (Moncalvo et al. 2002, Co-David et al. 2009). Although it is unquestionably an under-sampled genus, monophyly of *Pouzarella* is supported by molecular data (from only two collections, the European *Entoloma araneosum* and Indian *E. violaceovillosum*; Co-David et al. 2009). In this paper, we include a phylogenetic analysis of two European (Co-David et al. 2009) and ten Australian *Pouzarella* species; given the exclusion of other entolomataceous genera, we understand that our phylogeny neither refutes nor supports the monophyly of *Pouzarella.* Nonetheless, our sequence analyses support delineation of the ten Australian species based on micromorphological features, and features common to these clades are highlighted as characters that may form the basis of a natural classification for other genera.

One interesting pattern apparent in the phylogeny is the strong support for the monophyletic clade containing *P. lageniformis*, *P. parvula,* and *P. pilocystidiata*. All species possess long cylindro-clavate pileocystidia, abundant thin-walled lageniform to ventricose-rostrate cheilocystidia, and lack aborted

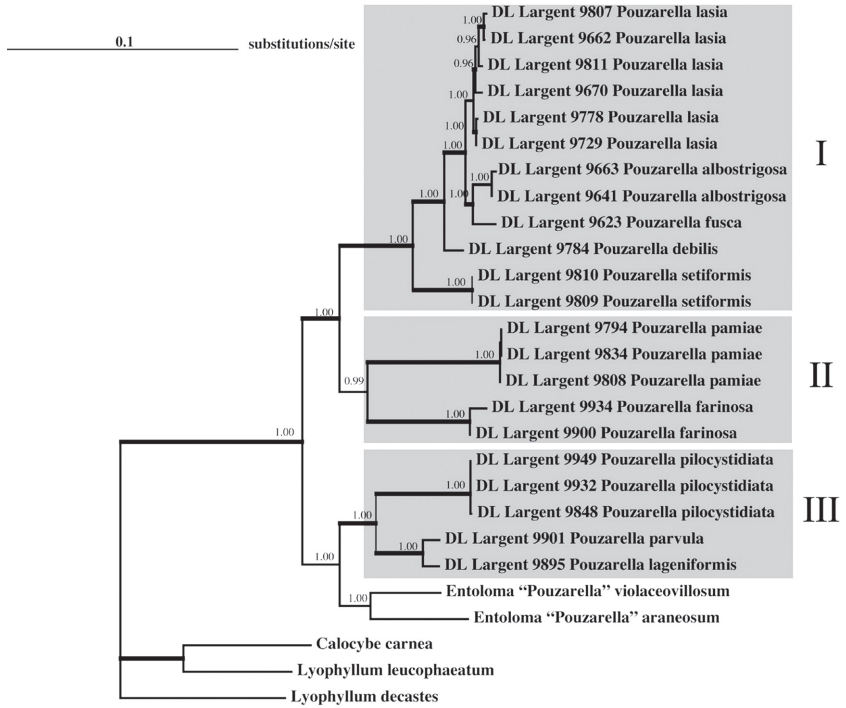


Plate 21 – Phylogenetic relationships of *Pouzarella* based on Maximum Likelihood analysis of mtSSU, LSU, and RPB2 of 10 Australian, one European, and one Indian species. Tree topologies based on Maximum Likelihood and Bayesian estimation were congruent. Thick branches represent nodes that are supported by bootstrap values of 70% or greater and values above each branch are the Bayesian posterior probability values computed from the 50% majority rule consensus tree. Shaded areas represent the three clades recovered. Taxa are labeled with the collection identification number and name of taxon.

basidia (unusual in *Pouzarella*). Strong support was also observed for another clade comprising *P. albostrigosa*, *P. debilis*, *P. fusca, P. lasia,* and *P. setiformis*. One unique morphological feature common to this clade is the conspicuous absence of cheilocystidia, a feature shared by other tropical species including *Pouzaromyces minutus* (Horak 1980), *Pouzaromyces napaliensis* (Desjardin & Baroni 1991), and *Entoloma testaceostrigosum* (Manimohan et al. 2006). *Pouzarella pamiae* and *P. farinosa* formed a monophyletic group, although with low ML BS support. Both possess a squamulose-hispid stipe surface and ± thick walled cheilocystidia — features also encountered in the tropical *P. ferreri* and temperate *P. dysthales* (Peck) Mazzer (Baroni et al. 2008, Noordeloos 2004). Future studies that include these and other representative *Entolomataceae* taxa are needed to better assess phylogenetic relationships and diagnostic

morphological characters that could form the basis of a natural classification of *Pouzarella*.

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Key to *Pouzarella* species from central New South Wales (N) and northeastern Queensland (Q)

1. Cheilocystidia present; aborted basidia absent or if present, rare and colorless in 3% KOH ..... 2 (Clade II, III)
1. Cheilocystidia absent; aborted basidia present and typically abundant and staining dark reddish-brown in 3% KOH ..... 6 (Clade I)
2. Cheilocystidia acicular, aculeate, narrowly acuminate, finally rostrate ventricose; pileocystidia cylindro-clavate, with Q = 7.0 or more ..... 3 (Clade III)
2. Cheilocystidia globose, napiform, vesiculose pyriform or clavate to broadly acuminate; pileocystidia versiform, with a Q <5.0 ..... 5 (Clade II)
3. Pileus orange white to grayish-orange; stipe apex pruinose; taste mild; 4
3. Pileus squamules and fibrils dark brown on dark blond to brown background; stipe apex tomentulose to densely hirsute or with abundant bundles of fibrils; taste farinaceous ..... 1. *P. pilocystidiata* (N)
4. Pileus hispid-squamulose; stipe entirely pruinose; pileocystidia at most acuminate ..... 2. *P. lageniformis* (N)
4. Pileus entirely densely matted squamulose; stipe pruinose at the apex, glabrous elsewhere; at least some pileocystidia setiform ..... 3. *P. parvula* (N)
5. Pileus at first entirely reddish-golden to brownish-orange, then darkening to medium teak brown to burnt umber; cheilocystidia broadly clavate to broadly acuminate ..... 4. *P. pamiae* (Q)
5. Pileus with densely packed upright tufts of orange gray squamules on a grayish-brown background, overall light grayish-brown; cheilocystidia at first nearly globose then napiform, vesiculose, or pyriform.. 5. *P. farinosa* (N)
6. Stipitipellis with scattered to abundant setiform caulocystidia; pileipellis with setiform pileocystidia, most evident at the margin ..... 6. *P. setiformis* (Q)
6. Stipitipellis with cylindric to clavate to obclavate caulocystidia; setiform caulocystidia and pileocystidia absent 7
7. Pileus broadly convex to convex; stipe up to 22 mm long; aborted basidia embedded in the hymenium or rarely extending beyond the hymenium by 6–7 μm 8
7. Pileus conic, campanulate or parabolic; stipe ≤50 mm long; aborted basidia extending beyond the hymenium by ≤17 μm ..... 10. *P. lasia* (Q)
8. Caulocystidia of one type, similar to the pileocystidia ..... 7. *P. debilis* (Q)
8. Caulocystidia of two types: 1) cylindro-clavate, the end cells of loosely entangled hyphae, similar to the pileocystidia; 2) clavate, obclavate, or broadly napiform and typically solitary and not in loose hyphal clusters 9
9. Pileus minutely but entirely squamulose, black to a very dark gray-brown, without off-white squamules when young; pileus opaque when mature; stipe base pallid and obscurely strigose ..... 8. *P. fusca* (Q)
9. Pileus entirely hispid-squamulose and with off-white (5A-B1-2) squamules when young, base color dark grayish-brown; pileus striate when mature; stipe base white and distinctly strigose ..... 9. *P. albostrigosa* (Q)