A NEW CORTICOLOUS SPECIES OF *MYCENA* SECT. *VISCIPELLES* (BASIDIOMYCOTA: AGARICALES) FROM THE BARK OF A LIVING AMERICAN ELM TREE IN TEXAS, U.S.A.

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ABSTRACT

A *Mycena* species new to science was obtained from moist chamber cultures of trunk bark of a living American elm tree (*Ulmus americana*) located in the Fort Worth Botanic Garden, Tarrant County, Texas. This discovery was part of an ongoing study of corticolous myxomycetes on larger American elm trees occurring in Fort Worth nature parks. More than 15 American elm trees were sampled for trunk bark but only a single tree yielded *Mycena* basidiomes. Collections of bark began in the summer of 2017 and continued until the beginning of 2020. Bark samples from the north, south and west side of the tree yielded fruit bodies of the mushroom in moist chamber culture. No fruit bodies were observed in nature nor were early formation stages on the underside of the bark. Crystals previously described in another study were present on the bark surfaces, edges, and undersides. Habit and morphological development were photographed using light microscopy applying multifocal imaging and computer stacking to increase depth of field. Basidiome development was observed and photographed from the earliest primordial beginning stage, the button stage, intermediate stage and the final emerging stalk elongation and mature cap formation stage. Mature mushroom development took from 9 to 21 days after wetting the bark in moist chamber cultures. Scanning electron microscopy was used to illustrate development of the button stage, emerging stalk and pileus stage, and the fully mature pileus, lamellae, and pseudocollarium. Morphological features and DNA sequence data confirmed that this *Mycena* species was undescribed and distinct from other *Mycena* taxa. Morphological features suggest placement of the novel taxon in *Mycena* sect. *Viscipelles*, distinct from other members based on morphological characters and phylogenetic analysis.

Key Words: fungal diversity, moist chamber cultures, morphological development, multifocal imaging/computer stacking, mushroom, mycenoid fungi, pseudocollarium, taxonomy

RESUMEN

Se obtuvo una especie nueva para la ciencia de *Mycena* en cultivos en cámara húmeda de corteza de *un olmo americano vivo* (*Ulmus americana*) localizado en el Jardín Botánico de Fort Worth, Condado Tarrant, Texas. Este descubrimiento es parte de un estudio en marcha de myxomycetes cortícolas en los árboles más grandes de olmo americano en los parques naturales de Fort Worth. Se muestrearon cortezas de más de 15 olmos americanos, pero solo un único árbol dio basidiomas de *Mycena*. Las colecciones de corteza empezaron en el verano de 2017 y continuaron hasta el principio de 2020. Las muestras de corteza tomadas de los lados norte, sur y oeste del árbol dieron cuerpos fructíferos de los hongos en la cámara de cultivo húmeda. No se observaron cuerpos fructíferos en la naturaleza ni estadios tempranos de formación en la parte interior de la corteza. Los cristales descritos previamente en otro estudio estaban presentes en las superficies de la corteza, bordes, y lado interno. El hábito y el desarrollo morfológico se fotografiaron usando microscopio óptico aplicando escaneo multifocal y apilado por ordenador para incrementar la profundidad de campo. El desarrollo del basidioma se fotografió desde el estadio primordial más temprano, estado de botón, estado intermedio y la elongación final emergente del estipe y estadio de formación del píleo maduro. El desarrollo de la seta madura tomó de 9 a 21 días desde el riego de la corteza en las cámaras de cultivo húmedas. Se usó el microscopio electrónico de barrido para ilustrar el desarrollo del estadio de botón, estadio de emergencia del estipe y píleo, y el píleo completamente maduro, laminillas, unión de las laminillas, y pseudocollarium. Las características morfológicas y datos de secuencias de ADN confirmaron que esta especie de *Mycena* estaba sin describir y era distinta de otros taxa de *Mycena*. Las características morfológicas sugieren el emplazamiento del nuevo taxon en *Mycena* sect. *Viscipelles*, distinto de otros miembros por caracteres morfológicos y análisis filogenético.

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INTRODUCTION

Ulmus americana L. (American elm) trees were selected for an ongoing study of corticolous myxomycete biodiversity because their trunk bark (Fig. 1B) has furrowed rough surfaces, is spongey, readily absorbing and retaining water after rain, and has bark in the neutral pH range of 7.0. In addition, collections of corticolous myxomycetes over a 45-year period has confirmed *Ulmus americana* ranks in the top group of tree species, also including *Juniperus virginiana* L. and *Acer rubrum* L., yielding the highest species diversity of corticolous myxomycetes based on field collections and moist chamber bark cultures (Keller & Braun 1999; Snell & Keller 2003; Keller 2004; Keller et al. 2009). Moist chamber bark cultures rarely yield fleshy agaric mushroom species, but in July 2018 a corticolous species of *Mycena* fruited profusely from the trunk bark of a single *Ulmus* tree (Figs. 1C, D) from the Fort Worth Botanic Garden (Fig. 1A). Examination of this taxon and comparison with similar taxa indicated that this is a novel species, herein proposed as *Mycena ulmi*.

Field study sites in the Fort Worth, Texas area

Mature American elm trees were dying by the thousands from Dutch elm disease in the 1950s and 1960s in cities and towns throughout the mid-western U.S.A., including along creeks, streams, and river bottoms (H.W. Keller, pers. obs.). American elms often were planted close together as a monoculture, lining both sides of streets and avenues especially in Iowa and Kansas. When one tree became infected with *Ophiostoma ulmi* (Buisman) Nannf., all nearby trees became infected through root grafts, such that every tree on the block often had to be destroyed. Fortunately, few American elm trees were planted in the Fort Worth residential neighborhoods, and therefore Dutch elm disease did not spread to the surrounding areas, including the Elmer W. Oliver Nature Park, Fort Worth Botanic Garden, and the Fort Worth Nature Center and Refuge, where trees were sampled as part of this study. None of the American elm trees sampled in this study show signs of Dutch elm disease nor had dead or dying areas on the trunks or limbs.

History and collection of bark samples at study sites

Nine American elm trees were sampled at Elmer W. Oliver Nature Park within the city limits of Mansfield, one tree in the Fort Worth Botanic Garden (Fig. 1A), and six trees in the Fort Worth Nature Center and Refuge, all within Tarrant County, Texas. These are large trees, with approximate trunk diameter at breast height (DBH) of l.2 m and heights of 16 to 25 m. None of these trees had significant mosses, liverworts, or lichens present on the bark surface. Additional site details are available in an earlier publication (Keller & Marshall 2019).

Corticolous macrofungi on living healthy trees in U.S.A.

Comprehensive studies of corticolus Basidiomycete taxa in North America are currently lacking, even though numerous species of *Mycena* and other genera are known to be restricted to growth on the bark of various hardwood and coniferous trees. In previous research by Keller (2004) and Keller et al. (2009) examining the corticolous species of macrofungi found on large, healthy trees from the temperate deciduous forests of Great Smoky Mountains National Park, only five taxa were encountered: *Lentaria byssiseda* Corner (on *Quercus velutina* Lam.); *Mycetinis opacus* (Berk. & M.A. Curtis) A.W. Wilson & Desjardin; *Mycena supina* (Fr.) P. Kumm. (on *Acer rubrum* L.); *Ramaricium polyporoideum* (Berk. & M.A. Curtis) Ginns, and *Cheimonophyllum candidissimum* (Sacc.) Singer (on *Quercus alba* L.). Additionally, approximately 100 bark collections from urban nature parks and residential areas in Fort Worth, Texas included *Dendrothele* species, mostly *Dendrothele jacobi* Duhem & H. Michel on living American elm and cedar elm (*Ulmus crassifolia* Nutt.), and *Dendrothele nivosa* (Berk. & M.A. Curtis ex Höhn. & Litsch.) P.A. Lemke on living Eastern red cedar (*Juniperus virginiana* L.) (HWK pers. obs.). However, at no time were large fruitings of any fleshy basidiomycete fungi observed on trunks of living healthy trees in this area. For this reason, it was very surprising when the taxon herein described as *Mycena ulmi* fruited so abundantly from American elm bark placed in moist chambers.

MATERIALS AND METHODS

Field study site description and collection of bark samples

The Fort Worth Botanic Garden (FWBG) was selected, in part, because of its close proximity to the Botanical

Fig. 1. A. Front entrance to Fort Worth Botanic Garden highlighted by evergreen *Ligustrum sinense* shrub with bright yellow foliage. **B.** Bark surface of living *Ulmus americana* tree at Fort Worth Botanic Garden. **C.** Living *Ulmus americana* with summer foliage, holotype site for *Mycena ulmi* located in the Fort Worth Botanic Garden maintenance area. **D.** Wintertime habit of deciduous *Ulmus americana* tree shown in Figure 1C; note dichotomous branching pattern typical of American elms.

Research Institute of Texas (BRIT), a distance of several hundred meters (Fig. 1A). Large, mature American elm trees are present in sufficient numbers in the 17–24 m height and 1.2–1.8 m DBH size range. The southern portion of what is now the botanic garden featured natural springs that supplemented the nearby Trinity River as a water source for the Native American population, early pioneers, and the army in the early settlement of Fort Worth (Anonymous 1983, 1987). Ed Terrell and John P. Lusk were early traders who are believed to have camped at this site in the early 1840s. The Garden's original 15 ha were purchased on 15 June 1912. Prior to that purchase, a cotton gin was operated on this site in 1868, and horse stables to the west continued in business until the purchase. What was named Rock Springs Park became the FWBG on 18 December 1934. The Garden currently comprises 13 ha located in the heart of the Fort Worth Cultural District at coordinates 32°44'18"N 97°21'42"W. The FWBG is the oldest botanic garden in Texas with the southern portion listed on the National Register of Historic Places on 13 February 2009 (Anonymous 1983, 1987).

Only live American elm trees free of dead branches and wounds, with no signs or symptoms of Dutch elm disease, were selected for trunk bark sampling. Where possible, fingers were used to pry smaller pieces of trunk bark, or a heavy bladed knife for larger pieces up to 25 cm in length and 0.8 cm to 10 cm in diameter.

Extreme caution was used to remove only the outermost portions of bark layers without injuring any living tissue. Trunk bark was collected from 1.2 m to 1.8 m in height from all sides of the tree except the east side from the one tree that produced *Mycena* fruit bodies. Multiple visits to the same tree after rainy periods gave no evidence of any mushroom fruit bodies at the collection site. This tree had no observable mosses, liverworts, or lichens present, appearing as a bare bark surface. However, scattered on the bark surface were crustose fruit bodies of the basidiomycete *Dendrothele jacobi*, a seldom collected species, but common on American elm trunk bark in the Fort Worth area (HWK, pers. obs.). Scanning bark surfaces, edges, and undersides with a dissecting microscope immediately after collecting provided no evidence of any stage of mushroom development. Additional details of American elm morphology and identification are described in Keller & Marshall (2019).

Moist chamber trunk bark batch cultures

This culture technique was described in detail and illustrated by Keller and Marshall (2019). A circular shallow aluminum pie tin 25 cm across was lined with non-sterile paper toweling covering the bottom. Bark pieces were larger and fewer in number, usually 6–8 per culture, lining the bottom of the pie tin without overlapping. Water used to wet the bark was de-ionized and sterile, with 30–40 ml poured around the bark. Any water remaining after 48 hours was decanted, leaving only saturated bark and moist toweling. Each pie tin was tightly wrapped with a thin, transparent plastic material that was labeled with the following information: tree location, tree species, and direction (n,s,e,w) on tree, date bark collected, and date bark wetted. Once produced, the developing *Mycena* basidiomes could be seen through the transparent plastic wrap without unsealing, thus minimizing contamination from airborne spores. Bark pieces with developing basidiome stages were removed for photography. *Mycena* basidiomes started to develop after as few as nine days, but the majority of the cultures took 18 to 21 days to develop mature basidiomes. Bark that had dried out was rewetted after several months and another flush of basidiomes developed. Macromorphological data was derived from fresh specimens, and these were preserved for herbarium storage by drying in an Excalibur™ food dehydrator or air dried. Color terms and notations follow Kornerup and Wansher (1978).

Habit photographs

Specimens fruited in moist chambers were photographed in situ using an Olympus OM-D E-M1 MII digital camera under natural light, or with a Nikon Coolpix S9900 camera with and without flash. Both in-camera stacking capabilities and manual adjustment of focus increments were used to maximize depth of field, with resulting images stacked via Helicon Focus (HeliconSoft, Kharkiv, Ukraine) and adjusted in Adobe Photoshop (Adobe, San Jose, CA).

Light microscope observation of macromorphological features

Growth and development of *Mycena* basidiomes presented different sizes that required different microscopes. An Olympus AX70 light microscope with a D71 camera system was used to record specimen measurements, and an Olympus BH2 compound microscope with epi-illumination and extra-long working distance objectives was used for observation. Images were recorded with a Sony A6000 digital camera. Helicon Focus stacking software Version 7.6.1 increased depth of field for all images. The number of selected images photographed was a function of specimen size and how finite the steps are for focusing the microscope. The Olympus AX70 microscope enabled one-micron step focusing. One image was taken for every micron of specimen dimension as a separate photograph. Depending upon specimen size, magnification, and working distance, this resulted in various numbers of individual images (15–100) that were stacked into the final image.

Scanning electron microscope (SEM) preparation and observation

A new genomics facility at BRIT includes a Hitachi SU 3500 high resolution SEM which was used to observe and photograph the *Mycena* specimens. Basidiomes in various developmental stages were removed from the American elm bark using a chisel-shaped blade. Vertical cuts were made around the specimen about 1–2 mm deep and the bark sample was then cut horizontally beneath the *Mycena* specimen which facilitated removal. Due to the fragile and top-heavy nature of the *Mycena* pileus, selection was made favoring specimens with the

lamellae facing up. In this position the stalk was cut 3–4 mm below the pileus with a micro-scissors and the tissue placed lamellae side up on the SEM stub. Forceps were used to place the pileus on the SEM stub to enable a clear view of the lamellae.

SEM observations were made from 50× up to 6000× image magnifications at a pressure of 30 Pa and accelerating voltage between 5kV and 10kV. SEM circular specimen stubs used were the pin type with diameters of 12.5 mm and 25 mm. Specimens were mounted on double-sided adhesive conductive tape both with and without sputter coating. Sputter coating was used to reduce charging effects when operating at higher acceleration voltages. SEM photographic images have a label strip at the bottom that contains the following data, reading from left to right: model number of the instrument, accelerating voltage in kV, working distance in mm, magnification, electron detector used for observation (SE=secondary electron detector), date and time of observation. A calibrated measurement scale which is divided into 10 parts is provided. The length of the scale is indicated below the 11 division marks in millimeters or microns. Images were adjusted in Photoshop to optimize brightness and contrast.

Light microscope observation of micromorphological features

Micromorphological data were derived from dried material rehydrated in 95% EtOH followed by distilled water, 3% KOH or Melzer's reagent. Micromorphological terminology follows Vellinga (1988). Basidiospore statistics include: x_m, the arithmetic mean of spore length by spore width (± standard deviation) for n spores measured; Q, the ratio of spore length to spore width, expressed as a range for all spores measured; Q_m, the mean of all Q values (±SD); and n, the total number of spores measured. Specimens were examined on an Olympus BX-53 light microscope equipped with Differential Interference Contrast (DIC) optics and *camera lucida* drawing attachment. All cited specimens are deposited at the Botanical Research Institute of Texas (BRIT) and the H.D. Thiers Herbarium, Department of Biology, San Francisco State University (SFSU).

Molecular methods

Total genomic DNA was extracted from dried material using the Extract-N-Amp Plant PCR Kit (Sigma-Aldrich, St. Louis, MO) following manufacturer instructions. PCR protocols followed those outlined in Perry et al. (2007). For two specimens the nuclear ribosomal internal transcribed spacer region (ITS) was amplified using primers ITS1-F/ITS4 (Gardes & Bruns 1993; White et al. 1990). Amplification products were cleaned using the Exo-SAPit kit (Affymetrix, Santa Clara, CA), and sent to Elim Biopharmaceuticals (Hayward, CA) for sequencing with the same primers used for amplification. Sequencing products were edited and assembled in Geneious 9.0 (Biomatters Ltd., Auckland, New Zealand). To place the new taxon within a phylogenetic context, ITS sequences were aligned within a limited sampling of *Mycena* sequences obtained from Genbank. Initial ITS alignments were performed using ClustalX 2.1 (Larkin et al. 2007) with default parameter settings, followed by manual adjustment in Mesquite (Madison & Madison 2015). All sequences generated as part of this study have been deposited in GenBank (MT790766–MT790768).

Phylogenetic analyses

Maximum likelihood analyses were run in RAxML 8.2.9 (Stamatakis 2014) under a GTRGAMMAX model and consisted of 500 alternative runs using default parameters, with node support estimated by 100 RAxML bootstrap replicates. Bayesian analyses were performed using Metropolis Coupled MCMC methods as implemented in MrBayes 3.2.6 (Huelsenbeck & Ronquist 2001; Ronquist & Huelsenbeck 2003) under an HYK+I+G model of sequence evolution as determined under the Bayesian Information Criterion in PAUP*4.0a.167 (Swofford 2002). Bayesian analyses consisted of two parallel searches, run for 6 million generations, initiated with random starting trees and default chain temperature and swaps per generation parameters. Eight chains were sampled every 3000 generations for a total of 2000 trees each, sampled from the posterior distribution. Those trees sampled prior to the analysis reaching an average standard deviation of split frequencies of 0.01 were discarded as the burn-in, while the remaining trees were used to calculate the posterior probabilities of the individual clades. Default settings were used in MrBayes to set unconstrained branch lengths and uninformative topology priors.

Bioluminescence

Fresh specimens were checked for bioluminescent properties by measuring any potential light production from entire basidiomes and pilei only, on a Berthold FB12 single tube luminometer (Pforzheim, Germany), and with the naked eye in total darkness. No bioluminescence was detected with the naked eye, and measurement of basidiomes and pilei on the luminometer detected no significant production of relative light units.

TAXONOMY

Mycena ulmi B.A. Perry & H.W. Keller, **sp. nov.** (**Figs. 2–3, 5–8**). TYPE: U.S.A. TEXAS. Tarrant Co.: Fort Worth Botanic Garden, specimens collected from bark of live American elm (*Ulmus americana*), silver tag #2002, Fig. 1B,C,D, N32°44.8'W 97°21.9', cultured in batch moist chambers, bark collected 10 December 2019, wetted 17 Jan 2020 at California State University, East Bay, harvested 29 Jan 2020, *Brian A. Perry 1085* (HOLOTYPE: BRIT, barcode BRIT425241, MycoBank MB836222, Genbank MT790767).

Diagnosis.—Pileus 1.5–5 mm diam., dark gray to gray or gray-brown; surface sulcate-striate, minutely white-pubescent, covered with a separable gelatinous pellicle. Lamellae ascending-adnate, typically seceding from the stipe to form pseudocollarium in mature specimens, subdistant, gray with a pale edge, the edge not separable as a gelatinous thread. Stipe 17–38 × 0.5–1 mm, central; surface densely minutely white-pubescent, glabrescent towards apex, pale gray, gray-brown below, lacking any basal tomentum, radiating hyphae or basal disc. Basidiospores broadly ellipsoid, amyloid. Basidia 4-spored. Pileipellis an ixocutis, epicutis hyphae and terminal cells ornamented. Pluerocystidia absent. Cheilocystidia clavate, giving rise apically to simple or slightly coarse excrescences. Stipe cortical hyphae smooth to ornamented, terminal cells irregular-clavate to irregular-cylindric, often with exceedingly long, tapered apices or excrescences. Clamps present.

Description.—Pileus 1.5–5(–7) mm diam., hemispheric to broadly hemispheric and convex with age, with or without a small umbo, the disc flattened to shallowly depressed; margin entire to slightly fimbriate or scalloped; surface minutely white-pubescent, appearing almost granular in young specimens, partially to entirely glabrescent with age, dry, covered with a separable gelatinous pellicle, sulcate-striate, the striations distinctly darker than the rest of the pileus, disc and striations dark gray (6–7F3) initially, gray-brown (5–6F4) elsewhere, hygrophanous, fading with moisture loss to pale gray and with or without brown tones, the disc and striations often retaining darker coloration. Context very thin. Lamellae ascending-adnate, seceding from the stipe to form a small pseudocollarium in most mature specimens, subdistant, 9–12(–14) lamellae reaching stipe, with 0–2 series of lamellulae, moderately broad, pale gray; the edges paler, not separable as a gelatinous, elastic-like thread. Stipe 17–38 × 0.5–1 mm, central, terete, hollow, filled with hyaline fluid in young specimens, the base broader at point of attachment; surface entirely covered with minute white-pubescence, glabrescent towards apex and more densely pubescent in the lower portion such that base often appears white, apex pale gray, gray-brown (5–6E3–4) below; inserted on substrate, lacking any basal tomentum, radiating hyphae or basal disc. Odor indistinct, fungal. Bioluminescence absent.

Basidiospores (Fig. 3A) 7.0–8.5(–9) × 5.0–6.0(–6.5) µm [x_m = 7.7 ± 0.4 × 5.6 ± 0.3; Q = 1.3–1.6; Q_m = 1.4 ± 0.07; n = 100 spores], ellipsoid to broadly ellipsoid, smooth, amyloid, thin-walled. Basidia (Fig. 3B) 15–22.5 × (4–)7.5–9 µm, 4-spored, clavate, stout, clamped, with sterigmata 3–6 µm in length; basidioles similar. Cheilocystidia (Fig. 3C,D) forming a sterile lamellar edge, clavate to broadly clavate or irregular clavate, with a tapered base or nearly sessile, clamped, thin-walled, hyaline, giving rise apically to few or numerous, simple to branched, cylindric to slightly coarse excrescences, main cell 13–35 × 7–12 µm, excrescences 1.5–25.5(–44) × 1–2.5 µm, often with 1–2 significantly longer excrescences and the remainder shorter. Pleurocystidia absent. Pileipellis an ixocutis, hyphae (Fig. 3E) embedded in gelatinous matrix and easily disassociated, subcutis hyphae highly branched, smooth, thin-walled, clamped, cylindric and often with slightly swollen regions, 2–6 µm diam., giving rise to an epicutis of highly ornamented hyphae and terminal cells that form dense, often difficult to resolve masses that protrude through gel layer, epicutis hyphae cylindric, often with swollen portions, 1.5–6 mm diam., clamped, thin-walled, terminal cells (Fig. 3F) cylindric to clavate or irregular-clavate, 20–38 × 3–10 µm, abundant, especially near pileus margin, excrescences simple to branched, cylindric to slightly coarse, 1.5–13(–21) × 1–3 µm. Hypodermium composed of inflated, globose to ellipsoid cells, 25–52 × 16–25 µm, thin-walled, brownish-vinescent in Melzer's reagent. Pileus and lamellar trama similar in composition to hypodermium. Stipe cortical hyphae (Fig. 3G) 2–3(–7) µm diam., clamped, thin-walled, smooth for the

Fig. 2. A. Basidiomes of *Mycena ulmi* (*BAP 1088*). **B.** Basidiomes of *Mycena ulmi* (*BAP 1085*, HOLOTYPE). **C.** Light micrograph image of *Mycena ulmi* pileus demonstrating a pseudocollarium that typically develops in mature specimens.

most part, but some portions often with simple to furcate, cylindric to slightly coarse excrescences, 1.5–10 × 1.5–3 µm; giving rise to numerous terminal cells (Fig. 3H,I) that may be quite variable in size and shape, ranging from irregular-cylindric to irregular-clavate, 12–53 × 5–11 µm, covered entirely to apically with simple to branched, short to quite long, cylindric to slightly coarse excrescences, 2–37 × 1.5–3 µm, some forms more or less irregular-cylindric, often tapered, giving rise to one or more apical to lateral, long, often branched, often tapered, flexuous excrescences that tend to form a loose network over stipe surface, these forms up to 125 µm in length; medullary hyphae broader, 10–19 µm diam., brownish-vinescent in Melzer's reagent; lactiferous hyphae present, 2–4 µm diam., with hyaline, refractive contents.

Habit, habitat, and known distribution.—Solitary to densely gregarious or subcespitose on the bark of *Ulmus americana*. Thus far only encountered on bare trunk bark collected from a single living tree at the Fort Worth Botanic Garden in Texas, and fruited in moist chambers in the laboratory at the Botanical Research Institute of Texas and California State University, East Bay.

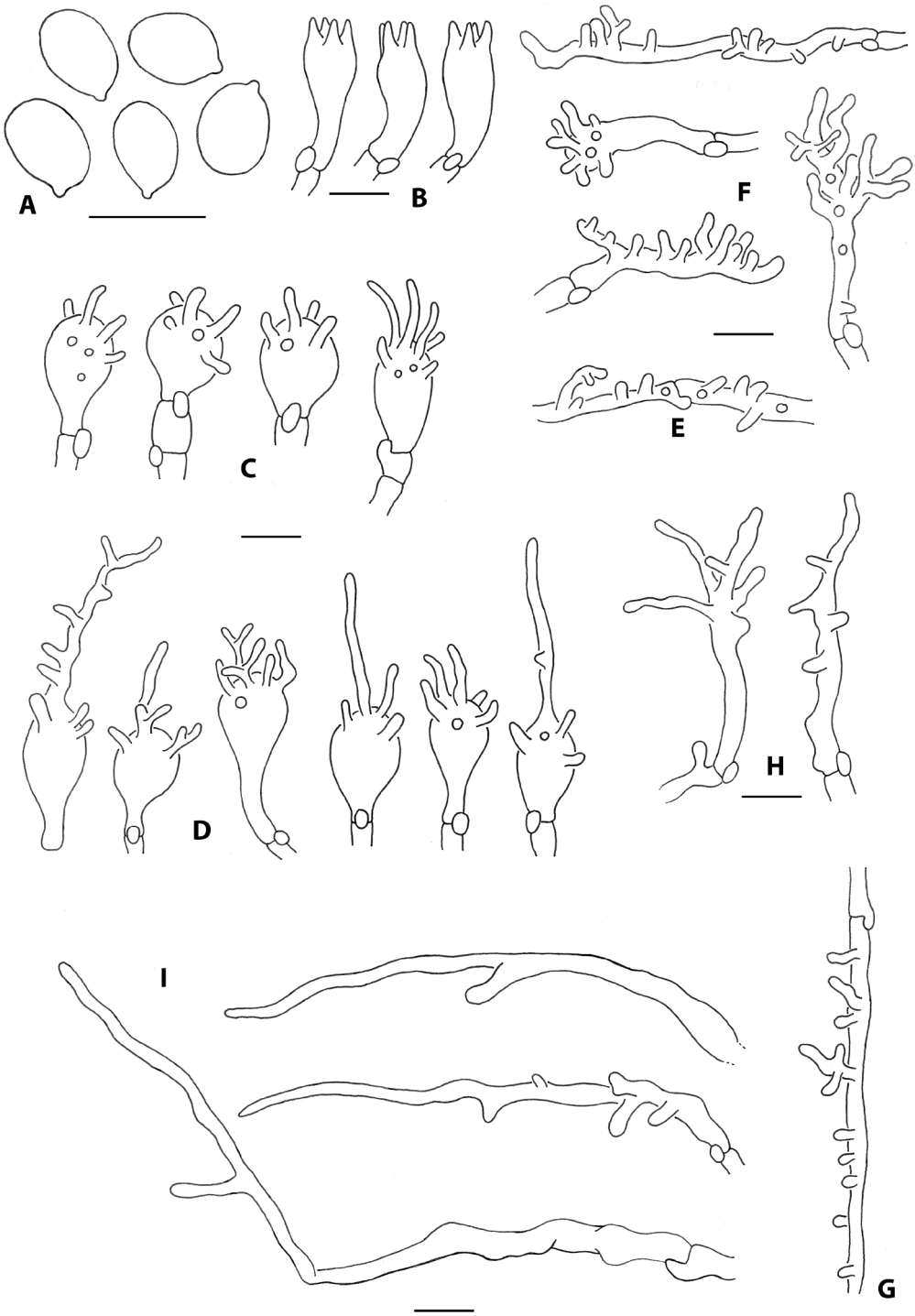

Fig. 3. Micromorphological features of *Mycena ulmi*. **A.** Basidiospores (*BAP 1084*). **B.** Basidia (*HWK 4937*). **C.** Cheilocystidia (*HWK 4839*). **D.** Cheilocystidia (*BAP 1084, BAP 1085*). **E.** Pileus epicutis hyphae (*HWK 4839*). **F.** Pileus epicutis hyphae terminal cells (*BAP 1085, HWK 4837, HWK 4839*). **G.** Stipe cortical hyphae (*HWK 4839*). **H.** Stipe cortical hyphae terminal cells (*HWK 4943*). **I.** Stipe cortical hyphae terminal cells (*HWK 4937*). Scale bars = 10 µm.

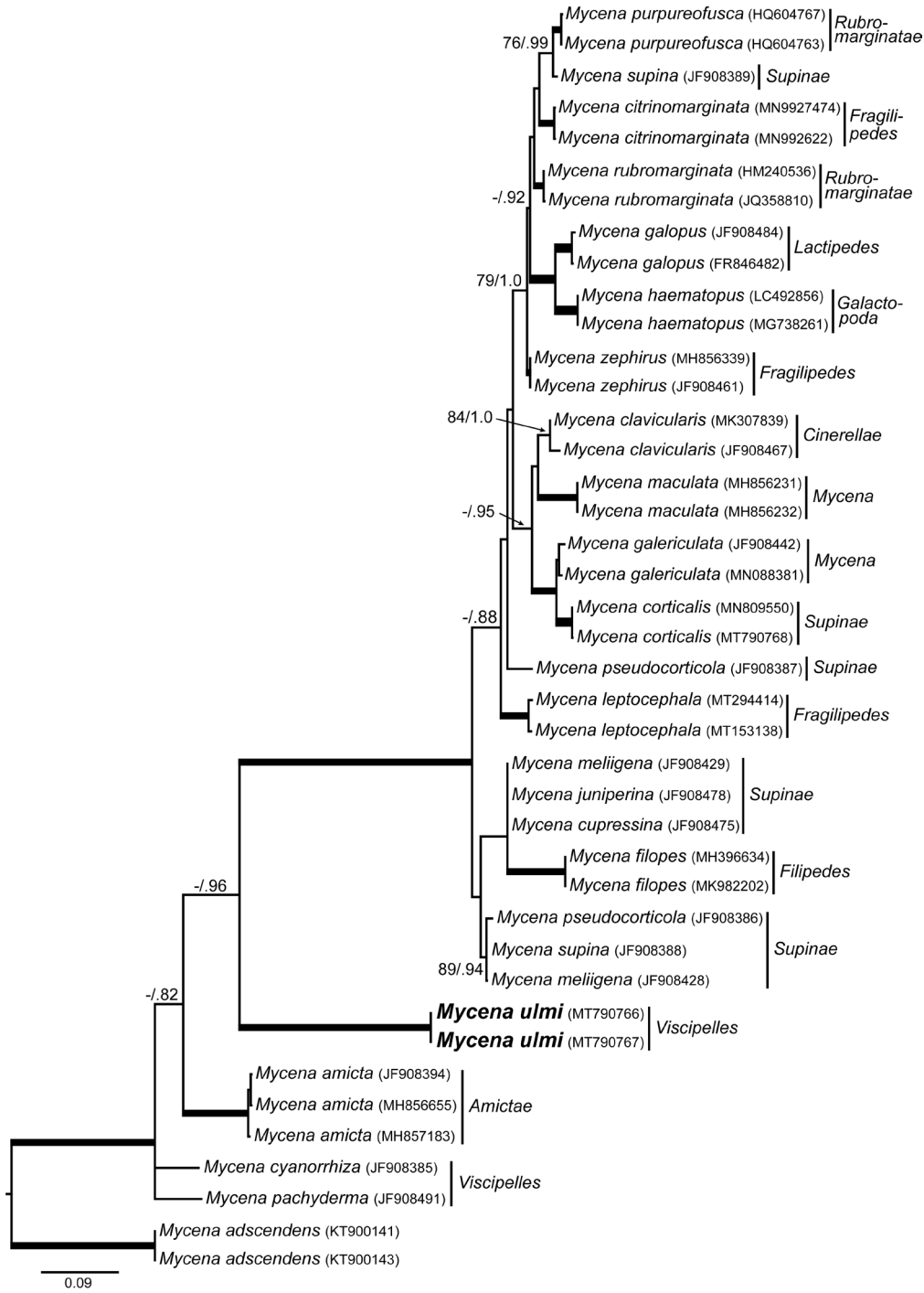

Fig. 4. Maximum likelihood phylogeny of *Mycena ulmi* and related taxa based on ITS sequence data (-ln*L* = 3762.3899). Sequences of *Mycena ulmi* indicated in bold type. Values separated by / refer to nonparametric ML bootstrap proportions and Bayesian posterior probabilities, respectively. Only values greater than 70/0.70 are shown (- designates a value below 70% or 0.70). Nodes receiving support values greater than 90/0.95 are highlighted in bold.

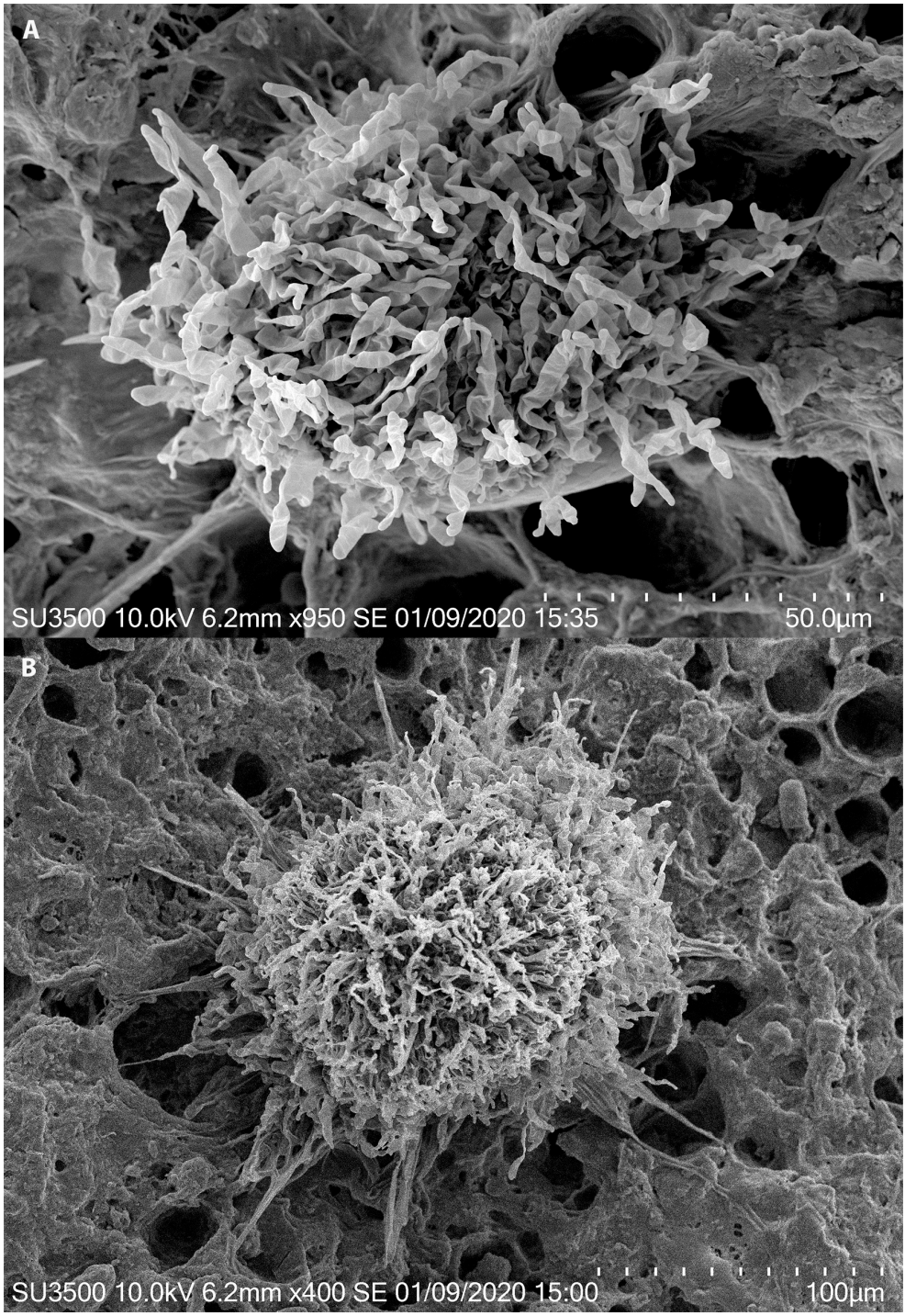

Fig. 5 A, B. *Mycena ulmi*, SEM. **A.** Close-up of primordial initial growing on bark of *Ulmus americana*; visible portion represents region that will develop into pileus surface. Scale bar = 50 µm. **B.** Primordial initial growing on bark of *Ulmus americana*; visible portions include developing pileus surface and stipe base with anchoring hyphae. Scale bar = 100 µm.

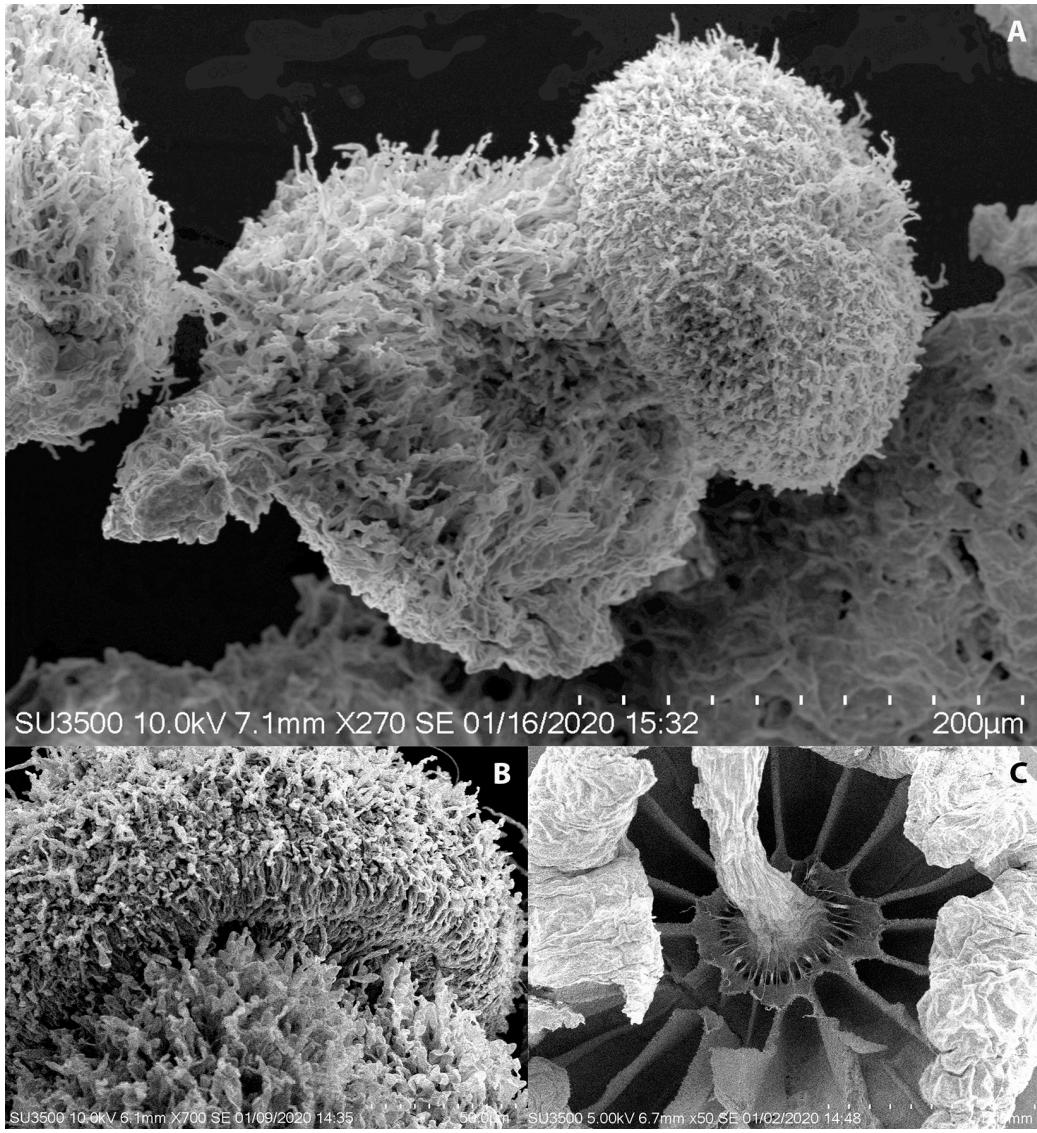

Fig. 6 A, B. ***Mycena ulmi*****, SEM. A. Intermediate stage with distinct zone separating the pileus and stipe surface hyphae, with no suggestion of an organized layer of wrapping hyphae. Scale bar = 200 µm. B. Close-up of intermediate stage with distinct zone separating the developing pileus and stipe regions. Scale bar = 50 µm. C. Close-up of lamellae separating from stipe surface to form a distinct pseudocollarium. Scale bar = 1.0 mm.**

Etymology.—*ulmi* is in reference to growth on the bark of live *Ulmus americana*, the only known substrate for this taxon.

Additional specimens examined.—**U.S.A. Texas. Tarrant Co.:** Fort Worth Botanic Garden, specimens collected from bark of live American elm (*Ulmus americana,* silver tag #2002) cultured in batch moist chambers, bark collected 3 Apr 2018, wetted 3 Jul 2018, harvested 12 Jul 2018, *HWK 4839* (paratype: BRIT, barcode BRIT507957); same location, bark collected 26 Jul 2019, wetted 29 Jul 2019, harvested 21 Aug 2019, *HWK 4906* (paratype, BRIT, barcode BRIT521895); same location, bark collected 20 Jul 2019, wetted 7 Aug 2019, harvested 10 Dec 2019, *HWK 4915* (paratype: BRIT, barcode BRIT521904); same location, bark collected 20 Jul 2019, wetted 7 Aug 2019 at BRIT, harvested 10 Dec 2019, *HWK 4916* (paratype: BRIT, barcode BRIT521905); same location, bark collected 4 Dec 2019, wetted 6 Dec 2019, harvested 26 Dec 2019, *HWK 4935* (paratype: BRIT, barcode BRIT425240); same location, bark collected 3 Apr 2019, wetted 3 Jul 2019, harvested 29 Jul 2019,

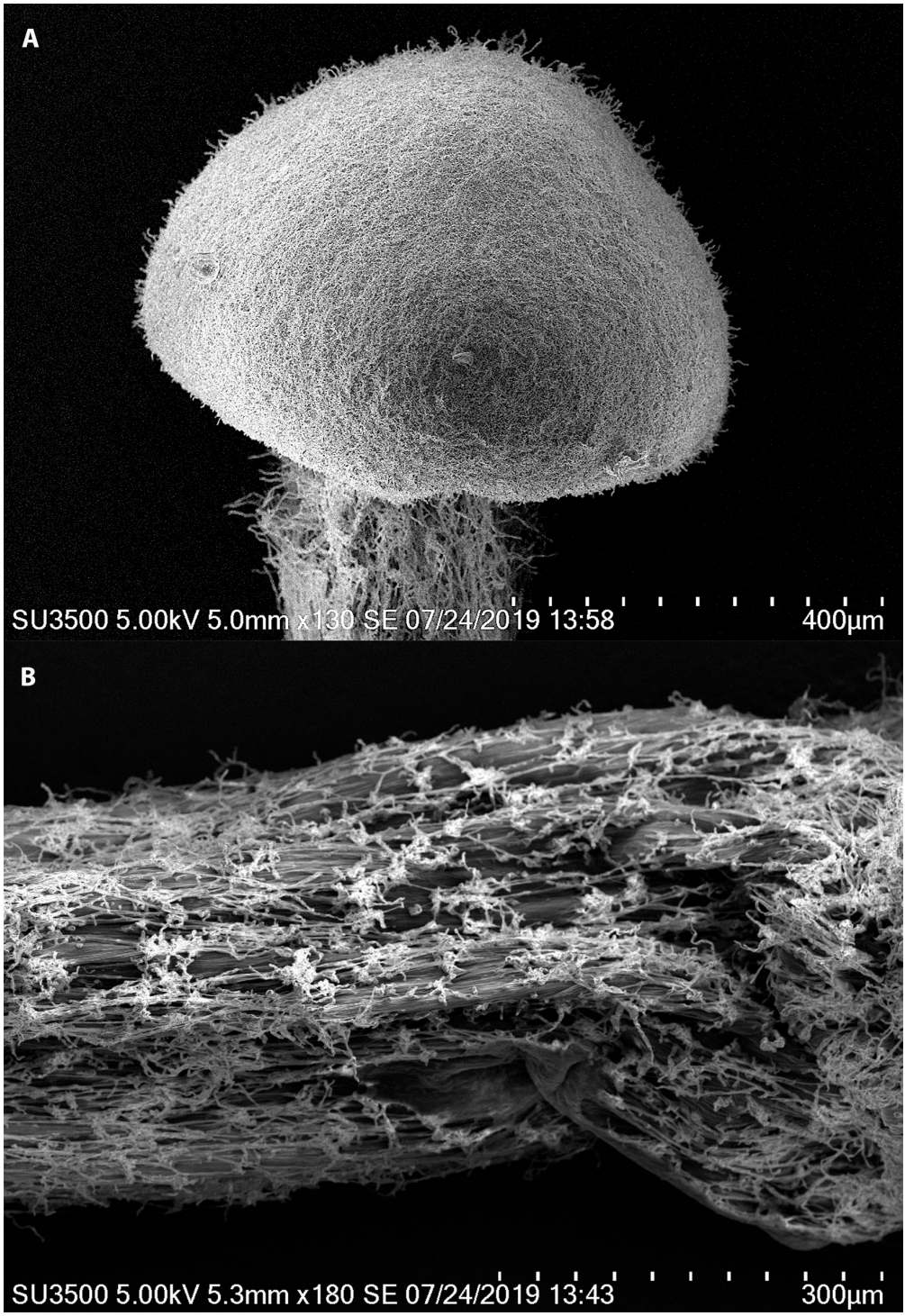

Fig. 7 A, B. *Mycena ulmi*, SEM. **A.** Densely minutely pubescent pileus surface of developing basidiome. Scale bar = 400 µm. **B.** Densely minutely pubescent stipe surface of developing basidiome. Scale bar = 300 µm.

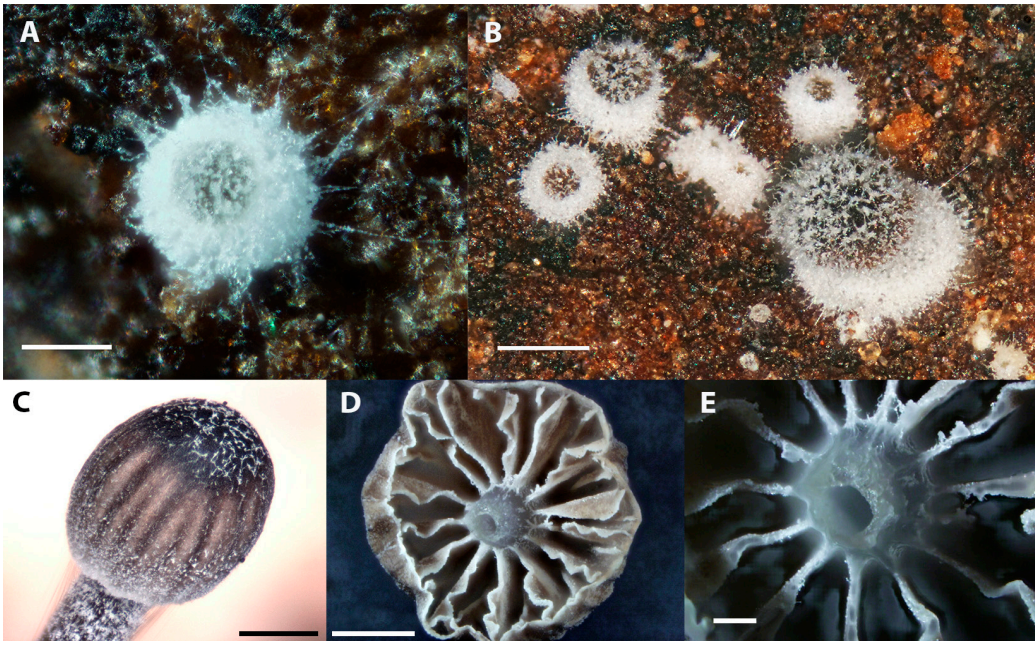

Fig. 8 A, B. ***Mycena ulmi*****, light micrographs. A. Primordial initial growing on bark of** ***Ulmus americana*****. Scale bar = 100 µm. B. Primordial initial and early intermediate stage growing on bark of** ***Ulmus americana*****. Scale bar = 100 µm. C. Densely minutely pubescent pileus of developing basidiome. Scale bar = 500 µm D. Lamellae and hollow stipe of young basidiome before lamellae have seceded from stipe to form a pseudocollarium; note droplet of fluid being exuded by stipe where it has been cut. Scale bar = 500 µm. E. Close-up of D. Scale bar = 200 µm.**

HWK 4936 (paratype: BRIT, barcode BRIT378113); same location, bark collected 4 Dec 2019, wetted 6 Dec 2019, harvested 28 Dec 2019, *HWK 4937* (paratype: BRIT, barcode BRIT425239); same location, bark collected 4 Dec 2019, wetted 6 Dec 2019, harvested 31 Dec 2019, *HWK 4943* (paratype: BRIT, barcode BRIT425238); same location, bark collected 8 Jan 2020, S side of tree, wetted 2 Feb 2020, harvested 21 Feb. 2020, *HWK 4944* (paratype: BRIT, barcode BRIT528580); same location, bark collected 8 Jan 2020, N side of tree, wetted 2 Feb 2020, harvested 20 Feb 2020, *HWK 4946* (paratype: BRIT, barcode BRIT59721); same location, bark collected 10 Dec 2019, wetted 17 Jan 2020 at CSUEB, harvested 23 Jan 2020, *BAP 1084* (SFSU, barcode SFSU-F-034628); same location, bark collected 10 Dec 2019, wetted on 17 Jan 2020 at CSUEB, harvested 4 Feb 2020, *BAP 1088* (paratype: SFSU, barcode SFSU-F-034629).

Commentary.—*Mycena ulmi* is herein proposed as a new species of sect. *Viscipelles* Kühner, based upon the presence of a separable gelatinous pellicle covering the pileus, ellipsoid to broadly ellipsoid basidiospores, clavate cheilocystidia that give rise apically to excrescences, and stipe cortical hyphae terminal cells (i.e., caulocystidia) that are often quite long and flexuous. It should be noted that the presence of a separable gelatinous pellicle is easily overlooked in fresh material due to the dry, minutely pubescent nature of the pileus. This character can, however, be determined even in dried material by completely rehydrating the pileus in water and using fine tipped forceps to peel this layer off.

Mycena sect. *Viscipelles* currently contains three taxa known only from Europe: *M. cyanorrhiza* Quél., *M. pseudocyanorrhiza* Robich, and *M. pachyderma* Kühner. While *M. ulmi* is quite similar in overall stature to *M. cyanorrhiza*, it can be distinguished from this species macroscopically by the lack of blue coloration at the stipe base as well the absence of a lamellar edge that is separable as a gelatinous, elastic-like thread. *Mycena pseudocyanorrhiza* is characterized by relatively larger basidiomes (pileus 6–10 mm diam, stipe 35–75 in length, 12–16 lamellae reaching stipe) in comparison to *M. ulmi*, and is also distinguished by the presence of blue pruina and/or pubescence on the lower portions of the stipe (Robich 2003).

Micromorphologically, *M. ulmi* can be distinguished from these taxa by the slightly narrower spores of *M. cyanorrhiza* (3.6–5 µm, as reported by Robich 2003 and Maas Geesteranus 1984), and the broader, subglobose

spores of *M. pseudocyanorrhiza* (9–11 µm, as reported by Robich 2003). Ecologically, *M. cyanorrhiza* is found primarily on the wood and bark of conifers, while *M. pseudocyanorrhiza* is described from soil, grass, ferns, and other herbaceous debris, among mosses or on twigs on the ground in mixed forests (Robich 2003). Neither *M. cyanorrhiza* nor *M. pseudocyanorrhiza* have been reported from the bark of *Ulmus*, the only substrate known thus far for *M. ulmi*.

Mycena pachyderma, also known only from Europe, appears to be a relatively rare and poorly understood species. Kühner (1938) described the species from French collections reported as growing on the bark of *Ulmus* and *Salix*. Maas Geesteranus (1984) was unable to locate Kühner's material, but noted both significant similarities and differences between this taxon and *M. cyanorrhiza* based on Kühner's original description. Material identified as *M. pachyderma* has since been collected from Belgium and Germany (according to Aronsen & Læssøe 2016), Italy (Robich 2003) and Spain (GenBank JF908491). Robich (2016) provided a detailed examination based on two collections from Italy, and retained the taxon in sect. *Viscipelles* despite the presence of subglobose to nearly globose basidiospores. Aronsen and Læssøe (2016) and Aronsen (https://www.mycena.no/cyanorrhiza.htm) question Robich's recognition of this material as *M. pachyderma*, suggesting instead that the material in Robich's photographs likely represents *M. clavularis* (Batsch) Sacc. due the presence of a small basal disc, a feature not indicated in Kühner's original description. However, Robich's (2016) illustrations and description indicate the presence of clamp connections and ornamented pileipellis terminal cells, features not known in *M. clavularis*. Additionally, an ITS sequence of a Robich collection from Spain identified as *M. pachyderma* falls close to *M. cyanorrhiza* in our analyses (Fig. 4), albeit on a collapsed branch without significant support. It is obvious that additional material needs to be examined before there is a clear concept of *M. pachyderma*. Although it is one of the few *Mycena* species that has been reported from the bark of *Ulmus*, this taxon as currently understood is easily distinguished from *M. ulmi* by paler coloration, shorter stipe, and presence of subglobose to globose basidiospores. A comparison of the taxonomically informative characters for all species currently treated in sect. *Viscipelles* can be found in Table 1.

Phylogenetic analysis of ITS sequences for collections made by Robich and identified as *M. cyanorrhiza* and *M. pachyderma* suggest a possible relationship between these taxa, but sequences of *M. ulmi* are resolved in an isolated position from these species (Fig. 4). Comparison of the aligned, overlapping ITS sequence data indicates 85–85.8% pairwise similarity between the sequences of *M. pachyderma* and *M. ulmi*, and 84.8–85.6% between *M. cyanorrhiza* and *M. ulmi*, with differences consisting primarily of polymorphic nucleotide positions, as well as eleven indels 1–7 nucleotides in length. In comparison, the sequences of *M. pachyderma* and *M. cyanorrhiza* show 92.6% pairwise similarity, with differences consisting of polymorphic positions and nine indels 1–2 nucleotides in length. While these data support the recognition of these taxa as independent species, they do not reveal anything additional regarding their phylogenetic relatedness nor the integrity of sect. *Viscipelles*.

Additional species found in North America that superficially resemble *M. ulmi* in stature or habitat include *M. amicta* (Fr.) Quél. and *M. subcaerulea* Sacc. from sect. *Amictae* A.H. Smith ex. Maas Geest., as well as *M. pseudocorticola* Kühner and other taxa from sect. *Supinae* Konr. & Maubl. *Mycena amicta* was originally included in sect. *Viscipelles* by Kühner (1931), but this taxon has since been transferred to sect. *Amictae*, with *M. subcaerulea*, based upon morphological differences between the taxa of these sections. Both *M. amicta* and *M. subcaerulea* are characterized by growth on woody substrates, blue coloration to the stipe base and often pileus, and a separable gelatinous pellicle covering the pileus. These taxa can be distinguished from *M. ulmi* and the other species of sect. *Viscipelles*, however, by their relatively larger basidiomes, cylindric to sublageniform and smooth cheilocystidia, and predominantly smooth pileipellis hyphae and terminal cells. Species of sect. *Supinae* (= sect. *Corticolae* (Kühner) A.H. Smith 1947), including *M. pseudocorticola*, *M. corticola* (Pers.) Gray, *M. corticalis* A.H. Sm., *M. meliigena* (Berk. & Cooke) Sacc., and *M. supina* in North America, are all characterized by growth on the bark of standing trees. However, all of these taxa are typically smaller in stature than *M. ulmi* and lack a separable gelatinous pellicle. With the exception of *M. corticalis*, which has been reported from Texas (Smith 1947), all of these taxa are also characterized by globose to subglobose spores.

TABLE 1. Comparison of taxonomically informative morphological features of *M. ulmi* and other species of sect. *Viscipelles*. Superscript indicates source from which data were adapted as follows: [1]Robich 2003, [2]Aronson and Læssøe 2016, [3]Maas Geesteranus 1984, [4]Kühner 1938. [5]Robich 2016.

	M. ulmi	*M. cyanorrhiza*	*M. pseudocyanorrhiza*[1]	*M. pachyderma*
Pileus color	Dark gray to gray-brown, the sulcations and disc darker	– White, white-gray, white-brown, cream-brown, pale brown, with brown disc[1] – Pale brown to pale gray, almost white with age[2] – Grayish brown to grayish white[3]	Dark gray to sooty gray, pale creamy brown, the margin white	– White, the disc watery white[5] – Glaucous gray, becoming white[4]
Pileus diameter	1.5–5(–7) mm	6–9 mm [1] 2–5(–10) mm [2] 1–5 mm [3]	6–10 mm	1–5 mm [5] 3–6 mm [4]
No. lamellae reaching stipe	9–12(–14)	10–18 [1] 9–14 [2] 10–12 [3]	16–20	11–14 [5] 12–14 [4]
Stipe length	17–38 mm	14–32 mm [1] 5–30(–70) mm [2] 3–20 mm [3]	35–75 mm	3–8 mm [5] 5–9 mm [4]
Stipe base color	gray-brown (covered with white pubescence)	blue or with a blue tinge[1] sky blue[2&3]	distinctly dotted with blue granules or hairs	hyaline white[5] watery white to gray[4]
Spore size (LxW)	7.0–8.5(–9) × 5.0–6.0 (–6.5) µm	7–9(–10) × 4–5 µm [1] 6.5–9 × 4–5 µm [2] 6.7–8 × 3.6–4.5 µm [3]	9–11 × 6–7 µm	(7–)8–9.5 × (6–)7–8.5 µm [5] 7.5–9.5 × 6.5–8.5 µm [4]
Cheilocystidia size (LxW)	13–35 × 7–12 µm	12–18 × 6–12 µm [1] 9–20 × 5.5–7 µm [2] 11.5–16 × 3.5–10 µm [3]	12–20 × 7–13.5 µm	10–20 × 6–12 µm [5] 15–16 × 9–10 µm [4]
Cheilocystidia excrescence length	1.5–25.5(–44) µm	up to 25 µm [1] 3–14 µm [2] up to 9 µm [3]	2–22 µm	2–28 µm [5]
Stipe terminal cell length	up to 125 µm	up to 60 µm [1&2] up to100 µm [3]	up to 40 µm (regular) up to 300 µm (pigmented)	up to 45 µm [5]

Phylogenetic analysis of ITS sequence data for members of sections *Amictae* and *Supinae* resolve sequences of *M. ulmi* as isolated and distinct from the taxa included from these sections. Sequences of *Mycena amicta* (sect. *Amictae*) are resolved on a branch subtending *M. ulmi*, while sequences for taxa from sect. *Supinae* are resolved in a weakly supported clade with *M. filopes* (sect. *Filipedes* (Fr.) Quél.) or scattered among taxa from other sections. Again, these data support the recognition of *M. ulmi* as an independent taxon, but reveal little regarding the phylogenetic relatedness of this taxon to members of these sections.

KEY TO THE SPECIES OF *MYCENA* SECT. *VISCIPELLES*

1. Stipe base with distinct blue coloration; lamellar edge separable as a gelatinous elastic-like thread, or not; basidiospores ellipsoid.
   2. Lamellar edge separable as a gelatinous elastic-like thread; stipe base with blue coloration in the stipe flesh; stipe generally up to 32 mm in length; basidiospores 3.6–5 µm in diameter _____ **M. cyanorrhiza** Quél.
   2. Lamellar edge not separable as a gelatinous, elastic-like thread; stipe base coloration due to presence of blue granules and/or hairs; stipe generally up to 75 mm in length; basidiospores 6–7 µm in diameter _____ **M. pseudocyanorrhiza** Robich
1. Stipe base lacking blue coloration; lamellar edge not separable as a gelatinous elastic-like thread; basidiospores ellipsoid, globose or subglobose.
   3. Pileus and stipe with distinct gray to gray-brown component; stipe length greater than 15 mm in mature specimens; basidiospores ellipsoid (5–6 µm diam); stipe terminal cells (caulocystidia) up to 125 µm in length _____ **M. ulmi** B.A. Perry & H.W. Keller
   3. Pileus and stipe white to pale gray; stipe length less than 10 mm in mature specimens, basidiospores subglobose to globose (6.5–8.5 µm diam); stipe terminal cells (caulocystidia) up to 45 µm in length _____ **M. pachyderma** Kühner

RESULTS AND DISCUSSION

Phylogenetic analyses

To resolve the placement of *M. ulmi* among morphologically similar members of sections *Viscipelles*, *Amictae*, and *Supinae*, ITS sequences for the holotype (*BAP 1085*) and paratype (*HWK 4839*) collections were analyzed phylogenetically within a limited sampling of representative sequences from these and other sections of the genus obtained from Genbank. As *Mycena* is an exceedingly large genus (ca. 500 species, Kirk et al. 2001) with a very high degree of ITS variability (B.A. Perry, pers. obs.), only Genbank BLASTn (Altschul et al. 1990) matches with percent identity scores of 85% or greater were included in the data set for alignment purposes. Two sequences of *Mycena adscendens* Mass Geest. (=*Mycena tenerrima* (Berk.) Quél., sect. *Sachariferrae*) were selected as the outgroup for rooting purposes based upon phylogenetic evidence that this section falls outside the bulk of *Mycena* sensu stricto (B.A. Perry, unpublished).

Maximum likelihood analysis of the ITS sequence data (Fig. 4) resolves the two collections of *M. ulmi* in an isolated grade subtending a clade composed of members of sections *Cinerellae* Sing. ex Maas Geest., *Filipedes*, *Fragilipedes* (Fr.) Quél., *Galactopoda* (Earle) Mass Geest., *Lactipedes* (Fr.) Quél., *Mycena* (Pers.) Roussel, *Supinae,* and *Rubromarginatae* Sing. ex Maas Geest. The sequences of *Mycena amicta*, the only representative of sect. *Amictae* with available data, fall out together in a well-supported clade basal to *M. ulmi*. *Mycena cyanorrhiza* and *M. pachyderma*, the only additional species of sect. *Viscipelles* for which sequence data currently exists, fall out on a collapsed branch basal to the other ingroup taxa. With the exception of *Lactipedes*, *Galactopoda*, *Cinerellae*, *Filipedes,* and *Amictae*, which are only represented by a single taxon each, the ITS data do not support the recognition of the included sections within the genus as they are currently circumscribed (and in several cases underscores the challenge of successfully identifying the small corticolous member of sect. *Supinae*). This is not surprising given that all sections within *Mycena* are based entirely on morphological data. While ITS is clearly not an adequate genetic marker for addressing questions of sectional integrity, it does serve to support the recognition of *M. ulmi* as a distinct taxon from morphologically and ecologically similar species (Fig. 4).

Fruit body growth displays negative geotropism

Most *M. ulmi* basidiomes developed on external bark surfaces or along edges in moist chamber cultures. However, some basidiomes developed on the bark underside from a single midpoint. The stipes grew laterally around the bark edges eventually curving upward, reorienting at right angles in an upright position.

Mature basidiomes had stipes up to 38 mm in length with mostly bell-shaped pilei up to 7 mm in diameter. Lamellae re-oriented downward exposing spores. Typically, spores are discharged into air currents unimpeded. Negative geotropism was well documented more recently by Bunyard (2012) in a wide spectrum of different basidiomycetes, *Pleurotus dryinus* (Pers.) P. Kumm., *Amanita muscaria* (L.) Lam., and *Ganoderma* species. More than 250 mature *M. ulmi* basidiomes were measured and observed throughout this study. Unfortunately, basdiospores were not observed in any moist chamber bark cultures on bark surfaces or overlapping pileus surfaces. Numerous basidiospores were observed, however, during the microscopic examination of the dried material.

Morphogenesis of *Mycena* basidiome development

The most recent study of mycenoid basidiome development is that of Walther et al. (2001) using light and scanning electron microscopy (SEM). These authors used field collected primordia/basidiomes of *Mycena stylobates* (Pers.) P. Kumm. from fallen beech leaves representing various developmental stages. Light microscopy (LM) and SEM analysis suggest that *M. stylobates* primordia are enveloped in a layer of differentiated "wrapping hyphae" soon after emerging from the substrate tissue. Sectioned tissue also revealed synchronous development of the pileus, hymenophore and stipe features. A pattern of development superficially similar to that of *M. stylobates* was observed in primordia of *M. ulmi* using both LM and SEM (Figs. 5–8). One major difference between these taxa, however, is that in *M. stylobates* the wrapping hyphae ultimately form the marginal hyphae of a distinct basal disc which displays the imprints of the developing lamellae. In *M. ulmi*, which lacks a basal disc, wrapping hyphae are absent. Instead, what is visible covering young primordia is in fact the pileus hyphae. The pileus appears to completely envelope any developing stipe tissues, with these only being revealed when the stipe initiates elongation and effectively lifts the pileus off the substrate. A distinct zone can clearly be seen separating the pileus and stipe surface hyphae, with no suggestion of an organized layer of wrapping hyphae (Fig. 6A,B). The SEM and LM images clearly demonstrate the dense, minute pubescence of the stipe and pileus surfaces (Fig. 8A,B).

Moist chamber trunk bark cultures yielded basidiomes in various stages of morphological development over a period of 9 to 21 days. Complete development from the earliest visible stages of primordial initials to mature basidiomes typically occurs within 48 hours, and can be divided into three stages: the first observable earliest primordial initials (Figs. 8A,B); intermediate or "button" stage with a short stalk and rounded unexpanded pileus (Fig. 8B) and a fully mature third stage with elongated stalk and expanded pileus with mature lamellae (Figs 2A,B). The earliest primordial initials are the first observable sign of development as a whitish, circular, filamentous, undifferentiated area about 50 µm in diameter. The primordia develop over a period of 8–12 hours into the intermediate stage, with the pileus becoming obvious as a dark gray mound covered with minute white pubescence, and the developing stipe visible as a white region below (Fig. 8B). Over the intervening 36–40 hours, the stipe elongates and the pileus continues to expand, eventually forming a fully mature basidiome, characterized by a long narrow, hollow stipe (Figs. 2A,B; 8D,E) and expanded pileus. As they age these mature specimens often become top heavy, eventually falling over on the substratum (Figs. 2A,B) before drying out.

CONCLUSIONS

Trees in urban areas support a diverse biodiversity of life forms, especially the microscopic corticolous myxomycetes and more rarely macromorphological corticolous fungi. Individual mature trees may take 50 to 100 years to provide enough time for organisms to colonize bark surfaces. Trees have more value than just aesthetic beauty as they also create microhabitats for hidden life forms that are lost every time a tree is cut down. As highlighted by the current research, the beneficial value of trees in urban settings includes the potential harboring of cryptic or rarely observed species that are new to science. Managing urban trees should include the assessment and evaluation of species biodiversity before the removal of such trees to create space for construction or other projects.

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