A NEW IRIDESCENT CORTICOLOUS MYXOMYCETE SPECIES (*LICEA*: LICEACEAE: LICEALES) AND CRYSTALS ON AMERICAN ELM TREE BARK IN TEXAS, U.S.A.

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ABSTRACT

A *Licea* species new to science is described on the bark surface of living American elm (*Ulmus americana* L.) cultured in moist chambers. It is characterized by an iridescent peridium on the sides of the sporangium, a black apical circular patch of globular debris, and dark reddish black spores that are smooth over half the surface and ridged-reticulate over the other half with the paler thinner wall collapsing into a coffee-bean shape. This combination of morphological characters is distinct and separates this taxon from all other species of *Licea*. The history of moist chamber culture use and field collection of corticolous myxomycetes is reviewed. The discovery of crystals of unknown origin on the bark surface of American elms associated with *Licea* species are illustrated with light microscope photography and scanning electron microscopy. Light microscope images and habit photographs were made using multi-focus imaging and computer stacking to increase depth of field and provide illustrations in color of sporangial structures of the new *Licea* species. This tiny short-stalked *Licea* approximately 100 µm in height, and with distinctive external and internal morphological characters, was photographed using scanning electron microscopy. Dark-spored versus light-spored species of *Licea* are reviewed and compared with the most recent molecular analysis as this relates to the genus *Licea*. This paper is the first in a series that will document the discovery of *Licea* fruiting bodies of four new species on American elms in nature parks near Fort Worth, Texas.

Key Words: Fruiting bodies, moist chamber cultures, multi-focus imaging, peridium, plasmodial slime molds, scanning electron microscopy, sporangia, spores, software stacking

RESUMEN

Se describe una nueva especie para la ciencia de *Licea* de la superficie de la corteza de olmo americano vivo (*Ulmus americana* L.) cultivada en cámaras húmedas. Se caracteriza por un peridio iridiscente en los lados del esporangio, una mancha circular negra apical en los restos globulares, y esporas negro-rojizo oscuras que son lisas en la mitad de su superficie y crestado-reticuladas en la otra mitad con la pared más pálida y fina colapsándose en forma de grano de café. Esta combinación de caracteres morfológicos es distintiva y separa este taxon de todas las otras especies de *Licea*. Se revisa la historia del uso de la cámara de cultivo húmeda y la colección de campo de mixomycetes cortícolos. El descubrimiento de cristales de origen desconocido en la superficie de la corteza de olmo americano y asociado con especies de *Licea* se ilustra con fotografías de microscopio lumínico y microscopio electrónico de barrido. Las imágenes de microscopio lumínico y fotografías del hábito se hicieron usando representación multi-foco y apilado para incrementar la profundidad de campo y dar imágenes en color de las estructuras esporangiales de la nueva especie de *Licea*. Esta *Licea* menuda de pedúnculos cortos aproximadamente de100 µm de altura, y con caracteres morfológicos distintivos externos e internos, se fotografió usando un microscopio electrónico de barrido. Se revisaron especies de *Licea* de esporas oscuras contra las claras y se compararon con los análisis moleculares más recientes y esto la relaciona con el género *Licea*. Este artículo es el primero de una serie que documentará el descubrimiento de cuerpos fructíferos de *Licea* de cuatro nuevas especies sobre olmos americanos en parques naturales cerca de Fort Worth, Texas.

"Nature is to be found in her entirety nowhere more than in her smallest creatures."—*Pliny the Elder*

INTRODUCTION

The Family Liceaceae contains the genus *Licea* with about 75 species (Lado 2005–2019) and the monotypic genus *Listerella paradoxa* Jahn (Lado & Eliasson 2017). The world monograph by Martin and Alexopoulos (1969) listed 19 *Licea* species, thus adding about one new species per year for the last 50 years. The majority of *Licea* species have come from the bark of living trees placed in moist chamber cultures.

Liceas are characterized by their tiny fruiting bodies, usually less than 1 mm, and the lack of structural characters such as a columella and capillitium. Therefore, surface features of the sporangial wall (peridium),

spores, measurements of sporangial size, spore diameters, plus colors serve as the main descriptors of the different species. Developmental stages such as the tiny protoplasmodia often go unobserved and are typically not key characters in species descriptions.

The lack of *Licea* morphological characters that are present in other myxomycete taxa places increasing emphasis on the use of scanning electron microscopy (SEM). Thus, outer and inner surface features of the sporangial wall and external morphology of spores become more critical in species descriptions and in the construction of keys to identify and separate different species. Modern instrumentation such as SEM has increased the ultrastructural fine details that are observable when compared to light microscopy (LM) used for species descriptions. Species descriptions and illustrations were seen at approximately 430× in Martin and Alexopoulos (1969).

Licea minima, which Fries described in 1829, is the oldest taxon in the genus. Few new species were discovered and described until Gilbert and Martin (1933) introduced the moist chamber culture technique used to study and isolate myxomycetes from the bark of living trees. Henry C. Gilbert was a student of Professor George W. Martin while working in the Mycology Laboratory at the University of Iowa (Keller 2012). In another paper, Gilbert (1934) described three corticolous myxomycete species new to science: *Macbrideola scintillans*, *M. decapillata* (*Macbrideola* is a commemorative generic name for Thomas H. Macbride a world famous myxomycetologist and former President of the University of Iowa), and *Hymenobolina pedicellata* currently considered *Licea pedicellata* (H.C. Gilbert) H.C. Gilbert. All three species form tiny plasmodia, each one producing a tiny stalked sporangium on American elm bark. However, where and when these species occurred in nature continued to evade collectors for many years until Dr. Travis E. Brooks developed collecting techniques in the 1960s while at the University of Kansas (Brooks 1967).

Field-collection of Liceas is a challenge because their small size requires at least a 20× hand lens to detect sporangia. Brooks targeted certain tree species such as *Juniperus virginiana* L. (Eastern red cedar) and *U. americana* with systematic and careful searching of bark following extensive rainy periods during summer months (Keller 1979; Keller & Braun 1999). This resulted in the discovery of many new species in the genus *Echinostelium* and *Licea* (Keller & Brooks 1976b; Keller & Brooks 1977).

Objectives

The broad objectives of this study are to describe a new *Licea* species, compare morphological differences between some Liceas, and introduce the discovery of tree bark crystals. Through an ongoing study of American elm bark and the community of corticolous myxomycete assemblages, four species of *Licea* new to science have been discovered (Marshall & Keller 2018). The discovery of species new to science and the search for new myxomycete records for the state of Texas represents another priority. Collections of myxomycete species made over the course of 45 years from *U. americana* from states in the Midwest will be compared with *U. americana* trees sampled in the Tarrant County, Fort Worth, Texas area. Older, large-sized *U. americana* trees that have survived unscathed from Dutch elm disease will serve as a source of bark samples. Previous research has shown that American elm tree bark in moist chamber cultures absorbs water and provides a favorable substratum source for the development of myxomycete fruiting bodies (Keller & Braun 1999).

Corticolous myxomycetes

A series of papers introduced the term corticolous myxomycetes, defined as myxomycete species that develop, grow, and form fruiting bodies on the bark surface of living trees and woody vines (Keller & Braun 1999; Keller & Brooks 1973; Keller & Brooks 1975; Keller & Brooks 1976a,b; Keller et al. 1977; Keller 1980). The majority of these corticolous myxomycetes only occur on the bark surface of living trees and woody vines as opposed to ground sites such as decaying or rotting logs or leaf litter. Many corticolous myxomycetes were collected in the field primarily from *J. virginiana* and *U. americana* (Keller & Braun 1999; Keller et al. 2009).

The first tree canopy study of corticolous myxomycetes used the doubled rope climbing method in the Great Smoky Mountains National Park (Snell & Keller 2003; Keller 2004). More recently, moist chamber bark cultures from various tree species show corticolous myxomycete fruiting bodies are associated with either a

more acidic pH near 4.0, or a more neutral pH of 7.0. Such pH-specific myxomycetes are considered specialists, while those that occur on a broader range of pH values are considered generalists (Everhart & Keller 2008; Everhart et al. 2008; Everhart et al. 2009; Keller et al. 2008; Keller et al. 2009; Kilgore et al. 2008; Kilgore et al. 2009; Scarborough et al. 2009). Furthermore, many of these species are tiny (less than 1 mm) and produce small protoplasmodia that give rise to single fruiting bodies, often resulting in a patchy distribution (Everhart & Keller 2008; Everhart et al. 2008; Everhart et al. 2009). Some of these corticolous species have a short colonization time, becoming established on the living stems of herbaceous plants or on tree fruits within a year (Kilgore et al. 2009).

Field study site

Elmer W. Oliver Nature Park, (ONP) was selected within the city limits of Mansfield, Texas because sizable *U. americana* trees were present (Fig. 1A). The 80 acres of ONP contain remnants of the eastern Cross Timbers and the northern Blackland Prairies ecoregions. Nine American elm trees, 18–24 m in height, were selected for bark samples. None of these trees had dead branches or any signs or symptoms of Dutch elm disease. The new *Licea* species developed on bark collected from American elm tree #8 located near a viewing deck and trail marker #11 (Figs.1B–F).

American elm field characteristics

Ulmus americana was described by Linnaeus (1753) in his *Species Plantarum*. However, elms have been around since the Miocene geological period 20 to 40 million years ago. They are native to eastern U.S.A., occurring from Nova Scotia west to Alberta, Canada; along the eastern seaboard south to Florida; westward to the midwestern states; and south to North Central Texas.

American elms are one of the dominant forest tree species reaching the top of the forest canopy. They are prized as ornamental shade trees due to their beautiful stature, generally characterized by a straight columnar trunk, vase-shaped silhouette, and symmetrical crown with spreading dichotomous or y-shaped branching patterns (Fig.1G). The dark green leaves in the summer turn bright yellow-gold in the fall eventually becoming deciduous (Fig. 1H). There are more than 30 species of elms, but the leaves of *U. americana* are distinctive and should not be mistaken for other tree species. American elm leaves measure 10–15 cm in length and 7 cm in width, occurring singly and alternating on two sides of the stem. Their oval shape comes to a sharp point at the apex and the margins are doubly serrate (saw-toothed). The leaves have prominent midribs, lateral veins (pinnate venation), and a strongly unequal base (Burns & Honkala 1990). The bark is grayish in color with irregular ridges of spongy texture separated by deep furrows (Fig. 1F, I). A voucher specimen was prepared and deposited in the Botanical Research Institute of Texas Herbarium and given a digital barcode BRIT 531101 (Fig. 2).

American elm tree bark crystals

Tree bark collected from living *U. americana* displayed crystals freely scattered on the surface or concentrated in crystal fields (Figs. 3A, B). Almost every piece of bark from tree #8 at Oliver Nature Park had crystals of irregular shapes and two broadly different size categories. Larger crystals measured 25–75 µm in diameter and smaller crystals were 7–15 µm (Figs. 3C, D). Individual crystals did not show a characteristic surface feature of regular facets which would indicate a specific mineral such as calcium oxalate (often found in compartmentalized cells in internal tissues of plants). Crystals were observed in clear lactophenol which was used to make microscopic mounts of myxomycete fruiting bodies.

Calcium carbonate crystals, typically found on the peridial surface of the myxomycete genus *Didymium* species (Fig. 3E), dissolve in lactophenol because the lactic acid reacts with the carbonate resulting in the visible release of bubbles. The observed tree bark crystals did not dissolve in clear lactophenol. Crystals on the bark surface of living trees apparently have not been described and published in the scientific literature. Some of these crystals were adhering to or next to myxomycete fruiting bodies (Fig. 3F). Additional bark samples were observed in moist chamber cultures as part of an ongoing study to determine the source and identity of these crystals

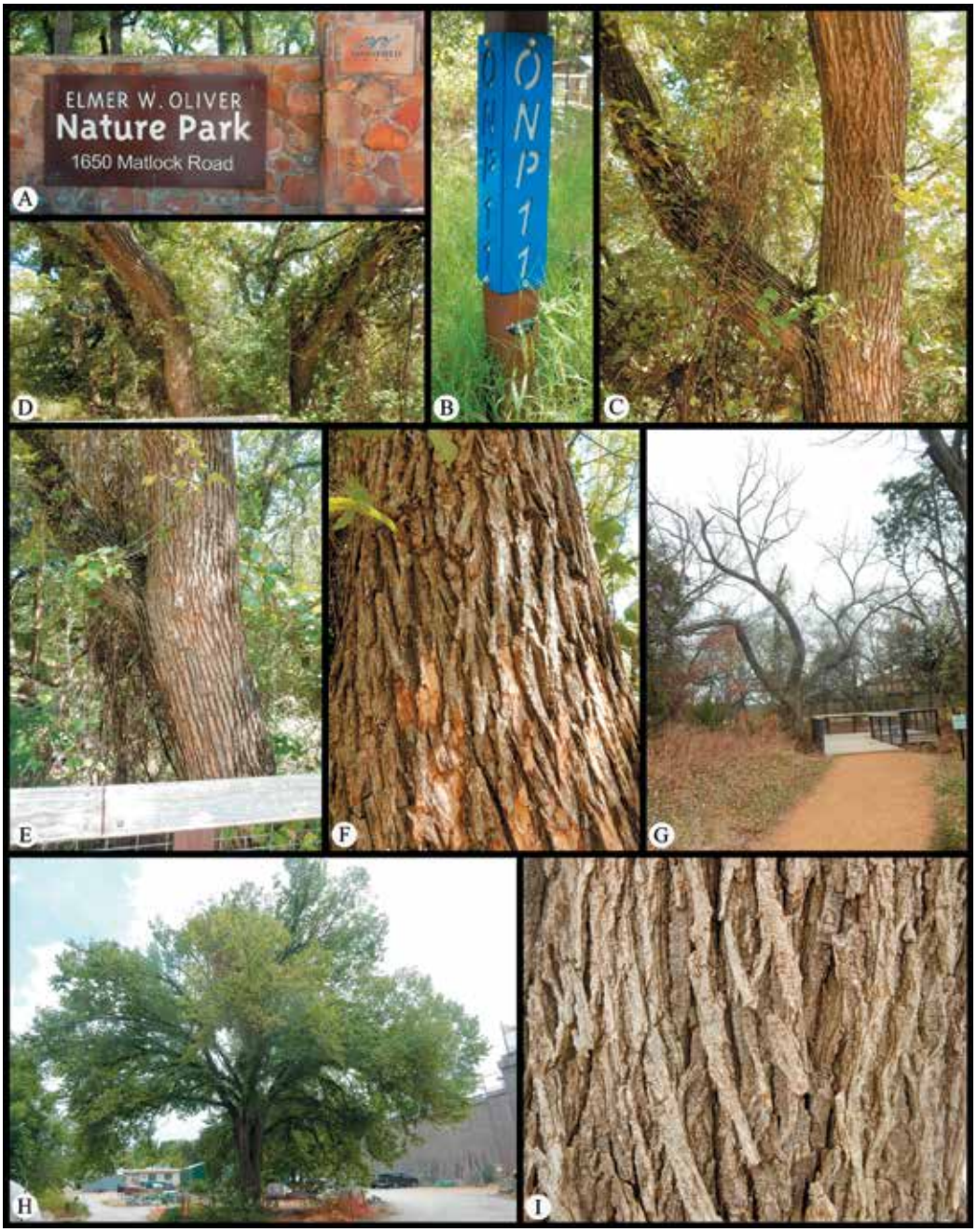

Fig. 1. **A.** Sign near front entrance to Oliver Nature Park. **B.** Trail Marker ONP 11 near viewing stand and American elm tree #8. **C, D, E, F.** Trunk of American elm tree type locality source of the bark samples for the new *Licea* species. This tree trunk branched below the viewing stand deck nearby. Samples were gathered at ca. 3–4 m from the viewing stand. **G.** American elm tree #8 during wintertime. Note leaves have fallen showing the dichotomous or y-shaped branching pattern in the tree canopy. **H.** General habit of American elm tree located at the Fort Worth Botanic Garden near the BRIT campus. Note single trunk and spreading canopy. **I.** Close up of bark showing deep fissures characteristic of American elm.

Fig. 2. Herbarium specimen of American elm twig showing leaf arrangement and upper and lower leaf surface features (*O'Kennon 32036 & Keller,* BRIT).

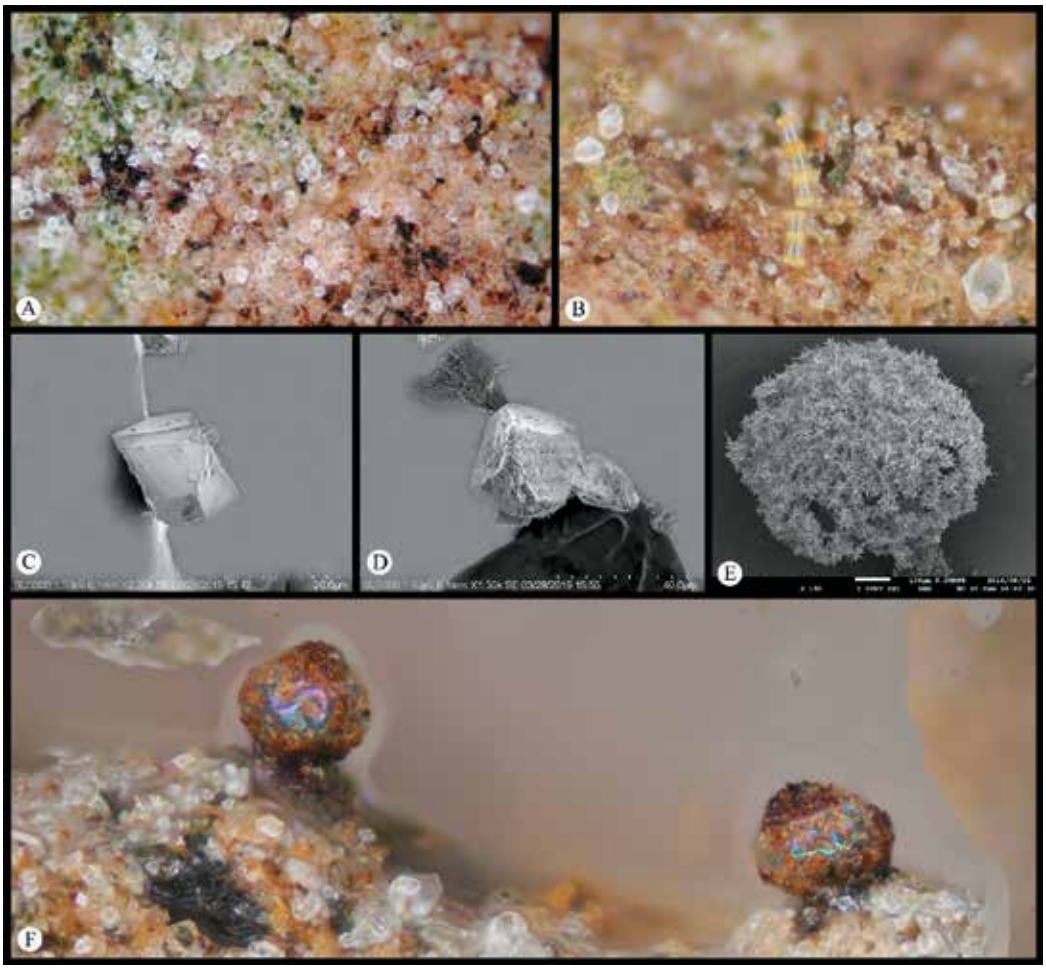

Fig. 3. **A.** White glittering crystals of irregular shapes scattered on bark surface as seen with LM and stacked images. **B.** Scattered irregular shaped larger and smaller crystals with three rod-like, yellow-banded crystals as seen with LM and stacked images. **C.** SEM of smaller irregular shaped crystal. **D.** SEM of larger crystal. **E.** SEM of *Didymium leoninum* sporangium showing stellate calcium carbonate crystals covering the upper half of the fruiting body (Photo by Shouya Yamazaki, Japan). **F.** Concentrated field of larger and smaller irregular white crystals associated with two *Licea iridescens* sporangia. Note the convex thick black area of debris centered apically and the iridescent sides of bluish and red sparkles as seen with LM and stacked images.

Future questions that will be addressed are: What is the identity of these crystals? What is their origin? What is their chemical composition, for example, silicon dioxide or quartz? How did crystals get on the bark surface? Are these crystals embedded in the bark layers suggesting an internal developmental origin? How many different tree species have surface bark crystals? How abundant are crystals on neighboring trees?

Iridesence in myxomycetes

The *Licea* species described herein is iridescent on the outer surface mostly on the sides of the peridium (sporangial wall). When natural or ambient light is used to view fruiting bodies the sporangia appear dull with no apparent color. Iridescence was observed by directed incident light using an external fiber optical system with an optical illuminator oriented at various angles. Structural iridescence occurs when white light passes through the sporangial wall and is reflected off the back wall of that same structure. Thickness of the medium,

angle of the light relative to the surface, and intensity of the illumination are variables contributing to iridescence. Observed iridescence included bluish, reddish or pinkish colors that sparkle giving a combination of colors on some myxomycete sporangia (Fig. 4A).

Iridescence is a property found in many different life forms. This includes butterfly scales and metallic bright green beetles, as well as hummingbird feathers. Myxomycete structural iridescence is exhibited strikingly by species of *Diachea*, such as *D. arboricola* H.W. Keller & M. Skrabal (Keller et al. 2004; Keller & Barfield 2017; Fig. 4B), *D. leucopodia,* and *D. splendens* (Keller 2011). *Lamproderma* species (Poulain et al. 2011) are mostly bluish colors, while Diacheas are silvery, bluish, and spectral colors of the rainbow. These colors vary depending on the surface structure of the fruiting body and angle of the direct light source (Fig. 4C). Most *Licea* species descriptions simply refer to iridescence as part of peridial surface features.

Species of *Licea* that exhibit iridescence include (Lado 2005–2019): *L. atricapilla* Nann.-Bremek. & Y. Yamam. (silvery, iridescent, colors not described); *L bryophila* Nann.-Bremek. (silvery, shiny or blue-green iridescent); *L. iridis* Ing & McHugh (bronze-brown, shining, iridescent colors not described); *L. kleistobolus* Martin, bright coppery iridescent (colors not described) mostly confined to operculum; *L. lilacina* Nann.-Bremek , T.N. Lakh. & R.K. Chopra (iridescent blue-violet); *L. margaritacea* Ing (rosy ochraceous slight iridescence colors not described); *L. metallica* D. Wrigley, T.W. Ko Ko, W.C. Rosing, & S.L. Stephenson (shiny, black, iridescent colors not described but Fig. 2 appears bluish); *L. perexigua* Brooks & Keller (more or less iridescent blue and red hues); *L. pseudoconica* Brooks & Keller (shiny somewhat iridescent, colors not described); *L. scintillans* McHugh & D.W. Mitch. (strongly iridescent colors not described). These eight *Licea* species exhibit varying degrees of iridescence compared to the conspicuous, bright sparkling colors seen in *Licea iridescens* (Figs. 4C–F).

MATERIALS AND METHODS

Collection of bark samples

The American elm trees selected for this study were the largest at the study site with a minimum trunk diameter of 60 cm at Diameter at Breast Height=DBH 1.5 m) and minimum total height of 25 m. Nine trees were sampled at ONP. A heavy bladed knife was used to pry tree bark samples, avoiding any damage to underlying tissues. Strips of bark from all sides of the trunk were collected at approximately 1.5–1.8 m until enough samples per tree were gathered to half-fill a paper bag (ca 1000 cm^3). Every bag was labeled with the identifying tree number and collection date.

Moist chamber tree bark cultures

Standard size moist chamber culture Petri dishes.—Two moist chamber cultures per tree were prepared using American elm bark samples to document the corticolous myxomycetes present. Each sterile, disposable plastic Petri dish (150 × 25 mm) was lined with one sterilized P8-creped filter paper fitted to cover the bottom. Bark samples, 3–6 pieces and 4–8 cm in length, were selected randomly from the bag, then placed in the Petri dish bark side up close together, but not overlapping or touching (Fig. 5A). If the Petri dish lid was unable to close completely due to bark thickness, a knife was used to thin the bark by slicing off pieces from the backside. Each Petri dish had the tree number, collection site, and date wetted marked on the lid and bottom side. Approximately 30 ml of deionized and autoclaved sterile water was added so that each piece of bark and the filter paper was thoroughly and evenly wetted. After 24 hours any excess water was decanted. An Orion 610 pH flat probe meter was used to measure pH on the filter paper surface. Details of the pH measurements will be published elsewhere.

Cultures were placed near a window, exposed to indirect natural light and ambient room light, remaining in the same location unless they were examined under a microscope. Incubation occurred with light and dark cycles throughout the year while remaining at room temperature at approximately 22–25 C. On day two and day six after wetting and every two to four days thereafter for four weeks the plates were examined under a Nikon dissecting microscope with a MKII Fiber Optic Light at magnifications of 50×–150×.

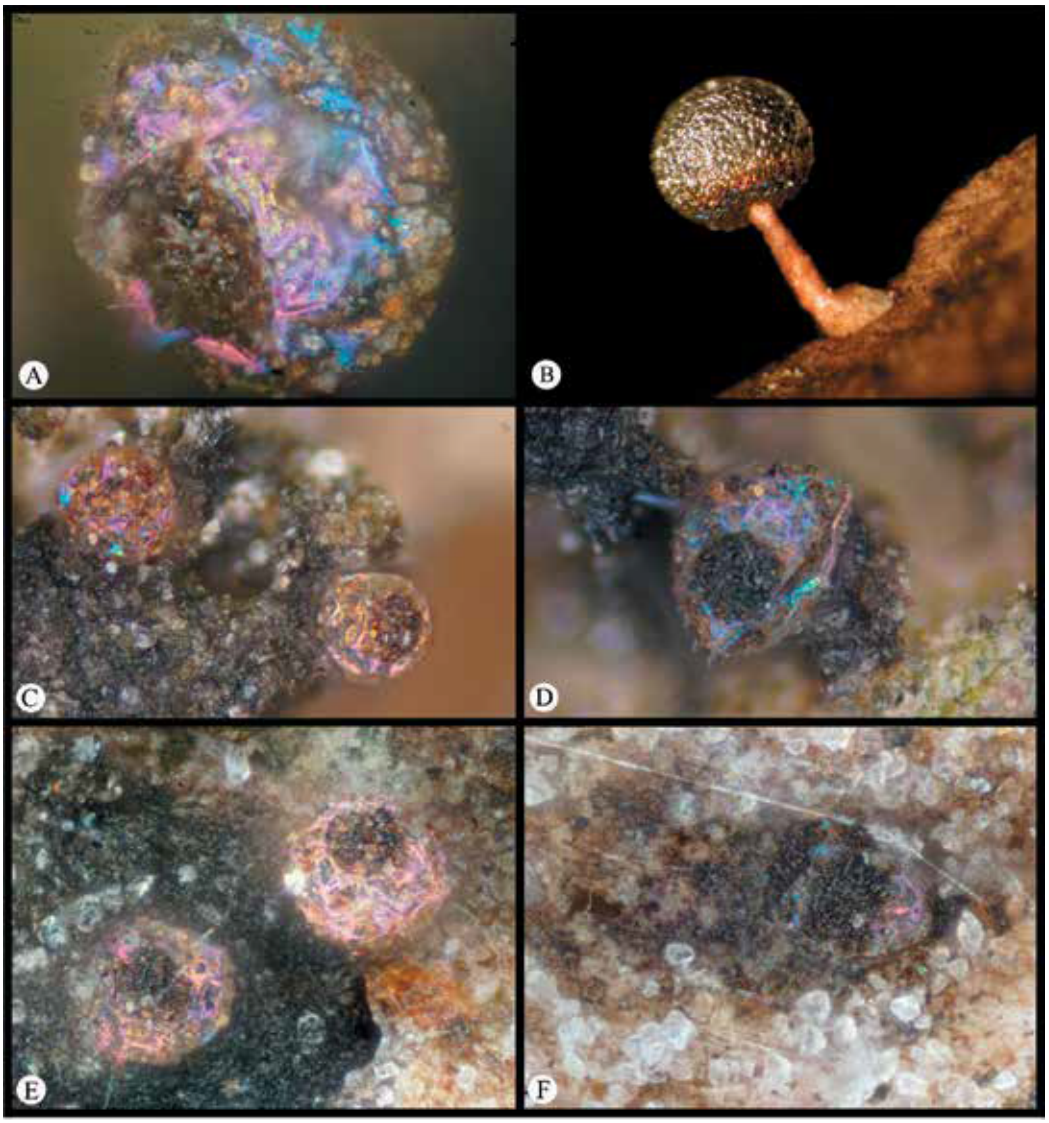

Fig. 4. **A.** Close-up of *Licea iridescens* sporangium showing black, apical, thickened patch of debris and smoother iridescent sides with bluish, reddish sparkles. **B.** Stalked sporangium of *Diachea arboricola* showing silvery metallic peridium and basal iridescent colors of the rainbow. Discovered in the tree canopy on the bark surface of a living White Oak tree (Photo by Sydney E. Everhart). **C, D, E, F.** Sporangia of *Licea iridescens* species showing variable iridescent color images. **C.** Two sporangia, one on right with circular black patch of debris, slightly recessed, and sides with golden-pinkish iridescence. **D.** Single sporangium showing circular black apical debris and iridescent bluish sides. **E.** Two sporangia with pinkish iridescent sides surrounded by large crystals of various shapes. **F.** Sporangium with flattened apical black debris extending to margin. Note bluish- pinkish iridescence confined to sides surrounded by crystals.

Each dish was scanned systematically from side to side to ensure that all bark was examined thoroughly. However, exposure to aerial contaminates should be minimized to avoid filamentous fungi overgrowing fruiting bodies such as *Trichoderma*, a commonly encountered green mold. Tiny fruiting body size and scattered habit made it difficult to find Liceas. Therefore, more and larger bark samples were needed to harvest additional *Licea* specimens for type collections. To accomplish this, pie tins were used as moist chamber cultures

Fig. 5. A. Moist chamber bark culture plastic Petri dish. **B.** Batch moist chamber culture pie tin. **C.** Holotype specimen boxed and labeled.

rather than Petri dishes. Additionally, to save time, insect pins were used to identify fruiting body locations. When moist chambers are re-scanned, pins facilitate specimen location. Pins may interfere with photographic techniques and must be removed when orienting the specimen under the microscope for photography.

Moist chamber batch cultures.—Shallow circular aluminum pie tins 25 cm across were fitted with paper toweling and prepared for moist chamber bark cultures. American elm bark recently collected from ONP tree #8 were 102 mm–15 cm in length and 1.3 cm–2.5 cm in diameter and 8–12 pieces were arranged to fit in the bottom of a pie tin without overlapping (Fig. 5B). No attempt was made to keep the cultures sterile with the exception of water used to wet the bark which was de-ionized and sterilized. These cultures were made with

less costly materials readily available to anyone interested in using moist chamber cultures to search for myxomycetes (Braun & Keller 1977; Keller et al. 2008; Keller & Everhart 2010). Batch cultures afforded the opportunity to collect multiple bark specimens of Liceas for herbarium collections. This was of paramount importance because sporangia were present in such few numbers that more specimens were needed to accurately describe a new species. In addition, many more fruiting bodies were observed in various developmental stages, especially variable structures such as stalks, sporangial overall size, and surface features of the peridium.

Light microscope observations.—Tools for preparing fruiting bodies for slide-making such as dissecting needles, air blowers, and forceps are described in detail elsewhere (Sundberg & Keller 1996; Keller & Braun 1999). Microscope glass slides (75 mm × 25 mm × 1 mm) with a frosted end were used to make mounts of myxomycete structures for identification. Small pins attached to wooden handles were used to remove fruiting bodies from the bark. A droplet of clear lactophenol was placed in the center of the microscope slide, sporangia were added, and a square cover slip (22 × 22 mm and 0.16 mm thick) was gradually lowered at an inclined angle over the specimen to avoid creating air bubbles. Permanent slides were made for future use by sealing the edge of the cover slip with ZUT, a resiniferous slide-ringing compound. Identification labels were written in pencil on the frosted end of the slide then covered with clear tape to prevent future smudging. Slides were wrapped with lens paper and placed in properly labeled collection boxes. A Nikon Alphaphot light compound microscope was used to scan slides at 100×, 430×, and 1000× with a calibrated ocular micrometer to make measurements to identify myxomycete species.

Light microscope photography.—An Olympus AX70 Microscope with an Olympus DP71, 12 mega-pixel camera was used for taking all slide images. The AX70 internal microscope software calibration was verified using the classical stage micrometer measurement technique. An Olympus BH2 microscope, used with 50× and 100× objectives (7.5 mm and 2.5 mm working distances respectively) and vertical illumination, was used for habitat images along with a Sony A5000 20-megapixel camera. Image stacking was performed for both slide and habitat images using Helicon Focus software version 6.8.0. Each image stack varied between 10 to 75 individual images depending upon specimen size and magnification. Image stacking increased depth-of-field of sporangia so that the entire sporangium (spore case and stalk) were in sharp focus. Images were optimized using Adobe Photoshop CC 19.0.

Scanning electron microscope observations.—The newly acquired analytical field emission Hitachi SU3500 high resolution Scanning Electron Microscope (SEM) facilitated observation up to 5000× image magnification without the need for sputter coating the specimen. SEM specimen stubs used were the pin type with circular diameters of 12.5 mm and 25 mm where specimens were mounted on double-sided adhesive conductive mounting tabs without sputter coating. Observations were made at a pressure of 30 Pa and accelerating voltage between 1.5 kV and 5 kV with variable magnifications up to 5000×. 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, and calibrated measurement scale comprised of 11 marks, the combined length indicated below the marks in millimeters or microns. Dried bark harvested from moist chamber cultures was the substratum source for myxomycete sporangia. Final images were photoshopped to obtain the best contrast and image quality.

Sporangia about 100 µm in height were difficult to handle on dried tree bark because of their brittle and fragile condition. A sharp needle attached to a wooden dowel was used to tease the sporangium from the bark surface. This was facilitated with a droplet of saliva on the needle tip and moistened bark that facilitated adherence of the sporangium to the tip of the needle. (Water does not work, apparently due to its lower surface tension or viscosity). This required a steady hand to rotate the needle into the desired position and transfer the sporangium properly oriented to the surface of the sticky adhesive on the surface of the SEM specimen stub.

Approximately 10 sporangia were placed in different positions on the SEM specimen stub to maximize desired viewing surfaces. Sporangia of different species often developed in close proximity or were intermixed

on the bark surface. Careful searching was required to select the desired species separated some distance from other look-a-like species.

Preparation of herbarium myxomycete specimens.—Moist chamber cultures of tree bark often have scattered sporangia of *Echinostelium* and *Licea* species (usually less than 100 µm) that occur more frequently on the edges and in crevices, and less on the bark surface. Their tiny size and scattered habit make it difficult to find these sporangia.

The presence of myxomycete fruiting bodies on dried tree bark can best be located by using a white paint permanent waterproof liquid opaque marker with an extra fine (0.7 mm) tip (Fig. 5C). A white dot is placed equidistant on either side of the fruiting body and close enough in a straight line so that both the white dots and specimen are in the field of view simultaneously. This technique should prove valuable in marking the location of myxomycete fruiting bodies in type collections especially when their presence is obscured by look-alike life forms.

Pieces of dried bark with fruiting bodies of myxomycete sporangia were glued to the bottom of a collecting box (4.5 × 10.5 × 2 cm). Hand-written labels included species name, state, county, collection locality, habitat, geographical coordinates, collection date, collector's name (*legit*) and accession number, person identifier name (*fide*) were affixed to the box top. The practice of hand-written labels used by Professor George W. Martin at the University of Iowa was followed, rather than typed labels, which indicated personal observation of the specimen. All bark and slide specimens were assigned a BRIT barcode number and deposited in the BRIT Herbarium. The **holotype** was assigned a MycoBank number, see Fig. 5C.

TAXONOMY

Licea iridescens H.W. Keller & V.M. Marshall, **sp. nov.** (**Figs. 3F, 4A, C–F, 6A–J, L–M; Figs. 7A, B; 8A, B; 9A, B; 10A, B SEM**). TYPE: U.S.A. TEXAS. Tarrant Co.: Oliver Nature Park, Mansfield, specimen (Fig. 5C) collected from bark of live American elm (*U. americana*) tree #8, elev. 167 m, 32.587.542–97.095.708, cultured in batch moist chambers, bark collected 4 Feb 2019, wetted 8 Feb 2019, harvested 19 Feb 2019, *Harold W. Keller 4836* (HOLOTYPE: BRIT 478988, MycoBank, MB 831218).

Sporangia sessile, spherical on a constricted base or more often short-stalked, widely scattered, solitary or in pairs, less often in small groups, often occurring along bark edges and in moist fissures, stalks variable, sometimes stout, thickened, solid, surface roughened, lacking grooves, (10)–42–(60) µm in height, width (47)–74–(105) µm, about half or equal the sporangial width, total fruiting body height, (100)–120–(156) µm, diameter of sporangium, (62)–122–(139) µm, height (27)–93–(131) µm; outer peridium with bluish-reddish-pinkish sparkles, iridescence confined to wrinkled, smooth sides, with a blackish, circular, apical spot of spherical globules and irregular debris, usually centered, mostly convex and more nipple-like or thickly flattened, less often concave then slightly depressed with a circumscissile distinct rim; appearing somewhat thickened as a translucent single layer with debris of particulate matter, sometimes with entrapped crystals and fungal spores, dehiscence irregular at maturity, with roughened markings conical to irregular on the inner surface; spores free, shiny reddish-black, dark in mass, reddish-brown by transmitted light, collapsed, enfolded, and coffee-bean shaped due to a conspicuous paler or colorless thin-walled area on one side with ridged-reticulate markings, forming a wide partial meshwork, occupying about ⅓–½ of the spore surface, the remainder with a thicker-walled smooth surface area, L (12)–14–(16) × W (10)–12–(14) µm in diameter. Plasmodium not observed.

Etymology.—Derived from the Latin *iris* meaning a lustrous rainbow-like display of colors caused by the differential refraction of light waves by microstructures and *escens* meaning becoming, tending to be, that refers to the iridescence of the peridium.

Distribution.—Only known from type locality. Texas: Tarrant County, Oliver Nature Park, 1650 Matlock Road, Mansfield, 76063, coordinates 32.587.542–97.095.708, at trail marker 11 next to viewing stand, elevation 167 m.

Terminology.—Myxomycete terms used in this species description and throughout this paper follow Keller et al. (2017).

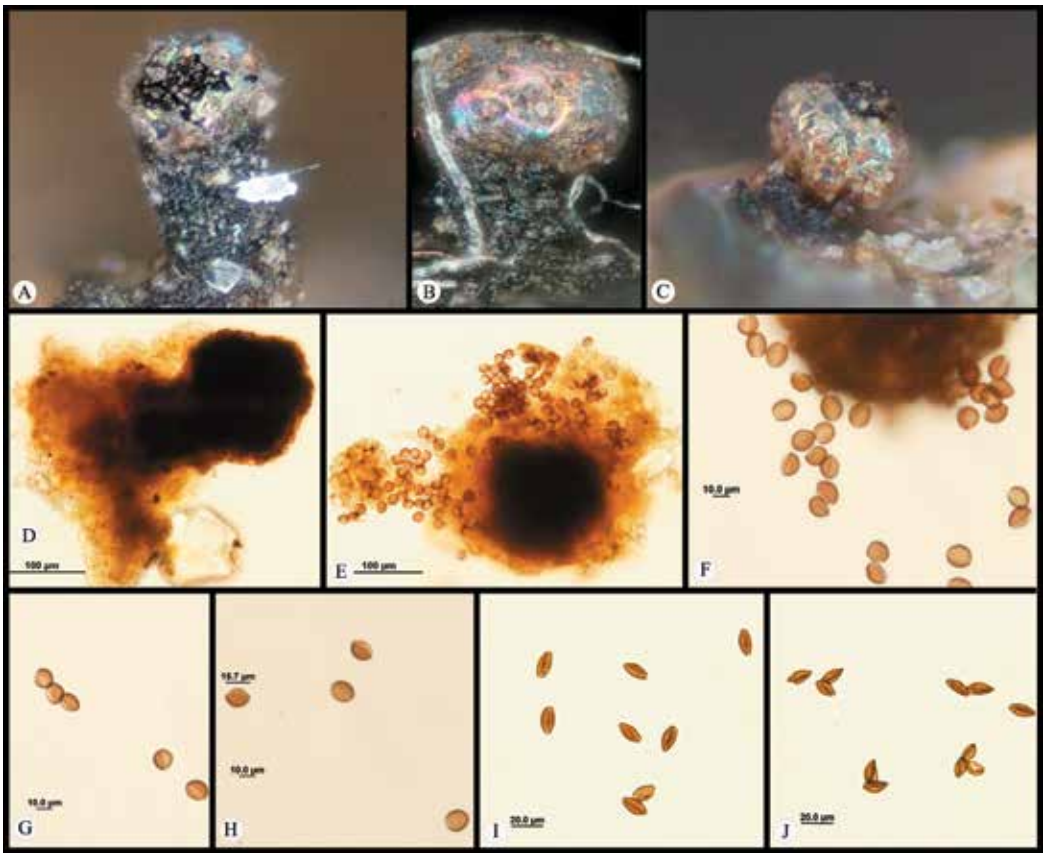

Fig. 6. A–J. *Licea iridescens*. A. Stalked habit with dark spores in mass. B. Stout stalk with side of sporangium iridescent. C. Sporangium with short stalk and apical nipple-like black convex debris. D. Entire opaque sporangium seen with LM. E. Reddish-brown spores seen with transmitted light (LM) undergoing swelling with some thicker spore walls in optical section. F. Portion of dark apical patch surrounded by spores in different optical planes of focus showing darker thicker wall half and paler thinner wall half, LM. G. Five spores partially swollen showing darker thicker walls and paler thinner walls, LM. H. Four spores partially swollen showing plane of focus highlighting darker half and paler half, LM. I. Eight spores photographed immediately after mounting in clear lactophenol showing enfolded and collapsed coffee-bean shape. Paler thinner wall not visible, LM. J. Spores collapsed coffee-bean shapes. Note cluster of three spores lower right with one spore partially swollen showing membrane-like paler wall, LM.

Paratype bark collections examined (5) (HWK=Harold W. Keller): *HWK 4831*, collected 4 Feb-2019, wetted 8 Feb 2019, harvested 19 Feb 2019, BRIT 478965; *HWK 4832*, collected 4 Feb 2019, wetted 8 Feb 2019, harvested 19 Feb 2019, BRIT 478968; *HWK 4833*, collected 4 Feb 2019, wetted 8 Feb 2019, harvested 19 Feb 2019, BRIT 478967; *HWK 4835*, collected 4 Feb 2019, wetted 8 Feb 2019, harvested 19 Feb 2019, BRIT 478986, *HWK 4837*, specimen with abundant crystals, collected 4 Feb 2019, wetted 8 Feb 2019, harvested 19 Feb 2019, BRIT 64949.

Microscope slides examined (4): *HWK 4834* collected 4 Feb 2019, wetted 8 Feb 2019, harvested 19 Feb 2019, BRIT 478987; *HWK 4821*, collected 16 Jul 2018, wetted 16 Jul 2018, harvested 6 Aug 2018, BRIT 533869; *HWK 4822*, collected 16 Jul 2018, 16 Jul 2018, 6 Aug 2018, BRIT 533867; *HWK 4823*, collected 16 Jul 2018 wetted 16 Jul 2018, 6 Aug 2018, BRIT 533866.

Taxonomic Commentary

Specimens of *L. iridescens* were difficult to find in moist chamber cultures due to lack of abundance, scattered habit, and microscopic sporangia. Many pieces of American elm bark had only one or two sporangia or none at all. Another iridescent *Licea* species was also sometimes present that was, sessile, smaller, with finely spinulose spore ornamentation not seen with LM. Microscopic sporangia of other *Licea* species in greater abundance usually developed in close association to *L. iridescens*, including *L. perexigua*, *L. pseudoconica*, and *L. pedicillata*.

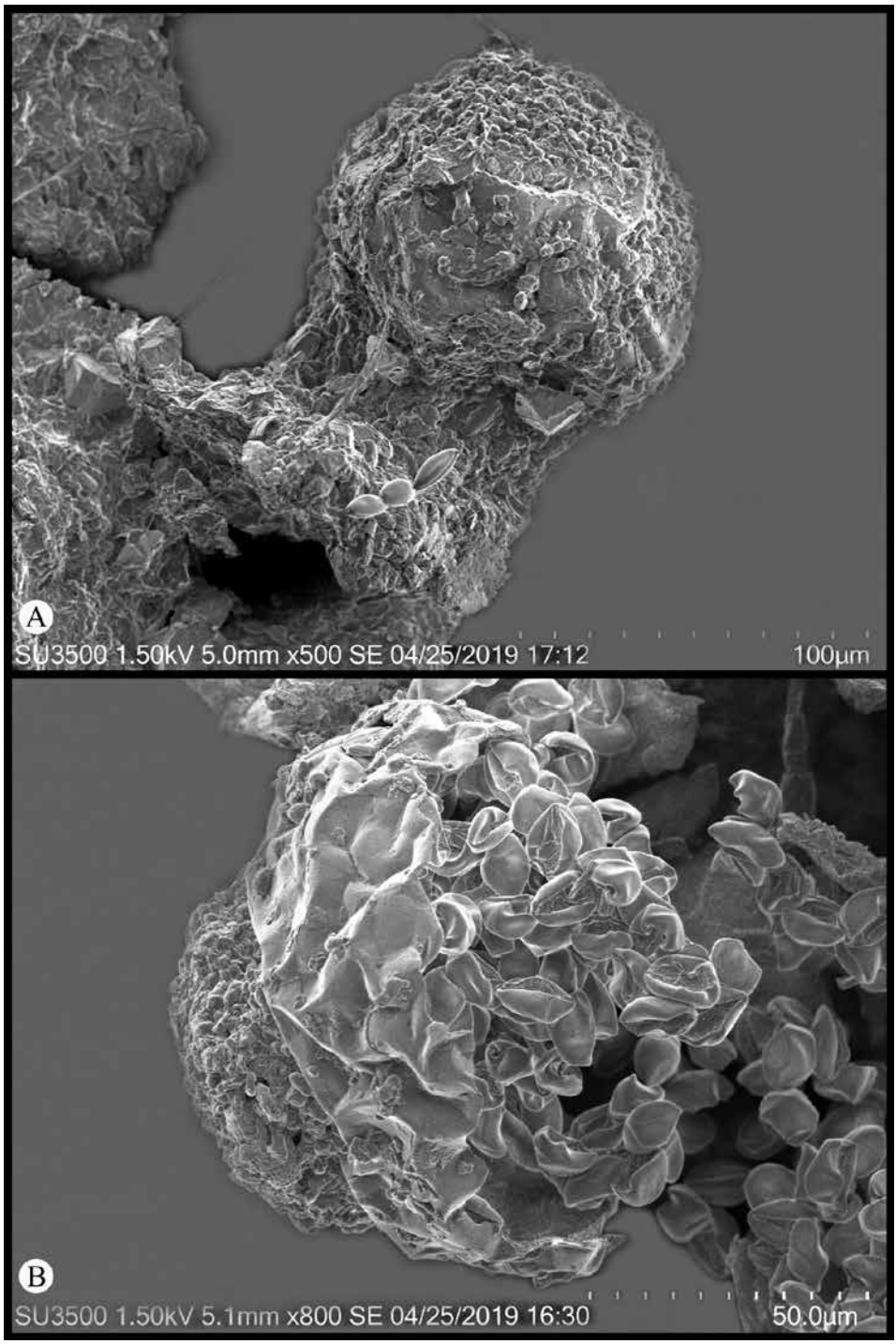

Fig. 7 A, B. *Licea iridescens*, SEM. **A.** Whole stalked sporangium. Note apical region with globular debris, smoother sides, base of stalk with adhering crystals, and three spores. **B**. Sporangium top half showing convex, globular, irregular debris, and wrinkled smooth sides (location of iridescence) with coffee-bean collapsed spores showing both smooth thicker wall surface and enfolded thinner wall surface.

Nostoc balls, a cyanophycean blue green alga, also were sometimes confused with developing spherical, dark, sessile *Licea* species. When bright greenish in early developmental stages, *Nostoc* is easily identified, but more mature developments are much darker, almost black, and difficult to distinguish from developing *Licea* species. Many tiny *Licea* species are patchy and confined to small areas of the tree bark.

Measurements of *L. iridescens* sporangia were based on the holotype collection (3) and paratype collections (5) on American elm tree bark. Spores (100) were measured when swollen to a more spherical shape (length L and width W) in clear lactophenol. The colorless portion of the spore was thin and membrane-like, the rest of the spore was much thicker about 1 µm. This thin-wall spore area probably represents the germination pore (Figs. 6E–H).

Only two sessile species of *Licea*, *L. metallica* and *L. pseudoconica*, have refuse debris deposited outside the sporangium on the surface not structurally part of the peridial wall. In *L. metallica*, the debris forms a mound at the base and apex (Wrigley de Basanta et al. 2017) and in *L. pseudoconica* debris forms a false cone of debris apically that can be removed exposing the sporangial wall and spores underneath (Keller & Brooks 1977). In *L. iridescens* the dark apical portion of the sporangium is structurally part of the peridial wall (Figs. 7A, B; 8A, B; 9A, B).

RESULTS AND DISCUSSION

Four *Licea* species that appear remotely similar morphologically to *L. iridescens* are compared in Tables 1–3. Some morphological details cannot be seen with LM, especially the inner surface markings of the peridium and spore surface ornamentation. *Licea iridescens* differs from all other *Licea* species in the combination of peridial surface features (Table 1). When habit and spore characters are taken together *L. iridescens* is distinct and is easily separated from the other *Licea* species (Tables 1–3).

Licea iridis and *L. scintillans* are both iridescent as their name implies (Table 1). The former species compared to *L. iridescens* have slightly larger sporangia but differ in spore characters: "transmitted light dark smoky-grey, uniformly coloured and prominently and evenly warted" (Ing & McHugh 1988; Table 2). *Licea scintillans* is slightly larger than *L. iridescens* but also differs: "transmitted light, pale ochraceous, 10–10.5 µm diameter, smooth, thick-walled and with a conspicuous germinating pore occupying 25–33% of the circumference" (Mitchell & McHugh 2000; Table 2). Unfortunately, there is only a line drawing illustration of the short-stalked habit of *L. scintillans* and no other sporangial structures are illustrated (Table 2).

Licea metallica appears most similar to *L. iridescens* morphologically in the spores. Spores of both species are in the same size range and the spore characters for both species have thinner, paler walls. The thinner spore wall of *L. iridescens* is membrane-like and the thicker portion of the wall is 1 µm or more in thickness which accounts for the darker spore wall (Figs. 6E–H). Differences in *L. iridescens* spore wall thickness result in the spores collapsing into a coffee-bean shape (Figs. 6I, J).

Fresh microscope slide mounts of dried *L. iridescens* spores mounted in clear lactophenol are collapsed and enfolded into a coffee-bean shape. It takes 2–7 days for spores of *L. iridescens* to swell into more globose shapes. Collapsed spore diameters measure approximately 2–3 µm larger (15–17 µm) than the nearly globose spores (L (12)–14–(16) × W (10)–12–(14) µm), (Figs. 6E–H). Thus, for this type of spore with variable wall thickness, measurements should be made after spores have assumed a more globose shape in order to avoid introducing sources of measurement errors. This perhaps accounts for spores of variable sizes based on wall thickness. Another possible source of spore measurement error is "giant spores" that form from incomplete protoplasmic cleavage during spore development, but it is obvious that these "aberrant spores" should not be measured (HWK, pers. obs.).

Dark-spored versus light-spored Liceas in mass.—Traditional myxomycete classification recognized five orders divided into three bright-spored orders (Echinosteliales, Liceales, and Trichiales) and two dark-spored orders (Physarales and Stemonitales), (Martin & Alexopoulos 1969). These color differences emphasized the lighter, brighter colors represented by the whites, greys, reds, and yellows and the darker colors represented by the browns and blacks as seen in the mature fruiting bodies. The family Liceaceae, as circumscribed by Martin

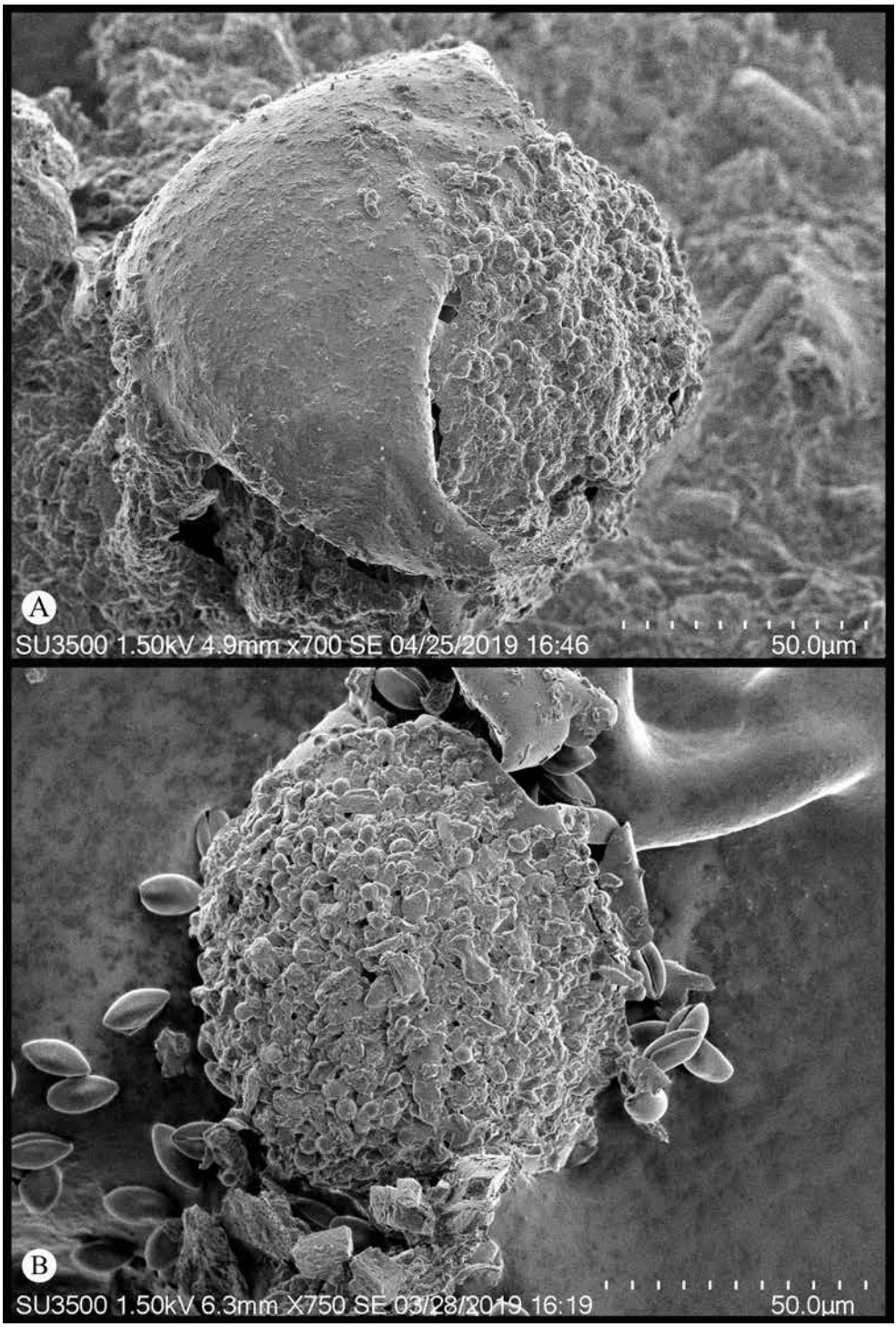

Fig. 8 A, B. *Licea iridescens*, SEM. A. Sessile sporangium showing centered apical debris with sharp marginal edge and smooth sides. **B.** Top portion of sporangium showing apical debris with a mixture of globular and irregular matter. Note cluster of crystals at bottom surrounded by collapsed spores.

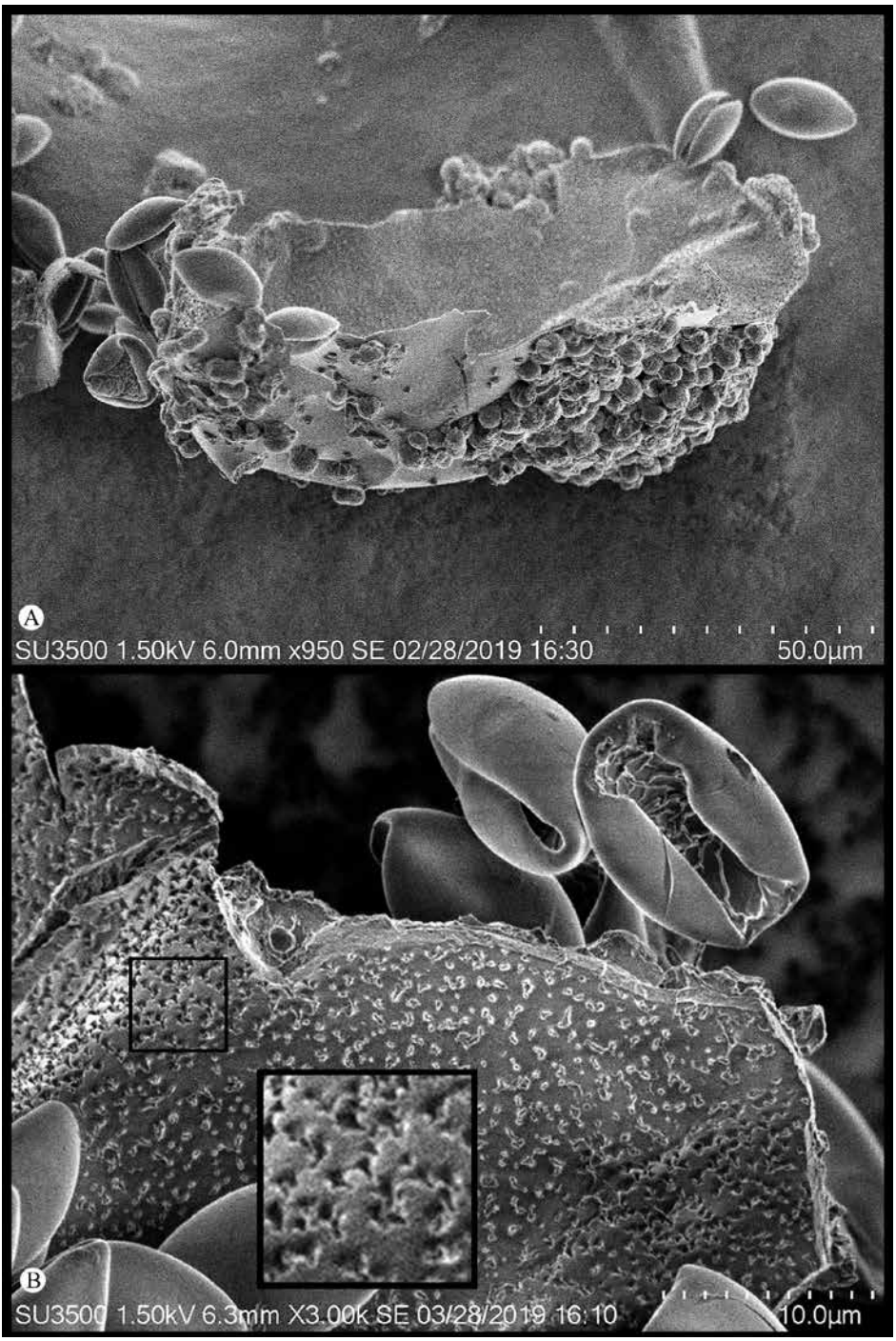

Fig. 9 A, B. *Licea iridescens*, SEM. A. Top portion of sporangium upside down showing globular debris and roughened inner peridial surface. Collapsed spore at left side slightly open exposing reticulate meshwork on thinner wall surface. B. Portion of peridium showing inner surface ornamentation of irregular markings and distinct, upright, conical projections in profile (see inset from left side). Spores with smooth outer surfaces and ridged-reticulate meshwork on collapsed thin wall of spore (upper right).

Table 1. Sporangial habit seen from directed light source.

Species Name	Iridescence/Color	Habit/Size/Stalked/Sessile	Peridium/Dehiscence/Outer Surface	Spore Mass Color
L. iridescens	yes, sides bluish, reddish, pinkish sparkles	globose, sessile mostly short stalked, average overall height, 120 µm, sides wrinkled, smooth, iridescent	apical dark black patch of globular debris, convex or concave, part of outer wall, irregular	reddish-black, dark
L. iridis	yes, interference patterns, no	globose, sessile, overall height 200–300 µm, bronze-brown	two membranous layers, irregular	very dark
L. metallica	yes, sides bluish	subglobose, sessile, up to 240 µm overall height, black, shiny	refuse matter yellow-orange white at top and bottom, separate from outer wall, irregular	reddish-brown, dark
L. pseudoconica	yes, no	globose, sessile, 70–120 µm in total height, brown-black	conical false mass of pale debris separate from outer wall, irregular	dark brown to almost black
L. scintillans	yes, strongly, no	globose, sessile, short-stalked, 180–220 µm in diameter	single, membranous wall, hyaline	reddish-brown, dark

Table 2. Sporangial structures seen by light microscope transmitted light 430×–1000×.

Species Name	Peridium Inner Surface	Spore Color	Spore Diameter Size	Spore Ornamentation	Spore Optical Section
L. iridescens	smooth, single layer	reddish-brown	L (12)–14–(16) µm × W (10) –12–(14) µm	smooth	thicker wall half darker, smooth, thinner wall half, colorless, smooth
L. iridis	no obvious markings	dark smoky-gray	10–12 µm	prominently and evenly warted	uniformly colored
L. metallica	membranous, hyaline, single layer	reddish-brown	12–14 µm	smooth	thicker wall half smooth, thinner wall half wrinkled
L. pseudoconica	single, membranous, minutely and closely papillose	olivaceous-brown	9.5–11–(13) µm	smooth	pale thinner area on one side
L. scintillans	single, membranous, hyaline, smooth	pale ochraceous	10–10.5 µm	smooth	thicker wall ⅔ with a thinner wall ⅓

Table 3. Sporangial structures seen with scanning electron microscope.

Species Name	Peridium Outer Surface	Peridium Inner Surface	Spore Ornamentation Outer Surface
L. iridescens	globular debris on top, smooth and wrinkled on sides	roughened with conical markings	smooth thicker wall half, thinner wall half ridged-reticulate with partial meshwork, collapsed, enfolded, coffee-bean shape
L. iridis	none	none	none
L. metallica	none	irregular shaped warts	smooth surface half, wrinkled half
L. pseudoconica	none	none	none
L. scintillans	none	none	none

and Alexopoulos (1969), noted spore color in the genus *Licea* as "spores mostly dingy to black in mass, then smoky by transmitted light, sometimes bright-colored in mass, then tinted yellow or ochraceous by transmitted light." This quotation suggests there were more dark-spored Liceas than bright-spored Liceas at that point in time.

Recent molecular data analysis by Fiore-Donno et al. (2013) proposes a monophyletic *Licea* tree based on three *Licea* bright-spored species, *L. castanea* G. Lister, *L. marginata* Nann.-Brem, and *L. parasitica* (Zukal) Martin that form a separate clade of pale light-spored species. In contrast, there are black or reddish black-spored *Licea* species that suggest the *Licea* genus may be polyphyletic. Indeed, Eliasson (2015) and Dmitry et

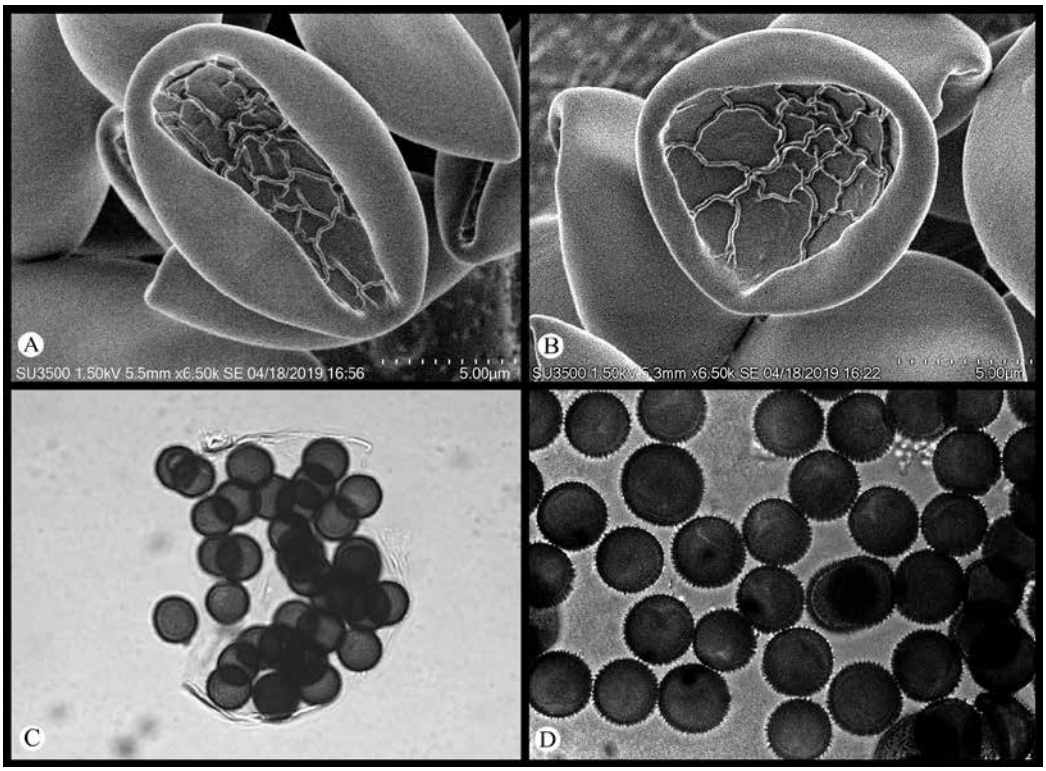

Fig. 10 A, B. *Licea iridescens*, SEMs. A. Ridged-reticulate meshwork on collapsed thin wall spore surface. B. Irregular reticulate meshwork slightly raised in side view. C. Dark (black)-spored new *Licea* species surrounded by transparent peridium in LM transmitted light. D. Spiny black spores from Figure C in optical section with transmitted light (LM) at approximately 2000×. Note spores are spherical due to uniform wall thickness.

al. (2019), propose that the genus *Licea* is polyphyletic, the former highlighting the light-spored Liceas associated with the genus *Perichaena* and the latter the dark-spored Liceas with platelets representing a distinct group. Another *Licea* that represents a species new to science obtained from ONP American elm tree #8 had black spores with extremely long spines and a uniformly thick spore wall lacking a pale thin walled area surrounded by a transparent peridium (Fig. 10C), the spores appearing black with LM transmitted light (Fig. 10D). More dark-spored *Licea* species must be included in any phylogenomics study of the genus, and many more species of light-spored Liceas, before a more natural classification is possible based on solid molecular evolutionary evidence.

CONCLUSIONS

Current trends in myxomycete research and taxonomy will result in the discovery of many more new species of myxomycetes, especially the exploration of understudied geographical areas, for example, in Southeast Asia. Additional habitats that have been more productive, and are still relatively unexplored, in deserts and semi-arid environments. Discovery of microhabitats on the bark of living trees and woody vines extending into the canopy has produced new taxa. Myxomycete species with microscopic fruiting bodies in the genus *Licea* that go unnoticed in moist chamber cultures, especially on the bark of living trees and woody vines, will undoubtedly be another source of new species. Cryptic species that are buried in molecular data eventually will be detected and described. SEMs with higher magnifications will provide fine structural details not seen or even possible with LM, especially for the inner surface of the peridium and ornamentation on external spore

surfaces. The next generation of creative taxonomic students of myxomycetes will discover new places to search and collect where others have not gone before.

There will always be taxonomic puzzles that will defy evolutionary pathways and confound even the long-term myxomycete taxonomists. *Trichioides iridescens* Novozh. Hoof & Jagers, (Novozhilov et al. 2015) will challenge older and newer monographic publications and test molecular evidence posed by a combination of morphological characters possibly represented by three different myxomycete orders, Liceales, Physarales, and Trichiales. (pers. obs., HWK).

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