

Calonema foliicola a new myxomycete from Mexico

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Abstract: A new species of myxomycete, *Calonema foliicola* Estrada, J. M. Ramírez & Lado, recorded in the Mexican states of Chihuahua, Hidalgo and Tlaxcala is described. The most relevant characters of this species are the scattered, minute and stalked sporocarps, the red color of the sporotheca and the capillitium, with a faint and irregular reticulum.

Key words: leaf litter, morphology, *Quercus*, taxonomy, Trichiales

INTRODUCTION

During the course of a survey of the myxobiota of Mexico, we found sporocarps of an undescribed species in the genus *Calonema* that had developed on decayed oak (*Quercus* spp.) leaves. Additional collections were obtained from moist chamber cultures, which confirmed the stability of the characters of the new species.

MATERIALS AND METHODS

Specimens were collected directly in the field from two places in central Mexico (Hidalgo and Tlaxcala) and another two in northern Mexico (Chihuahua), on dead oak (*Quercus rugosa*) leaves. All the collections are deposited in the herbaria TLXM or MA-Fungi, with duplicate of the type collection at BPI.

In addition, 150 moist-chamber cultures were prepared in three sets, consisting of 50 plastic Petri dishes (90 × 15 mm). One filter paper disk and then a circular fragment of a dead oak leaf approximately 5 cm diam were placed in each dish. Enough sterile water was added to moisten the contents thoroughly, and the excess water was poured off

after a day. Cultures were maintained for approximately two months.

Agar cultures were prepared with 2% water oatmeal agar in sterile glass Petri dishes (90 × 20 mm), and one sporocarp was washed with 0.5 mL of a 0.1% solution of Tween 80, rinsed with sterile water, crushed and the spores spread on the agar surface (Kalyanasundaram and Venkataramani 1974). These cultures were maintained approximately a month.

Macroscopic measurements were taken from at least 170 mature field-collected sporocarps and from moist chambers. Microscopic measurements and observations were made with material mounted directly in Hoyer's medium. At least 30 spores, stalk cysts or capillitial threads from each collection were measured. The ratio of height/width of the sporothecae and the average values for each set of measurements were calculated for a more accurate description of this taxon. In the description, we included the minimum, average and maximum measurements.

The description was based on differential interference microscopy and scanning electron microscopy (SEM). All SEM preparations were critical-point dried. Species description follows the terminology of Lado and Pando (1997). Color notations in parentheses are from the Munsell Soil Color Charts (Anonymous 1992). Myxomycete names, follow the nomenclature compiled by Lado (2001).

TAXONOMY

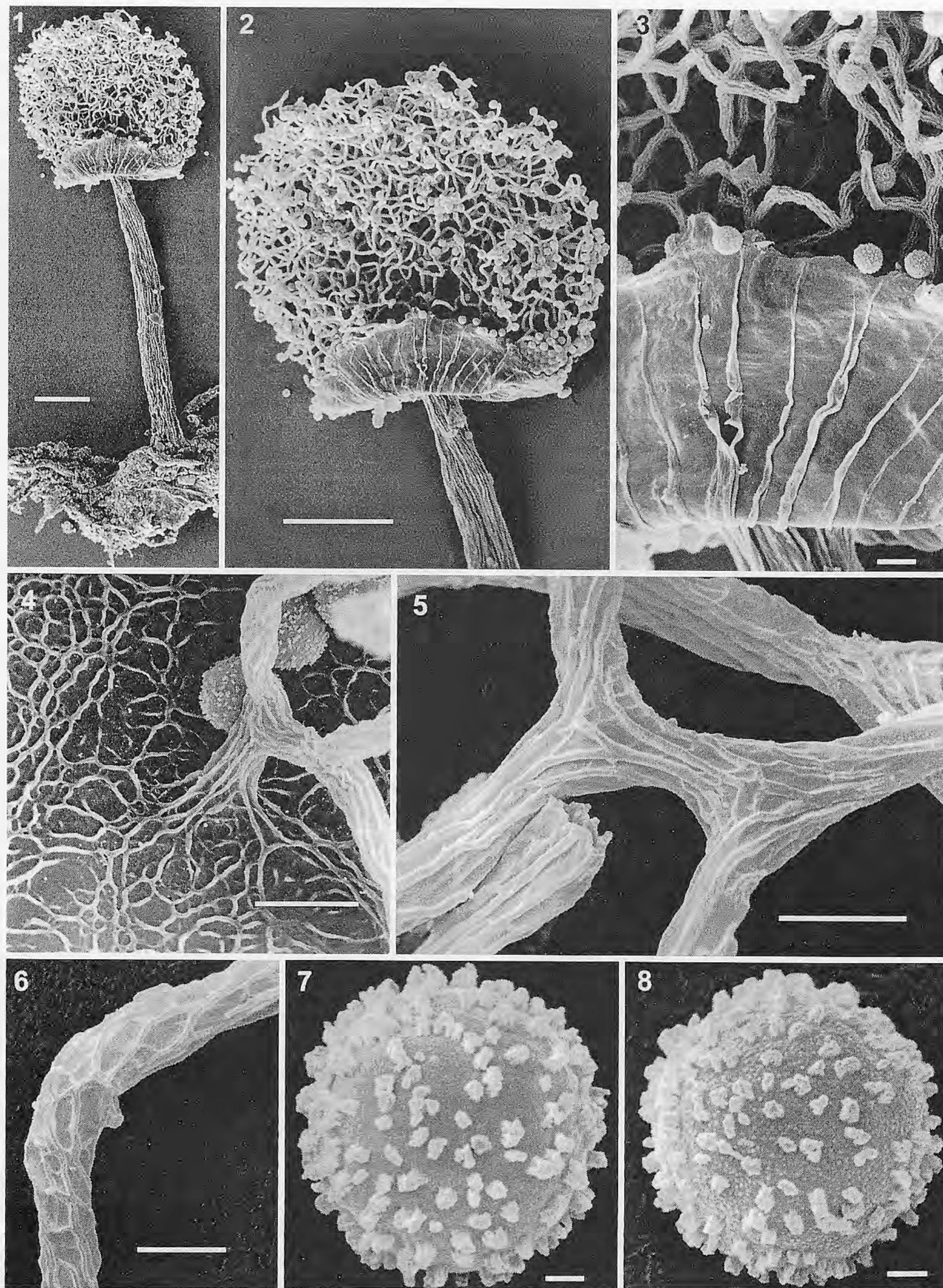
Calonema foliicola Estrada, J. M. Ramírez & Lado,
sp. nov. FIGS. 1–8

Sporocarpi sparsi, stipitati atque toti 0.4–1.4 mm alti, rubri. Sporothecae subglobosae (0.3–0.6 mm diam) aut late ellipticae (0.3–0.9 × 0.2–0.7 mm). Peridium evanidum superne, basi vero instar calyculi perstans. Capillitium elasticum, tubulare, tubulis quidem efformatum ramosis atque anastomosantibus (intrincatis), lineolis laevibus, tenuibus, crebro subparallelis percursis, quae item ramosae atque anastomosantes plerumque apparent. Sporae subglobosae, 7.9–9.5 µm diam, verrucosae.

Sporocarps scattered, stalked, 0.5–(1.0)–1.4 mm in total height (FIG. 1). Sporotheca subglobose, 0.3–0.6 mm diam, to ovoid or broadly elliptical, 0.3–(0.5)–0.9 × 0.2–(0.4)–0.7 mm, occasionally slightly elongated, sporotheca height and width ratio 0.82–(1.13)–1.71, red (2.5YR4–5/8) to light red (2.5YR6/6–8) occasionally yellowish-red (5YR5/6–8) to reddish-brown (5YR5/4) or pinkish (5YR7/4), fading to yellowish (10YR7/6) when old. Hypothallus ranging from colorless to dark reddish-brown (2.5YR3/4),

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FIGS. 1-8. *Calonema foliicola* (SEM). FIGS. 1-3, 7 (MA-Fungi 50720, ISOTYPE), FIGS. 4-6, 8 (MA-Fungi 50721). 1. Sporocarp. 2. Sporotheca showing the capillitium and calyculus. 3. Detail of calyculus, showing the radial folds. 4. Inner surface of the peridium, with a capillitial thread attached to it and showing the same type of ornamentation. 5. Capillitium with subparallel and reticulate bands. 6. Capillitium with reticulate bands. 7-8. Spores ornamented with bacula and small crestae. Scale bars: 1-2 = 100 μm , 3-4 = 10 μm , 5-6 = 5 μm , 7-8 = 1 μm .

bright, yellowish red (5YR5.5/8) by transmitted light, membranous, discoid. Stipe cylindrical (FIG. 1), slightly expanded toward the base, erect, 0.2–(0.5)–0.8 × 0.04–(0.07)–0.14 mm, minutely striate (FIG. 2), translucent, dark reddish brown (2.5YR2.5–3/4) to dusky red (2.5YR3/2) or red (2.5YR4/6–8), occasionally brown to yellow (7.5YR5–6/6; 10YR4/6; 10YR7–8/6), yellow (2.5 Y8/6–8) by transmitted light, yellow (10YR8/8) to light red (2.5YR6/8) toward the base, filled with subglobose cysts 7.9–(11.8)–19.7 µm diam. Peridium single, membranous, partially evanescent, remaining as a basal, shallow calyculus (FIG. 2), translucent, radially plicate (FIGS. 2–3), pale yellow (2.5Y8/4) to yellow (2.5Y8/8) by transmitted light, the inner surface ornamented with 0.5 µm broad veins, forming a reticulum of isodiametric or elongated mesh, 0.5–5.0 µm diam by SEM (FIG. 4), occasionally remaining as small fragments attached to the top of the sporotheca; dehiscence irregular and slightly circumscissile. Columella absent. Capillitium of tubular threads, elastic, slightly or not expanded, pale yellow (2.5Y8/3) to yellow (2.5Y8/8) by transmitted light; threads 2.4–(3.4)–4.7 µm diam, flexuous, branched and anastomosed, with few free ends, entangled, attached to the calyculus, decorated with smooth, subparallel, faint, irregular veins, usually branched and anastomosed in a reticulate pattern (FIGS. 5–6), occasionally resembling spirals or half rings (only seen in ET-5187); by SEM this reticulate venation is continuous with the peridial inner surface at the base of the sporotheca (FIG. 4). Spores free, reddish (2.5YR5/8, 2.5YR6/8, 5YR6/6) in mass, pale yellow (2.5Y8/3) to very pale brown (10YR8/3) by transmitted light, subglobose, 8.0–(9.0)–10.5 µm diam, warted, the warts dispersed and irregularly distributed; baculate by SEM, the bacula up to 0.6 µm height, sometimes fused and forming small crestae (FIGS. 7–8), occasionally with small warts, up to 0.15 µm height between the bacula. Plasmodium not observed.

HOLOTYPE. MEXICO. TLAXCALA: Municipality of Tlaxco, El Rosario, Cerro El Peñón, 19°41'30" N, 98°14'00" W, 2800 m, on the lower surface of dead leaves of *Quercus rugosa*, 17 Nov 1998, A. Estrada-Torres 5187 (TLXM; ISOTYPE MA-Fungi 50720, and BPI)

Specimens examined. MEXICO. CHIHUAHUA: Municipality of Bocoyna, El Pinabetal, 27°45'38" N, 107°41'41" W, 2360–2380 m, on the lower surface of dead leaves of *Quercus rugosa*, 30 Jul 2001, A. Estrada-Torres, C. Lado & M. Ramírez-Ortega 8146 (TLXM, duplicate at MA-Fungi 50729), 8147 (TLXM), 8159 (TLXM, duplicate at MA-Fungi 50730), 8160 (TLXM). Ibidem, 15 Aug 2001, A. Estrada-Torres & M. Ramírez-Ortega 8186 (TLXM), 8190 (TLXM), 8192 (TLXM), 8194 (TLXM). Ejido de San Ignacio Ara-

reko, Cañón de la Pista de Aviación, 27°42'20" N, 107°37'03" W, 2382 m, on dead leaves of *Quercus rugosa* and *Quercus sideroxyla*, 20 Aug 2001, A. Estrada-Torres & M. Ramírez-Ortega 8282 (TLXM), 8286 (TLXM), 8290 (TLXM). HIDALGO. municipality of Zacualtipan, km 91.5 road Pachuca-Tampico, 20°36'56" N, 98°37'20" W, on the lower surface of dead leaves of *Quercus rugosa*, 28 Nov 1998, A. Velasco Ramírez 499 (TLXM). TLAXCALA: Tlaxco, El Rosario, Cerro El Peñón, 19°41'30" N, 98°14'00" W, 2800 m, on the lower surface of dead leaves of *Quercus rugosa*, 20 Aug 1998, A. Estrada-Torres 4532 (TLXM), 4546 (TLXM). Ibidem, 13 Nov 1998, A. Estrada-Torres 5117 (TLXM). Ibidem, 17 Nov 1998, A. Estrada-Torres 5155 (TLXM), 5178 (TLXM, duplicate at MA-Fungi 50721). Specimens from moist chamber culture: Ibidem, collected on 16 Apr 2000, obtained on 12 Jul 2000, A. Estrada-Torres 6338. Ibidem, obtained on 24 Jul 2000, A. Estrada-Torres 6354. Ibidem, harvested on 3 Aug 2000, A. Estrada-Torres 6360. Ibidem, collected on 20 Aug 2000, harvested on 18 Sep 2000, A. Estrada-Torres 6375. Ibidem, obtained on 25 Sep 2000, A. Estrada-Torres 6484. Ibidem, harvested on 26 Oct 2000, A. Estrada-Torres 7343. Ibidem, harvested on 11 Dec 2000, A. Estrada-Torres 7405.

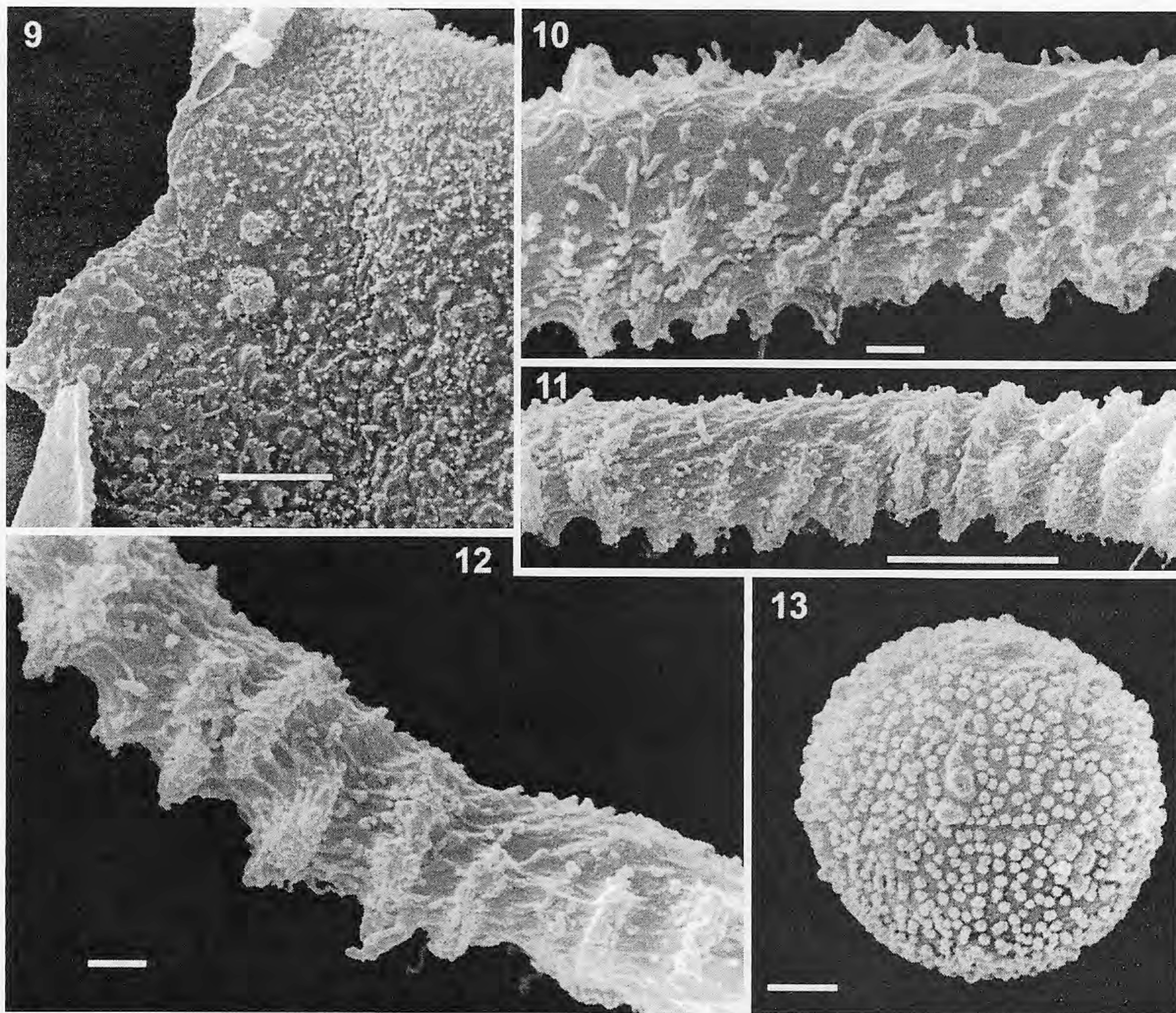
Etymology. Named after the substrata upon which it occurs.

Known distribution. Mexico

Habitat. On the lower surface of dead leaves of *Quercus* spp., in conifer-oak forests.

Commentary. *Calonema foliicola* is characterized by its capillitium of tubular threads with subparallel veins, branched and anastomosed in a reticulate pattern continuous with the peridial inner surface at the base of the sporotheca, the character that includes it in the genus *Calonema*. In Morgan's description of the genus the capillitium is described as "of slender tubules, arising from the base of the sporangium" (Morgan, 1893) and more recently Rammeloo (1984) comments on the reticulate-venose ornamentation on parts of the capillitium as a very typical feature of *Calonema aureum* Morgan, the type species of the genus. *Calonema foliicola* also is characterized by small stipitate sporocarps with a scattered habit, reddish color and warted spores, which distinguish it from the other species of the genus. The stipitate habit and the reddish color of the sporocarps extend the generic concept of *Calonema*.

This species differs from *C. aureum* Morgan, *C. cornuvioides* Chassain & Nann.-Bremek. and *C. geesinskii* Nann.-Bremek. because these species are sessile, have crowded sporocarps, are yellow and have reticulate spores (Martin and Alexopoulos 1969, Chassain and Nannenga-Bremekamp 1982, Rammeloo 1984, Nannenga-Bremekamp 1985). In addition, the capillitium in both *C. cornuvioides* and *C. geesinskii* has characteristic rings, well defined as viewed under the light microscope, as well as by SEM (Chassain and



FIGS. 9–13. *Arcyria stipata* (VR-474) (SEM). 1. Inner surface of the peridium. 10–12. Capillitium with helicoidal crest poorly developed, more prominent on one side, and small warts. 13. Spore ornamented with small warts and groups of larger coalescent ones. Scale bars: 9, 11 = 5 μm , 10, 12–13 = 1 μm .

Nannenga-Bremekamp 1982, Rammeloo 1983, Nannenga-Bremekamp 1985), that are not observed in *C. foliicola*. The presence of these rings is not a diagnostic character of the genus, because they are absent from the type species, *C. aureum*, which Rammeloo (1984) illustrated with SEM micrographs.

Calonema dissipatum Nann.-Bremek., R.K. Chopra & T.N. Lakh., is another stipitate species described in this genus that has a scattered habit without reticulate spores. This species, however, has yellow sporocarps with an irregular dehiscence, spores with a border in optical section, the capillitium is free of the calyculus and has thickened areas 6–20 μm in extent, numerous free ends, and is ornamented with warts, spines and scattered teeth (Chopra et al 1992).

The macroscopic view of *C. foliicola* is reminiscent of some species of *Arcyria*. Because of the color of the sporocarps and the fact that the ornamentation of the capillitial threads occasionally resembles spi-

erals, it could be confused with scattered stipitate forms of *A. stipata* (Schwein.) Lister, but the capillitium of the latter forms an elastic network of freely branching threads marked with close-set blunt-ended transverse bands and numerous spinules (Lister 1925), while none of these characters has been observed in *C. foliicola*. In addition, the spores of *A. stipata* have groups of warts on a finely punctate or practically smooth surface, whereas in *C. foliicola*, the spore ornamentation is evenly warted. The differences between the two species are more apparent when viewed by SEM. In *A. stipata*, poorly developed helicoidal crests can be seen in the capillitial threads and are usually more prominent on one side (FIGS. 11–12). These crests sometimes are somewhat reticulate, as result of being beset over the entire surface with small and regular warts (Rammeloo 1986) (FIG. 10). In *C. foliicola* only the subparallel veins frequently forming a reticulum and occasionally resembling spi-

rals can be observed (FIGS. 5–6). In addition, the surface of the spores of *A. stipata* has small warts, with groups of larger coalescent ones (Rammeloo 1986) (FIG. 13), but in *C. foliicola* there are sparsely and evenly distributed bacula of uniform size (FIGS. 7–8). The inner surface of the calyculus also shows some differences. In *A. stipata*, it is subreticulate with warts or spines (Rammeloo 1986) or densely papillate (FIG. 9), while in *C. foliicola* it is completely reticulate and continuous with the ornamentation of the capillitial threads and it lacks warts or spines (FIG. 4).

Other species of small *Arcyria* with reddish tones in the sporocarp and the capillitium adhering to the calyculus, such as *A. helvetica* (Meyl.) H. Neubert, Nowotny & K. Baumann, *A. denudata* (L.) Wettst. and *A. minuta* Buchet, can be distinguished easily from *C. foliicola* by the characteristics of the capillitium. The first two have a capillitium ornamented with cogs or half-rings (Martin and Alexopoulos 1969, Neubert et al 1989), and *A. minuta* has uniformly arranged spines (Martin and Alexopoulos 1969); in the capillitium of the new species, none of these elements (cogs, half rings or spines) are present.

Calonema foliicola does not have a birrefringent capillitium. Nannenga-Bremekamp (1982) emphasized the importance of this character in delimiting the families and genera of the order Trichiales, but Lado and Pando (1997) discussed the doubtful taxonomic value of it because many exceptions have been found in such genera as *Arcyria* (e.g., *A. major*, *A. ferruginea* and *A. versicolor*) and *Hemitrichia* [= *Hyporhamma* (Lado 2001)], for example *H. abietina* and *H. imperialis*.

The microhabitat in which *C. foliicola* develops—the top layers of semidecomposed oak-leaf litter—also could cause this species to be confused with old, immature or decolored forms of *Arcyria cinerea* (Bull.) Pers. or *A. afroalpina* Rammeloo, but both differ in the ornamentation of the capillitial threads, which comprise warts instead of a reticulum of veins (Martin and Alexopoulos 1969, Rammeloo 1981).

The branched and anastomosing capillitial network with few free ends, the capillitial ornamentation that occasionally resembles spirals and the similarity in habit could lead to confusion with some species of *Hemitrichia*, such as *H. leiocarpa* (Cooke) Lister or *H. pseudoleiocarpa* Illana, G. Moreno, Lizárraga & A. Castillo (Lister 1925, Lado and Pando 1997, Illana et al 1999). However, the capillitial threads with reticulate-venose ornamentation, continuous with the peridial inner surface at the base of the sporotheca, and the absence of true spiral bands, separate it from this genus.

In moist-chamber culture, *C. foliicola* required 8–18 days to develop. The number of sporocarps was

variable from one leaf to another, forming from 5–38 sporocarps per moist chamber. A succession of three harvests of sporocarps was obtained over a period of 23 days. The frequency of appearance of *C. foliicola* in the series of cultures was only 2–6%, or 1–3 fruitings in each set of 50 leaf fragments cultured. Therefore, it seems to be a somewhat rare species, and this was corroborated in the field, where most of the fruiting of 6–40 sporocarps collected was dispersed over a few leaves. Plasmodia were not observed in any of the moist chambers in spite of daily observations. The sporocarps appeared in fewer than 24 hours, without evidence of a plasmodium, which suggests that the latter must be very small and probably hyaline to escape notice against the complex texture of the downy underside of the leaf.

Spores of collections, obtained from the field or from moist-chamber cultures, were sown on 20 plates of 2% water-oatmeal agar, but all of these spore-culture attempts failed.

The most immature sporocarps observed in moist-chamber cultures were white and already had well-defined stalks. At this stage, fragmentation of the protoplasm to form the spores and capillitium was evident, and the immature sporotheca was delimited by a fine membrane that widened in the basal portion of the sporotheca to later form the calyculus. The spores darkened gradually as they matured from reddish brown (2.5YR5–6/3–4) to red (10R4/6), finally turning reddish brown to light red (2.5YR4–6/6–8). If the sporocarps dried before maturing, they remained reddish brown (2.5YR5/3) or light reddish brown (2.5YR6/4) and never acquired the characteristic red color. Each plasmodium appears to give rise to a single sporocarp, because in every case the latter developed in isolation, with a discoid hypothallus and in different developmental stages. The type of sporocarp ontogeny observed is defined as subhypothallic (Alexopoulos 1973), myxogastroid (Ross 1973), or nonstemonitoid (Mims 1973), because a continuous membrane covers the whole fructification.

In the moist chambers, *C. foliicola* developed alongside species of *Physarum*, *Didymium* and *Trichia*, and in field collections the accompanying species were from the genera *Craterium*, *Diderma*, *Didymium* and *Physarum*.

Calonema foliicola has been collected only in two localities in Hidalgo (Sierra Madre Oriental) and Tlaxcala (Neovolcanic Axis), in central Mexico, and in another two in Chihuahua (Sierra Madre Occidental), in northern Mexico, about 1200 km apart. In most cases, *C. foliicola* appeared on the lower leaf surface of the dead leaves of *Quercus rugosa* and never on the upper leaf surface, and this was true in the positive moist-chamber cultures. When it appeared

on dead leaves of *Q. sideroxyla*, these were mixed with leaves of *Q. rugosa*. The latter are strongly concave, leathery, with lustrous and sparsely pubescent, upper leaf surfaces, and densely tomentose, becoming nearly glabrate or pubescent persistent lower leaf surfaces (Nixon 1997). These structural characteristics could create a favorable microhabitat for spore germination and development of species of small myxomycetes such as *C. foliicola*. The leathery texture of the leaf adaxial surface would allow the water to run off and dry the surface quickly, while the downy concave abaxial surface would retain water. It is possible that *C. foliicola* is a species particularly adapted to this microenvironment and that its distribution corresponds to the areas where *Q. rugosa* grows—from high wooded slopes in Texas, New Mexico and Arizona, throughout most of the mesic montane parts of Mexico, to southern Guatemala (Nixon 1997) and probably to other places where *Quercus* species with similar leaf structure occur. However, its size and dispersed habitat have caused it to be overlooked until now.

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