DESCRIPTION AND CULTURE OF A NEW MYXOMYCETE,
LICEA SUCCULENTICOLA

by
JUAN MOSQUERA¹, CARLOS LADO², ARTURO ESTRADA-TORRES³,
ESPERANZA BELTRÁN TEJERA⁴ & DIANA WRIGLEY DE BASANTA⁵

¹ Department of Medicine, University of Manchester, CSB, Hope Hospital, Salford M6 8HD, UK
² Real Jardín Botánico, CSIC. Plaza de Murillo, 2. E-28014 Madrid
³ Centro de Investigación en Ciencias Biológicas, Universidad Autónoma de Tlaxcala.
Ctra. Texmelucan-Tlaxcala, km 10.5. Ixtacuixtla. 90122 Tlaxcala (México)
⁴ Departamento de Biología Vegetal (Botánica), Universidad de La Laguna.
E-38071 La Laguna
⁵ American School of Madrid. Apartado 80. E-28080 Madrid

Resumen

Se describe una nueva especie de Myxomycetes, perteneciente al género Licea, que se desarrolla en plantas suculentas. Los materiales proceden de España (Islas Canarias), México y Estados Unidos. Se parece a L. biforis pero difiere en sus pequeños esporóforos y en sus esporas mayores, amarillo-naranjas, con una gruesa pared que gradualmente hacia un lado se adelgaza y se vuelve más pálida. La estabilidad de estos caracteres ha quedado demostrada por el cultivo de espora a espora en laboratorio.


Abstract

A new succulenticolous myxomycete of the genus Licea is described based on material from Spain (the Canary Islands), Mexico and the USA. It is very similar to L. biforis but differs in its smaller sporophores, and its bigger orange-yellow spores. The spores have a thick wall that is thinner and lighter towards one side. These differentiating characters were stable in spore to spore culture on agar.

Key words: Canary Islands, Licea, life cycle, ecology, Mexico. Spain, succulenticolous myxomycetes, taxonomy. USA.
**INTRODUCTION**

*Licea* (Liceales, Myxomycetes) is a genus described in 1797 by Schrader in *Nova Genera Plantarum*, and currently includes more than 65 species (LADO, 2001) from all around the world. More than 80 % of the recognized species have been described in the last three decades, after KELLER & BROOKS (1977) made popular the use of the moist chamber technique to study corticolous species. The minute size of the sporophores (about 0.01-0.5 mm) and the fact that they predominate mainly on the bark of living trees meant that they were easily overlooked. Because of the number of species described recently, and the simplicity of the sporophores, the systematics of the genus has become very controversial, and the limits of the genus with others of different orders, like *Perichaena* (Trichiales), are not clear (GILERT, 1994).

For more than four years, we have been studying the succulenticolous myxomycetes associated with decaying plant debris in semi-arid lands and deserts in the Canary Islands and Mexico (LADO & al., 1999; MOSQUERA & al., 1999, 2000a, 2000b). During these surveys we have abundantly collected a *Licea* that was initially identified as *L. biforis* but that presents some differences, mainly in the spores. When we compared our material with the type of *L. biforis*, we confirmed the existence of consistent differences. We have also been able to complete its life cycle from spore to spore in the laboratory, verifying the genetic stability of those distinguishing features. Thus we describe it here as a new species.

**MATERIALS AND METHODS**

This study is partially based on material collected directly in the field on different decaying succulent plants (*Aeonium, Agave* and *Opuntia*) from Spain (the Canary Islands), and from Mexico; other samples were obtained in moist chamber cultures of different succulent substrata (*Agave, Austrocylindropuntia, Euphorbia, Opuntia, Myrtillocactus, Nolina, Stenocereus* and *Yucca*), and others from spore to spore cultures on agar.

Moist chamber cultures, were prepared using plastic Petri dishes (90 × 15 mm) fitted with one filter paper disc and little pieces of the substrata. Enough sterile distilled water was added to moisten the contents thoroughly, and the surplus water was removed after one day. Agar cultures were prepared with 2 % oatmeal agar in sterile plastic Petri dishes (90 × 15 mm). Two sporocarps were crushed, and the spores spread on the agar surface and inoculated with a thin layer of distilled water. Plates were incubated in the dark at room temperature and checked daily.

The material studied has been deposited in the herbaria TFC Mic., MA-Fungi, TLXM and the private collection of D. Wrigley de Basanta (dwb). All microscopical measurements were made with material directly mounted in Hoyer's medium. For descriptive data and micrographs we used a microscope with Differential Interference-Contrast. For all SEM-pictures the critical-point technique was employed. Color notations in parentheses are from the ISCC-NBS Color-Name Charts Illustrated with Centroid Colors (Anonymous, 1976).

**RESULTS**

*Licea succulenticola* Mosquera, Lado, Estrada-Torres & Beltrán-Tej., sp. nov. (figs. 1-10)

*Speciei Licea biforis Morgan proxima, differens vero sporophoris minoribus –sporocarpicis 40-110 um diam., plasmodiocarpicis autem 40-100 × 55-170 um– et sports maioribus (11-14.5 × 12-17 um) atque pariete quoad partem incrassatis, sed tenuioribus et pallidioribus quoad aliam partem.*


Sporophores sporocarpic to plasmodiocarpic, dispersed or grouped, sessile, strong yellowish brown (74. s. y Br) to deep yellow-
Figs. 1-8.—_Licea succulenticola_. 1-2. Sporophores (TFC-Mic 8225), the lighter longitudinal area is visible (arrows), on the left side of fig. 2 an open sporophore is visible; 3. Closed sporophore (SEM) (MA-Fungi 47346), longitudinal area free of refuse matter can be clearly seen. Inner membrane holds together the two halves of the peridium; 4. Dehisced sporophore (SEM) (MA-Fungi 47347), dehiscence happens along the inner membrane which remains as torn fragments. On the inner surface of the top membrane verrucae can be seen; 5. Inner view of peridium (SEM) (MA-Fungi 47346), depressions formed by impression of the spores are visible; 6. Verrucae of the inner edge of the membrane (SEM) (MA-Fungi 47346); 7. Spores seen by LM (TFC-Mic 8225); 8. Spore (SEM) (MA-Fungi 47347), densely and minutely warded.
ish brown (75. deep y Br). Sporocarps subglobose, (40)65-170 µm diam. Plasmodiocarps ellipsoid, (37)47-150(200) x (55)75-260(400) µm, somewhat laterally compressed. Hypothallus inconspicuous. Peridium double, without peridial platelets, light yellow (86. 1. Y) by LM. Inner part persistent, membranous, thin. Outer part gelatinous when moist. composed of granular and occasionally some crystalline material, lacking in an upper longitudinal, lighter area which is occasionally forked; inner peridial surface densely punctate, with dispersed verrucae on the inner side of the lighter longitudinal area; by LM granules can be noticed and are seen clearly by SEM. Columella absent. Capillitium absent. Spores free, strong yellowish brown (74. s. y Br) in mass, brilliant orange-yellow (67. brill. OY) by LM. Subglobose to irregular, 11-14.5 x 12-16(17) µm diam., minutely and densely punctate (mainly appreciable with immersion oil and SEM; fig. 8); spore wall thick on the 1/2-2/3 of the surface (0.8-1.2 µm), gradually thinner and lighter towards one side (less than 0.8 µm). Protoplastidium colourless.

The name derives from Latin: succulentus (fleshy) and cola (dweller), referring to the characteristic ecology of this species.

**Habitat.** On decaying succulent plants such as *Agave americana* and *A. atrovirens* leaves, *Aeonium* sp. leaves and pith skeletons of *Austrocylindropuntia exaltata*, *Euphorbia canariensis*, *Myrtillocactus geometrizans*, and *Myrtillocactus tomentosa* sp. leaves, and bark of *Yucca filifera* and *Opuntia streptacantha*, and *Nolina laxiflora*.

**Distribution.** At the moment known from Tenerife (Canary Islands, Spain), the States of Hidalgo, Morelos, Puebla and Tlaxcala (Mexico) and New Jersey (USA). Probably occurring in other regions of the world where succulent plants are present.

**Specimens examined**


Agar culture specimens: MA-Fungi 46913, 46914 (spores obtained from TFC Mic. 8390).

*Licea biforis* Morgan

**USA. Ohio:** Preston. 1893, Rex 163, A.P.M. (BPI 826016, Lectotype).

**Canada. Ontario:** London. 1893, J. Dearness, det. A.P.M. (BPI 826043, Syntype).
Culture. Five oatmeal-agar Petri dishes were sown with spores from the type material of *L. succulenticola*. Myxamoebae appeared one day after sowing the spores and a dense population could be observed the next day. Plasmodia appeared three days after sowing and were very similar to those of *L. biforis* described by McManus (1966), and of the proplasmodium type. They moved as rounded or elongated masses, almost colourless, and fructified overnight, generally after migrating to the edges of the agar. They produced single sporophores slightly smaller in size than those directly collected in the field (40-60 × 55-65 μm) and uncoated with refuse matter. Spores could be seen through the colourless peridium and the typical lighter longitudinal area was difficult to observe due to lack of refuse matter and the lack of contrast against the agar. Spore characteristics were the same as in those of material directly collected in the field.

In moist chamber culture the colourless proplasmodia gave rise to transparent sporocarps (fig. 9) with the thinner area of dehiscence already visible between the edges of the slightly opened halves of the peridium, the whole structure reminiscent of a tiny hyaline bivalve (figs. 1-3). Spores were visible forming inside (fig. 10) until the sporocarp darkened with age and the lighter longitudinal area became less obvious. On drying, many of the sporocarps remained closed, but those that opened appeared like tiny circles of golden yellow spores, with the halves of the peridium bent back against the substrate (fig. 2). The incubation period on bark was from 26 to 33 days.

**Discussion**

*Licea succulenticola* is similar in sporotheca form and spore colour to *L. pumila* G.W. Martin & R.M. Allen. We examined the type material of *L. pumila* and found several differences between it and *L. succulenticola*. The spores of *L. pumila* are very pale yellow, with a border that is almost hyaline, there is a clear proplast visible in the centre of each spore, and the spore walls have no thinner area. The spores of *L. succulenticola* are uniformly orange-yellow or golden-yellow throughout, and show a tendency to collapse around a thinner area of the wall. Some of the spores of *L. pumila* are round
Licea succulenticola also has similarities to L. tenera E. Jahn and L. punctiformis G.W. Martin, but both lack the lighter longitudinal area of dehiscence. In addition, L. tenera differs in its smooth spores (minutely and densely punctate in L. succulenticola). The size of the sporocarps of L. punctiformis is similar to that of the species we describe but the spores are smaller (8-10 μm diam) and with rather sparsely distributed large warts (MARTIN & ALEXOPOULOS, 1969).

Licea succulenticola is very similar to L. biforis Morgan in having a somewhat compressed sporotheca with a paler longitudinal area free of refuse material. The differences however, distinguishing L. succulenticola from L. biforis are the spore size, spore color and wall thickness of the spores. Licea is a morphologically simple genus that presents few taxonomically helpful morphological characters. Nevertheless, shape, size and colour of spores are considered to be valuable taxonomic characters in the Myxomycetes (MARTIN & ALEXOPOULOS, 1969). In order to check the validity of the different characters between both species, we studied the lectotype of L. biforis and a syntype kept at the BPI, and found clear and constant differences in the spore characteristics. Licea biforis type had brilliant yellow (83. brill. Y) spores in mass and they were strong yellowish brown (74. s. y Br) in L. succulenticola. By LM L. biforis spores were light yellow (86.1. Y), and orange-yellow (67. brill. OY) in L. succulenticola. The more variable shape of the spores of L. biforis, their smaller size (7.2-8 × 8.8-11.2 μm vs. 11-14.5 × 12-17 μm), and a homogeneously thin spore wall also differentiate the two, whereas the spores have thick walls which are thinner and lighter towards one side in L. succulenticola (fig. 7). GILERT (1997) reported that in TEM longitudinal sections, L. biforis lacks a thinner portion on the spore wall, it being of equal thickness.

In addition to the spore differences, L. succulenticola differs from L. biforis in its smaller sporotheca (47-150 × 75-260 μm vs. 100-300 × 200-1500 μm), in not having as long a fusiform shape, and in never being long plasmodiocarpous (MARTIN & ALEXOPOULOS, 1969; McMANNUS & GIRONEN, 1966). It has a smaller length to width ratio. Both species share the possession of granules all over the inner side of the peridium (GILERT, 1997) and the possession of warts on the inner surface of the lighter longitudinal area (NANNENGA-BREMKAEMP, 1965) (figs. 4-6). Granules also occur in L. retiformis Nawawi as shown by GILERT (1987), so are not an exclusive character.

We have also found material of L. succulenticola within the BPI myxomycete collection (BPI 826021). It was collected in New Jersey (USA) from Opuntia leaves and labelled as “Licea biforis Morg.”. Inside the box a quote stated “See letter of R.M. Allen to Farr 5 IV 1963.”. We tried to find that letter within the BPI archives but we were unsuccessful. It seems likely that R.M. Allen noticed that material from Opuntia was different to some extent from L. biforis and asked for help from M.L. Farr. We examined Allen’s specimen and it concurred with the characteristics of the new species described here.

In order to check that the differing characters between L. biforis and L. succulenticola are due to genetic differences and not to different environmental growing conditions, we cultured L. succulenticola from spore to spore in controlled conditions. In our cultures, laboratory conditions present from germination throughout fructification were: pH = 7, temperature of about 20 °C and no chemical compounds or microflora from decaying Opuntia cladodia present. These conditions are very different from those in the decaying cladodia: alkaline pH, temperatures higher than 20 °C normally, presence of complex chemical compounds from the plant and of a complex microflora composed of different species of
bacteria and yeasts (Mosquera et al., 2000a). Even so, the spores obtained from sporophores in culture presented the same characteristics as those from specimens collected in the field confirming their characters as constant.

*Licea biforis* has been cultured in laboratory by other authors and spore characteristics were typical of that species (McMannus, 1966; McMannus & Gronen, 1966; Wollman & Alexopoulos, 1964, 1967). Wollman & Alexopoulos (1967) reported that sporophores obtained from spore to spore on agar produce spores that were pale yellow under oil immersion, and were about 10.5 µm diam. Thus it seems that *L. succulenticola* has morphological differences to *L. biforis* that are stable, and reflect genomic variation and not environmental responses.

Ecology. Myxomycetes from the genus *Licea* occur frequently on bark of living trees (Keller & Brooks, 1977). They have protoplasmidia, the same as *Macbrideola* and *Echinostelium*, other genera that fructify abundantly in their habitat. The possession of this type of Plasmodium seems to be an adaptation to a habitat with a high risk of desiccation (Lado et al., 1999). Succulent plants usually grow in arid lands, where the environmental conditions, in which succulenticolous myxomycete plasmodia develop, can be considered to be similar to those on the bark of some living trees. Thus the appearance of *Licea* species on this substratum is not surprising. Blackwell & Gilbertson (1980a) already cited *L. parasitica* (Zukal) G.W. Martin and *L. pedicellata* (H.C. Gilbert) H.C. Gilbert on *Opuntia* spp. from the Arizona Desert, and we have found *Licea kleistobolus* G.W. Martin on *Stenocereus* pith skeletons. *Licea succulenticola* appeared abundantly in moist chamber from material collected from large arid areas of Mexico and the Canary Islands.

The abiotic parameters of decaying succulent plants, however, are different from those of the bark of living trees. The pH of the former substrata was usually alkaline in the moist chamber cultures from which we have obtained *L. succulenticola* fructifications: *Stenocereus*, pH 10; *Opuntia ficus-indica*, pH 8-10; *Euphorbia canariensis*, pH 8.5-9; *Agave*, pH 7.5-10.3. Blackwell & Gilbertson (1984) reported a pH of 8.7-10.4 for dead saguaro tissue (*Carnegiea gigantea*) although *Yucca filifera* bark pH 7.0, *Nolina laxiflora* bark pH 6.8, and *Opuntia streptacantha* bark pH 7.2 were more neutral. Almost all these values are higher than those obtained for bark of living trees (Harkonen, 1977, 1978; Ukkola, 1998). Other important differences between both substrata include the porosity of the material, the water-holding capacity, temperature and nutrient concentrations. In addition, the microbial biota is very different (Fogleman & Foster, 1989) and there may be competition for food among myxocells and different invertebrates (Mosquera et al., 2000a). *Licea succulenticola* seems to be specialised for this particular habitat, as are the other succulenticolous species *D. eremoophilum* Blackwell & Gilbertson (Blackwell & Gilbertson, 1980b), *Cribraria zonatispora* Lado, Mosquera & Beltrán-Tej. (Lado & al., 1999), *Trichia agaves* (G. Moreno, Lizárraga & Illana) Mosquera, Lado, Estrada & Beltrán-Tej. (= *T. perichaenoides* Mosquera, Lado, Estrada & Beltrán-Tej.) (Lado, 2001; Mosquera et al., 2000b) and *Didymium subreticulosporum* Oltra, G. Moreno & Illana (Moreno et al., 1996).

We have found *L. biforis* in the same environment as *L. succulenticola* (decaying Agave), each species showing its different characters. After four years studying the succulenticolous habitat, however, we have only found one sample of *L. biforis* on decaying succulent plants, in contrast to the numerous and abundant samples of *L. succulenticola*, showing that this new species is better adapted to the succulenticolous habitat. We have collected it on nine different decaying succulent plants and the bark of three other species in xeric habitats. It has also been found on two continents, off the coast of Africa (the Canary Islands) and in America (Mexico and USA), although it probably occurs in other regions of the world where succulent plants are present.
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