ISSN (print) 0093-4666 ISSN (online) 2154-8889

MYCOTAXON

DOI: 10.5248/113.111

Volume 113, pp. 111–117 *July–September 2010*

New record of *Circinella muscae* from a hydrocarbon polluted sand beach of Tabasco, Mexico

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Abstract — During a survey of fungal biodiversity from Mexican sand beaches, an uncommon fungus of the subphylum *Mucoromycotina* was isolated from the intertidal area of Playa Paraiso, State of Tabasco. A study of culture isolates demonstrated that it is a mucoraceous species belonging to the genus *Circinella* characterized by sporangiophores bearing circinate branches terminated by globose sporangia with persistent sporangial walls. Several sandy soil samples placed in sterile re-sealable plastic bags were processed in the laboratory within 4 h. Plates of corn meal agar inoculated with 0.5 g of sandy soil were incubated 15 d. The fungus produced sympodially branched sporangiophores with fertile circinate branches bearing one or two sporangia, or a single sporangium and a sterile spine. Sterile spines were light in color and the globose sporangia had persistent walls bearing globose, hyaline sporangiospores. The characters of the Mexican isolate agree with those described for *C. muscae*. Few zygomycete studies have been conducted in Mexico, making this the first recorded mucoraceous fungus isolated from a sand beach environment in the country.

Key words — arenicolous fungi, endopsammon, Gulf Coast of Mexico, tropical seashore

Introduction

At the national level, little is known about fungal communities that inhabit the endopsammon in Mexico (González et al. 1998, 2000). During a survey of fungal biodiversity from Mexican sand beaches, an uncommon fungus of the phylum *Zygomycota* was isolated from the intertidal area of Playa Paraiso, State of Tabasco. A study of the characteristics of this isolate on culture media demonstrated that it is a mucoraceous species belonging to the genus *Circinella*

Tiegh. & G. Le Monn., characterized by the production of sporangiophores bearing circinate branches terminated by globose multispored sporangia with persistent sporangial walls. After the genus was monographed by Hesseltine & Fennell (1955), several additional species were described (Hesseltine & Ellis 1961, Faurel & Schotter 1965, Patil & Kale 1981, Arambarri & Cabello 1996). *Circinella* currently includes nine species with an apparently worldwide distribution (Benny 2006). Members of this genus usually have been isolated from soil, dung, fermented cacao beans, musty nuts, and more recently, *C. lacrymispora* was described from hydrocarbon-polluted soil from the coast of Argentina (Arambarri & Cabello 1996). The biotechnological potential of *Circinella* has been explored by Nakagawa et al. (1995) who performed the biochemical conversion of milbemycins with *C. umbellata*.

Materials & methods

Playa Paraiso is located on the southeast coast of the Gulf of Mexico (18°24'00"N 93°13'59"W) in the County of Paraiso, a petroleum extraction zone characterized by numerous lagoons, estuaries, and swamps. It receives 1,751 mm of annual precipitation with a median annual temperature of 26°C. In this study, the beach was sampled August 18, 2001, during low tide; three sandy soil samples of 50 g each were collected and placed in sterile Ziploc® bags and were processed in the laboratory within 4 h. The surface of six plates of corn meal agar (Difco) prepared with artificial seawater (Instant Ocean®) with antibiotics (chloramphenicol 1 mg/ml, penicillin 500 µ/ml, streptomycin 300 µg/ml added after autoclaving) were inoculated each one with 0.5 g of sandy soil and incubated 15 d at 25°C. After this incubation, a mucoraceous fungus was transferred to mucor agar (Hesseltine 1954, Hesseltine & Fennell 1955), potato dextrose agar, malt agar and V8 vegetable juice agar without antibiotics for descriptive purposes. The fungus was identified with the keys published by Hesseltine & Fennell (1955) and Arambarri & Cabello (1996). The culture and slide of this isolate are deposited in the fungal collection of Herbario Nacional (MEXU) of the Institute of Biology, Universidad Nacional Autónoma de México.

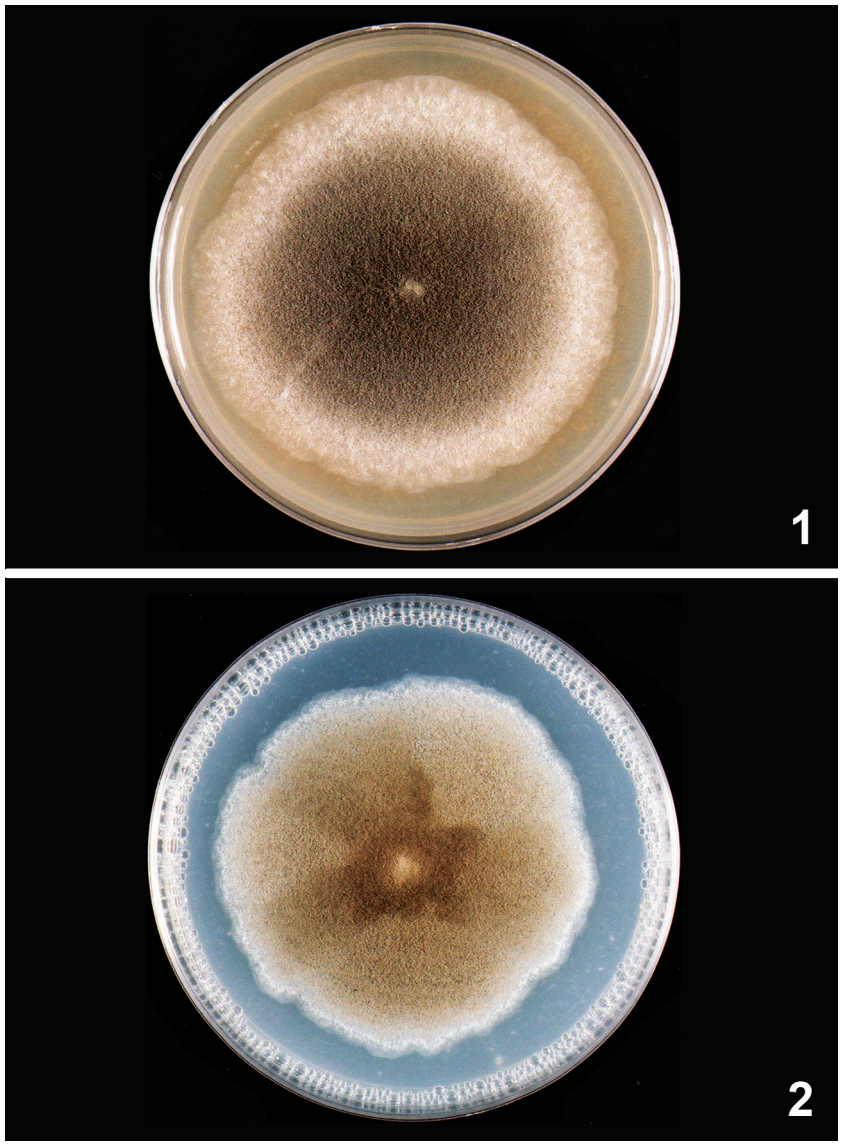
Results

A total of 28 fungal isolates were recovered from the sandy soil samples of Paraiso Beach, and 42 colony-forming units were obtained per gram of soil. *Circinella muscae* was an uncommon species with a low relative abundance value (0.14%).

Circinella muscae (Sorokīn) Berl. & De Toni, Sylloge Fungorum 7: 216. 1888.

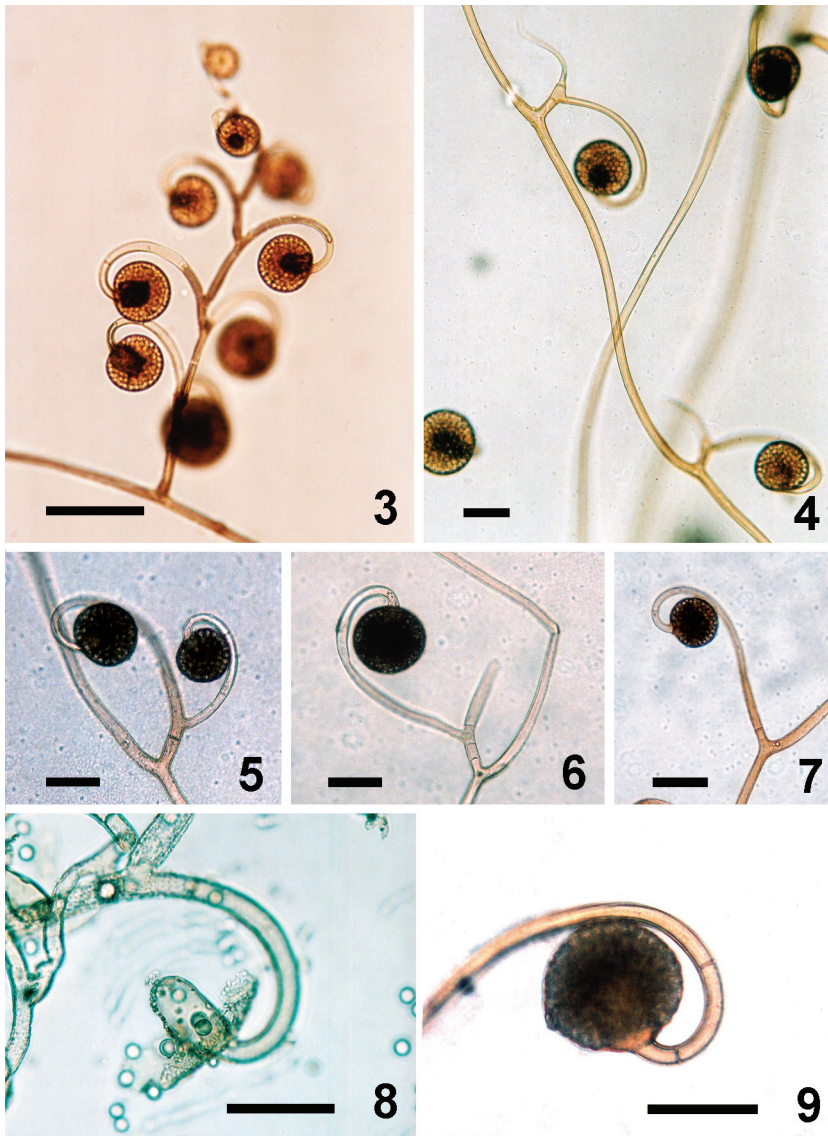
Figs 1–9

Specimen examined: MEXICO. State of Tabasco: Paraiso Co., Paraiso Beach (18°24'00"N 93°13'59"W), from intertidal soil sand, 18 Aug 2001, *N. Murueta-Figueroa, M.C. González*. MEXU 25512, dehydrated specimen, slide and culture MGSW20.



Figs. 1, 2. *Circinella muscae.* 1. Colony appearance on V8 vegetable juice agar after 7 days at 25°C. 2. Colony appearance on potato dextrose agar after 7 days at 25°C. Petri dishes: 100 mm diam.

Mycelium forming a uniform, dense colony on the surface of the agar, relatively slow-growing, reaching a diam. of 75 mm after 7 days at 22–23°C. Colony



Figs. 3–7 *Circinella muscae*. 3. Lateral branch of aerial sporangiophore with a sympodial arrangement of small sporangia. 4. Aerial sporangiophore showing lateral branches with sterile spines and circinate sporangia. 5–7. Aerial sporangiophore with lateral branches bearing two sporangia, a sporangium and a sterile spine, or one sporangium. 8. Columella of sporangium with basal collar and sporangiospores. 9. Characteristic two septa near end of lateral sporangial branch. All bars = 50 μm.

initially white, then gray, becoming cinnamon brown with age (Figs 1, 2). Colony consisting of a layer of short sporangiophores overgrown by longer, aerial sporangiophores. Short sporangiophores erect, up to 1 cm high, arising from hyphae on the agar surface, nonseptate, recurving at apex to form a terminal sporangium. Short sporangiophores with closely spaced, sympodial branches, each with an apical circinate sporangium, frequently with a few large sporangia and a cluster of smaller sporangia. Aerial sporangiophores very long, up to 6 cm in length, sometimes with two main branches, straight to wavy, but never spirally coiled. Aerial sporangiophores with scattered, alternate lateral branches terminated by a large circinately attached sporangium, or dichotomously branched, with two sporangia, a sporangium and a sterile spine, or occasionally with two sterile spines; a few lateral branches bear a sympodial arrangement of small sporangia and without spines (Figs 3–7). Sporangial branches usually with a basal septum and sometimes with one or two additional septa near the sporangium (Fig. 9). Sporangia globose, nonapophysate, with a columella and persistent wall, at first hyaline, then dark gray and appearing black in reflected light. Large sporangia 46–72(–92) µm diam (sd = 12.9, n = 25), small sporangia 18–28 µm diam (sd = 4.9, n = 25), the different sizes readily discernable under the dissecting microscope. Sporangiophores and sporangial branches light brown in color. Columellae variable in shape, subglobose, oblong or conical, smooth, sometimes with a short, hyaline apical protuberance, 21–42 µm high × 15–32 µm wide. A large, broad collar often remains around the base of the columella after breakdown of the sporangial wall (Fig. 8). Sporangia filled with numerous sporangiospores that are liberated when the wall eventually breaks up. Sporangiospores globose, subglobose to ovoidal, 5–7 × 5–6.6 µm, hyaline and smooth, appearing dark brown to black in mass. Zygospores not observed.

Discussion

The tropical species *Circinella mucoroides* Saito is morphologically close to *C. muscae* in producing subglobose, multispored sporangia on circinate sporangiophores, globose sporangiospores, and the presence of sterile spines. However, *C. mucoroides* has spirally twisted sporangiophores that often bear branches that only form spines, whereas *C. muscae* has straight to wavy sporangiophores with branches that typically bear sporangia along with sterile spines. The change in colony color from gray to brown with age also is characteristic of *C. muscae*.

Although no extensive studies of zygomycetes have been conducted in Mexico, several other species of zygomycetes have been reported from the country. Benny & Benjamin (1975, 1976) isolated *Backusella ctenidia* (Durrell & M. Fleming) Pidopl. & Milko ex Benny & R.K. Benj., *Benjaminiella poitrasii*

(R.K. Benj.) Arx (as *Mycotypha poitrasii* (R.K. Benj.) Benny & R.K. Benj.), *Chaetocladium brefeldii* Tiegh. & G. Le Monn., *Cokeromyces recurvatus* Poitras, *Dichotomocladium elegans* Benny & R.K. Benj., *D. robustum* Benny & R.K. Benj., *Thamnostylum lucknowense* (J.N. Rai et al.) Arx & H.P. Upadhyay, *T. nigricans* (Tiegh.) Benny & R.K. Benj. and *Zychaea mexicana* Benny & R.K. Benj. from rodent and lizard dung in northern Mexico. Earlier, Zenteno-Zevada et al. (1955) reported *Rhizopus* sp. on *Annona* sp. in Veracruz and *R. stolonifer* (Ehrenb.) Vuill. (as *R. nigricans* Ehrenb.) on potato from Chihuahua and Guanajuato. Ulloa & Herrera (1971) isolated *R. stolonifer* and *Mucor racemosus* Fresen. from pozol; and Pérez-Silva (1976) isolated *Thamnostylum piriforme* (Bainier) Arx & H.P. Upadhyay (as *Helicostylum piriforme* Bainier) from cow dung from the Distrito Federal. Samaniego et al. (1988) isolated *Actinomucor elegans* (Eidam) C.R. Benj. & Hesselt., *Mucor* sp., *Phycomyces* sp., and *Rhizopus arrhizus* A. Fisch. from soils in Coahuila State. Moretti & Robledo (1988) isolated *Mucor* sp., *Rhizopus* sp., and *Syncephalastrum* sp. from air samples in Mexico City in the Distrito Federal; and Ramírez-Guíllen & Guzmán (2003) reported *Thamnidium elegans* Link on a decaying basidioma of *Lepiota* sp. from Veracruz. Trigos et al (2008) isolated *Circinella minor* Lendn. from "ejote" (*Phaseolus vulgaris* L.), also in Veracruz.

The physical and chemical properties of a soil may determine the fungal diversity that inhabits that ecosystem. The fungal diversity of coastal sand beaches still is unknown for the most part. The sandy soil of beaches probably has a high and characteristic mycobiota composed of species adapted to that particular marine environment where the ascomycetes are the more common and best studied group (Kohlmeyer & Kohlmeyer 1979, Dunn & Baker 1983). This is the first record of a mucoraceous fungus isolated from a sand beach environment in Mexico. Because this fungus was isolated from hydrocarbon polluted sand, chemical studies need to be performed to investigate its potential biotechnological value.

Acknowledgments

The authors would like to thank Drs. Kerry O'Donnell and Anthony E. Glenn for reviewing the manuscript and for their valuable comments. The authors thank Carmen Loyola for assistance with photomicrographs of Figs 1, 2. This work was supported by grant of Consejo Nacional de Ciencia y Tecnología (CONACYT-SEP 60502) to Instituto de Biología of the Universidad Nacional Autónoma de México, and Museum of Natural History, University of Georgia, USA.

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