

# Aquatic fungi from estuaries in Puerto Rico: Mouth of the Manatí River<sup>1, 2</sup>

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## ABSTRACT

Aquatic fungi were isolated from sea foam, leaf litter, beach sand, and driftwood in an estuary known as "La Boca" (river mouth) of the Manatí River in Barceloneta, northern Puerto Rico. Observations of the baits (sterilized balsa wood) and incubated organic debris, revealed the presence of 28 species of aquatic fungi, 13 of which belong to the ascomycetes and 15 to the mitosporic fungi. The species *Arenariomyces triseptatus*, *Corollospora* cf. *colossa*, *C. filiformis*, *Halosphaeria* sp., *Kirschsteiniothelia* sp., *Astrosphaeriella* aff. *mangrovei*, *Torpedospora radiata* (Ascomycota), *Brachiosphaera tropicalis*, *Campylospora* sp., and *Clavatospora bulbosa* (Mitosporic fungi) were the most common in the samples. Eleven species are new records for Puerto Rico; six fungal isolates could not be identified.

**Key words:** marine fungi, aquatic hyphomycetes, estuary, river mouth, Puerto Rico, Caribbean

## RESUMEN

### Hongos acuáticos de los estuarios en Puerto Rico: Boca del Río Manatí

Se aislaron hongos acuáticos de la espuma de mar, hojarasca, arena playera y madera a la deriva de un estuario conocido como "La Boca" (boca de río) del Río Manatí en Barceloneta, al norte de Puerto Rico. Las observaciones de los cebos (madera de balsa esterilizada) y material orgánico incubado, revelaron la presencia de 28 especies de hongos acuáticos, de los cuales 13 pertenecen a los ascomicetos y 15 a los hongos mitospóricos. Las especies *Arenariomyces triseptatus*, *Corollospora* cf. *colossa*, *C. filiformis*, *Halosphaeria* sp., *Kirschsteiniothelia* sp., *Astrosphaeriella* aff. *mangrovei*, *Torpedospora radiata* (Ascomycota), *Brachiosphaera tropicalis*, *Campylospora* sp. y *Clavatospora bulbosa* (Hongos Mitospóricos) fueron las más comunes en las muestras. Once especies son registros nuevos para Puerto Rico y se aislaron seis hongos que no pudieron ser identificados.

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**Palabras clave:** hongos acuáticos, estuario, boca de río, Puerto Rico, Caribe

## INTRODUCTION

Fungi are of widespread occurrence in the oceans, seas, and estuaries as parasites in marine plants or animals or as saprobes on timber, algae, sea grasses, protozoans, corals, sea foam, and other substrata (Kirk et al., 2001). The distribution of fungi in the seas and estuaries has been studied in several Caribbean localities: 1) Belize (Kohlmeyer and Volkmann-Kohlmeyer, 1987a; Minter et al., 2001). 2) Cuba (Capó-de Paz, 1986a, b; Minter et al., 2001; González et al., 2003). 3) Lesser Antilles (Kohlmeyer and Kohlmeyer, 1971; Fell and Master, 1975; Stevenson, 1975; Kohlmeyer, 1981; Kohlmeyer and Volkmann-Kohlmeyer, 1987b, 1988; Minter et al., 2001). 4) Mexico (González et al., 1998, 2000, 2001; Minter et al., 2001). 5) Puerto Rico (Rossy-Valderrama, 1956; Meyers, 1957; Kohlmeyer, 1968; Carvajal-Zamora, 1971; Hernández-Vera, 1972, 1975, 1980; Stevenson, 1975; Galler-Rimm, 1982; Hernández-Vera and Almodóvar, 1983, 1984; Acevedo, 1987, 2001; Valdéz-Collazo et al., 1987; Kohlmeyer and Volkmann-Kohlmeyer, 1987c; Calzada, 1988; Lodge, 1996; Minter et al., 2001; Nieves-Rivera et al., 2002). 6) Venezuela (Dennis, 1970; Minter et al., 2001). Although aquatic fungi have been extensively studied worldwide, in many Caribbean islands the estuarine and marine mycobiota is poorly known. Most estuarine and marine collections are in some cases sporadic and interrupted by many years. The purpose of this study was to report the incidence of aquatic fungi in marine foam from a river mouth (estuarine conditions) in Puerto Rico, a subtropical island located between 18°00' and 18°30'N, 65°35' and 67°15'W, in the northeastern Caribbean Sea.

## MATERIALS AND METHODS

On 25 November 1998, intertidal foam and sand were collected from the Manatí River (also known as "Río Grande de Manatí"). Samples were baited in the laboratory with pieces (10 cm long) of sterilized balsa wood. Washed-up beach debris (leaf litter and drift wood) was also collected for incubation, following the methods described by Kohlmeyer and Kohlmeyer (1979).

A sandy beach of the Manatí River mouth, also known as "La Boca" or "Boca" (Spanish for mouth), located next to road PR-684 in the Barceloneta municipality, about 36 km west (18°28'81.8"N and 66°32'09.2"W) of San Juan, was selected as the study site. The Manatí River is about 64 km long, rises in the Cordillera Central just north of Barranquitas, flows northwest, past Ciales to the Atlantic Ocean 6.4

km northwest of Manatí. Mean annual precipitation is less than 1,650 mm; mean annual temperature is 25.3°C (Ravaló et al., 1986; Anonymous, 2003). The climate is described as that of the subtropical dry life zone (Ewel and Whitmore, 1973). Surface seawater salinity ranges from 19 to 31 g/L, with temperatures of 27 to 31° C, a pH of 7.4 to 8.7, a total alkalinity of 87 to 116 mg/L, and a dissolved oxygen (DO) value of 7.2 to 8.6 mg/L (Nieves-Rivera, unpublished data). At the collection site, the river mouth beach sand composition is dominated by dark minerals, volcanic rock fragments, quartz, and feldspar (Morelock et al., 1985). The geomorphology of La Boca was studied by Lobeck (1922), Wood et al. (1975), Morelock et al. (1985), and Barreto-Orta (1997).

The Manatí River estuary has been found to be highly stratigraphic, with little or no mixture in the interphase between fresh- and seawater (Carvajal-Zamora, 1977). The sea wedge extends five to six km up river. The intrusion of seawater into the river usually causes a decrease in DO, which is related to a high microbiological activity and chemical demand in the sea wedge and in sediments; this decrease in DO creates anoxic conditions during the year (Carvajal-Zamora, 1977). Slightly high phosphate ( $\text{PO}_4^{3-} = 0.08$  to  $0.12 \mu\text{M}$ ) and nitrate ( $\text{NO}_3^{2-} = 16.72 \mu\text{M}$ ) values have been detected in near shore surface waters, especially near the Manatí River mouth, probably because of agricultural runoff (Wood et al., 1975: Station PMA-3A at 0 m depth). Carvajal-Zamora (1977) found that the concentrations of heavy metals and nutrients in the sediments of the Manatí River were greater in the estuarine part than in the riverine portion.

The procedure used in the present study for collection and preservation of marine foam on the sandy beach follows Kohlmeyer and Kohlmeyer (1979); their illustrated keys are described elsewhere (Kohlmeyer and Volkmann-Kohlmeyer, 1991; Hyde and Sarma, 2000). For the isolation and identification of aquatic hyphomycetes, we followed Santos-Flores and Betancourt-López (1997). Voucher specimens (slides) of all species were deposited at the University of Puerto Rico at Río Piedras herbarium (UPRRP).

## RESULTS AND DISCUSSION

A total of 28 fungi were found, 13 of which belong to the ascomycetes and 15 to the mitosporic fungi (Table 1; Figures 1 to 8). The most common fungi in the samples were the species *Arenariomyces triseptatus* Kohlm. (Figure 1), *Corollospora* cf. *colossa* Nakagiri & Tokura (Figure 2), *C. filiformis* Nakagiri, in Nakagiri & Tokura (Figure 3), *Halosphaeria* sp., *Kirschsteiniothelia* sp. (Figure 4), *Astrosphaeriella* aff. *mangrovei* (Kohlm. & Vittal) Aptroot & K. D. Hyde (Figure 5), *Torpedospora radiata*

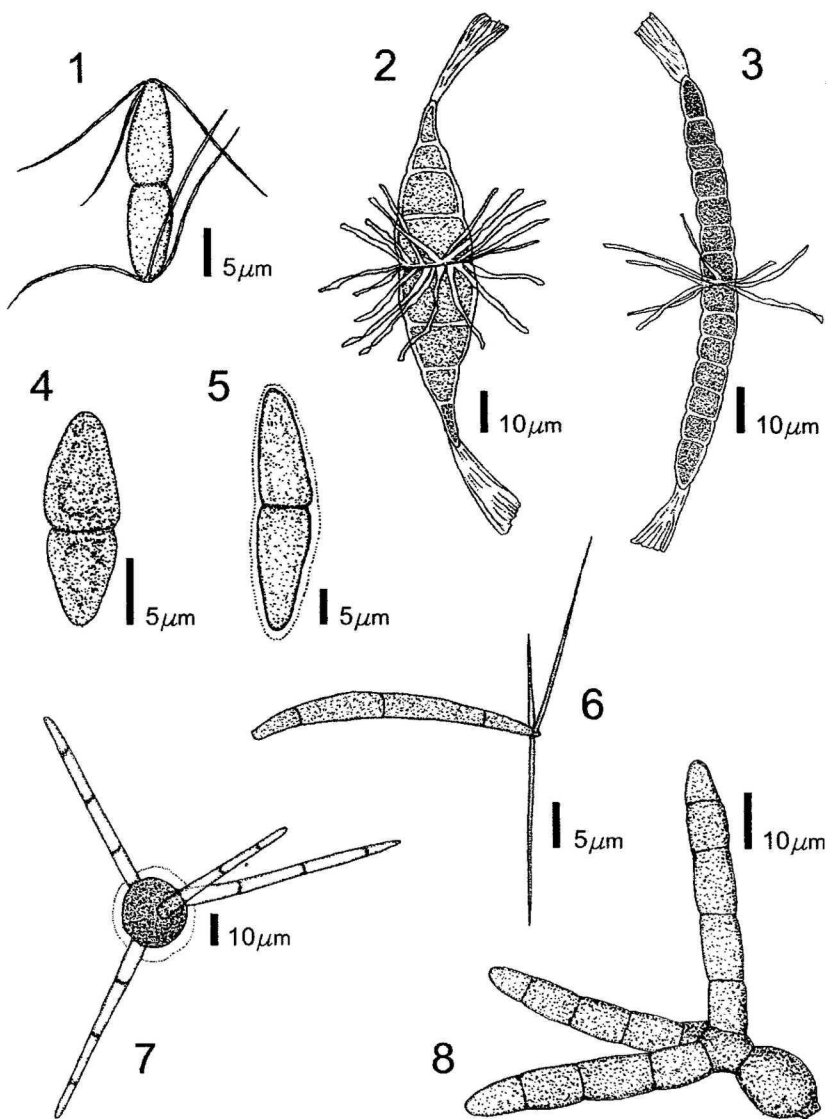
TABLE 1.—*Summary of the aquatic fungi (obligate and facultative marine fungi sensu Kohlmeyer, 1974) recovered from samples of sea foam, leaf litter, and wood in the mouth of the Manatí River, northern Puerto Rico.*

Fungus	Substrate <sup>1</sup>
ASCOMYCOTA	
<i>Arenariomyces</i> cf. <i>majusculus</i> Kohlm. & Volkm.-Kohlm.*	F, S
<i>A. triseptatus</i> Kohlm.	F, S
<i>Astrosphaeriella</i> aff. <i>mangrovei</i> (Kohlm. & Vittal) Aptroot & K.D. Hyde*	F, L, W
<i>Chaetomastia</i> cf. <i>typhicola</i> (Karst.) Barr*	F, S
<i>Corollospora</i> cf. <i>colossa</i> Nakagiri & Tokura*	F
<i>C. filiformis</i> Nakagiri, in Nakagiri & Tokura*	F
<i>C.</i> cf. <i>pseudopulchella</i> Nakagiri & Tokura*	F
<i>Coronopapilla</i> aff. <i>mangrovei</i> (K.D. Hyde) Kohlm. & Volkm.-Kohlm.*	F, W
<i>Halosphaeria cucullata</i> (Kohlm.) Kohlm.*	F, S
<i>Halosphaeria</i> sp.	F, S
<i>Halorossellinia oceanicum</i> (S. Schatz) Whalley, E.B.G. Jones, K.D. Hyde & T. Laessøe	F
<i>Kirschsteiniothelia</i> sp.*	F
<i>Kretzschmariella culmorum</i> (Cooke) Y.M. Ju & J.D. Rogers	F
<i>Lindra</i> sp.	F, S
<i>Lulworthia</i> sp.	F, S
<i>Torpedospora radiata</i> Meyers	F
Unknown sp. 1	F, S
Unknown sp. 2	F
MITOSPORIC FUNGI	
<i>Anguillospora</i> cf. <i>longissima</i> (Sacc. & P. Syd.) Ingold	F, L, S
<i>Articulospora tetracladia</i> Ingold	F
<i>Brachiosphaera tropicalis</i> Nawawi, in Descals	F
<i>Camposporidium</i> sp.	F
<i>Campylospora</i> sp. (s. str. Santos-Flores & Betancourt-López, 1997)	F, L, W
<i>Clavatospora bulbosa</i> (Anastasiou) Nakagiri & Tubaki*	F, S
<i>Curvularia</i> sp.	F, L, W
<i>Diplocladiella scalaroides</i> G. Arnaud	F, L
<i>Fusarium</i> sp.	F, L, W
<i>Lemonniera pseudofloscula</i> Dyko, in Descals, J. Webster & Dyko	F, L
<i>Triscelophorus acuminatus</i> Nawawi	F, S
<i>Triscelophorus</i> sp.	F
<i>Tubeufia cylindrothecia</i> (Seaver) Höhn.*	F, S
Unknown sp. 1	F, W
Unknown sp. 2	F, L
Unknown sp. 3	F
Unknown sp. 4	F, W

<sup>1</sup>Substrate: F = sea foam; L = leaf litter; S = beach sand; W = wood.

\* = New record for Puerto Rico.

Meyers (Figure 6) (Ascomycota), *Brachiosphaera tropicalis* Nawawi (Figure 7), *Campylospora* sp., *Clavatospora bulbosa* (Anastasiou) Nakagiri & Tubaki (Figure 8) (Mitosporic fungi). Few marine ascomycetes were iso-



FIGURES 1-8. Aquatic fungi isolated from samples collected of the mouth of Manatí River, northern Puerto Rico. Ascospores: 1. *Arenariomyces triseptatus*. 2. *Corollospora* cf. *colossa*. 3. *Corollospora filiformis*. 4. *Kirschsteiniothelia* sp. 5. *Lophiostoma* cf. *mangrovei*. 6. *Torpedospora radiata*. Conidia: 7. *Brachiosphaera tropicalis*. 8. *Clavatospora bulbosa*.

lated; this scarcity was probably due to the selectivity of the isolation method employed and the time of sampling. Ascomycetes and mitosporic fungi (mostly aquatic hyphomycetes) were found mixed in foam, leaf litter, and sand of the La Boca beach. Eleven species are new records for Puerto Rico, and six fungal isolates could not be identified (Table 1).

Along with ascospores of marine fungi, conidia of 10 species of aquatic hyphomycetes were detected: *Anguillospora* cf. *longissima* (Sacc. & P. Syd.) Ingold, *Articulospora tetracladia* Ingold, *Brachiosphaera tropicalis* Nawawi, *Camposporidium* sp., *Campylospora* sp., *Diplocladiella scalaroides* G. Arnaud, *Lemonniera pseudofloscula* Dyko, *Triscelophorus acuminatus* Nawawi, *Triscelophorus* sp., and *Tubeufia cylindrothecia* (Seaver) Höhn. (Santos-Flores and Betancourt-López, 1997) (Table 1). *Camposporidium* sp. conidia resembled *Camposporidium* spp. reported from Río Sonadora and Quebrada Jiménez near the El Verde LTER Field Station, Puerto Rico (Hamilton, 1973). Some specimens of *B. tropicalis* showed a translucent halo around the central cell (Figure 7); whereas other *B. tropicalis* were typical. Aquatic hyphomycetes are not uncommon in estuarine habitats (Johnson and Sparrow, 1961; Kohlmeyer and Kohlmeyer, 1979). For example, Kirk (1969) reported two lignicolous aquatic hyphomycetes (*Clavatospora stellatacula* Kirk and *Tetraploa aristata* Berk. & Br.) adapted to seawater conditions in the Chesapeake Bay.

Conidia of six terrigenous mitosporic fungi were also isolated from sea foam samples. These were *Curvularia* sp., *Fusarium* sp., and conidia of four unknown species, as listed in Table 1. The unknown spores (also referred to as *Ignotus* by Acevedo, 1987, 2001) are described as dematiaceous, aseptate or septate, ranging in sizes, aleurospore, dictyospore, phragmospore, lenticular with or without apical pore. These terrigenous species are apparently common to marine and estuarine habitats. Acevedo (1987) found 25 species of mitosporic fungi in sand, from the reefs and an offshore island (Isla Cuevas) from La Parguera, southwestern Puerto Rico. These mitosporic fungi were: *Alternaria* sp., *Aspergillus* spp., *Cephalosporium* sp., *Cladosporium* spp., *Curvularia* spp., *Diplodia* spp., *Fusarium* spp., *Helminthosporium* spp., *Penicillium* spp., *Scopulariopsis* sp., *Trichoderma* spp., and *Mycelia Sterilia* (Acevedo, 1987).

In this survey, we did not recover any species that are potentially pathogenic to humans, a finding different to that of González et al. (2000). The assemblage of species reported by González et al. (2000) also differs greatly from that recorded in the present study because of the methodology and bait used. González et al. (2000) isolated a total of 17 keratinophilic fungi, 13 of which were hyphomycetes and four ascomycetes. González et al. (2001) reported a larger collection

throughout Mexico; therefore, their checklist contains 47 ascomycetes, 14 mitosporic fungi, and one basidiomycete. In the Cuban survey, González et al. (2003) reported 29 marine fungi (25 ascomycetes and four mitosporic fungi), 19 of which were new records. In contrast, our collection from a single locality produced 15 mitosporic fungi and 13 ascomycetes (Table 1).

Most of the species collected are arenicolous (*Arenariomyces* cf. *ma-jusculus*, *A. triseptatus*, *Corollosopora* cf. *colossa*, *C. filiformis*, *C. cf. pseudopulchella*; see Kohlmeyer and Kohlmeyer, 1979). Marine ascomycetes (*Arenariomyces triseptatus*, *Lulworthia* sp., and *Torpedospora radiata*) have been recorded previously from Belize (Kohlmeyer and Volkmann-Kohlmeyer, 1987a), Cuba (González et al., 2003), Mexico (González et al., 2000, 2001), Puerto Rico (Kohlmeyer and Volkmann-Kohlmeyer, 1987c) and St. Croix (Kohlmeyer and Volkmann-Kohlmeyer, 1988). *Halorosellinia oceanicum*, *Lulworthia* sp., and *Torpedospora radiata* are lignicolous or algicolous species (Kohlmeyer and Kohlmeyer, 1979; Acevedo, 2001). All species isolated in this survey are saprobes living in parts of angiosperms, plant debris, or in the blades of seagrasses.

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