Diversity of marine ascomycetes from the disturbed sandy beaches of Tabasco, Mexico

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The coastline of Tabasco State in the Gulf of Mexico represents a highly deteriorated ecosystem, where densely populated human settlements and large offshore petroleum developments are negatively affecting the marine biodiversity. Previous work on marine ascomycetes reported that in the Gulf of Mexico the diversity of these fungi might be threatened by anthropogenic activities. Therefore we evaluated the diversity of marine ascomycetes in this area, and registered 19 taxa. Ceriosporopsis capillacea *was recorded for the first time for Mexico. The highest diversity was obtained in the beach of Sánchez Magallanes, which receives a great quantity and diversity of organic remains originating from El Carmen/Machona mangrove forests via the Santa Ana mouth, benefiting the proliferation of marine fungi. The lowest diversity was documented in the beach of Paraíso, which is close to the delta of one of the most polluted rivers in Mexico and to off-shore oil extraction platforms. We found a significant correlation between the community composition and abundance, implying that the overall abundance is defined by the community structure, perhaps as a result of competition. Additionally, our results indicated that there is no relationship between the grain size and the biodiversity observed.*

INTRODUCTION

The ascomycetes that inhabit the intertidal zone of sandy beaches constitute an ecological group of marine saprobic microorganisms that live between or on the surface of sand grains. These fungi represent a key component of the biodiversity in intertidal habitats, because they degrade organic matter containing lignin, cellulose or chitin deposited by the sea on marine beaches contributing to the process of remineralization of the nutrients (Kohlmeyer & Kohlmeyer, 1979; González, 2009). Additionally, other trophic levels depend on these saprotrophic fungi to cleave lignocellulose that can enter the food web through mycophagic invertebrates and bacteria (Newell & Porter, 2000). Marine ascomycetes have been reported to be important sources of novel bioactive metabolites that may have biotechnological applications; also they are useful as bioindicators, and potentially as bioremediators of beaches polluted with hydrocarbons since they have been proved to use n-hexadecane, n-tetradecane, l-hexadecene and pristane as sole carbon sources for growth (Kirk & Gordon, 1988; Kirk et al., 1991; Liberra et al., 1998; González & Hanlin, 2010).

Since the pioneering study of Barghoorn & Linder (1944) on marine lignicolous species, the study of marine fungi inhabiting sandy beaches was notably enhanced with the publication of Kohlmeyer & Kohlmeyer (1979), followed by several studies undertaken by Kohlmeyer (1960, 1962, 1963, 1968a) and Tubaki (1966, 1968). Subsequently, many papers on sand beach fungi have been published; some recent contributions are those of authors who have described new species of this group of fungi (Koch, 1986; Nakagiri & Tokura, 1987; Kohlmeyer & Volkmann-Kohlmeyer, 1989, 1997; Sundari et al., 1996; Abdel-Wahab et al., 2009; Jones et al., 2009). Presently, 530 species of marine fungi are described, and only 64 of them have been recorded in Mexico (González et al., 2001; Jones et al., 2009; Velez et al., 2013; Velez et al., in press).

The first study of marine fungi in Mexico was made by Kohlmeyer (1968b), and since then only few reports have been published (González & Herrera, 1993; González et al., 1998, 2000, 2001; González, 2009; González & Hanlin, 2010; Velez et al., 2013). So, the diversity of marine ascomycetes inhabiting around 70% of the Mexican coasts on the Pacific Ocean, Gulf of Mexico and the Caribbean Sea remains mostly unexplored (González et al., 2001; Velez et al., 2013). Consequently, the geographic and ecological patterns of these fungi remain poorly understood.

Moreover, Mexican beaches represent highly populated and deteriorated ecosystems (Merino, 1987). Especially, the littoral of the Tabasco State in the Gulf of Mexico where large offshore petroleum developments of the Petróleos Mexicanos, one of the world's largest petroleum companies, are negatively affecting the marine biodiversity (Botello et al., 1983; Rivera-Arriaga & Villalobos, 2001; Petróleos Mexicanos, 2013). This area is also characterized by high

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fecal pollution due to the discharge of wastewaters from human settlements (Rosas et al., 1985). Velez et al. (2013) conducted a preliminary evaluation of the diversity of marine fungi from one site in Tabasco State (Paraíso Beach) as part of a large-scale survey of the Gulf of Mexico, and barely reported two species concluding that the diversity of marine ascomycetes might be threatened by anthropogenic activities in this region. Several authors have noted the critical need to study the biodiversity inhabiting endangered ecosystems (i.e. Kerr et al., 2000). Considering that the coastline of Tabasco State represents a particularly highly deteriorated ecosystem, the importance of registering the mycobiota at a local scale in this area is evident.

Therefore, the aims of this work were (1) to estimate the diversity of marine arenicolous ascomycetes from 10 sandy beaches spread along the coast of the state of Tabasco, (2) to assess patterns of community differentiation given by geographic distance, (3) to determine the association between abundance and community structure and (4) to evaluate the relationship between the sand textural parameters and the biodiversity registered in the studied beaches.

MATERIALS AND METHODS

Tabasco State is located in the southeast of Mexico with 184 km of coastline in the Gulf of Mexico. For more than 60 years, the oil industry has been present in the littoral of this state, resulting in highly petroleum-polluted coastal ecosystems (Botello et al., 1991; Ponce-Vélez et al., 2012). Ten exposed sandy beaches were sampled during low tide (Figure 1). We collected a total of 200 samples in the intertidal zone consisting of washed-up detritus (woody debris, algae wrack and other organic remains) covered with moist sand from the collecting site placed in Ziploc® plastic bags. In
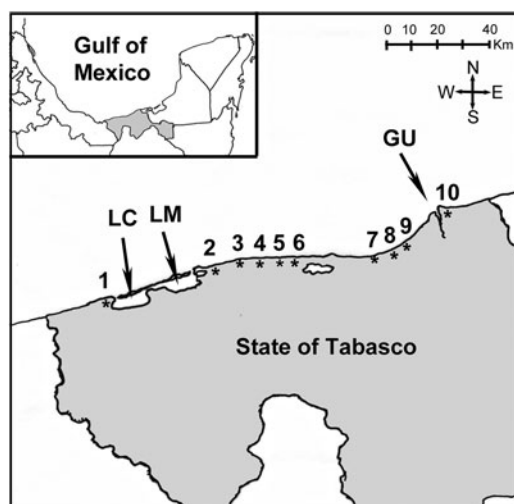

Fig. 1. Location of the studied beaches in the coast of the State of Tabasco in the Gulf of Mexico: (1) Sánchez Magallanes (18° 17′ 38.75″ N 93° 52′ 18.53″ W), (2) Tupilco (18° 25′ 47.1″ N 93° 25′ 18.3″ W), (3) La Unión (18° 26′ 03.2″ N 93° 22′ 22.3″ W), (4) Dorada (18° 26′ 12.7″ N 93° 18′ 16.9″ W), (5) Mar de Plata (18° 26′ 18.1″ N 93° 17′ 20.9″ W), (6) Paraíso (18° 26′ 19″ N 93° 13′ 4.3″ W), (7) Pico de Oro (18° 27′ 0.6″ N 92° 52′ 14.8″ W), (8) Unnamed beach (18° 30′ 10.47″ N 92° 46′ 27.70″ W), (9) Miramar (18° 29′ 40.9″ N 92° 47′ 04.4″ W), (10) El Bosque (18° 36′ 43.7″ N 92° 40′ 41.9″ W). Arrows: GU, Grijalva/Usumacinta delta; LC, Lagoon El Carmen; LM, Lagoon Machona.

the laboratory, the samples were incubated for 12 months in moist chambers at 25°C and a 12 h photoperiod under cool white lights (to induce the production of fungal reproductive structures), and examined monthly for the presence of fungi. The ascomata that developed on sand grains in moist chambers, were used for observation and isolation. To identify the recovered fungi, ascomata were removed from sand grains with a flame-sterilized needle, then opened in a drop of distilled water on a slide using dissecting needles, and covered with a cover slip. We identified the ascomycetes based on the morphology of ascomata, asci and ascospores, using the taxonomic keys of Kohlmeyer & Kohlmeyer (1979), Kohlmeyer & Volkmann-Kohlmeyer (1991), Hyde & Sarma (2000) and Jones et al. (2009). Fungi were measured and photographed using a Nikon Eclipse 80i. For preservation, dehydrated specimens, slides and cultures were deposited at the Colección de Hongos del Herbario Nacional (MEXU) of Instituto de Biología, Universidad Nacional Autónoma de México.

Species richness (N) was considered as the number of species registered in a site. The frequency of occurrence (F) was calculated from number of occurrences of a specific taxa divided by the total number of samples ×100 (Magurran, 2004). The species diversity of each beach was calculated using Shannon–Wiener (H′) species diversity index. Correlations between distance matrices for taxon composition and geographic location, and taxon composition and overall abundance were analysed using the Mantel test. The diversity data and distance matrices of marine ascomycetes recorded from the beaches of the Gulf of Mexico were analysed utilizing the statistical software R version 2.15.2 (R Development Core Team, 2012) using the packages: ade4, vegan, gclus, FD, FactoMineR, perturb, Hmisc, ape and raster (Paradis et al., 2004; Dray & Dufour, 2007; Laliberté & Shipley, 2011; Hendrickx, 2012; Hurley, 2012; Hijmans & Etten, 2013; Husson et al., 2013; Oksanen et al., 2013; Harrell Jr & Dupont, 2014).

Lastly we analysed the sand textural characteristics of the studied beaches as proposed by Carranza-Edwards (2001) and compared the results with the biodiversity recovered from each site.

RESULTS

We observed a wide variation in the amount and assemblage of substrata in the studied beaches. Sánchez Magallanes Beach was dominated by a great amount of plant debris, principally mangrove and water lily remains; in Tupilco Beach we mainly recorded palm leaf remains; in the beaches of La Unión, Dorada, Mar de Plata, Miramar and unnamed we registered palm leaf and driftwood remains, though in the beaches of Miramar and unnamed the amount of litter was greater than in the beaches of La Unión, Dorada and Mar de Plata since the latter are located near a complex of private beach houses where organic litter is cleaned away periodically; the beaches of Paraíso and Pico de Oro represent important beach destinations in the State of Tabasco, consequently the organic litter is cleaned away very often, so we were only able to find a few palm and timber remains; in El Bosque Beach substrata was primarily water lily debris. Moreover, we detected an association between a small amount of substrata in the beach (in touristic sites where wrack is cleaned)

and low diversity values of marine fungi. However, high diversity values were obtained from beaches where the wreckage was diverse, containing water lily, plant, driftwood and mangrove remains.

Out of 200 samples, 160 presented fungal development and supported 19 ascomycetes (Table 1). The beaches of Paraíso ($H = 0.11$) and Pico de Oro ($H = 0.29$) obtained the lowest values of diversity, whereas Sánchez Magallanes Beach exhibited the highest value ($H = 0.91$). In the studied beaches we registered strict marine arenicolous fungi (*Corollospora* and *Arenariomyces* members), core mangrove fungi (*Lulworthia grandispora*), freshwater species (*Ascosacculus heteroguttulatus*) and cosmopolitan marine species (*Corollospora maritima*, *Torpedospora radiata*, *Ceriosporopsis halima* and *Savoryella lignicola*) (Jones & Pang, 2012). The hydrocarbonoclastic species *C. maritima* was the most dominant marine fungus. The rare ascomycete *C. capillacea* was recorded for the first time in Mexico.

The geographic distance among the studied sites was not correlated with the species composition ($r = 0.1393$, $P = 0.2103$). Moreover, our results showed that diversity and overall abundance were positively correlated ($r = 0.5909$, $P = 0.0002$). Finally, we found no clear trend in the relationship between grain size and the diversity observed (Table 1). In the beaches with the lowest diversity values, the grain size composition varied, comprising mostly fine sand (Paraíso Beach) and medium sand (La Unión Beach and Mar de Plata Beach). Whereas in the sites with the highest diversity values, the grain size composition also differed, finding mostly coarse sand and medium sand (Sánchez Magallanes) and fine sand (El Bosque).

DISCUSSION

The 200 sampling units examined supported a total of 19 ascomycetes belonging to the families of *Halosphaeriaceae* and *Lulworthiaceae* (Table 1). The *Halosphaeriales* is the largest order of marine fungi and is comprised of saprophytic ascomycetes occurring on a wide range of substrata whereas the *Lulworthiaceae* are typically found growing on submerged wood or seaweed (Jones *et al.*, 1983, 2009; Kohlmeyer *et al.*, 2000). Correspondingly, we recorded members of the *Halosphaeriales* occurring in diverse organic substrata. However, the members of the *Lulworthiaceae* were strictly found in wood substrata.

The beaches of Paraíso ($H = 0.11$) and Pico de Oro ($H = 0.29$) exhibited the lowest values of diversity. This finding agrees with previous observations of low levels of marine mycodiversity in Paraíso beach (Velez *et al.*, 2013). This result might be associated with the geographic proximity of these beaches to the discharge delta of the Grijalva/Usumacinta Rivers, the biggest and one of the most polluted in Mexico (Yáñez-Arancibia *et al.*, 2009). Furthermore, this hydrological

Table 1. Diversity of arenicolous marine ascomycetes from deteriorated beaches in the coast of Tabasco State, Mexico, and sand textural parameters of the collection sites

Taxa	Beaches										
	1	2	3	4	5	6	7	8	9	10	F
Arenariomyces trifurcatus Hönk	2	0	0	0	0	0	0	0	0	0	1.3
Ascosacculus heteroguttulatus (S.W. Wong, K.D. Hyde et E.B.G. Jones) J. Campb J.L. Anderson et Shearer	0	0	0	0	0	0	0	0	0	2	1.3
Corollospora gracilis Nakagiri et Tokura	0	0	0	0	2	0	0	0	2	0	2.5
Corollospora maritima Werderm.	2	4	2	8	0	24	10	4	8	2	40.0
Corollospora pseudopulchella Nakagiri et Tokura	0	0	0	2	0	0	0	0	0	0	1.3
Corollospora pulchella Kohlm., I. Schmidt et N.B. Nair	2	0	0	2	0	0	0	0	0	0	2.5
Corollospora sp. A	0	0	0	4	0	2	12	0	2	0	12.5
Corollospora sp. B	0	0	0	0	0	0	0	0	2	0	1.3
Corollospora sp. C	2	0	0	0	0	0	0	0	0	0	1.3
Ceriosporopsis capillacea Kohlm.*	4	0	0	0	0	0	0	0	0	0	2.5
Ceriosporopsis halima Linder	6	0	0	0	0	0	0	0	0	0	3.8
Halenospora varia (Anastasiou) E.B.G. Jones	2	0	2	2	2	0	0	2	2	4	10.0
Lulworthia grandispora Meyers	0	2	0	0	0	0	0	0	0	0	1.3
Lulworthia sp. A	2	0	0	0	0	0	0	0	0	0	1.3
Lulworthia sp. B	0	0	0	0	0	0	0	2	0	0	1.3
Remispora sp.	0	0	0	0	0	0	0	0	2	0	1.3
Savoryella lignicola E.B.G. Jones et R.A. Eaton	0	2	0	0	0	0	0	0	0	0	1.3
Torpedospora radiata Meyers	2	4	0	0	0	0	0	4	0	0	6.3
Unidentified anamorph	0	0	0	0	0	0	0	4	2	4	6.3
TO	24	12	4	18	4	26	22	18	20	12	100
N	9	4	2	5	2	2	2	5	7	4	
H′	0.91	0.57	0.30	0.62	0.30	0.11	0.29	0.75	0.75	0.57	
Coarse sand ($\frac{1}{2}$ to 1 mm)	50	0	0	0	0	0	0	0	0	10	
Medium sand ($\frac{1}{4}$ to $\frac{1}{2}$ mm)	45	85	60	95	90	0	0	80	25	0	
Fine sand ($\frac{1}{8}$ to $\frac{1}{4}$ mm)	5	15	40	5	10	85	60	20	75	60	
Very fine sand ($\frac{1}{16}$ to $\frac{1}{8}$ mm)	0	0	0	0	0	10	30	0	0	30	
Silt ($\frac{1}{16}$ to $\frac{1}{256}$ mm)	0	0	0	0	0	5	10	0	0	0	

Sampled beaches: (1) Sánchez Magallanes, (2) Tupilco, (3) La Unión, (4) Dorada, (5) Mar de Plata, (6) Paraíso, (7) Pico de Oro, (8) Unnamed beach, (9) Miramar, (10) El Bosque. F=frequency, *TO* = total number of occurrence, *N* = species richness, *H′* = Shannon's diversity index, *new record for Mexico. Sand textural parameters are given in percentages.

system is related to the second largest oceanic region disturbed with a severe and prolonged oxygen deficiency, referred to as a 'dead zone' (Rabalais *et al.*, 2002; Day *et al.*, 2003; Yáñez-Arancibia & Day, 2004). The low diversity values might also be related to the impact of the local petroleum industry, since the beaches of La Unión, Mar de Plata and Paraíso are located opposite the off-shore oil extraction platforms Teocalli and Yaxche, and close to Dos Bocas, an important petroleum storage and distribution site (Petróleos Mexicanos, 2009). Furthermore, these two beaches represent important tourist destinations, where debris (including mollusc and crustacean exoskeletons, plant material, driftwood, algae and seaweeds, seagrasses, etc.) is cleaned from the intertidal zone. Therefore, marine fungi inhabiting the coastline are left with no substrata for degradation, which decreases their diversity since these organisms are saprobes.

The highest diversity was obtained from the beach of Sánchez Magallanes (0.91). This result may be related to the geographic location of this beach, which is close to Santa Ana mouth, positively influencing the beach by contributing to a greater quantity and diversity of organic remains originating from El Carmen/Machona mangrove forests (lagoon/estuarine system). Perhaps the greater quantity of diverse substrata benefits the proliferation of marine fungi. Hence, substrata diversity and abundance is an important driver of the diversity of marine fungi inhabiting sandy beaches.

The diversity recovered from the 10 beaches of the State of Tabasco was typical of what has been reported in tropical beaches, with the important presence of the arenicolous genus *Corollospora* (Jones, 1993; Volkmann-Kohlmeyer & Kohlmeyer, 1993). The members of this genus characteristically represent obligate marine arenicolous ascomycetes. These fungi form their ascomata attached to grains of sand or other hard substrates, and have distinctive and exclusive adaptations to the beach environment conferring on them advantages over

exogenous marine fungi (Kohlmeyer & Volkmann-Kohlmeyer, 1987; González & Hanlin, 2010).

The marine species *Arenariomyces trifurcatus*, *C. halima*, *Corollospora gracilis*, *C. pseudopulchella*, *C. pulchella*, *L. grandispora*, *S. lignicola*, *T. radiata* and *Halenospora varia* are recorded for the first time in the State of Tabasco. However, these species have been previously reported in marine sandy beaches in Mexico (González *et al.*, 2001). Furthermore, *C. capillacea* was recorded for the first time in Mexico (Table 1, Figures 2–11). This uncommon ascomycete was described from subtidal wood in Martinique (French Antilles), and has been recorded occurring in intertidal driftwood from Taiwan, and from Indian beaches (Kohlmeyer, 1981; Nambiar & Raveendran, 2010; Pang & Jheng, 2012). The presence of this marine taxon in the beach might be due to the occurrence of the spores in water and wood remains originating from the adjacent mangrove forest of El Carmen lagoon.

A collection resembling *A. heteroguttulatus* was observed in the El Bosque beach, the nearest sampling site to the Grijalva/Usumacinta delta. This freshwater fungus was previously recorded as a dominant species from a heavily polluted urban freshwater lagoon located far from the Gulf coast (Rosique-Gil *et al.*, 2008). Nonetheless, the information about the viability and ecological function of freshwater ascomycetes in the marine ecosystems is still unclear.

Corollospora maritima was present in almost all of the beaches, having the highest abundance ($F = 40.0$). The presence of this species in nine of the 10 studied beaches, as well as its high frequency of occurrence, suggests that this species has a wide range of adaptation that permits it to inhabit and fulfil its ecological function in the studied beaches (González & Hanlin, 2010). Additionally, the ability of *C. maritima* to break down hydrocarbon compounds has granted tolerance to petroleum pollution, allowing this species to inhabit oil-contaminated beaches.
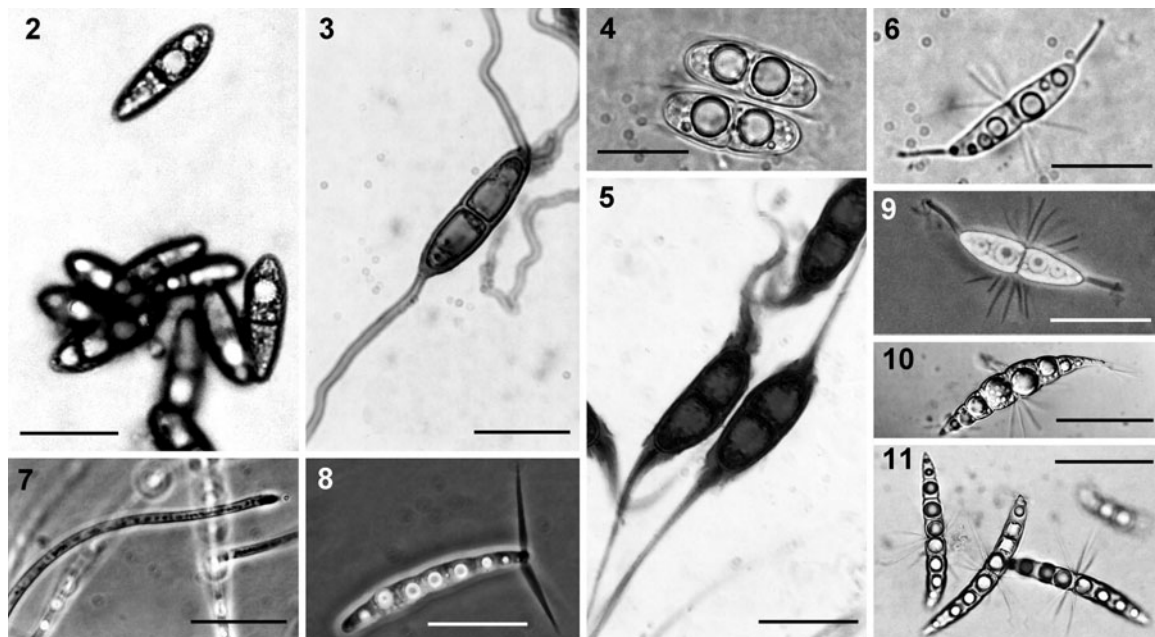
Figs 2–11. Ascospores of marine ascomycetes from the coast of Tabasco State, Mexico. (2) *Ascosacculus heteroguttulatus*. (3) *Ceriosporopsis capillacea*. (4, 5) *Ceriosporopsis halima*. (6) *Corollospora gracilis*. (7) *Lulworthia grandispora*. (8) *Torpedospora radiata*. (9) *Corollospora maritima*. (10) *Corollospora pulchella*. (11) *Corollospora pseudopulchella*. Figs 2, 3, 5, stained with gentian violet; 7, 8, 9 with dark field microscopy. Scale bars: 2, 30 μm; 3, 4, 5, 8 and 9, 20 μm; 6 = 25 μm, 7, 75 μm; 10 = 40 μm; 11 = 50 μm.

According to the first law of geography, the similarity between two observations often decreases as the distance between them increases (Tobler, 1970). However, our results showed that the geographic distance among the studied sites was not correlated to the species composition ($r = 0.1393$, $P = 0.2103$). This finding suggests that geographic proximity is not a strong factor shaping marine fungal communities in the coastline of Tabasco, and might be more related to the amount and variety of debris present in each site, since we observed that in beaches with greater quantity and heterogeneity of debris the fungal diversity was higher. Therefore, future work should focus on the importance of considering the debris type as a fundamental factor determining fungal diversity. Additionally, it has been reported that biotic factors (i.e. competition) play an important role in marine fungal communities (Jones, 2000). Hence, we tested the relationship between abundance and species composition in the studied sites. Our results showed that diversity and overall abundance were positively correlated ($r = 0.5909$, $P = 0.0002$), implying that the presence of some species inhibits the occurrence of others, thus influencing the overall abundance. Even though literature on antagonistic relationships of marine fungi is scarce, some marine species found in this study such as *A. trifurcatus* and *C. maritima* have been reported to have antifungal activity (Strongman et al., 1987; Panebianco et al., 2002). Additionally, our findings support the observations made by Miller (2000) demonstrating that antibiosis could be a major determinant of population structure of terrestrial fungi.

According to the qualitative examination of the textural parameters in the sand, there is no clear trend in the relationship between grain size and the diversity observed (Table 1). In the beaches with the lowest diversity values, the grain size composition varied, comprising mostly fine sand (Paraíso Beach) and medium sand (La Unión Beach and Mar de Plata Beach). In the sites with the highest diversity values, the grain size composition also differed, finding mostly coarse sand and medium sand (Sánchez Magallanes) and fine sand (El Bosque). Although grain-size parameters might give some indication of environmental conditions and transport dynamics, studies on macroinvertebrates have proved there is no significant effect of grain size on taxa microdistribution (Culp et al., 1983). It is possible that substrate composition is a major determinant of the diversity and distribution of marine fungi rather than textural parameters of the sand.

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