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# Targeted ITS1 sequencing unravels the mycodiversity of deep-sea sediments from the Gulf of Mexico

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## Summary

**Fungi from marine environments have been significantly less studied than terrestrial fungi. This study describes distribution patterns and associated habitat characteristics of the mycobiota of deep-sea sediments collected from the Mexican exclusive economic zone (EEZ) of the Gulf of Mexico (GoM), ranging between 1000 and > 3500 m depth. Internal Transcribed Spacer 1 (ITS1) amplicons were sequenced by Illumina MiSeq. From 29 stations sampled across three annual campaigns, a total of 4421 operational taxonomic units (OTUs) were obtained, indicating a high fungal richness. Most OTUs assignments corresponded to Ascomycota, unidentified fungi and Basidiomycota. The majority of the stations shared a mere 31 OTUs, including the worldwide reported genera *Penicillium*, *Rhodotorula* and *Cladosporium*. Both a transient and a conserved community were identified, suggesting their dependence on or adaptation to the habitat dynamics, respectively. The differences found in fungal richness and taxonomic compositions were correlated principally with latitude, carbon and carbonates content, and terrigenous content, which could be the potential drivers that delimit fungal distribution. This study represents an expansion of our current knowledge on the biogeography of the fungal community from deep-sea sediments, and identifies the geographic and physicochemical properties that delimit fungal composition and distribution in the GoM.**

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## Introduction

In marine environments, fungal organisms are represented by a wide diversity of lineages (Richards *et al*., 2012) that can be found on the surface, in the photic zone of the water column, in deep-sea sediments at 3000 m (Raghukumar, 2006), or in the deep-subsurface up to 2 Km below the sea floor (Edgcomb *et al*., 2011; Orsi *et al*., 2013a; Orsi *et al*., 2013b; Redou *et al*., 2014). Fungi play fundamental ecological roles in the ocean, including mutualistic relationships with other organisms (Wegley *et al*., 2007; Peixoto *et al*., 2017) and participating in biogeochemical processes (Edgcomb *et al*., 2011; Singh *et al*., 2012b). Understanding and studying the fungal diversity of marine sediments constitutes a great challenge because: (i) marine sediments occupy a much larger area (~65%) than terrestrial habitats, (ii) the habitat is highly inaccessible, (iii) DNA and RNA are recovered at low concentrations, (iv) there is a high level of cryptic diversity, and (v) fungal isolation leads to the recovery of morphologically similar organisms (Richards *et al*., 2012; Raghukumar, 2017a; Raghukumar, 2017b). Marine fungal diversity has been assessed in the Arabian Sea, the Central Indian Basin, the South China Sea, the Pacific Ocean, the Atlantic Ocean, the Canterbury Basin New Zealand, and the Gulf of Mexico (USA waters), among other ecosystems (Raghukumar and Raghukumar, 1998; Raghukumar *et al*., 2004; Damare *et al*., 2006; Lai *et al*., 2007; Burgaud *et al*., 2009; Le Calvez *et al*., 2009; Nagano *et al*., 2010; Singh *et al*., 2010; Edgcomb *et al*., 2011; Singh *et al*., 2011; Widger *et al*., 2011; Bik *et al*., 2012; Thaler *et al*., 2012; Redou *et al*., 2014; Xu *et al*., 2014; Redou *et al*., 2015; Zhang *et al*., 2015; Xu *et al*., 2016; Zhang *et al*., 2016; Nagano *et al*., 2017; Xu *et al*., 2018a; Xu *et al*., 2018b). In the studies above, fungal diversity was established by both cultivation-dependent and cultivation-independent methods (cloning/sequencing and target gene sequencing). However, all studies showed a great abundance of ascomycetes and

basidiomycetes, a prevalence of yeast forms, and a relatively high number of fungi with undetermined taxonomy. Only a few of these studies took into account the potential drivers of the distribution of marine fungi. In sediments from the high Arctic, the fungal diversity was mostly influenced by salinity, organic carbon, silicates and phosphates content (Zhang *et al*., 2015). The fungal community and activity from the subsurface sediments of the Peru margin was correlated with dissolved and total organic carbon, and sulphides (Orsi *et al*., 2013a). At a global scale the distribution of marine fungi has been correlated with temperature and salinity (Booth and Kenkel, 1986); in sediments it has been correlated with geographic (particularly depth) and environmental physicochemical characteristics (i.e. oxygen and nitrate content) (Tisthammer *et al*., 2016). Consequently, the distribution of marine fungi might depend on their interaction with multiple environmental factors. Nevertheless, there is no consensus yet on which drivers shape the fungal community composition and diversity from regional to global spatial scales. Therefore, a major effort in the characterization of the mycobiota in deep-sea sediments at regional scales is needed to decrease uncertainties in distribution models associated with these organisms.

The Gulf of Mexico (GoM) is divided into different areas, with the deepest sediments (at more than 3000 mbsl) found in the continental slope and the Sigsbee deep, which together represent 40% of the total area of the GoM (Lara-Lara *et al*., 2008). These areas receive a large amount of particles from the water column and the rivers that discharge into the GoM (Escobar Briones, 2004). In addition, the GoM is a natural source of minerals, oil and gas. These characteristics make the GoM a rich, dynamic ecosystem and an ideal region to study the spatio-temporal dynamics of fungi.

A handful of culture independent studies have evaluated the microbiota of coastal sediments from the United States waters of the GoM. The abundance of species such as *Candida dubliniensis*, *Lodderomyces elongisporus* and *Penicillium chrysogenum* as well as species belonging to the genera *Cladosporium* and *Alternaria* increased in areas impacted by oil spills in the U.S. (Widger *et al*., 2011; Bik *et al*., 2012). Their dominance in the microbial communities of contaminated shorelines suggests that fungi can thrive in these impacted environments and may play an important ecological role in hydrocarbon degradation. Therefore, there is a need to better characterize the fungal diversity and habitat in order to comprehend the functional role and community dynamics of fungi that have experienced disturbance events.

The development of cultivation independent molecular methods has enabled the characterization of microbial communities (including fungi) in marine systems, providing unprecedented insight into spatial distribution patterns, their functional role in ecosystems and their potential response to natural and anthropogenic disturbance.

As part of the Gulf of Mexico Research Consortium (CIGoM), in the present study we characterized the fungal community from deep-sea sediment samples. Three oceanographic campaigns named XIXIMI 4, 5 and 6, were conducted in the Mexican Exclusive Economic Zone (EEZ) of the GoM. The Internal Transcribed Spacer (ITS) 1 region was used as barcode and Illumina MiSeq as sequencing platform. In addition, we evaluated whether the geographic location and sediment physicochemical characteristics of the sampled stations influence the distribution of the characterized mycobiota.

## Results

### *The deep-sea sediments from the GoM harbour a high fungal richness*

A total of 8,563,339 raw sequences were obtained from Illumina sequencing. From those, 562,261 sequences corresponded to the controls included during the sampling campaigns and the experimental procedures and the remaining 8,001,078 sequences corresponded to the samples from the three campaigns. The XIXIMI 4 campaign contained the fewest raw sequences (2,435,016), followed by XIXIMI 5 with 2,717,128 sequences and XIXIMI 6 with 2,848,934 sequences. After processing the reads in the ITSx program to obtain the ITS 1 region and after applying quality control steps, a total of 2,584,642 sequences remained for subsequent analyses. The number of sequences per station ranged from a minimum of 5077 (A5.4) to a maximum of 229,518 (D26) (Table S1).

In total, 8949 OTUs were obtained at 97% similarity threshold. Singletons, OTUs more abundant in the controls than in the samples, and OTUs identified as *Neurospora crassa*, amounting to a total of 1293 OTUs, were deleted. A total of 7656 OTUs remained after all the above quality filters were applied. From the resulting OTUs, 4421 were identified as fungi and the remaining 3235 OTUs as Metazoa, Viridiplantae and No Blast Hit assignments. The stations A5.4 and B14 contained some of the lowest numbers of OTUs, while the stations C22.6, D26 and G44.6 contained the highest numbers of OTUs (Table 1).

### *Ascomycota and unidentified fungi dominate the seafloor of the GoM*

Overall, the taxonomically assigned OTUs from all the sediment samples belonged to six phyla, 25 classes, 51 orders, 98 families, and 148 genera. From the 4421 fungal OTUs analysed, 2872 (64.96%) correspond to Ascomycota, 638 (14.43%) to Basidiomycota, 17 (0.38%)

**Table 1.** Richness and diversity indexes.

| Campaign | Station | Number of sequences[a] | Observed number of OTUs[b] | Shannon Wienner |
|---|---|---|---|---|
| X4 | A1 | 32,630 | 286 | 2.41 |
| | A5 | 2851 | 93 | 2.77 |
| | A7 | 50,309 | 322 | 2.12 |
| | B14 | 3802 | 87 | 2.95 |
| | B18 | 8969 | 142 | 1.54 |
| | C22 | 36,667 | 231 | 1.84 |
| | E31 | 69,178 | 515 | 2.12 |
| | G44 | 51,644 | 332 | 2.25 |
| | H45 | 64,235 | 370 | 1.27 |
| | H47 | 23,784 | 242 | 1.83 |
| X5 | TS1 | 119,249 | 719 | 2.56 |
| | A3 | 21,118 | 183 | 0.87 |
| | A5 | 89,396 | 482 | 1.61 |
| | A8 | 10,087 | 164 | 2.28 |
| | B11 | 171,726 | 714 | 2.34 |
| | B15 | 62,699 | 414 | 2.49 |
| | B18 | 35,724 | 290 | 2.20 |
| | C22 | 55,329 | 451 | 1.92 |
| | D28 | 125,952 | 613 | 2.31 |
| | G44 | 78,528 | 484 | 2.21 |
| X6 | B12 | 120,400 | 555 | 1.99 |
| | B18 | 7203 | 141 | 2.56 |
| | C22 | 181,168 | 717 | 1.71 |
| | D26 | 218,599 | 861 | 2.19 |
| | D27 | 100,808 | 520 | 1.85 |
| | E33 | 72,014 | 491 | 1.93 |
| | G44 | 114,582 | 784 | 2.34 |
| | G44R | 117,525 | 606 | 2.20 |
| | H48 | 98,850 | 556 | 1.85 |

**a**. Number of sequences after quality control and identified as fungi.
**b**. Number of operational taxonomic units (OTUs) identified as fungi.

to Chytridiomycota, 37 (0.84%) to Glomeromycota, three (0.07%) to Mucoromycota, one (0.02%) to Neocallimastigomycota and 853 (19.29%) to unidentified fungi (Fig. S1A). Approximately 50% of the BLAST results from the unidentified fungi assignments revealed that the highest hit similarity corresponds to sequences belonging to studies from marine sediments. The Ascomycota phylum and unidentified fungi were the most abundant in the majority of the stations, and the Basidiomycota was the most abundant phylum in some sub-samples of a few stations such as A5.5, C22.5, and D28 from the abyssal plain (AP), and B11 from the Tamaulipas-Veracruz continental slope (TVCS), with abundances between ~50% and 90% (Fig. 1A and B; Fig. S1).

Some fungal groups were identified in all stations. The Eurotiales, Saccharomycetales and Capnodiales were the most abundant orders and were present in all stations (Fig. 1A). The unidentified fungi presented a greater abundance in stations from the TVCS (A1 and D26), the Yucatan continental slope (YCS; B18.A) and the AP (A8, A5.4, B14); the Eurotiales order presented a high abundance in stations from AP (C22.6 and E33), the TVCS (D27), Campeche canyon (CC; G44.6 and H48) and Campeche saline canyon (CSC; H47); the Saccharomycetales abundance was higher in the AP and TVCS stations (B12 and E31 respectively); and the Capnodiales were the dominant order in AP (A3) and Coatzacoalcos canyon (CZC; H45) (Fig. 1A).

Stations from the AP were found clustering with stations from other regions (dendrogram in Fig. 1B), while TVCS stations clustered only with stations from AP and CC. This organization seems to be influenced by the most abundant groups, including unidentified fungi, Dothideomycetes, Eurotiomycetes, Saccharomycetes and Microbotryomycetes (Fig. 1B).

### *Temporal variation in fungal community composition*

Differences in abundance and composition were found among the same stations sampled in different campaigns. A5.4 harboured primarily members of Ascomycota as well as unidentified fungi, while members of the Basidiomycota predominated in the following year (A5.5). For B18, unidentified fungi dominated in all three campaigns. However, B18.6 presented a greater abundance of Eurotiomycetes and Dothideomycetes than B18.4 and B18.5, while B18.5 had a greater abundance of Saccharomycetes, than B18.4 and B18.6 (Fig. 1A and B; Table S2). Samples C22.4, C22.5 and C22.6 differed mostly in the abundance of the order Eurotiales. Among samples G44.4, G44.5, G44.6 small differences were found in terms of unidentified fungi, but the composition of other fungal groups largely differed; for instance, the Sordariomycetes was the most abundant class in G44.4, while the Tremellomycetes was the most abundant in G44.5. Replicate samples G44.6 and G44R.6 differed in terms of composition; while G44.6 was dominated by Eurotiomycetes, Sordariomycetes and Cystobasidiomycetes, G44R.6 was dominated by Dothideomycetes and Eurotiomycetes (Fig. 1A and B).

### *Deep-sea sediments harbour both, a transient and a conserved OTU composition*

Network analysis displayed how the OTUs are partitioned across stations (Fig. 2A); this analysis revealed that just nine OTUs were common in all the 29 stations, while there are some specific OTUs (i.e. from 1 to 5) shared among less than 28 stations.

Most stations were compositionally distinct. The stations that shared the largest number of OTUs were B12 and D26, followed by A3 and H45, A5.5 and B11, and TS1 and E31 (Fig. 2A). Stations D26, G44.6 and E31 showed the largest number of unique OTUs, while A5.4, B14 and B18.4 showed the lowest number of unique OTUs (Fig. 2A).

The highest Shannon Wiener diversity index was found for B14 and A5.4 stations, while the lowest values were obtained for A3 and H45 stations (Table 1). Differences in the observed richness were found among the same

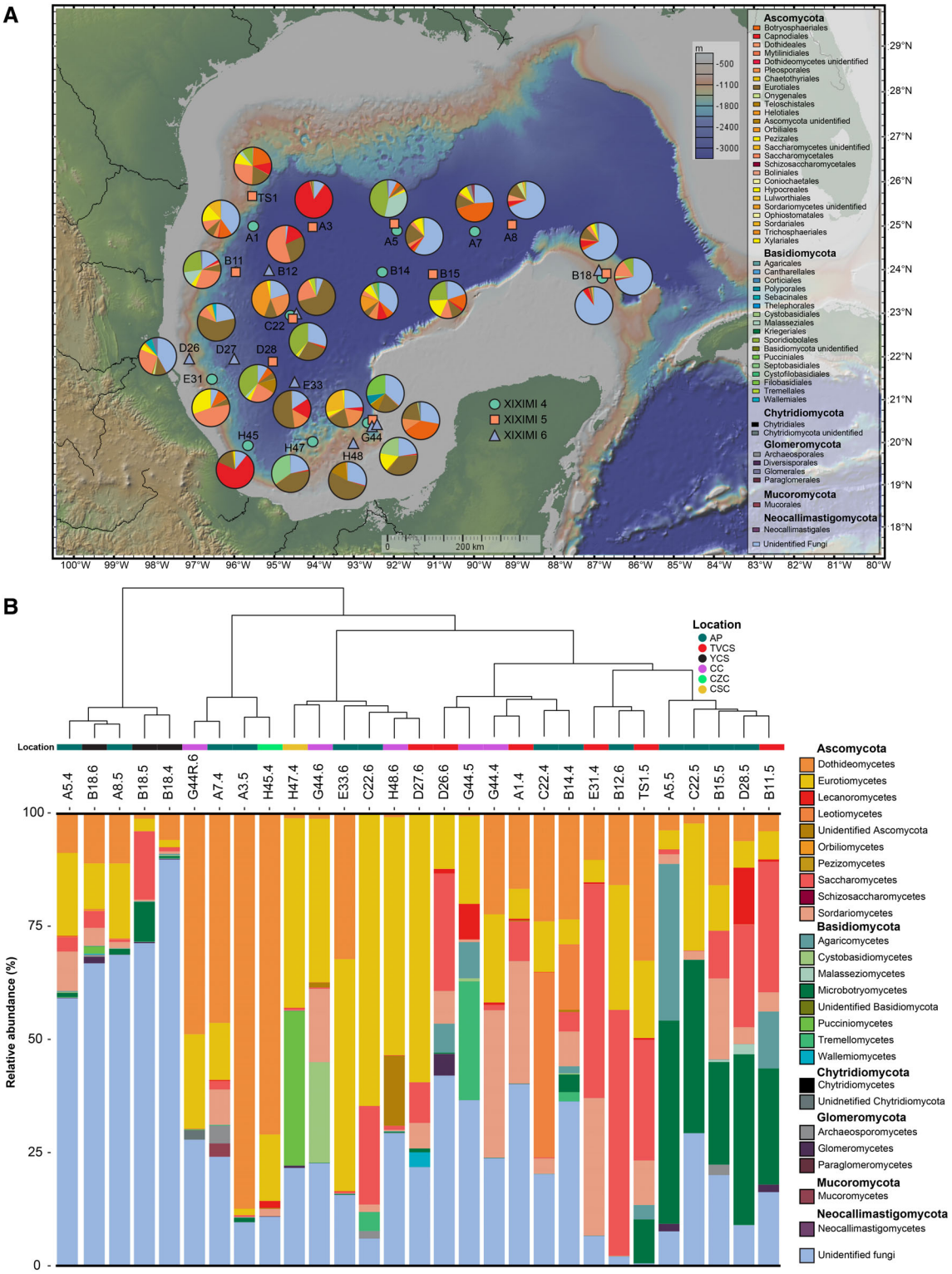


**Fig. 1.** Taxonomic profiles of fungi among sampling stations in the Gulf of Mexico.
A. Distribution map of the relative abundance at order level among the sampling stations.
B. Hierarchical clustering represented as a dendrogram coupled to a bar plot of relative abundance at the class level among the sampling stations. The dendrogram was calculated using Euclidean distance and Ward clustering.

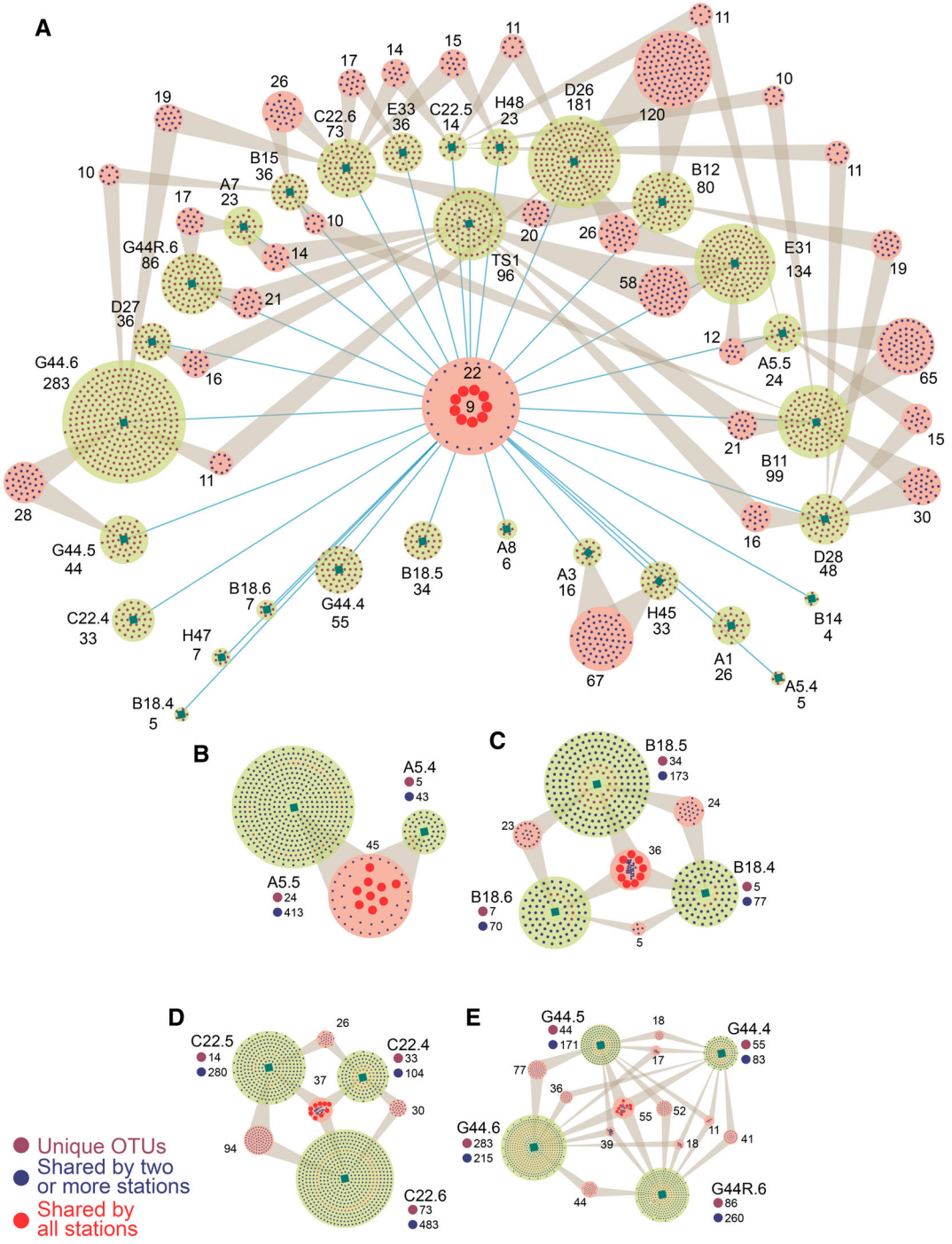


**Fig. 2.** Cytoscape OTUs network illustrating the 4421 fungal OTUs.
A. OTUs network highlighting the number of unique and shared OTUs among stations.
B. Shared OTUs between the A5 station sampled in XIXIMI 4 and XIXIMI 5.
C. Shared OTUs in the station B18 sampled in XIXIMI 4, 5 and 6.
D. Shared OTUs in the station C22 sampled in XIXIMI 4, 5 and 6.
E. Shared OTUs in the station G44 sampled in XIXIMI 4, 5 and 6; and shared OTUs between G44 and replica G44R. In the B, C, D and E panels, the purple circles represent the unique OTUs in each station, while the blue circles represent the OTUs that each station shared with other stations. The red circles indicate the 9 OTUs depicted in A shared by all stations. Edges with less than 10 shared OTUs were discarded.

stations sampled in different campaigns (Table 1). Those differences were more evident in A5.A, C22.A and G44. A, where the number of OTUs differed in more than three-fold across years (Table 1; Fig. 2B, D, E). Across the different campaigns, A5.A and G44.A shared more OTUs than B18.A and C22.A (Fig. 2B–E).

*Alpha diversity is correlated with longitude and sediment physicochemical properties*

Significant correlations were found among the number of observed OTUs with longitude, water content, carbonate, nitrogen and terrigenous content (Table S3; $P < 0.05$). It is worth noting that despite the significant relationships, the explained variance ranged between $R^2 = 0.13$ and 0.30. No significant correlations were found with Shannon diversity index (Table S3).

*The geographic characteristics influenced the fungal community structure*

The NMDS analysis (stress = 0.1358) did not reveal an obvious clustering of stations (Fig. 3A). Similarly, additional NMDS analyses arranging stations by categorical groups indicated no significant correlations between fungal community composition and regions, latitude, longitude or depth (Fig. S2B–D; Table 2; Table S4).

However, when analysing the variance of OTU composition of stations arranged by categorical groups, the beta dispersion analysis indicated significant differences by region, latitude and longitude ($P < 0.05$; Fig. 3B–D; Table 2). Additionally, a Pairwise analysis indicated significant differences within each categorical group: stations from the TVCS differed from those of the YCS ($P < 0.05$; Fig. 3B; Table S5), latitude 21 stations differed from latitude 25 stations ($P < 0.05$; Fig. 3C, Table S5), and longitude 273 stations differed from those of longitude 264, 265 and 267 ($P < 0.05$; Fig. 3D, Table S5). The beta dispersion analysis did not show significant differences in the variance of OTU composition of stations categorized by depth ($P > 0.05$, Fig. S2D; Table 2).

NMDS and beta dispersion analyses indicated an effect of the type of corer (box corer or multicorer) in the OTU composition (Fig. 2E, G). However, the corer type did not have any effect on the observed OTUs composition or on their correlation with the different sediment physicochemical properties when the stations were analysed separately according to the corer (Table S6).

The effect of the geographical location (latitude and longitude) on the OTU composition among stations was further tested with a distance decay analysis. A significance distance decay relationship was found for the fungal community composition along the geographical distance (Mantel r = 0.1308, $P < 0.05$; Fig. 4).

*Carbon, carbonate and terrigenous content influence the fungal community in the GoM*

For the canonical correspondence analysis (CCA), a variable selection was made by the Akaike information criterion (AIC), where the lowest AIC values were for a model that included only carbon, carbonates and terrigenous content (Table S7). The model with the selected variables was statistically significant ($P < 0.05$; Fig. 5). The final CCA model accounted for a total inertia equalling 7.92 in community and environment associations, and the environmental variables explain a total of 1.21 of the constrained inertia, representing 15.3% of the total variability.

## Discussion

The major aim of this study was to explore the fungal diversity from deep-sea sediments of the GoM, and identify potential drivers shaping the fungal community structure. This study describes the previously unknown fungal community of the GoM sediments at a large-scale, allowing the identification of differences of the mycobiota across a wide range of geographic locations with different sediment characteristics and depths. A diverse OTU composition (4420 OTUs) was detected by Illumina sequencing. The taxonomic composition of the deep-sea sediments comprises, in its majority, fungi from the Dikarya and unidentified fungi, and in its minority, fungi from basal lineages. These results could be associated to the set of primers used, which preferentially amplify Ascomycota and Basidiomycota. Carbon, carbonate and terrigenous content were identified as the most important physicochemical properties influencing the distribution of the fungal communities in the GoM. Surprisingly, no effect or correlation was found between fungal community and depth. Furthermore, the geographic location of the sampling stations suggests a latitudinal effect on the fungal community composition.

The Mexican EEZ represents 55% (90 million ha.) of the total area of the GoM. The XIXIMI campaigns tried to cover most of the deep waters of this area by designing a course that included launching the corer in different zones with depth and ecological variability. Only a handful of studies on deep-sea sediments highlight the fungal community composition and their correlation with geographic location and habitat characteristics (Zhang *et al*., 2015). However, those studies cover small areas (<10,000 ha.), where the samples were taken near each other, ultimately hindering further analyses towards understanding whether the differences in fungal community could be correlated with the geography or the sediment physicochemical properties. This study represents an effort to understand the fungal diversity in a complex habitat, where the geographic, physical and chemical properties delimit their composition and distribution.

The 4421 fungal OTUs obtained from the 29 samples of the deep-sea sediments from the GoM represent a high recovery rate of OTUs and suggest that the deep-sea sediments from the GoM harbour an extraordinarily high fungal richness. In another study, where the same primers set and sequencing platform were used, the

 

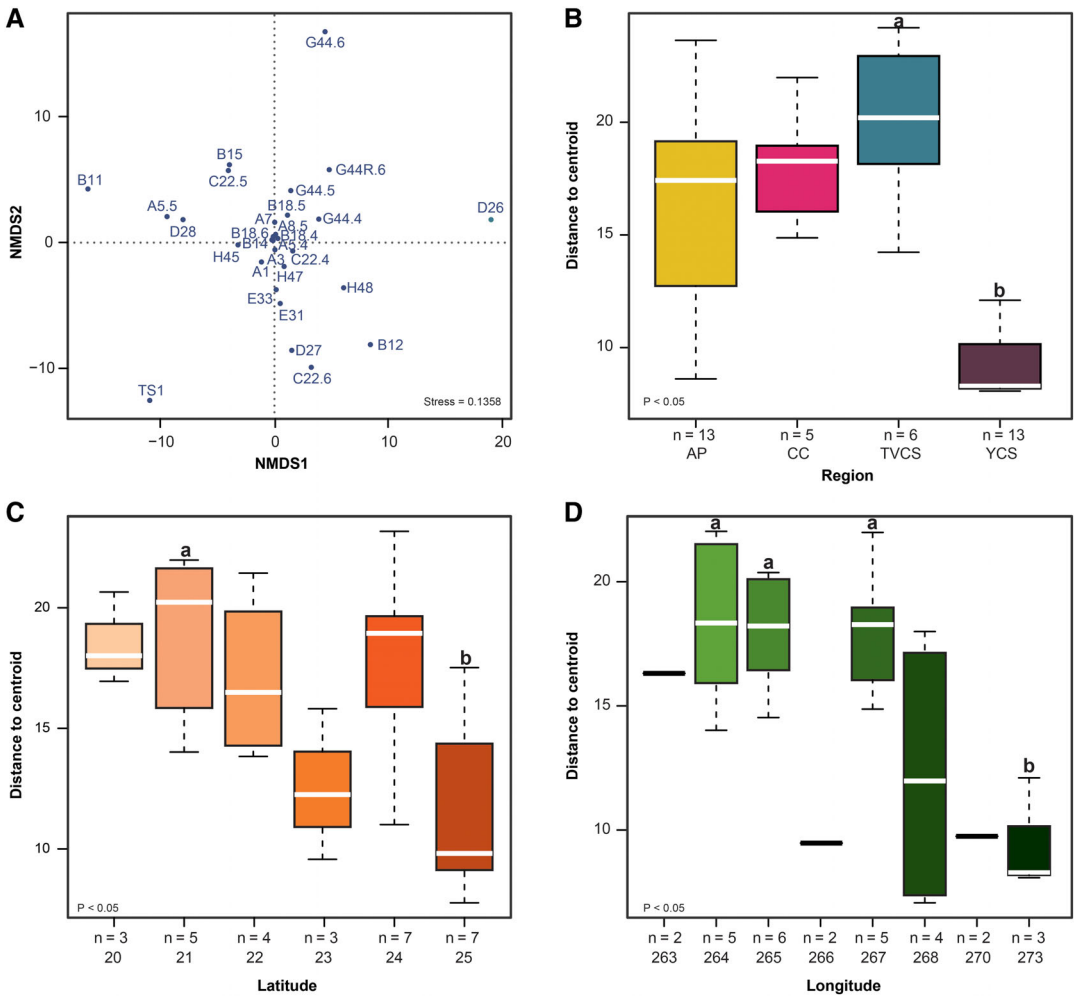


**Fig. 3.** Non-metric Multidimensional Scaling (NMDS) ordination of the similarities in fungal composition and beta dispersion across stations.
A. NMDS, based on Euclidean distance matrix.
B. Box plot of multivariate dispersions (beta dispersion) of stations similarities grouped by region.
C. Latitude and (C) longitude. NMDS and beta dispersion significant values refer to pairwise analysis after P-adjusted values by Bonferroni correction, to test differences in community composition among the different station characteristics.

number of OTUs identified as fungi were relatively low: 420 OTUs from four samples in Okinawua in Japan (Zhang *et al*., 2016). In other studies, where a different set of universal primers and sequencing platforms were used, the number of recovered OTUs were 113 OTUs from samples in the High Arctic (Zhang *et al*., 2015) and 1752 OTUs in 130 samples from six European sites (Richards *et al*., 2015). Although it is difficult to make comparisons with the results obtained in those studies, given the differences in sequencing platforms, primers, and data treatments, our results provide a new data set of the fungal community from the GoM at a large spatial scale.

The above comparisons stress that a better-standardized analysis on the course of sampling design and the samples processing among studies is needed. It is difficult to draw comparisons among studies of marine fungi in deep-sea sediments due to the differences in the approach used to assess their diversity. The majority of the studies use culture-dependent methods (Takami, 1999; Raghukumar *et al*., 2004; Damare *et al*., 2006; Burgaud *et al*., 2009; Jebaraj *et al*., 2010; Redou *et al*., 2015), clone libraries (Nagano *et al*., 2010; Singh *et al*., 2011, 2012a; Xu *et al*., 2014), or a combination of both techniques (Singh *et al*., 2010; Singh *et al*., 2012b; Zhang *et al*., 2016). Moreover, just a few of them use high throughput sequencing, previously via 454 pyrosequencing and currently with Illumina platforms (Redou *et al*., 2014; Richards *et al*., 2015; Zhang *et al*., 2015; Zhang *et al*., 2016). The increased use of high throughput sequencing to analyse marine fungal communities from deep-sea sediments is notably extending our current knowledge on the marine mycobiota.

The taxonomic assignment of the 31 OTUs shared in the majority of the stations (which represented only 0.70% of the 4421 OTUs) revealed they correspond to

**Table 2.** PERMANOVA analysis on NMDS ordination and ANOVA analysis on beta dispersion.

| PERMANOVA | Df | Sums of Sqs | Mean Sqs | F. Model | $R^2$ | *P* (>F) | *P* adjusted |
|---|---|---|---|---|---|---|---|
| Latitude | 6 | 5 | 1864.6 | 372.93 | 1.0985 | 0.19277 | *P* > 0.05 |
| Residuals | 22 | 23 | 7808.1 | 339.48 | 0.80723 | | |
| Total | 28 | 28 | 9672.7 | | 1 | | |
| Longitude | 9 | 7 | 2513 | 358.99 | 1.053 | 0.2598 | *P* > 0.05 |
| Residuals | 19 | 21 | 7159.7 | 340.94 | 0.7402 | | |
| Total | 28 | 28 | 9672.7 | | 1 | | |
| Depth | 3 | 1002.3 | 334.11 | 0.96338 | 0.10363 | 0.664 | *P* > 0.05 |
| Residuals | 25 | 8670.4 | 346.81 | | 0.89637 | | |
| Total | 28 | 9672.7 | | | 1 | | |
| Region | 5 | 1682.1 | 336.42 | 0.96835 | 0.1739 | 0.546 | *P* > 0.05 |
| Residuals | 23 | 7990.6 | 347.42 | | 0.8261 | | |
| Total | 28 | 9672.7 | | | 1 | | |
| Core | 1 | 633.6 | 633.58 | 1.8925 | 0.0655 | 0.001 | 0.024[a] |
| Residuals | 27 | 9039.1 | 334.78 | | 0.9345 | | |
| Total | 28 | 9672.7 | | | 1 | | |
| ANOVA | Df | Sums of Sqs | Mean Sqs | F. Model | | *P* (>F) | *P* adjusted |
| Latitude | 5 | 246.15 | 49.23 | 3.9103 | | 0.01035 | 0.0206[a] |
| Residuals | 23 | 289.56 | 12.59 | | | | |
| Longitude | 7 | 396.02 | 56.574 | 5.5781 | | 0.000977 | 0.0003[a] |
| Residuals | 21 | 212.98 | 10.142 | | | | |
| Depth | 3 | 49.71 | 16.571 | 0.717 | | 0.5511 | 1 |
| Residuals | 25 | 577.75 | 23.11 | | | | |
| Region | 3 | 233.52 | 77.841 | 4.5741 | | 0.01182 | 0.0236[a] |
| Residuals | 23 | 391.41 | 17.018 | | | | |
| Core | 1 | 102.71 | 102.707 | 4.9414 | | 0.03479 | 0.0695 |
| Residuals | 27 | 561.2 | 20.785 | | | | |

**a**. Significant values after Bonferroni correction.

the genera *Penicillium*, *Rhodotorula*, *Cladosporium*, *Aspergillus*, *Meyerozyma*, *Schizophyllum*, *Trichoderma*, *Alternaria*, *Clavispora* and *Candida*. The first five genera have been found distributed in other deep-sea sediments worldwide and at different depths, and are considered ubiquitous in this habitat (Roth *et al*., 1964; Takami, 1999; Nagano *et al*., 2010; Nagahama *et al*., 2011; Singh *et al*., 2011; Zhang *et al*., 2014; Redou *et al*., 2015; Xu *et al*., 2018a; Xu *et al*., 2018b). Some of them, such as *Penicillium* and *Rhodotorula*, have been even found in the Mariana Trench, classified as the deepest zone of the ocean (Takami *et al*., 1997; Nagano *et al*., 2010). Furthermore, the OTU network showed the unique OTUs present in each station. These unique OTUs may represent the community with the highest turnover rate, that is, a rapidly changing community fully dependent on the physicochemical properties unique to that location. In contrast, the shared OTUs represent a more stable fungal community well adapted to the habitat dynamics. Also, these OTUs might reflect the divergence among all the stations, and the genetic variability in the ITS 1 region sequences for the same taxonomic assignment.

The presence of hydrocarbonoclastic microbes in the GoM is expected given the presence of natural hydrocarbon seeps in the northern (U.S.) and southern (Mexico) regions (MacDonald *et al*., 1989; MacDonald *et al*., 2004;

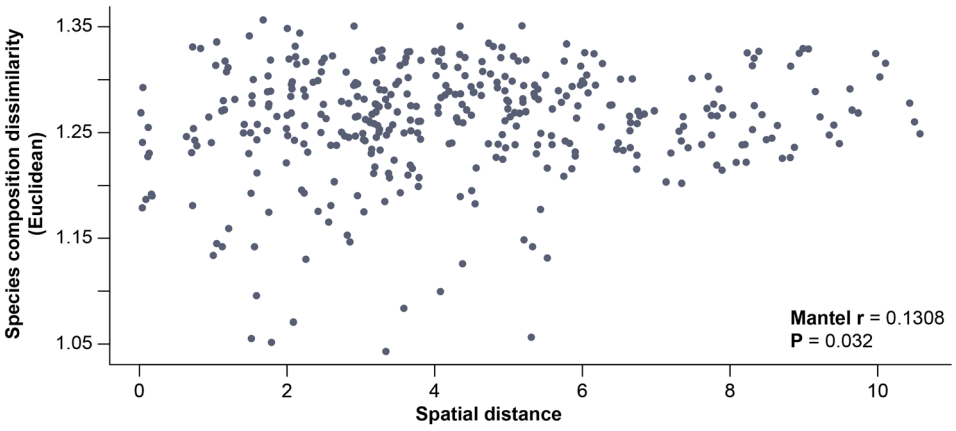


**Fig. 4.** Distance-decay for fungal community along environmental distance. Relationship between geographic distance and fungal community distance based on Euclidean dissimilarity.

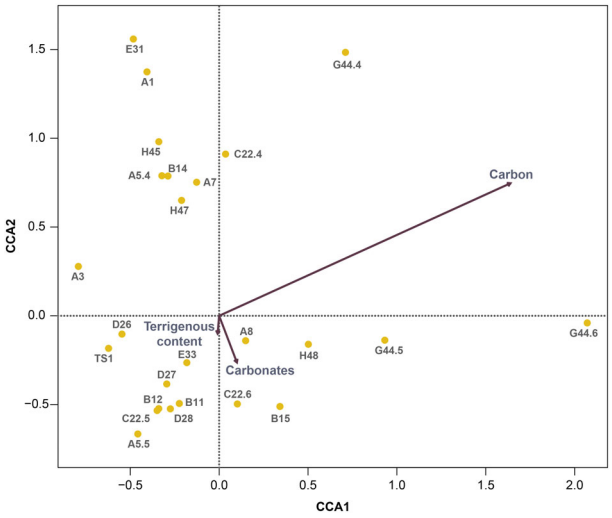


**Fig. 5.** Canonical correspondence analysis (CCA) showing the relationship between sediment characteristics and fungal community composition. Ordination diagram of the fungal community of stations together with sediment physicochemical characteristics: carbon, carbonate and terrigenous content. Direction and length of arrows show the degree of correlation between fungal community composition and the variables.

Canet *et al*., 2006; Binniger and Allen, 2014). Some fungal genera reported in the present study, such as *Candida*, *Penicillium*, *Yarrowia*, *Pichia* and *Aspergillus* have been described as capable of assimilating hydrocarbons (Polman *et al*., 1994; Chrzanowski *et al*., 2005; Kutty and Philip, 2008; Gofar, 2011). In parallel studies, we have isolated some of these fungal genera and are testing their capacity to degrade hydrocarbons.

The different analyses tested in this study suggest an influence of the geographic location of the stations on the fungal community. The distance decay analysis indicated increasing dissimilarity of fungal community composition as the distance between stations became larger (Fig. 4). This is easily observed when comparing the taxonomic composition of stations from latitude 25 (belonging to AP) with stations from latitude 21 (belonging to CSC and TVCS): i.e. the abundance of fungal orders such as the Eurotiales is larger in latitude 21 than in latitude 25, and the abundance of unidentified fungi is larger in latitude 25 than in latitude 21 (Fig. 1A). In terrestrial ecosystems it has been shown that there is a great variability among samples separated by short distances (Vargas-Gastélum *et al*., 2015), and the same can occur in marine sediments, due to the vertical and horizontal heterogeneity across the sediments. This short distance variability was observed in G44.6 and the replicate G44R.6 (sampled at the same geographic location during the same campaign). These spatial differences were also found among sub-samples from the same station, with changes in abundances of different fungal genera within the same core, where only 1 or 2 cm separates the sub-samples (Fig. S1). Micro-spatial distribution has been attributed to biotic (competition and mutualism) rather than abiotic interactions (Langenheder and Lindstrom, 2019). At large spatial scales, abiotic characteristics of the environment and dispersal limitation might be influencing the distribution of fungal communities in marine sediments from GoM. The composition of fungal communities can fluctuate depending on the fungal groups and the variability across the landscape (Tian *et al*., 2018). It has been suggested that the biogeography of microorganisms in deep-sea sediments can be influenced by physical processes such as deep-ocean circulation or biotic processes such as bioturbation by macrofauna (Orcutt *et al*., 2011), and at the same time, regulating the fungal dispersion in this environment. Common stations sampled in different years, were compared to analyse whether fungal communities are temporally structured. The OTU network analyses indicated that G44.A shared a larger number of OTUs than A5.A, C22.A or B18.A. Stations A5.5 and B11 shared more OTUs with other stations (outside the common group) than with the corresponding stations sampled in different years (65 OTUs). Variability among common stations was also observed in terms of the taxonomic composition and abundance of classes and orders for G44.A, A5.A and C22.A, while B18.A stations had a similar composition and abundance of unidentified fungi. These results might suggest variability among the stations across years. However, the complexity of the sampling makes it difficult to analyse these temporal variations, and results should be interpreted carefully. Due to ocean currents, it is practically impossible to sample the same exact geographic location twice, therefore some spatial variance was unavoidable within and across campaigns. Therefore, it is hard to interpret whether the differences observed in common stations across years are due to a real temporal variation of the fungal community, small-scale spatial variability, or both.

The Ascomycota is commonly reported as the most abundant phylum in deep-sea sediments (Zhang *et al*., 2016; Barone *et al*., 2018), and this agrees with the results obtained in the present study. However, a large abundance of unidentified fungi was also obtained. A recent study in the Mediterranean sea reported a total of 1742 fungal OTUs, recognizing the most abundant group as unidentified fungi (Barone *et al*., 2018). The continuous detection of these unidentified fungi suggest that deep-sea sediments harbour a largely unknown fungal community, which could include indigenous fungi and species of potential biotechnological importance (Zhang *et al*., 2015; Barone *et al*., 2018). On the other hand, the high recovery rate of unidentified fungi, may reflect the insufficient coverage of ITS sequences in databases (Khomich *et al*., 2017). Taxonomic groups other than

Ascomycota and Basidiomycota were also present, but in low abundance. This low detection of Chytridiomycota, Glomeromycota, Mucoromycota and Neocallimastigomycota could be the result of the bias from the primers used for the amplification of the ITS1 region (Tedersoo *et al*., 2015). The universal primers ITS1F and ITS2 are more likely to amplify ITS1 regions from Ascomycota and Basidiomycota, rather than other fungal groups (Op De Beeck *et al*., 2014; Amend *et al*., 2019). This bias is probably enriching the Dikarya group in databases and sequencing libraries and masking the presence of other fungal groups. Using multiple or group-specific primers might overcome this artefact (Singh *et al*., 2012a).

From the groups detected, *Malassezia* is considered ubiquitous in deep-sea sediments (Raghukumar, 2017a), but in the present study it was detected in low abundance (less than 1% per station) in only 10 stations, the majority of them sampled in the XIXIMI 5 and 6 campaigns. Among all the genera found, the OTUs related to the novel fungal DFS Group 1 was detected only in 5 stations (B14, C22.5, C22.6, D26, and G44.4), representing an abundance of ~0.7% among all the stations. This group was first described by Nagano *et al*. (2010) from deep-sea sediments from Japanese islands, and is closely related to the species *Metschnikowia bicuspidate*, which is parasitic on planktonic organisms in deep-sea environments. The DSF Group 1 has been reported in deep-sea sediments collected from methane cold-seeps of Japan Sagami-Bay (Nagahama *et al*., 2011), methane seeps of the GoM (American waters) (Thaler *et al*., 2012), the Mariana Trench of the Pacific Ocean (Xu *et al*., 2014) and the Chinese Seas (Li *et al*., 2016). Generally, this group is found in oxygen-depleted deep-sea sediments. The Neocallimastigomycota phylum was found also in low abundance (~0.0002%) in A7 and B11 stations. The members of this taxonomic group are identified as obligate anaerobes (Raghukumar, 2017a). Only the first 10 cm of the sediments are thought to be oxygenic. Therefore, these groups—indicators of anoxic habitats—would be expectedly more abundant in deeper sediment layers. The low abundance encountered of these groups in top layers of the sediments might be caused by a mixing of the layers during sampling.

The vast majority of the identified OTUs in all stations are fungi described from terrestrial environments, demonstrating the capacity of these organisms to adapt to this habitat and to extreme conditions (Lai *et al*., 2007). The input of sediments from diverse terrestrial origins may contribute to enlarge the diversity of fungi in marine sediments (Takishita *et al*., 2006). For instance rivers are some of the largest sources of organic matter and terrestrial organisms (Li *et al*., 2016). The Mexican EEZ from the GoM receives sediments from at least six rivers, which could help to increase the input of terrestrial organisms, including fungi. The CCA analysis indicated that terrigenous content has an effect in the fungal community from the analysed sediments. This sediment characteristic represents an important input of organic material from river discharge to the GoM (Díaz-Asencio *et al*., 2019). The capacity of adaptation of these organisms to extreme habitats may provide a fitness advantage.

In addition, it is important to consider that some of the terrestrial fungi identified in the marine sediment samples could come from other sources (Nagano *et al*., 2010). It is rather difficult to detect and confirm potential contaminations in this type of sampling. Commonly, culture-dependent and culture-independent reports do not include whether controls were used during the manipulation of the samples. In this study, we used different controls during material preparation for the sampling and during the samples processing: syringe preparation, sucrose buffer preparation, sterile sucrose buffer, control in research vessel where the samples were prepared for storage, DNA extraction, and the PCR blank. The main genera identified in the negative controls were *Cladosporium*, *Phenoliferia*, *Aspergillus*, *Rhodotorula*, *Candida*, *Pichia*, *Trichoderma* and *Neurospora*. While these genera can be commonly found in marine and terrestrial habitats (Nguyen *et al*., 2015), they were removed from the samples when identified as contaminants after applying our quality filters. It is important to clarify that the detection of fungi from terrestrial environments can also be influenced by the enrichment of terrestrial fungal sequences in the available databases, which may have an influence in the taxonomic assignment. Currently, there is not an ITS database that comprises sufficient marine fungal data to improve the taxonomic assignment. A larger effort is needed to enrich the databases of fungi from marine environments.

Surprisingly, the canonical correspondence analysis revealed that no significant correlation was found between fungal composition and depth, contradicting others studies where the fungal community composition and abundance changes with depth (Roth *et al*., 1964; Gong *et al*., 2015; Zhang *et al*., 2015). But the absence of correlation agreed with other studies carried out in Japanese islands (Nagano *et al*., 2010), where no correlation was found with depth, but a significant correlation was found between the fungal community composition, carbon, carbonate and terrigenous content. These correlations suggest that the carbon component of the sediments (carbon and carbonate content) is a limiting resource for the fungal community. The carbonate content is important since fungi participate in the degradation of calcareous material (Gleason *et al*., 2017).

We explored the fungal diversity of the EEZ from the GoM and found an unexpectedly high fungal diversity, in comparison with other studies in deep-sea sediments worldwide. This suggests that the GoM may serve as a

big reservoir for marine fungi, as well as terrestrial fungi with adaptative mechanisms, which remain poorly studied. Further investigation of the diversity and activity of fungi would be necessary to uncover the ecology of these organisms in this extreme habitat.

## Experimental procedures

### *Sampling strategy and sediment collection*

Three sampling campaigns named XIXIMI 4, 5 and 6 (*xiximi*: traditional Nahuatl for '*spill*'), were conducted between June and September (Summer season) of 2015, 2016 and 2017, respectively, in the Mexican EEZ of the Gulf of Mexico. All campaigns were operated on board of the Research vessel *Justo Sierra* from UNAM (Universidad Nacional Autónoma de México).

A total of 29 stations (10 for XIXIMI 4, 10 for XIXIMI 5, and 9 for XIXIMI 6), including depths ranging from 966 to 3734 m, were sampled (Table S8). These stations belonged to different regions of the Gulf of Mexico: Abyssal plain, Yucatan continental slope, Tamaulipas-Veracruz continental slope, Campeche saline canyon and Coatzacoalcos canyon (Fig. S3).

The sediment samples were obtained using a multicorer or a box corer (Table S8). The multicorer was composed of eight corers, each of 9.5 cm in diameter. When a box corer was used, cylindrical corers (same size as the corers in the multicorer) were placed inside the box to follow the same sampling procedure (Fig. S4). To detect if temporal changes occur in the fungal community, selected stations were sampled in three (G44, C22 and B18) or two campaigns (A5). Stations were named by adding 0.4, 0.5 and 0.6 to designate whether they were sampled during the XIXIMI 4, 5 and 6 campaigns, respectively. When referring to stations sampled in all three campaigns, a letter A following the station was included. During the XIXIMI 6 campaign a second lance was carried out in station G44 to test for within sample replicate consistency (the replicate station was named G44R).

At each station, five sub-samples were obtained with 10 ml syringes. The sediment was carefully transferred to 15 ml tubes containing 5 ml of sucrose buffer 25% wt/vol (Mitchell and Takacs-Vesbach, 2008), and stored at −20°C until processing.

### *Sediment sample processing, DNA extraction and sequencing*

Before DNA extraction, samples were centrifuged (Eppendorf 5415D centrifuge, Hamburg, Germany) at 13,000 r.p.m for 1 min to precipitate the sample and discard the sucrose buffer. From each station, two of five sub-samples were stored at −80°C, and three sub-samples were selected and lyophilized for 2 days with a FreeZone 2.5 L Benchtop Freeze Dryer (Labconco Corporation, Kansas City, MO, USA). The genomic DNA was extracted from 0.15 g of sediment using a modified protocol of the Power Soil DNA Extraction Kit® (Mo Bio Laboratories Inc., Carlsbad, CA, USA), which included the incubation of the sediment for 2 min with 100 µl of phenol:chloroform:isoamyl alcohol, followed by 30 s vortex. DNA concentration was measured in a Qubit 4 Fluorometer using a high sensitivity dsDNA Qubit Kit (Thermo Fisher Scientific Inc., Waltham, MA, USA).

The ITS 1 region was selected as fungal barcode because it has higher sequence variability than the ITS 2 region (Monard *et al*., 2013; Wang *et al*., 2015), and this variability allows a better resolution of fungal groups. Additionally, since the ITS 1 region is more widely used to study fungal diversity, a higher amount of sequences for this region can be found in databases.

A two-step PCR approach was used in order to obtain the amplicon libraries. The first PCR to amplify the ITS 1 region was performed using a touchdown annealing approach and primers ITS1-F (5´-CTTGGTCA TTTAGAGGAAGTAA-3′; Gardes and Bruns, 1993) and ITS2 (5´-GCTGCGTTCTTCATCGATGC-3′; White *et al*., 1990) fused to corresponding sequencing primers, which allowed the attachment of the indexes in the second PCR. The thermal cycling conditions were as follows: an initial denaturation step at 95°C for 10 min, followed by 10 cycles of 94°C for 45 s, 65°C–55°C for 45 s (the annealing temperature decrease 1°C each cycle) and 72°C for 75 s, followed by 27 cycles of 95°C for 45 s, 58°C for 45 s and 72°C for 30 s, and a final extension step at 72°C for 10 min. Each PCR reaction (50 µl final volume) contained 10 ng of genomic DNA, 0.3 µM of each primer, 0.8 mM nucleotide mix, 3 mM $MgCl_2$, 1X Colourless GoTaq® Reaction Buffer, 0.03 u GoTaq® DNA Polymerase (Promega, Radnor, PA, USA), 0.8 mg $\mu L^{-1}$ of BSA and sterile HPLC water. The resulting PCR products were purified with Just-a-plate™ 96 PCR purification kit (Charm Biotech San Diego, CA, USA) and verified with 1% agarose gel. The cleaned PCR products were submitted to a second PCR for index and adapters attachment. The second PCR consisted in an initial denaturation step of 95°C for 3 min, followed by 8 cycles of 95°C for 30 s, 61°C for 30 s and 72°C for 30 s, a final extension step at 72°C for 5 min. Each reaction (25 µl final volume) contained 1 µl of purified product from the first PCR, 0.6 µl of each 10 µM index, 2.4 µl of 10 mM nucleotide mix, 3.6 µl of 25 mM $MgCl_2$, 6 µl of 5X Colourless GoTaq® Reaction Buffer, 0.25 µl of 5u $\mu L^{-1}$ GoTaq® DNA Polymerase (Promega, Radnor, PA, USA), 2.4 µl of 10 mg $\mu L^{-1}$ of BSA and sterile HPLC water. The resulting PCR products were purified and normalized with SequalPrep™ Normalization Plate Kit (Thermo Fisher Scientific Inc., Waltham, MA, USA). The

products were quantified using a Qubit 4 Fluorometer (Thermo Fisher Scientific Inc., Waltham, MA, USA) and pooled in equal amounts. The sequencing was performed on an Illumina MiSeq platform using a MiSeq® Reagent Kit v3 (600 cycles for a 300 bp paired-end sequencing; Illumina, San Diego, CA, USA).

A custom mock community was generated as a positive control, using DNA from a total of 18 fungal strains (Table S9). In addition, one blank PCR control and six negative controls were included during different steps of the samples processing in order to detect contamination. The six negative controls corresponded to (i) sterile sucrose buffer, (ii) water during filtration of sucrose buffer, (iii) water during syringe preparation, (iv) water in research vessel, (v) sterile sucrose buffer in research vessel and (vi) water during DNA extraction (Table S10). All controls were processed as the sediment samples.

### *Bioinformatic analysis*

Raw data obtained from the present study has been deposited at NCBI SRA (PRJNA515259).

The resulting demultiplexed fastq files from the sequencing were processed using ITSx 1.0.11 (Bengtsson-Palme *et al*., 2013) to remove portions of DNA sequences that did not originate from ITS 1 (18S and 5.8S). To process the fastq files, the open source script fastq-from-ITSx was used (Darcy, 2018; https://github.com/darcyj/fastq-from-ITSx). Next, each fastq file containing only ITS 1 sequences were subjected to quality filtering, using an expected number of errors of 0.5 and an ambiguous bases threshold of 0, followed by chimera analysis by using the open source VSEARCH tool (Rognes *et al*., 2016) and as reference sequences, the UCHIME reference dataset v28.06.2017 (Nilsson *et al*., 2015).

The operational taxonomic units (OTUs) were obtained by clustering the sequences with a 97% of identity threshold, using the UCLUST algorithm (Edgar, 2010). Singletons (OTUs represented by one sequence) were removed. The longest sequences were selected as representative for each OTU, and used to assign the taxonomy. The taxonomic assignment was carried out using the BLAST algorithm (Altschul *et al*., 1990), against the NCBI nucleotide database, which performs better to identify the fungi present in the mock community control; also, standard databases containing ITS sequences do not cover a large amount of fungi from marine habitats. Entrez limits were applied to our database in order to minimize the number of unculturable gut fungus (Neocallimastigomycota) and to remove all Bacteria. The above processing was conducted using the Quantitative Insights Into Microbial Ecology (QIIME) pipeline (Caporaso *et al*., 2010).

An OTU table containing the frequency of each OTU and the taxonomic identification was constructed. The sequences from each sub-sample were pooled by station to avoid pseudoreplication. For OTUs present both in controls and samples the proportion of the number of sequences in the controls and in the samples was calculated (Nguyen *et al*., 2015), and those contaminants representing more than 1% of the total sequences in the samples were removed. We first removed OTUs where the sequence counts in the controls was higher than in the samples, as the proportion would tend to zero. Then OTUs identified as *Neurospora crassa* (known as laboratory contaminant) were removed. The OTUs corresponding to Unidentified Fungi were submitted to a Blastn search in the NCBI database, in order to improve the taxonomy assignment; this assignment was based on the best Blastn match. For the final frequency table, only OTUs identified as fungi were kept.

### *Statistical analyses*

Shannon-Wienner diversity index was calculated for each sampling station. The index was calculated using the Phyloseq package v.1.22.3 (McMurdie and Holmes, 2013). To visualize the number of shared and unique (OTUs present only in one station) OTUs among stations and campaigns, an OTU network was constructed with Cytoscape v3.7.0 (Shannon *et al*., 2003). To identify patterns of community structure among stations, we constructed a bar plot based on the abundance of fungal classes, with a hierarchical dendrogram computed using Ward's clustering algorithm to matrices of Euclidean distances. The dendrogram was computed using the hclust function from Vegan package (Oksanen *et al*., 2013).

Multiple linear regressions were performed to identify the relationship between richness/diversity indices, and geographic location and sediment physicochemical properties.

A presence/absence OTU table was tested with the rankindex function to select the best dissimilarity index that fits better to the data, and once the index was selected, the vegdist function was used to compute the distance. To analyse the similarity of the fungal composition among stations, a Non-metric Multidimensional Scaling (NMDS) was performed using the metaMDS function, establishing three-dimension ordination; the NMDS results were visualized in a two-dimension plot. The stations were categorized by geographic location (latitude and longitude), depth, region (Table S3), and type of core (i.e. box corer, multicorer), to detect differences or patterns among groups. For variables such as latitude and longitude, the geographic location was grouped to the nearest degree (e.g. 25.00892°-north to 25; 265.98224°-east to 265). Similarly, for depth, the samples were grouped every 500 m. A permutational multivariate analysis of variance (PERMANOVA) was conducted using the adonis function and Pairwise comparisons and *P*-adjusted

values were computed by Bonferroni correction using pairwise.adonis function from pairwiseAdonis package (Martinez Arbizu, 2019).

In order to compare beta diversity of the fungal communities taking into account the categorical variables explained above, a beta dispersion analysis using the betadisper function from Betapart package was conducted (Baselga and Orme, 2012). The Betadisper analysis was is based on an Euclidean distance matrix, and significance was computed by analysis of variance (ANOVA) test using the ANOVA function and *P*-adjusted values were computed by Bonferroni correction using p.adjust function. Pairwise comparisons were made among groups using pairwise.t.test function. The hclust, rankindex, vegdist, metaMDS and adonis functions were performed with the Vegan package (Oksanen *et al*., 2013).

To test whether the type or corer (box corer or multicorer) had an effect on the OTU composition, the stations were arranged by type of corer, and NMDS and beta dispersion analyses were carried out as explained above. Moreover, to test whether the corer type could be masking a correlation between the fungal community and sediment physicochemical properties, a Mantel test was carried out using the Euclidean distance matrices of the numerical variables and community data (presence/absence OTU table).

Euclidean distance matrixes were calculated for the OTU table, the geographic location (latitude and longitude) and a distance decay analysis was performed in order to determine the significance of the relationship among community dissimilarity and geographic distance. A Mantel test was performed to corroborate the significance of the relationship with the distance decay analysis.

A CCA was undertaken to detect the potential interactions between OTU fungal data set (presence/absence OTU table) and the geographic location and sediment physicochemical properties as variables, using the CCA function. Model selection was performed with the ordiestep function using a stepwise linear regression, and variable selection using the AIC (Ripley *et al*., 2015). The significance of the model was tested by an ANOVA with the ANOVA function, followed by the calculation of *P*-adjusted values, which were computed by Bonferroni correction using p.adjust function. The OTU table used in the analysis did not include station G44R.6, because there is not sediment characteristic data available for this replicate station. B18 stations were not included either, because previous research had demonstrated that this sampling site in the Yucatan slope is an outlier in comparison with other areas across the GoM (Díaz-Asencio *et al*., 2019). For further information on the methodology to determine the sediment characteristics the reader is referred to Díaz-Asencio *et al*. (2019).

The hclust, rankindex, vegdist, metaMDS, Adonis, mantel, CCA, ordistep functions were performed with the Vegan package (Oksanen *et al*., 2013). All the statistical analyses were carried out in RStudio v. 1.1.456 (R Development Core Team, 2008).

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## Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

**Fig. S1.** Taxonomic profiles of fungi among campaigns and sub-samples from each station. (A) Bar plot of relative abundance at phylum level among campaigns. (B) Bar plot of relative abundance at genus level exhibiting the differences among sub-samples belonging to the same station.

**Fig. S2.** Non-metric Multidimensional Scaling (NMDS) ordination of the similarities in fungal composition among different variables and beta dispersion across depths. (A) Stress plot with NMDS values constructed to visualize the correlation based on the stress and the goodness of fit. NMDS based on Euclidean distance matrix of stations similarities grouped by (B) Latitude, (C) Longitude and (D) Depth. (E) Corer. Beta dispersion analysis of stations grouped by (F) Depth and (G) Corer. NMDS and beta dispersion significant values refer to P-adjusted values by Bonferroni correction, to test differences in community composition among the different station characteristics.

**Fig. S3.** Distribution of stations sampled in the Mexican EEZ from the Gulf of Mexico. Geographical location and region of stations sampled throughout the XIXIMI 4, XIXIMI 5 and XIXIMI 6 campaigns. The colour scale shows water depth (in meters). AP, Abyssal Plain; CC, Campeche canyon; CSD, Campeche saline domo; CZC, Coatzacoalcos canyon; TVCS, Tamaulipas-Veracruz cont. Slope; YCS, Yucatan continental slope.

**Fig. S4.** Sampling strategy when using the boxcorer or the multicorer. Multicorer: only one core (from the eight) was selected to collect sediment for metagenomic studies. One half of that core was used to insert the syringes (named one to five), which represented the sub-samples. Box corer: eight corers (same size as corers from multicorer) are placed inside the box, and only one half of one core is selected to collect the sediment with the syringes.

**Table S1.** Number of sequences and OTUs through samples processing.

**Table S2.** Relative abundance of fungal phyla, classes and orders in all sampled stations.

**Table S3.** Summary of parameter of linear relationships of the Number of OTUs and Shannon index with geographical location and sediment physicochemical properties.

**Table S4.** Pairwise comparisons among groups formed by categorical variables using presence/absence data.

**Table S5.** Pairwise comparisons among groups formed by categorical variables using the not shared OTUs among stations.

**Table S6.** PERMANOVA analysis on NMDS ordination, ANOVA analysis on Beta Dispersion, and Mantel test correlations, testing the influence of Corer type on the fungal community.

**Table S7.** Model selection by Akaike Information Criteria and Monte Carlo permutation test of the selected model.

**Table S8.** Sampling stations, geographical characteristics and region.

**Table S9.** List of species from the mock community control.

**Table S10.** Taxonomic composition at genera level from the controls.