Fungal communities in sediments along a depth gradient in the Eastern Tropical Pacific

Keilor Rojas-Jimenez[1*], Hans Peter Grossart[2,3*], Erik Cordes[4], Jorge Cortés[1,5]

[1]Escuela de Biología, Universidad de Costa Rica, 11501 San José, Costa Rica.
[2]Institute for Biochemistry and Biology, Potsdam University, Maulbeerallee 2, 14469 Potsdam, Germany.
[3]Department of Experimental Limnology, Leibniz-Institute of Freshwater Ecology and Inland Fisheries, Alte Fischerhuette 2, D-16775 Stechlin, Germany.
[4]Department of Biology, Temple University, Philadelphia, Pennsylvania, USA.
[5]Centro de Investigación en Ciencias del Mar y Limnología (CIMAR), Universidad de Costa Rica, San Pedro, 11501 San José, Costa Rica.

***Correspondence:**
Keilor Rojas-Jimenez, keilor.rojas@gmail.com
Hans Peter Grossart, hgrossart@igb-berlin.de

Keywords: Deep-sea, aquatic fungi, biodiversity, Metschnikowia, Costa Rica

Abstract

Deep waters represent the largest biome on Earth and the largest ecosystem of Costa Rica. Fungi play a fundamental role in global biogeochemical cycling in marine sediments, yet, they remain little explored. We studied fungal diversity and community composition in several marine sediments from 16 locations sampled along a bathymetric gradient (from a depth of 380 to 3474 m) in two transects of about 1500 km length in the Eastern Tropical Pacific (ETP) of Costa Rica. Sequence analysis of the V7-V8 region of the 18S rRNA gene obtained from sediment cores revealed the presence of 787 fungal amplicon sequence variants (ASVs). On average, we detected a richness of 75 fungal ASVs per sample. Ascomycota represented the most abundant phylum with Saccharomycetes constituting the dominant class. Three ASVs accounted for ca. 63% of all fungal sequences: the yeast *Metschnikowia* (49.4%), *Rhizophydium* (6.9%), and *Cladosporium* (6.7%). Although we distinguished a cluster dominated by yeasts and a second cluster dominated by filamentous fungi, we were unable to detect a strong effect of depth, temperature, salinity, dissolved oxygen, and pH on the composition of fungal communities. We highlight the need to understand further the ecological role of fungi in deep-sea ecosystems.

INTRODUCTION

Fungi existed in the oceans long before they conquered land, and within the oceans, the deep sea represents the largest biome on Earth. Therefore, it is crucial to study fungal diversity and ecology in deep-sea waters, for which there is a paucity of studies compared to the rest of the ocean. Detailed knowledge of deep-sea fungi is required to understand better the overall fungal contribution to marine food webs and biogeochemical cycles at the global scale (Manohar and Raghukumar, 2013; Barone et al., 2018; Drake and Ivarsson, 2018; Grossart et al., 2019; Román et al., 2019; Hassett et al., 2020).

Fungi are active members of deep-sea sediment communities (Pachiadaki et al., 2016; Morales et al., 2019), but in this ecosystem, they are far more poorly characterized than bacteria and archaea (Edgcomb et al., 2011; Nagano and Nagahama, 2012; Dekas et al., 2016; Xu et al., 2018). In deep

46 waters, fungi are well adapted to the total absence of light, low temperatures, and high
47 hydrostatic pressure. Fungal communities have been described in sediments of hydrothermal
48 vents, methane-cold seeps, oxygen-minimum zones, and associated with other macro-organisms
49 (Nagahama et al., 2011; Zhang et al., 2016; Batista-García et al., 2017). Furthermore, the
50 subseafloor has been shown to represent a vast ecosystem where micro-aerobic respiration
51 occurs and where large amounts of microbial life subsist, even hundreds of meters below the
52 seafloor (D'Hondt, 2002; Roy et al., 2012; D'Hondt et al., 2015; Ivarsson et al., 2016a; Nagano et
53 al., 2016).

54 Fungi in the deep-sea environment mainly survive on marine snow, which consists of organic
55 matter derived from photosynthesis that takes place in the photic layer. In addition to performing
56 aerobic respiration, fungi are capable of carrying out processes such as fermentation, sulfate
57 reduction, methanogenesis (Lenhart et al., 2012; Orsi et al., 2013; Bochdansky et al., 2017), and
58 possibly lithoautotrophy (López-García et al., 2003; Nealson et al., 2005; Ivarsson et al., 2016b).
59 These metabolic processes may be more critical for fungi in deep waters since it has been
60 observed that as depth increases, fungal populations exhibit a more multitrophic lifestyle (Li et al.,
61 2019).

62 In recent years, there has been a growing interest in studying fungal communities in deep-sea
63 environments using culture-dependent and, to an increasing extent, culture-independent
64 methods. Abundant fungal populations have been observed in a variety of deep-sea locations such
65 as asphalt seeps in Sao Paulo Plateau (Nagano et al., 2017), methane seeps in the Kuroshima Knoll
66 (Takishita et al., 2006), hydrothermal vents in the Mid-Atlantic Ridge (Le Calvez et al., 2009; Xu et
67 al., 2017), sediments of the Peru Trench (Edgcomb et al., 2011), the East Indian Ocean (Zhang et
68 al., 2014), the High Arctic (Zhang et al., 2015), the Mariana Trench (Xu et al., 2016, 2018), the
69 Yellow Sea (Li et al., 2016), the Mediterranean Sea (Barone et al., 2018), the Yap Trench (Li et al.,
70 2019), and subsurface sediments in Suruga-Bay (Nagano et al., 2016).

71 Considering the enormous area to be explored for fungal diversity and function in deep-sea
72 sediments, the existing studies are minimal and often lack an adequate spatial and temporal
73 resolution (Grossart and Rojas-Jimenez, 2016; Grossart et al., 2019; Morales et al., 2019).
74 Therefore, there is still a large number of geographical locations that have not yet been studied,
75 including the Eastern Tropical Pacific (ETP). The deep-sea waters of the ETP constitute a
76 particularly important ecosystem in Costa Rica since they represent about 90% of the whole
77 territory (Cortés, 2016, 2019).

78 The Costa Rican ETP comprises a chain of mountains and submarine volcanoes across the
79 subduction zone of the Cocos and Caribbean tectonic plates. Here, there is a high diversity of
80 microhabitats (Lizano, 2001; Protti et al., 2012; Rojas and Alvarado, 2012) including methane
81 seeps (Sahling et al., 2008; Levin et al., 2012, 2015). Previous studies have shown high endemism
82 and diversity of macro- and microorganisms in this region (Rusch et al., 2007; Cortés, 2008, 2019;
83 Rojas-Jiménez, 2018). Also, the Costa Rican ETP is part of a marine corridor that extends through
84 Isla del Coco to the Galapagos Islands in Ecuador, which represents an essential site for the
85 conservation and regeneration of marine species throughout the ETP (Cortés, 2012).

86 In this work, we have explored the diversity and composition of fungal communities in deep-sea
87 sediments of the Costa Rican ETP. Two expeditions were carried out with transects of

approximately 1500 km length each, and sediments were sampled at 16 locations at depths between 380 m and 3474 m. We extracted DNA from subsamples of each sediment core, sequenced the 18S rRNA gene of eukaryotes, and performed a subsequent bioinformatic analysis. This work confirms the high abundance and diversity of fungi in sediments of the ETP region. We expect that our results will support current efforts to conserve this region by providing a baseline of the high diversity of eukaryotic species and microhabitats found in its deep-sea waters.

MATERIALS AND METHODS

We used Illumina sequencing of the eukaryotic 18S rRNA gene to characterize the fungal community composition in sediments along a depth gradient in the ETP of Costa Rica, across two transects of ca. 1500 km length each (**Figure 1**). All samples were collected with the permission of the Ministry of Environment and Energy of Costa Rica (SINAC-CUSBSE-PI-R-032-2018; R-070-2018-OT-CONAGEBIO). The RV *Atlantis* surveyed the Pacific continental margin of Costa Rica from October 24th to November 5th, 2018, from the continental slope to the offshore seamounts across a subduction zone. In this region, several methane-rich seeps have been detected (Sahling et al., 2008; Levin et al., 2012, 2015). All sediment cores were collected by the human-occupied vehicle (HOV) *Alvin* equipped with mechanical, maneuverable arms. We analyzed eight sediment-cores from this expedition. The following year, the RV *Falkor* surveyed the seamounts extending from the mainland to the Isla del Coco National Park between January 6th-21st, 2019. This region comprises several seamounts and natural gas seeps and provides an important corridor for highly specialized biological communities occupying the area. The sediment cores were collected employing the remotely operated vehicle (ROV) *SuBastian*, which is also equipped with mechanical, maneuverable arms. We analyzed another eight sediment cores from this expedition. Further details of the sampling sites, dates, and environmental variables measured are shown in **Table 1**.

After collection, nearly one gram of the upper (1-2 cm), middle (6-7 cm), and lower (13-14 cm) parts of each 15 cm-core was deposited into a 1.5 ml tube, stored at -20 °C on board the vessel and at -80 °C in the laboratory. The sediment DNA was extracted with a DNA isolation kit (PowerSoil®, Qiagen) following the manufacturer´s instructions. From some subsamples, unfortunately, it was not possible to obtain enough DNA for subsequent analyzes, so in total, we retrieved 40 DNA samples (out of the 48 possible) from the 16 cores sampled in both transects. For the construction of the amplicon library, primers FF390 / FR1 were used to amplify the V7 and V8 regions of the 18S rRNA gene (Prévost-Bouré et al., 2011). The products were subjected to a 250 nt paired-end sequencing using Illumina MiSeq technology at MrDNA, TX, USA.

Sequences were tested for quality and analyzed using version 1.12 of the DADA2 pipeline (Callahan et al., 2016). The taxonomic assignment was performed on comparing sequences against the SILVA reference database v132 (Quast et al., 2013), and then curated by comparing sequences against NCBI. Global singletons were removed. This process resulted in an amplicon sequence variant table, a higher-resolution analog of the traditional OTU table, which records the number of times each exact amplicon sequence variant was observed in a sample. Sequence data were deposited in the sequence-read archive (SRA) under accession PRJNA632873.

Statistical analyses and their visualization were performed with the R statistical program (R-Core-Team 2019) and the Rstudio interface. Package Vegan v2.5-6 (Oksanen et al., 2019) was used to calculate alpha diversity estimators, non-metric multidimensional scaling analyses (NMDS). Data tables with the amplicon sequence variants (ASV) abundances were normalized into relative abundances and then converted into a Bray–Curtis similarity matrix. To determine if there were significant differences between the fungal community composition according to factors such as depth or transect, we used the non-parametric multivariate analysis of variance (PERMANOVA) and pairwise PERMANOVA (adonis2 function with 999 permutations). For the network analysis, we selected the 24 most abundant eukaryotic ASVs (10 classified as fungi), which corresponded to nearly 70% of the total number of eukaryotic sequences. We considered a valid co-occurrence event if the Spearman's correlation coefficient was >0.5 (Junker, 2008). The network inference and visualization were performed with package igraph v1.2.4.2 using a Fruchterman-Reingold layout (Csardi and Nepusz, 2006).

Environmental data were compiled from the measurements obtained from analyses of the water samples or sensors nearest to the sediment cores as possible. These data come from a variety of sources. Temperature and salinity data were obtained from the conductivity-temperature-depth (CTD) sensors on the HOV *Alvin* and ROV *SuBastian*, which were also equipped with niskin bottles for water sampling. There was a dissolved oxygen (DO) optode on the ROV *SuBastian* as well as the autonomous underwater vehicle (AUV) *Sentry* which was deployed over some of the sites during the 2018 *Atlantis* expedition. Niskin rosettes with attached CTDs were also deployed from the *Atlantis* and *Falkor* over the sites, and the *Falkor* CTD had a DO optode as well. DO data were compiled from a combination of these sources. DO data for the samples from the 2018 *Alvin* dives were derived from either the *Sentry* data (if available from the site) or calculated from a curve fitted to the DO data obtained from the ROV *SuBastian* and *Falkor* CTD DO-depth profiles. DO data for the 2019 SuBastian push core samples was deterimined from SuBastian optode. The pH data were exclusively from the water samples obtained by the rosette deployed from the ship or the niskin bottles on the submersibles. Water samples were brought to room temperature and the pHT (total scale) was measured using an Orion 5 Star pH meter and glass electrode (ROSS Ultra pH/ATC Triode 8107BNUMD) in triplicate within 4 h of collection (Dickson et al., 2007).

RESULTS AND DISCUSSION

Fungi constituted the most abundant group of eukaryotic organisms in the sediments of the ETP of Costa Rica, according to the analysis of the sequences of the 18S rRNA gene. We determined the presence of 787 fungal ASVs out of a total of 2707 eukaryotic ASVs. Fungi represented 59.72% of the 2,746,436 sequences analyzed (**Figure 2A**). Other abundant eukaryotic groups comprised Cercozoa and Ichthyosporea, which represented 24,1% and 6,75% of all eukaryotic sequences, respectively. The genus *Gymnophrys* was the most abundant within Cecrozoa, while an ASV related to *Pirum* was the most abundant within Ichthyosporea. It was not possible to provide a classification at the taxonomic level of the Kingdom for 2.63% of the sequences, which, however, contain 24.60% of all observed ASVs. This indicates the presence of a large number of rare species that have not yet been registered in reference databases, which suggests a high hidden eukaryotic diversity in the studied deep-sea sediments.

The most abundant fungal phylum in marine sediments of the ETP of Costa Rica consisted of Ascomycota, which represented 43% of all fungal sequences and 71% of the ASVs. The second most abundant fungal group comprised Basidiomycota, representing nearly 3% of the sequences but 22% of the ASVs, suggesting a very high phylogenetic diversity within this phylum. Chytridiomycota represented the third most abundant fungal group, with 3.5% of the sequences and 2.79% of the ASVs. Other less frequent fungal groups observed in this ecosystem were, Blastocladiomycota, LKM11, LKM15, Mucoromycota, and Zoopagomycota (**Figure 2B**). These results are consistent with earlier results obtained in deep-sea sediments from several parts of the planet confirming the general dominance of Ascomycota in deep-sea sediments together with the presence of Basidiomycota and Chytridiomycota in lower proportions (Li et al., 2016, 2019; Xu et al., 2016, 2019; Zhang et al., 2016; Nagano et al., 2017; Barone et al., 2018; Wang et al., 2019).

When analyzing the relative abundances at the class level, we detected a total of 32 classes in the deep-sea sediments, where Saccharomycetes was the most prominent in the majority of the samples. In samples where Saccharomycetes was dominant, they were typically accompanied by the presence of Chytridiomycetes. There were also groups of samples with high abundances of Eurotiomycetes, Dothideomycetes, and Agaricomycetes, but where Saccharomycetes were practically absent (**Figure 3**).

We also observed high variability in the composition within the horizons of some samples. In this regard, the homogeneity or heterogeneity of horizons could be related to the characteristics of the sampled habitat, but also with the sedimentation time. It will be necessary to further explore in more detail the variations that occur at the micro-scale in fungal communities in sediment profiles, as has been done to study variations in the physicochemical conditions of sediments in other deep-sea waters (Roy et al., 2012; D'Hondt et al., 2015; Román et al., 2019).

The samples of the deep-sea environment studied, characterized by high hydrostatic pressure, low temperatures, and the absence of light, presented an average richness of 75 fungal ASVs per sample (range 13-147), while the average value of the Shannon index was 1.77 (range 0.84-2.68). As with the community analyses, there were no significant differences in the alpha diversity estimations between depths and expeditions (Kruskal-Wallis, $P > 0.05$). The average value of the Pielou´s evenness was 0.42 (range 0.21-0.71), indicating a certain uniformity in the abundances of most of the observed phylotypes (**Supplementary Figure 1).**

The genus *Metschikowia* was the most abundant within the class Saccharomycetes and also the most abundant in the majority of the sediments analyzed. The genus *Metschikowia* comprises single-celled budding yeasts known for its participation in fermentation processes and wine production, reported mainly in terrestrial environments (Kang et al., 2017; Wang et al., 2017; Pawlikowska et al., 2019). In this study, we showed that this fungal genus was present in a wide depth gradient, from 380 to 3474 m, indicating that it can be highly tolerant to gradients in temperature, dissolved oxygen, food supply, and the hydrostatic pressure associated with this change in depth. However, in six of the studied sediment cores *Metschikowia* was almost absent, pointing more to microhabitat variability.

The most abundant genus within Chytridiomycetes was *Rhizophidium* which can function as parasite and decomposer (Letcher et al., 2006; Kagami et al., 2007; Frenken et al., 2017), while the most abundant genera of Eurotiomycetes and Dothideomycetes were *Aspergillus* and

Cladosporium, respectively. Within Agaricomycetes, the most abundant phylotype was related to the genus *Armillaria*. Previous studies have shown that *Aspergillus* and *Penicillium* are common inhabitants of deep-sea sediments; likewise, the presence of yeasts in this ecosystem has been frequently detected, but mainly related to genera such as *Pichia*, *Cryptococcus*, *Malassezia,* and *Rhodotorula* (Takishita et al., 2006; Zhang et al., 2015; Nagano et al., 2016, 2017; Grossart et al., 2019). To our knowledge, this is the first work showing a high abundance of *Metschikowia* in deep-sea ecosystems.

We used network analysis to explore possible relationships between eukaryotic microorganisms that coexist in deep marine sediments of Costa Rica (**Figure 4**). This technique allowed us to visualize positive associations not only between the members of the fungal taxa but also between fungi and other eukaryotes. For example, we confirmed the strong relationship between *Metschikowia*, the most abundant ASV from Saccharomycetes, and *Rhizophydium*, the most abundant ASV from Chytridiomycetes. Interestingly, in this yeast-dominated group, there were also associations with other eukaryotic ASVs belonging to Cercozoa, Ichthyosporea, Porifera, Annelida, and Cnidaria (**cluster 1, Figure 4**).

In a second group, strong associations were detected between fungal genera such as *Cladosporium*, *Aspergillus*, *Exophiala,* and *Armillaria*, belonging to the classes Dothideomycetes, Eurotiomycetes, Chaetothyriomycetes, and Agaricomycetes, respectively. However, in this cluster, we did not detect associations with other eukaryotic ASVs that could point to co-occurrence with specific environmental settings (**cluster 2, Figure 4**).

The statistical analyzes, at the ASV level, showed no significant differences (Permanova, $p > 0.05$) in the structure of the communities according to variables depth, salinity, dissolved oxygen, pH, or between seep/non-seep areas nor between expeditions (**Supplementary Table 1, Supplementary Figure 2**). For example, we showed that depth (and, consequently, hydrostatic pressure) does not have an apparent effect on the composition of communities, given the wide distribution range of species. Furthermore, we observe that cluster 1 and cluster 2 inhabit sites whose temperature, salinity, dissolved oxygen and pH ranges overlap each other (**Table 2**). Therefore, it seems that the conditions of the deep waters are not limiting for the growth of the fungi and that there could be other variables influencing the composition of the communities whose effects should be further explored in future studies.

As an empirical observation note, samples that contained a higher proportion of mud were the ones that exhibited a higher abundance of Saccharomycetes (cluster 1). In contrast, sandy samples showed higher abundances of Eurotiomycetes and Dothideomycetes, which are filamentous fungi (cluster 2). This observation suggests a possible relationship between fungal morphology and its ability to colonize substrates of different textures. For example, yeasts may directly depend on the type and concentrations of organic matter found in the habitat, but could also perform fermentation processes in muddy sediments (Takishita et al., 2006; Kutty and Philip, 2008; Zhang et al., 2015; Taube et al., 2018).

We highlight the high prevalence of fungi in deep-sea sediments of the ETP of Costa Rica. The high abundance of yeasts like *Metschikowia* should be further studied using cultivation-dependent methods to provide better insights into the physiology, genomic makeup, and their contributions to global biogeochemical processes. Since it was difficult to distinguish the association of specific

environmental variables with variations in the composition of fungal communities, particularly in the two clusters identified, further research will be necessary to determine how fungal communities in deep-sea waters are structured as well as to determine their ecological role in the largest biome on the planet.

DATA AVAILABILITY

The dataset generated for this study can be found in NCBI Sequence Read Archive under accession PRJNA632873.

AUTHOR CONTRIBUTIONS

KRJ, HPG, EEC and JC designed the study. JC and EEC collected the samples. KRJ, HPG, EEC and JC performed the analysis. KRJ wrote the manuscript. All authors helped to revise the manuscript.

CONFLICT OF INTEREST

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

ACKNOWLEDGMENTS

We would like to thank all of the officers and crew of the RV *Atlantis* and RV *Falkor* for their assistance, and the pilots of the HOV *Alvin* and ROV *SuBastian* for their efforts in the collection of samples. We thank the Ministry of Environment and Energy of Costa Rica for granting the permits for the collection of samples. Sample processing was carried out with assistance from Odalisca Breedy and environmental data were compiled by Steve Auscavitch, Jay Lunden, and April Stabbins at Temple University. We also thank Lisa Levin and Greg Rouse at Scripps Oceanography, UC San Diego, La Jolla, CA, and Victoria Orphan at the California Institute of Technology, Pasadena, CA for their support during the development of this project. We acknowledge the support of the Deutsche Forschungsgemeinschaft and Open Access Publishing Fund of University of Potsdam

FUNDING

This project was funded by Universidad de Costa Rica, DFG project GR1540/33-1 given to HPG, and NSF OCE 1635219 awarded to EEC.

REFERENCES

Barone, G., Rastelli, E., Corinaldesi, C., Tangherlini, M., Danovaro, R., and Dell'Anno, A. (2018). Benthic deep-sea fungi in submarine canyons of the Mediterranean Sea. *Prog. Oceanogr.* 168, 57–64. doi:10.1016/j.pocean.2018.09.011.

Batista-García, R. A., Sutton, T., Jackson, S. A., Tovar-Herrera, O. E., Balcázar-López, E., Sánchez-Carbente, M. del R., et al. (2017). Characterization of lignocellulolytic activities from fungi isolated from the deep-sea sponge Stelletta normani. *PLoS One* 12, e0173750. doi:10.1371/journal.pone.0173750.

Bochdansky, A. B., Clouse, M. A., and Herndl, G. J. (2017). Eukaryotic microbes, principally fungi and labyrinthulomycetes, dominate biomass on bathypelagic marine snow. *ISME J.* 11, 362–373. doi:10.1038/ismej.2016.113.

Callahan, B. J., McMurdie, P. J., Rosen, M. J., Han, A. W., Johnson, A. J. A., and Holmes, S. P. (2016). DADA2: High-resolution sample inference from Illumina amplicon data. *Nat. Methods* 13, 581–583. doi:10.1038/nmeth.3869.

Cortés, J. (2008). Historia de la investigación marina de la Isla del Coco, Costa Rica. *Rev. Biol. Trop.* (Suppl. 2), 1–18. doi:10.15517/RBT.V56I2.26934.

Cortés, J. (2012). Marine biodiversity of an Eastern Tropical Pacific oceanic island, Isla del Coco, Costa Rica. *Rev. Biol. Trop.* 60, 131–185.

Cortés, J. (2016). The Pacific coastal and marine ecosystems. *Costa Rican Ecosyst. Univ. Chicago Press. Chicago London*, 97–138.

Cortés, J. (2019). "Isla del Coco, Costa Rica, Eastern Tropical Pacific," in *Mesophotic Coral Ecosystems* (Springer), 465–475. doi:10.1007/978-3-319-92735-0_26.

Csardi, G., and Nepusz, T. (2006). The igraph software package for complex network research. *InterJournal, Complex Syst.* 1695, 1–9.

D'Hondt, S. (2002). Metabolic activity of subsurface life in deep-sea sediments. *Science (80-.).* 295, 2067–2070. doi:10.1126/science.1064878.

D'Hondt, S., Inagaki, F., Zarikian, C. A., Abrams, L. J., Dubois, N., Engelhardt, T., et al. (2015). Presence of oxygen and aerobic communities from sea floor to basement in deep-sea sediments. *Nat. Geosci.* 8, 299–304. doi:10.1038/ngeo2387.

Dekas, A. E., Connon, S. A., Chadwick, G. L., Trembath-Reichert, E., and Orphan, V. J. (2016). Activity and interactions of methane seep microorganisms assessed by parallel transcription and FISH-NanoSIMS analyses. *ISME J.* 10, 678–692. doi:10.1038/ismej.2015.145.

Dickson, A. G., Sabine, C. L., and Christian, J. R. (2007). *Guide to best practices for ocean CO2 measurements.* North Pacific Marine Science Organization Available at: https://www.nodc.noaa.gov/ocads/oceans/Handbook_2007.html.

Drake, H., and Ivarsson, M. (2018). The role of anaerobic fungi in fundamental biogeochemical cycles in the deep biosphere. *Fungal Biol. Rev.* 32, 20–25. doi:10.1016/j.fbr.2017.10.001.

Edgcomb, V. P., Beaudoin, D., Gast, R., Biddle, J. F., and Teske, A. (2011). Marine subsurface eukaryotes: the fungal majority. *Environ. Microbiol.* 13, 172–183. doi:10.1111/j.1462-2920.2010.02318.x.

Frenken, T., Alacid, E., Berger, S. A., Bourne, E. C., Gerphagnon, M., Grossart, H. P., et al. (2017). Integrating chytrid fungal parasites into plankton ecology: research gaps and needs. *Environ. Microbiol.* 19, 3802–3822. doi:10.1111/1462-2920.13827.

Grossart, H.-P. H.-P. P., and Rojas-Jimenez, K. (2016). Aquatic fungi: targeting the forgotten in microbial ecology. *Curr Opin Microbiol* 31, 140–145. doi:10.1016/j.mib.2016.03.016.

Grossart, H.-P., Van den Wyngaert, S., Kagami, M., Wurzbacher, C., Cunliffe, M., and Rojas-Jimenez, K. (2019). Fungi in aquatic ecosystems. *Nat. Rev. Microbiol.* 17, 339–354. doi:10.1038/s41579-019-0175-8.

Hassett, B. T., Vonnahme, T. R., Peng, X., Jones, E. B. G., and Heuzé, C. (2020). Global diversity and geography of planktonic marine fungi. *Bot. Mar.* 63, 121–139.

Ivarsson, M., Bengtson, S., and Neubeck, A. (2016a). The igneous oceanic crust – Earth's largest fungal habitat? *Fungal Ecol.* 20, 249–255. doi:10.1016/j.funeco.2016.01.009.

Ivarsson, M., Schnürer, A., Bengtson, S., and Neubeck, A. (2016b). Anaerobic Fungi: A Potential Source of Biological H2 in the Oceanic Crust. *Front. Microbiol.* 7, 674. doi:10.3389/fmicb.2016.00674.

Junker, B. H. (2008). Networks in Biology. *Anal. Biol. Networks*, 1–14. doi:doi:10.1002/9780470253489.ch1.

Kagami, M., de Bruin, A., Ibelings, B. W., and Van Donk, E. (2007). Parasitic chytrids: their effects on phytoplankton communities and food-web dynamics. *Hydrobiologia* 578, 113–129.

Kang, Y. M., Choi, J. E., Komakech, R., Park, J. H., Kim, D. W., Cho, K. M., et al. (2017). Characterization of a novel yeast species Metschnikowia persimmonesis KCTC 12991BP (KIOM G15050 type strain) isolated from a medicinal plant, Korean persimmon calyx (Diospyros kaki Thumb). *AMB Express* 7, 199. doi:10.1186/s13568-017-0503-1.

Kutty, S. N., and Philip, R. (2008). Marine yeasts—a review. *Yeast* 25, 465–483. doi:10.1002/yea.1599.

Le Calvez, T., Burgaud, G., Mahé, S., Barbier, G., Vandenkoornhuyse, P., Mahe, S., et al. (2009). Fungal diversity in deep-sea hydrothermal ecosystems. *Appl. Environ. Microbiol.* 75, 6415–6421. doi:10.1128/AEM.00653-09.

Lenhart, K., Bunge, M., Ratering, S., Neu, T. R., Schüttmann, I., Greule, M., et al. (2012). Evidence for methane production by saprotrophic fungi. *Nat. Commun.* 3, 1046. doi:10.1038/ncomms2049.

Letcher, P. M., Powell, M. J., Churchill, P. F., and Chambers, J. G. (2006). Ultrastructural and molecular phylogenetic delineation of a new order, the Rhizophydiales (Chytridiomycota). *Mycol. Res.* 110, 898–915. doi:https://doi.org/10.1016/j.mycres.2006.06.011.

Levin, L. A., Mendoza, G. F., Grupe, B. M., Gonzalez, J. P., Jellison, B., Rouse, G., et al. (2015). Correction: Biodiversity on the Rocks: Macrofauna Inhabiting Authigenic Carbonate at Costa Rica Methane Seeps. *PLoS One* 10, e0136129. doi:10.1371/journal.pone.0136129.

Levin, L. A., Orphan, V. J., Rouse, G. W., Rathburn, A. E., Ussler, W., Cook, G. S., et al. (2012). A hydrothermal seep on the Costa Rica margin: middle ground in a continuum of reducing ecosystems. *Proc. R. Soc. B Biol. Sci.* 279, 2580–2588. doi:10.1098/rspb.2012.0205.

Li, W., Wang, M., Burgaud, G., Yu, H., and Cai, L. (2019). Fungal community composition and potential depth-related driving factors impacting distribution pattern and trophic modes from epi- to abyssopelagic zones of the Western Pacific Ocean. *Microb. Ecol.* 78, 820–831. doi:10.1007/s00248-019-01374-y.

Li, W., Wang, M. M., Wang, X. G., Cheng, X. L., Guo, J. J., Bian, X. M., et al. (2016). Fungal communities in sediments of subtropical Chinese seas as estimated by DNA metabarcoding. *Sci. Rep.* 6, 26528. doi:10.1038/srep26528.

Lizano, O. (2001). Batimetria de la plataforma insular alrededor de las Isla del Coco, Costa Rica. *Rev. Biol. Trop.* 49 (Suppl., 163–170.

López-García, P., Philippe, H., Gail, F., and Moreira, D. (2003). Autochthonous eukaryotic diversity in

hydrothermal sediment and experimental microcolonizers at the Mid-Atlantic Ridge. *Proc. Natl. Acad. Sci.* 100, 697–702.

Manohar, C. S., and Raghukumar, C. (2013). Fungal diversity from various marine habitats deduced through culture-independent studies. *FEMS Microbiol. Lett.* 341, 69–78. doi:10.1111/1574-6968.12087.

Morales, S. E., Biswas, A., Herndl, G. J., and Baltar, F. (2019). Global Structuring of Phylogenetic and Functional Diversity of Pelagic Fungi by Depth and Temperature. *Front. Mar. Sci.* 6, 131. doi:10.3389/fmars.2019.00131.

Nagahama, T., Takahashi, E., Nagano, Y., Abdel-Wahab, M. A., and Miyazaki, M. (2011). Molecular evidence that deep-branching fungi are major fungal components in deep-sea methane cold-seep sediments. *Environ. Microbiol.* 13, 2359–2370. doi:10.1111/j.1462-2920.2011.02507.x.

Nagano, Y., Konishi, M., Nagahama, T., Kubota, T., Abe, F., and Hatada, Y. (2016). Retrieval of deeply buried culturable fungi in marine subsurface sediments, Suruga-Bay, Japan. *Fungal Ecol.* 20, 256–259.

Nagano, Y., Miura, T., Nishi, S., Lima, A. O., Nakayama, C., Pellizari, V. H., et al. (2017). Fungal diversity in deep-sea sediments associated with asphalt seeps at the Sao Paulo Plateau. *Deep Sea Res. Part II Top. Stud. Oceanogr.* 146, 59–67.

Nagano, Y., and Nagahama, T. (2012). Fungal diversity in deep-sea extreme environments. *Fungal Ecol.* 5, 463–471. doi:10.1016/j.funeco.2012.01.004.

Nealson, K. H., Inagaki, F., and Takai, K. (2005). Hydrogen-driven subsurface lithoautotrophic microbial ecosystems (SLiMEs): do they exist and why should we care? *Trends Microbiol.* 13, 405–410.

Oksanen, J., Blanchet, F. G., Friendly, M., Kindt, R., Legendre, P., McGlinn, D., et al. (2019). vegan: Community Ecology Package (R package version 2.5–6).

Orsi, W., Biddle, J. F., and Edgcomb, V. (2013). Deep sequencing of subseafloor eukaryotic rRNA reveals active fungi across marine subsurface provinces. *PLoS One* 8, e56335. doi:10.1371/journal.pone.0056335.

Pachiadaki, M. G., Rédou, V., Beaudoin, D. J., Burgaud, G., and Edgcomb, V. P. (2016). Fungal and prokaryotic activities in the marine subsurface biosphere at Peru margin and Canterbury Basin inferred from RNA-based analyses and microscopy. *Front. Microbiol.* 7, 846. doi:10.3389/fmicb.2016.00846.

Pawlikowska, E., James, S. A., Breierova, E., Antolak, H., and Kregiel, D. (2019). Biocontrol capability of local Metschnikowia sp. isolates. *Antonie Van Leeuwenhoek* 112, 1425–1445. doi:10.1007/s10482-019-01272-w.

Prévost-Bouré, N., Christen, R., Dequiedt, S., Mougel, C., Lelièvre, M., Jolivet, C., et al. (2011). Validation and Application of a PCR Primer Set to Quantify Fungal Communities in the Soil Environment by Real-Time Quantitative PCR. *PLoS One* 6, e24166. doi:10.1371/journal.pone.0024166.

Protti, M., González, V., Freymueller, J., and Doelger, S. (2012). Isla del Coco, on Cocos Plate, converges with Isla de San Andrés, on the Caribbean Plate, at 78mm/yr. *Rev. Biol. Trop.* 60, 33–41.

Quast, C., Pruesse, E., Yilmaz, P., Gerken, J., Schweer, T., Yarza, P., et al. (2013). The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. *Nucleic Acids Res.* 41, D590–D596. doi:10.1093/nar/gks1219.

Rojas-Jiménez, K. (2018). Microorganismos del corredor marino Isla del Coco-Galápagos: diversidad funcional y de especies. *Rev. Tecnol. en Marcha*. doi:10.18845/tm.v31i4.3974.

Rojas, W., and Alvarado, G. E. (2012). Marco geológico y tectónico de la Isla del Coco y la región marítima circunvecina, Costa Rica zone off the central Pacific coast of Costa Rica. *Rev. Biol. Trop.* 60, 15–32.

Román, S., Ortiz-Álvarez, R., Romano, C., Casamayor, E. O., and Martin, D. (2019). Microbial Community Structure and Functionality in the Deep Sea Floor: Evaluating the Causes of Spatial Heterogeneity in a Submarine Canyon System (NW Mediterranean, Spain). *Front. Mar. Sci.* 6. doi:10.3389/fmars.2019.00108.

Roy, H., Kallmeyer, J., Adhikari, R. R., Pockalny, R., Jorgensen, B. B., and D'Hondt, S. (2012). Aerobic Microbial Respiration in 86-Million-Year-Old Deep-Sea Red Clay. *Science (80-.).* 336, 922–925. doi:10.1126/science.1219424.

Rusch, D. B., Halpern, A. L., Sutton, G., Heidelberg, K. B., Williamson, S., Yooseph, S., et al. (2007). The Sorcerer II Global Ocean Sampling Expedition: Northwest Atlantic through Eastern Tropical Pacific. *PLoS Biol.* 5, e77. doi:10.1371/journal.pbio.0050077.

Sahling, H., Masson, D. G., Ranero, C. R., Hühnerbach, V., Weinrebe, W., Klaucke, I., et al. (2008). Fluid seepage at the continental margin offshore Costa Rica and southern Nicaragua. *Geochemistry, Geophys. Geosystems* 9, n/a-n/a. doi:10.1029/2008GC001978.

Takishita, K., Tsuchiya, M., Reimer, J. D., and Maruyama, T. (2006). Molecular evidence demonstrating the basidiomycetous fungus Cryptococcus curvatus is the dominant microbial eukaryote in sediment at the Kuroshima Knoll methane seep. *Extremophiles* 10, 165–169. doi:10.1007/s00792-005-0495-7.

Taube, R., Ganzert, L., Grossart, H.-P., Gleixner, G., and Premke, K. (2018). Organic matter quality structures benthic fatty acid patterns and the abundance of fungi and bacteria in temperate lakes. *Sci. Total Environ.* 610–611, 469–481. doi:https://doi.org/10.1016/j.scitotenv.2017.07.256.

Wang, C., Liu, Y., Zhang, T., Lu, C., Liu, Y., Zhang, D., et al. (2017). Metschnikowia persici sp. nov., a novel protease-producing yeast species from China. *Curr. Microbiol.* 74, 365–370.

Wang, Z.-P., Liu, Z.-Z., Wang, Y.-L., Bi, W.-H., Liu, L., Wang, H.-Y., et al. (2019). Fungal community analysis in seawater of the Mariana Trench as estimated by Illumina HiSeq. *RSC Adv.* 9, 6956–6964. doi:10.1039/C8RA10142F.

Xu, W., Gao, Y., Gong, L., Li, M., Pang, K.-L., and Luo, Z.-H. (2019). Fungal diversity in the deep-sea hadal sediments of the Yap Trench by cultivation and high throughput sequencing methods based on ITS rRNA gene. *Deep Sea Res. Part I Oceanogr. Res. Pap.* 145, 125–136. doi:10.1016/j.dsr.2019.02.001.

Xu, W., Guo, S., Pang, K.-L., and Luo, Z.-H. (2017). Fungi associated with chimney and sulfide samples from a South Mid-Atlantic Ridge hydrothermal site: distribution, diversity and abundance. *Deep Sea Res. Part I Oceanogr. Res. Pap.* 123, 48–55.

Xu, W., Luo, Z.-H., Guo, S., and Pang, K.-L. (2016). Fungal community analysis in the deep-sea sediments of the Pacific Ocean assessed by comparison of ITS, 18S and 28S ribosomal DNA regions. *Deep Sea Res. Part I Oceanogr. Res. Pap.* 109, 51–60. doi:10.1016/j.dsr.2016.01.001.

Xu, Z., Wang, M., Wu, W., Li, Y., Liu, Q., Han, Y., et al. (2018). Vertical Distribution of Microbial Eukaryotes From Surface to the Hadal Zone of the Mariana Trench. *Front. Microbiol.* 9. doi:10.3389/fmicb.2018.02023.

Zhang, T., Fei Wang, N., Qin Zhang, Y., Yu Liu, H., and Yan Yu, L. (2015). Diversity and distribution of fungal communities in the marine sediments of Kongsfjorden, Svalbard (High Arctic). *Sci. Rep.* 5, 14524. doi:10.1038/srep14524.

Zhang, X.-Y., Wang, G.-H., Xu, X.-Y., Nong, X.-H., Wang, J., Amin, M., et al. (2016). Exploring fungal diversity in deep-sea sediments from Okinawa Trough using high-throughput Illumina sequencing. *Deep Sea Res. Part I Oceanogr. Res. Pap.* 116, 99–105. doi:10.1016/j.dsr.2016.08.004.

Zhang, X., Tang, G., Xu, X., Nong, X., and Qi, S.-H. (2014). Insights into Deep-Sea Sediment Fungal Communities from the East Indian Ocean Using Targeted Environmental Sequencing Combined with

456 Traditional Cultivation. *PLoS One* 9, e109118. doi:10.1371/journal.pone.0109118.

457

458 **Table 1.** Sites of the Eastern Tropical Pacific of Costa Rica sampled in this study, with the
459 respective values of the environmental variables measured.

460

Sample	RV	Site	Date	Depth [m]	Temp [°C]	Salinity [PSU]	DO [mg/L]	pH	Data sources*
A1	Atlantis	Mound 12**	24/10/18	996	5,06	34,57	1,10	7,62	1, 6
A2	Atlantis	Quepos Slide**	25/10/18	380	11,75	34,76	0,20	7,71	1, 7
A3	Atlantis	Quepos Plateau	26/10/18	2200	2,06	34,60	3,73	8,06	2, 4, 6
A4	Atlantis	Seamount 3	28/10/18	1383	3,35	34,60	1,67	7,70	2, 4, 6
A5	Atlantis	Mound 11**	3/11/18	1024	4,83	34,57	1,24	7,67	6, 7
A6	Atlantis	Jaco Scar**	4/11/18	1788	2,54	34,63	2,42	7,61	1, 6
A7	Atlantis	Parrita Seep**	5/11/18	1410	3,41	34,60	2,21	7,71	6,00
A8	Atlantis	Quepos Plateau	26/10/18	1873	3,50	34,61	3,11	8,06	1, 3
F1	Falkor	The Thumb**	10/1/19	1072	4,54	34,58	1,22	7,69	4, 7
F2	Falkor	Parrita Scar	11/1/19	1419	3,35	34,61	2,08	7,67	4, 5
F3	Falkor	Rio Bongo	13/1/19	659	14,41	34,93	1,50	7,60	4, 7
F4	Falkor	Subduction Seep	14/1/19	3474	1,88	34,66	4,20	7,71	4, 5
F5	Falkor	Seamount 5.5	15/1/19	1540	3,00	34,62	2,64	7,70	4, 5
F6	Falkor	Seamount 7	16/1/19	1320	4,11	34,59	1,80	7,67	4, 5
F7	Falkor	Coco Canyon	18/1/19	950	5,02	34,57	1,40	8,12	4, 5
F8	Falkor	Mound Jaguar**	25/1/19	1903	2,43	34,63	3,13	7,75	4, 5

461 * 1. AUV Sentry sensors, 2. HOV Alvin sensors, 3. HOV Alvin Niskin bottle, 4. ROV SuBastian sensors, 5.
462 ROV SuBastian Niskin bottle, 6. RV Atlantis CTD, 7. RV Falkor CTD

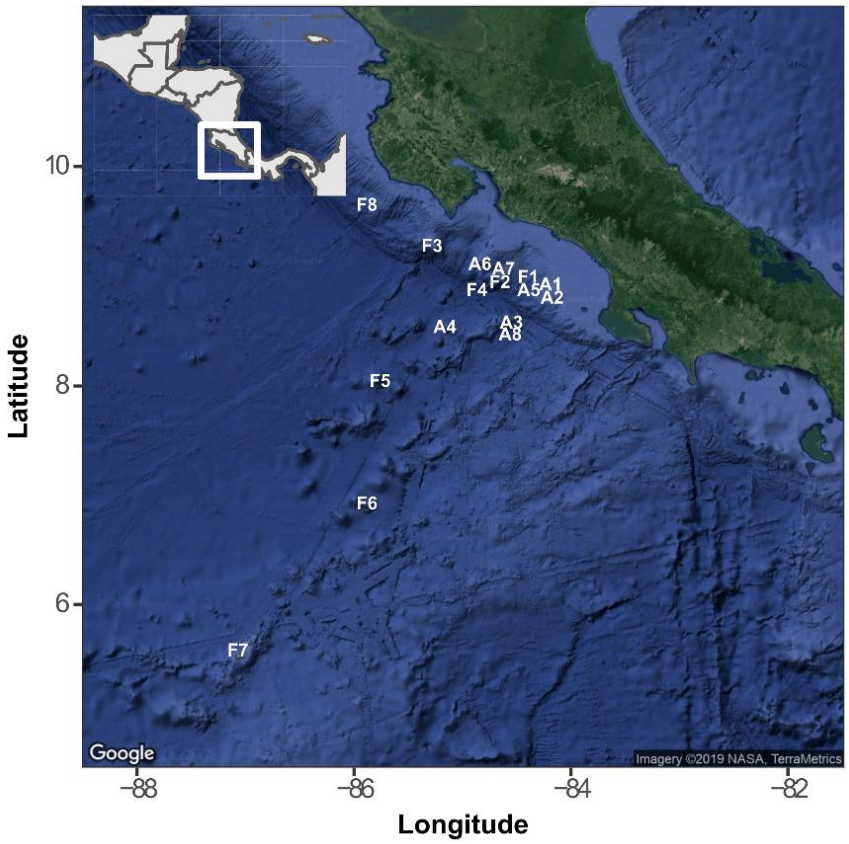
463 ** Seep areas

464 **Table 2.** Range of values of the environmental variables for each of the fungal clusters identified

Variable	Cluster 1 (yeast dominated)	Cluster 2 (Filamentous forms)
Depth [m]	380-1788	659-3474
Temperature [°C]	1.88-14.41	2.54-11.75
Salinity [PSU]	34.57-34.93	34.59-34.76
Dissolved Oxygen [mg/L]	1.10-4.20	0.2-2.64
pH	7.60-8.06	7.61-7.70

465

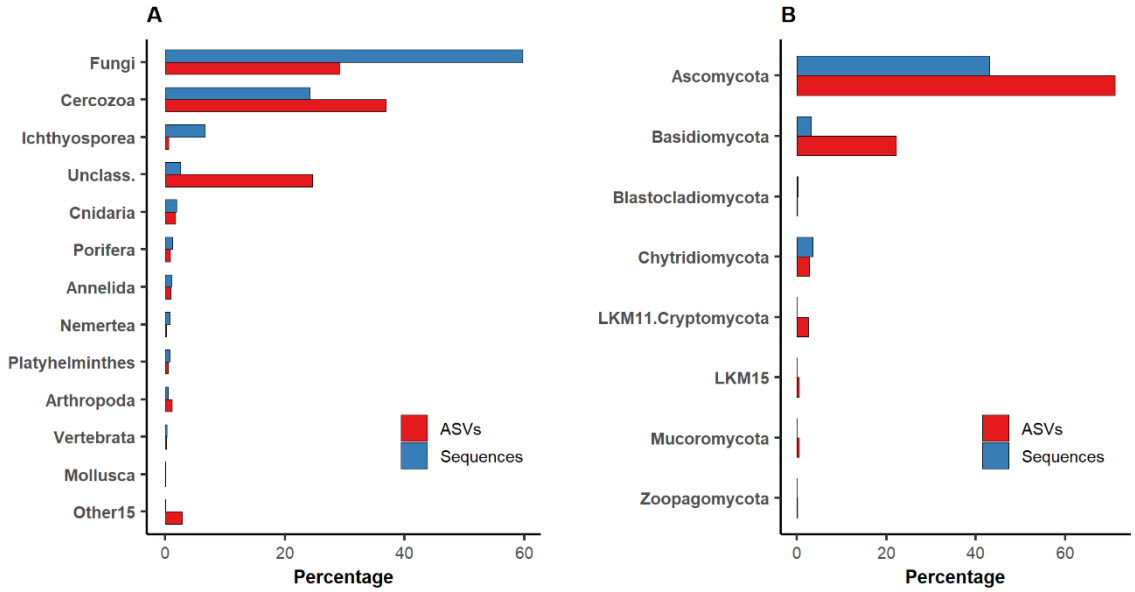
466 **Figure 1**



467

468 **Figure 1.** The geographical location of sampling points in the Eastern Tropical Pacific of Costa Rica. The
469 points indicated with the letter **A** correspond to the route followed by the *Atlantis* cruise and those with the
470 letter **F** to the *Falkor* cruise. The map was generated with the ggmap package using a Google satellite image.

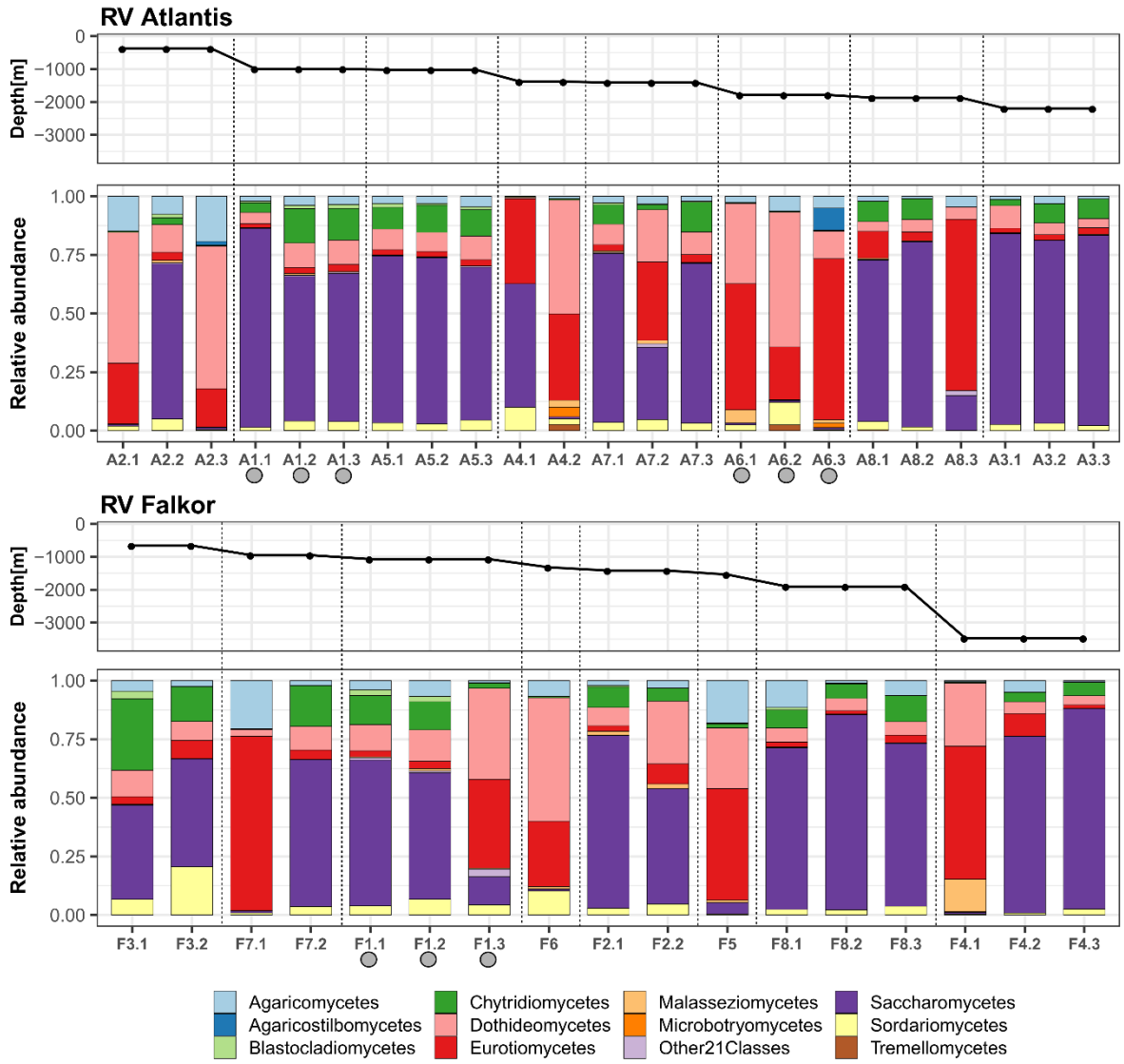
471 **Figure 2**



472

473 **Figure 2.** The relative abundance of eukaryotic groups (**A**) and fungal groups (**B**) in deep-sea sediments of
474 the Eastern Tropical Pacific of Costa Rica concerning the number of sequences and amplicon sequence
475 variants (ASVs).

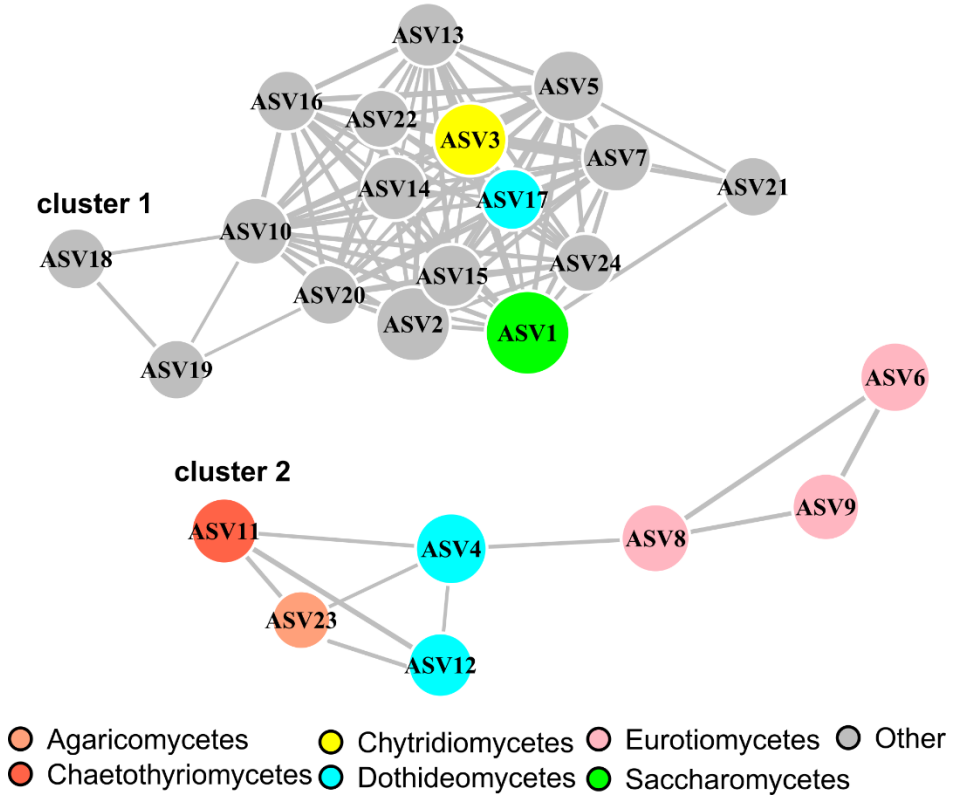
476 **Figure 3**



477

478 **Figure 3.** The relative abundance of fungi, at the taxonomic level of class, in deep-sea sediments of the
479 eastern tropical Pacific of Costa Rica. The proportions within sampling points of the core subsamples for
480 each of the cruise transects are shown. The samples were ordered according to the depth gradient. Gray
481 circles indicate active methane seeps.

482 **Figure 4**



483

484 **Figure 4.** Network analysis highlighting the relationships between the groups of fungi and also with respect
485 to other groups of eukaryotes present in 40 samples from deep-sea sediments. The analysis is based on the
486 24 most abudant eukaryotic ASVs (10 classified as fungi), which corresponded to nearly 70% of the total
487 number of eukaryotic sequences. Colors of the nodes represent the taxonomic afiliation of the ASVs, while
488 the size of the circles is proportional to their log-abundance. The network was generated and visualizaed
489 with package igraph. The taxonomic classification of the ASVs at the genus level and kingdom level are
490 shown as follows: ASV1: *Metschnikowia*(Fungi), ASV2: *Gymnophrys*(Cercozoa), ASV3: *Rhizophydium*(Fungi),
491 ASV4: *Cladosporium*(Fungi), ASV5: *Pirum*(Ichthyosporea), ASV6: *Aspergillus*(Fungi), ASV7:
492 *Pirum*(Ichthyosporea), ASV8: *Aspergillus*(Fungi), ASV9: *Aspergillus*(Fungi), ASV10:
493 *Cryothecomonas*(Cercozoa), ASV11: *Exophiala*(Fungi), ASV12: *Neophaeosphaeria*(Fungi), ASV13:
494 *Gymnophrys*(Cercozoa), ASV14: *Polymastia*(Porifera), ASV15: *Gymnophrys*(Cercozoa), ASV16:
495 *Pirum*(Ichthyosporea), ASV17: *Acidomyces*(Fungi), ASV18: *Cossura*(Annelida), ASV19:
496 *Tetrastemma*(Nemertea), ASV20: *Cryothecomonas*(Cercozoa), ASV21: *Pelagia*(Cnidaria), ASV22:
497 *Cryothecomonas*(Cercozoa), ASV23: *Armillaria*(Fungi), ASV24: *Quadricilia*(Cercozoa).

SUPPLEMENTARY MATERIAL

Fungal communities in sediments along a depth gradient in the Eastern Tropical Pacific

Keilor Rojas-Jimenez[1*], Hans Peter Grossart[2,3], Erik Cordes[4], Jorge Cortés[1,5]

[1]Escuela de Biología, Universidad de Costa Rica, 11501 San José, Costa Rica.

[2]Department of Experimental Limnology, Leibniz-Institute of Freshwater Ecology and Inland Fisheries, Alte Fischerhuette 2, D-16775 Stechlin, Germany.

[3]Institute for Biochemistry and Biology, Potsdam University, Maulbeerallee 2, 14469 Potsdam, Germany.

[4]Department of Biology, Temple University, Philadelphia, Pennsylvania, USA.

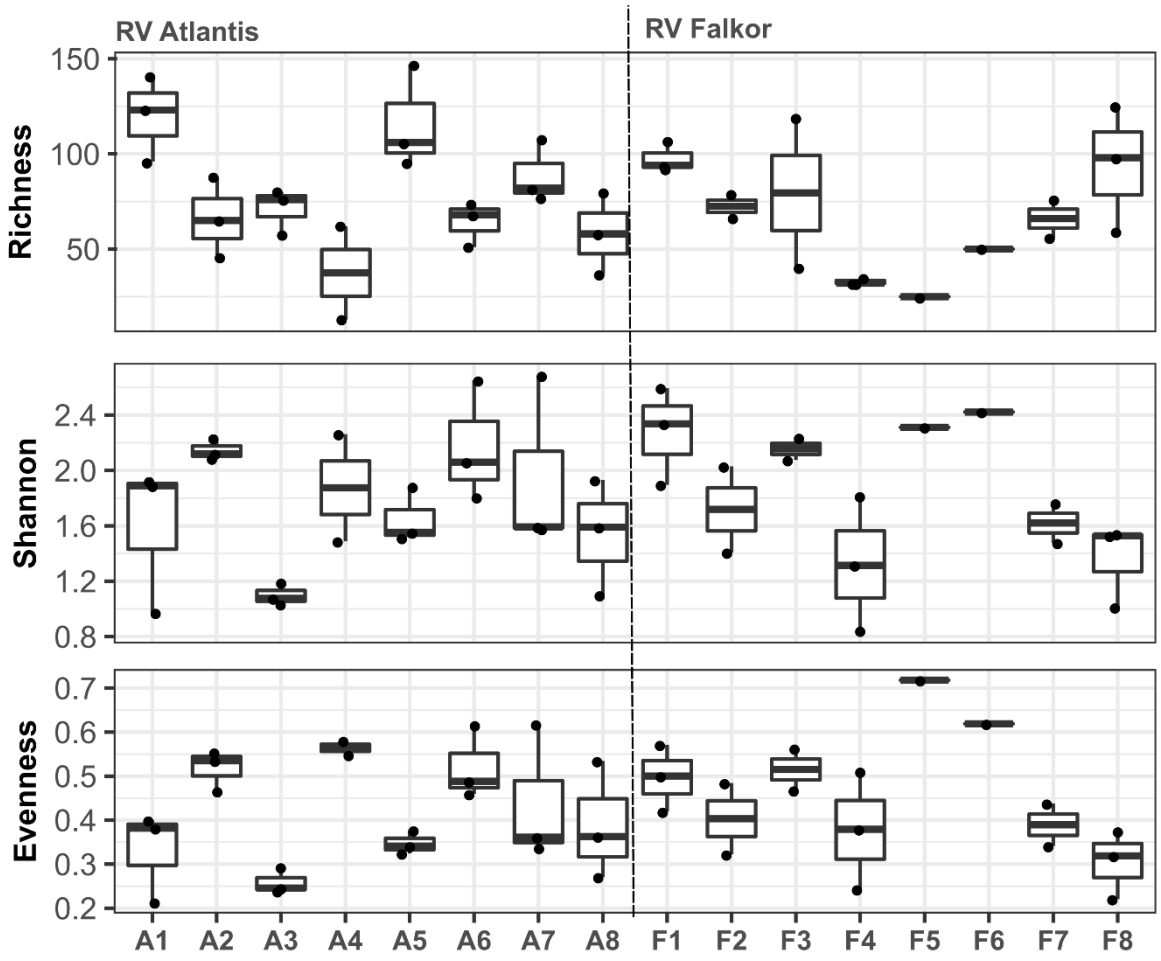
[5]Centro de Investigación en Ciencias del Mar y Limnología (CIMAR), Universidad de Costa Rica, San Pedro, 11501 San José, Costa Rica.

*Corresponding author

Keilor Rojas-Jimenez, keilor.rojas@gmail.com

Supplementary Table 1. Statistical analysis of the fungal community composition related to different variables. The PERMANOVA tests were performed using function adonis2 and implemented in Vegan package. Data were normalized by converting the ASV counts into relative abundances. Binning of continuous variables Depth, Temperature, Dissolved Oxygen and pH was performed with package Hmisc.

Variable	Comparison	R2	F.Model	p.value
Expedition	Atlantlis vrs Falkor	0.02092	0.8119	0.4555
Feature	Seep vrs no-seep	0.05148	1.0041	0.3506
Salinity	All (16 locations)	0.01851	0.7165	0.5275
Depth	All (16 locations)	0.02929	1.1467	0.2697
	380-1419 m vrs 1419-3474m	0.02233	0.8678	0.4046
Temperature	All (16 locations)	0.02843	1.1121	0.2947
	1.88-3.5 vrs 3.50-14.4	0.02003	0.7765	0.4585
Dissolved Oxygen	All (16 locations)	0.03454	1.3597	0.2118
	0.20-2.21 vrs 2.21-4.20 mg/L	0.02428	0.9456	0.3686
pH	All (16 locations)	0.03003	1.1765	0.2607
	7.60-7.71 vrs 7.71-8.12	0.02863	1.12	0.2697

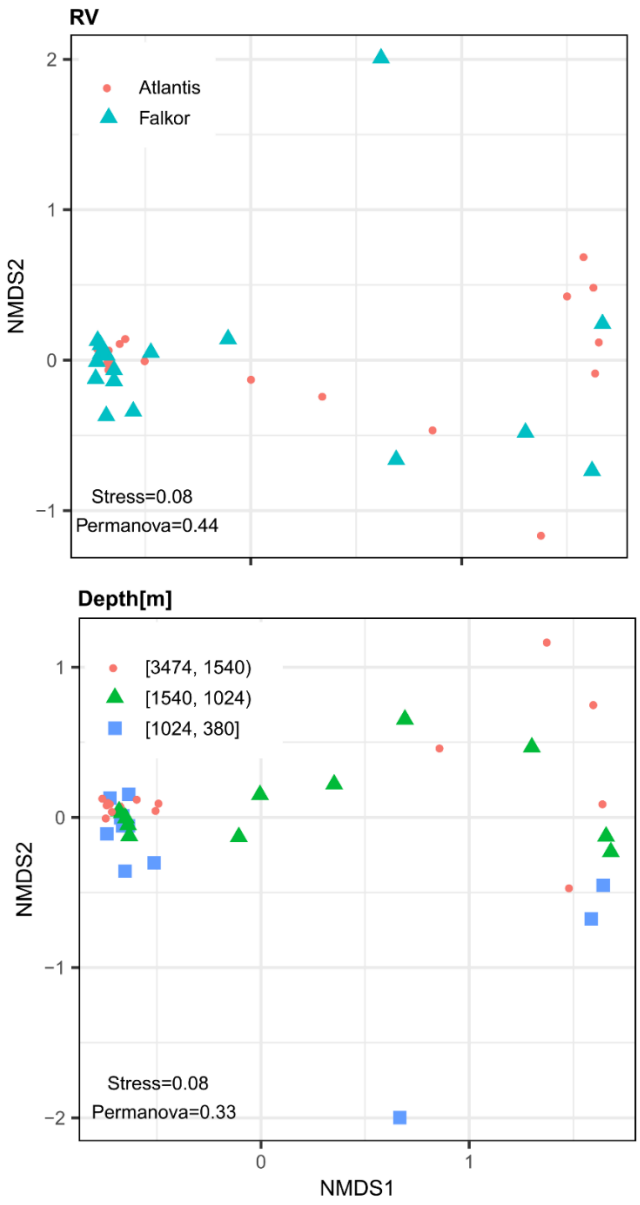


518

519 **Supplementary Figure 1.** Boxplots of the alpha diversity estimations of the sampling points in
520 deep-sea sediments of the Eastern Tropical Pacific.

521

522



Supplementary Figure 2. Non-metric multidimensional scaling (NMDS) analyses of the fungal communities in deep-sea sediments. The analysis include 40 samples from sediments obtained in a bathymetric gradient (from a depth of 380 to 3474 m) along two transects of about 1500 km length in the Eastern Tropical Pacific of Costa Rica. The upper panel shows the analysis by transect and the lower the analysis by depth. The stress values of the NMDS and the p value of the permanova analyses are also shown.