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Global biogeography of marine fungi is shaped by the environment

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ABSTRACT

Fungi are essential components of marine ecosystems, yet very little is known about their global distribution and diversity in the marine environment. In this study, we analyzed marine fungal community structure at a global scale using the International Census of Marine Microbes dataset. Marine fungal communities sampled from both the water column and sediments were compared. Based on the sequences of the nuclear ribosomal small subunit V9 region, 2200 operational taxonomic units (OTUs) were identified at 97% similarity. There was a significant distinction between the pelagic and benthic communities, with 15.4% OTUs shared between the two realms. Environmental factors, particularly sample depth, oxygen, and nitrate, strongly correlated with the fungal community composition and explained more variance than did geographic distance. This study represents the first global-scale analysis of marine fungal community structure, and highlights potential opportunities for research in marine fungal ecology and biogeography.

Introduction

Marine fungi are both diverse and important components of ocean ecosystems; however, they are understudied compared to other micro-organisms. Multiple lines of evidence indicate that fungi are critical and abundant components of nutrient cycling dynamics (Raghukumar 2004; Orsi et al., 2013a, b), exert top down control on phytoplankton communities (Gutierrez et al., 2011), are essential to marine food webs (Kagami et al., 2007; O'Rorke et al., 2013), and are symbiotic with marine macro-organisms (Yarden, 2014). It is clear that fungi are present, active and potentially significant players in marine biological processes across realms (Raghukumar 2004; Richards et al., 2012).

Diverse fungal assemblages have been enumerated from nearly every marine habitat searched, including open water (Gao et al., 2010), deep sea and hydrothermal vents (Bass et al., 2007; Edgcomb et al., 2011), anoxic environments (Jebaraj et al., 2010), wood substrata in the ocean (Rämä et al., 2014; Pang and Mitchell, 2005), as pathogens of macroorganisms (Nagahama et al., 2003), associated with healthy corals, sponges and other marine invertebrates (Amend et al., 2012;

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Yarden, 2014), and on various marine plants and algae (Zuccaro et al., 2008; Jones and Pang, 2011). However, little is known about the basic biogeographic patterning of marine fungi, and to what extent these habitats share fungal taxa.

Relative to fungi, patterns and processes of marine bacteria biogeography are better documented. Repeatedly, depth gradients (Giovannoni and Stingl, 2005; DeLong et al., 2006), habitat type (Zinger et al., 2011), and climate (Barberán and Casamayor, 2010) have been shown to contribute to biogeographic patterns in these microbes. Differences between sediment and water column bacteria are particularly stark. Zinger et al. (2011) found that bacterial communities of water column and sediment realms share only 7.1% of taxa on average. Comparatively rich, standardized datasets have enabled robust biogeographic inferences on a truly global scale, including the prevalence of macroecological patterns such as Rapoport's rule (Amend et al., 2013), latitudinal diversity gradients (Sul et al., 2013), isolation by distance and distance decay of community similarity patterns (Zinger et al., 2014).

Both space and environment also play a role in determining fungal community composition in the ocean. Studies of wood-inhibiting marine fungi show that community composition is driven by water temperature and salinity (Booth and Kenkel, 1986), as well as log attachment (whether secured or free-floating) and location (Rämä et al., 2014). A study of five subsurface sediments showed a high correlation between site physiochemical characteristics (dissolved inorganic carbon, total organic carbon and sulfide) and active fungal community composition as inferred from RNA analysis, suggesting a link between nutrients and fungal community composition (Orsi et al., 2013b). In a study of fungal communities associated with sea-cucumber aquaculture, both realms (benthic and water column) and sampling date significantly differentiated community composition (Guo et al., 2015).

Whereas both geographic distance and habitat are important predictors of fungal community composition it has not been determined which contributes more strongly to community differences. Of the handful of studies that have examined marine fungal community composition and community similarity patterns, the majority are limited to geographic scales <1000 km such as a depth gradient across 100 s of km within the North Pacific Gyre (Gao et al., 2010), fungal communities in the East (Zhang et al., 2014), or Central Indian Oceans (Singh et al., 2011). Fewer studies have examined fungal communities at greater geographic extent, and these have focused on specific habitats or substrata such as hydrothermal vent regions (e.g. Le Calvez et al., 2009, and Edgcomb et al., 2011), or Mangroves (Shmit and Shearer, 2003; Sarma, 2012). Further, differences in sampling methodology and primer choice among researchers have made robust synthetic analysis of fungal communities challenging. Analysis of fungal distributions based on taxonomy provide evidence for both narrow endemism (Sarma, 2012) and ubiquitous generalists (Hughes, 1986; Shearer et al., 2006; Amend et al., 2012; Richards et al., 2012; Amend, 2014), providing few clues as to which patterns predominate overall at the community level. To date, little is known about global biodiversity patterns and whether taxa overlap across habitats.

In this study we analyze a fungal community sequence dataset derived from the standardized collection by International Census of Marine Microbes (ICoMM) project (Amaral-Zettler et al., 2010). ICoMM was established in 2004 to facilitate the inventory of marine microbial diversity by cataloging all known diversity of marine single-cell organisms and exploring unknown microbial diversity. ICoMM provides one of the most geographically extensive datasets of marine microbial communities to date, enabling studies on the global distribution patterns of marine bacterial communities (see Zinger et al., 2011; Amend et al., 2012; Sul et al., 2013). Here, we use the dataset containing the eukaryotic environmental samples, processed in a single laboratory, using identical loci and protocols (Amaral-Zettler et al., 2009) and replicated amongst 56 water and sediment samples collected from various research groups from around the world's oceans. Previous analyses of portions of this dataset have focused on protists (Lie et al., 2014). Marine fungal community distributions have not been examined specifically from this dataset, and this study was the first attempt to understand them at a global scale.

The aims of our study were to determine whether community composition differs between fungal communities in sediments and the water column, to correlate composition with abiotic environmental variables, and to determine the extent to which realm, environment and distance shape fungal community dissimilarity patterns. We limited our analyses to fungal communities that are "free-living" (not associated with macroscopic host organisms) in marine sediments and water columns. We predicted that patterns of marine fungal biogeography would follow similar patterns to those detected amongst marine bacteria, and correlate with: (a) physiochemical characteristics of the environment, (b) geography, and (c) realm. We hypothesized that, similar to marine bacteria, fungal communities would more strongly correlate with environmental factors compared to geographic distance. Using a large, diverse and standardized dataset, we hoped to provide insight into the various factors that shape these diverse, little-studied assemblages.

Methods

The ICoMM data set

All samples were downloaded from the marine microbial database (MICROBIS, website: http://icomm.mbl.edu/microbis), which provides public access to the results of the ICoMM project (Amaral-Zettler et al., 2010), as well as detailed information about sampling sites and their associated projects and attributes. The dataset contains DNA sequence data from 454-sequencing technology, in which identical PCR, sequencing and bioinformatic processing were employed to ensure standardization. An ~65 bp fragment of the Eukaryotic nuclear ribosomal small subunit V9 region was amplified using 1380F/1389F and 1510R primers as described in Amaral-Zettler et al. (2009). Operational taxonomic units (OTUs) were determined at 97% similarity level. 2200 OTUs pertaining to Kingdom Fungi (2.7% of all OTUs in the dataset) were retained in a site-by-taxon matrix (Table S1). Taken together, 42 pelagic

and 14 benthic samples contained 10,793 fungal sequences. Associated metadata (e.g. latitude, longitude, depth) and various environmental parameters (e.g. temperature, salinity, and concentrations of phosphate, nitrate, dissolved oxygen and silicate) associated with each sample site were also obtained from MICROBIS. Incomplete data fields were estimated using the World Ocean Atlas 2001, 2005, and 2009 published by National Oceanographic Data Center of NOAA using the Ocean Data View 4.5.1. (Schlitzer, 2014), which completed depth-appropriate environmental parameters (temperature, salinity, phosphate, nitrate, dissolved oxygen and silicate) for 33 sites (Table S2). The samples were separated into two realms; those collected in water column (pelagic) and in sediment (benthic) based on sample locations reported in MICROBIS. Because reliable metadata were not available for some benthic samples, this realm was excluded from the environmental correlation analyses described below in the 'Fungal community analysis' section.

Whereas distance of pelagic samples from the equator was less (38.7°) on average compared to sediments (48.4°), the difference did not vary significantly from expectations of equal variance (t-test, df = 23, p = 0.15). Because most pelagic samples derived from the near-surface environment, the average depth of sediment samples was deeper. Thus, sample depth was partially confounded with realm.

Fungal community analysis

To visualize fungal community similarity patterns, OTU abundance tables were standardized by Hellinger transformation, and pairwise Bray–Curtis dissimilarity values were calculated for use in non-metric multidimensional scaling ordination. To account for different numbers of samples between the two realms, pelagic data were randomly subsampled 100 times. Each subsampled pelagic dataset was compared to the benthic samples using a permutational multivariate analysis of variances (function *adonis*) to determine whether community composition differed between the groups. Analysis was conducted using 1000 Monte Carlo permutation tests. R^2 and P values presented are the means of 100 random resamples. All analyses were conducted using the vegan package (Okansen et al., 2015) in the R programming environment.

To determine whether retention of singleton sequences would affect marine fungal community dissimilarity patterns we compared communities with and without singletons using a Procrustes correlation analysis. Procrustes test, or *protest* function in vegan, allows comparison of two ordinations using symmetric Procrustes analysis, assigning the significance via permutation tests (Okansen et al., 2015). The Procrustes correlation coefficient was 0.9991 (P = 0.001), indicating high similarity between the two ordination solutions. Therefore, singletons were retained. Samples containing fewer than 100 fungal reads were removed from all community analyses.

To evaluate the effects of environmental variables on marine fungal community structure in the pelagic realm, we conducted a Redundancy Analysis (RDA). Variance partitioning was conducted to estimate the pure effect of external environmental factors versus geography (longitude, latitude) on community similarity using partial RDA within the vegan package. We also assessed the order of importance of the explanatory variables using the marginal effects of each variable. The marginal effects were obtained by calculating the percentage of explained variance while using only one explanatory variable in RDA (Zuur et al., 2007).

Species richness

Sampling effort was assessed via rarefaction analysis using the *specaccum* function in the vegan package. Species accumulation-curves were constructed using sequences from all sites and realms, as well as all sequences excluding singleton OTUs. Additionally, separate species accumulation curves for the pelagic and the benthic samples were constructed using all available sequences and excluding singletons. Each sample was rarefied to an equal sequencing depth using the *Rarefy* function in the GUniFrac package in R, and total species richness was estimated using non-parametric asymptotic estimators (Chao1 and abundance-based coverage estimator (ACE)), using the function *estimateR* in the vegan package. The estimators of each sample met parametric assumptions, and within-sample diversity (α diversity) was compared between realms using a two-sample t-test. To account for variation in randomized subsampling, the procedure was repeated 1000 times, and we report mean estimator values and t-test statistics.

Results and discussion

The dataset

Our dataset contained a total of 56 samples of marine fungal assemblages, collected from all major ocean basins across broad ranges of environmental conditions (Fig. 1). The utility of the V9 region of the 18S locus lies in the fact that priming sites are relatively conserved across Eukarya, and that homology is maintained across multiple sequence alignments. The 18S in general (Schoch et al., 2012), and the V9 region in particular (Hartmann et al., 2010), are considerably more conserved than the ITS cistron. OTUs circumscribed in this study, therefore, likely correspond to a taxonomic rank higher than "species". The short sequence length and comparatively poor coverage of the V9 region in fungal reference databases hinders taxonomic assignments, which is why we purposely indicate taxonomy at relatively broad resolution. In total, 64.2% of the OTUs were identified to phylum, 46.3% were identified to class, and 38.7% were identified to subclass/order (Table S1).

Primer complementarity and taxonomic coverage

To assess the suitability of these primers for examining fungal communities, both primer pairs were queried against SILVA's small subunit database using SILVA's TestPrime tool (Quast et al., 2012). Analysis demonstrated that 997 of 6824 (14.6%) fungal sequences on the SILVA database (SSU r122) with coverage at this locus matched the most tolerant primer pair 100% identically, and 1452 of 7013 (20.7%) would be amplified allowing for up to four mismatches >3 base pairs beyond the

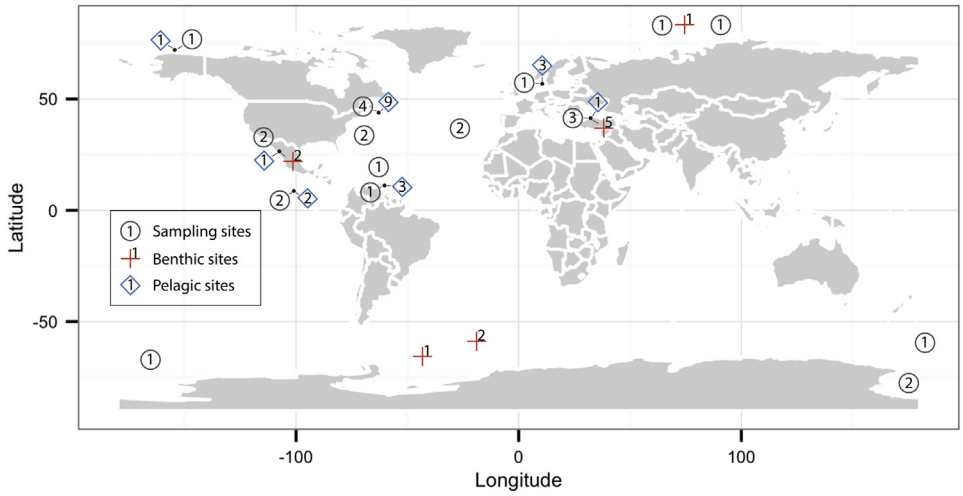

Fig 1 – Locations of sampling sites of International Census of Marine Microbes (ICoMM) that were analyzed as part of this study (n = 56). Sampling locations used for community structure analyses (n = 31) were indicated as follows; the pelagic samples in blue diamonds (◇), and the benthic samples in brown plus marks (+). The numbers indicate the number of samples collected near each location.

3′ terminus. However, all major fungal phyla contain representatives potentially amplified by these primers (Table S3) such that they provide broad, albeit sparse, coverage of Kingdom fungi *in silico*.

Taxonomic composition of marine fungal communities

Ascomycota and Basidiomycota were the most abundant phyla represented in our dataset (Fig S1A). Of the 23 OTUs identified to class, the three most abundant in our samples were Pezizomycetes, Agaricomycetes and Eurotiomycetes (Fig S1B). Pezizales, Hymenomycetidae and Eurotiales were the three most abundant subclass/order of the 27 OTUs identified to this level (Fig S1C). Basal fungal lineages such as Chytridiomycota and Cryptomycota, which have been detected in higher abundances elsewhere (e.g. Mohamed and Martiny, 2011; Guo et al., 2015), were notably sparse in our dataset. The dominance of Dikarya, however, generally agree with previous studies that find that this subkingdom tends to dominate marine habitats, whereas "basal" lineages predominate in fresh and brackish water (Richards et al., 2012, Shearer et al., 2006).

Community structure analysis

After discarding samples with low sequencing depth (<100 fungal reads), 31 samples were retained. These included 20 pelagic and 11 benthic samples, covering a broad range of environmental conditions (Fig. 1), consisting of 1995 OTUs and 9691 reads.

We found a clear distinction between the marine fungal community composition in the pelagic and benthic samples (*adonis*, $R^2 = 0.067$, $p = 0.008$). Similar to an equivalent analysis of marine bacteria (Zinger et al., 2011), relatively few OTUs were shared between the water column and sediment samples. Of 739 non-singleton OTUs present in the dataset, only 114 (15.4%) were shared between the two realms (Fig. 2). Composition of these "generalist" taxa was comparable at the class level with the communities found in the pelagic and benthic realms, all of which were dominated by Pezizomycetes, Agaricomycetes and Eurotiomycetes (Fig. 3). Thus, dissimilarity between realms was driven by differences between communities at low taxonomic ranks. This suggests that evolutionary transitions between realms are relatively frequent events.

As predicted, we found that environmental variables were strong and significant predictors of the pelagic marine fungal community structure. RDA analysis indicated that temperature, salinity, dissolved oxygen, nitrate, phosphate, silicate and depth together played a significant role in structuring marine fungal communities in the pelagic realm (RDA, $p < 0.001$; Fig. 4). Partial redundancy analysis also indicated that environmental variables, though not geographic location alone, significantly influenced pelagic fungal community structure; combined environmental variables accounted for

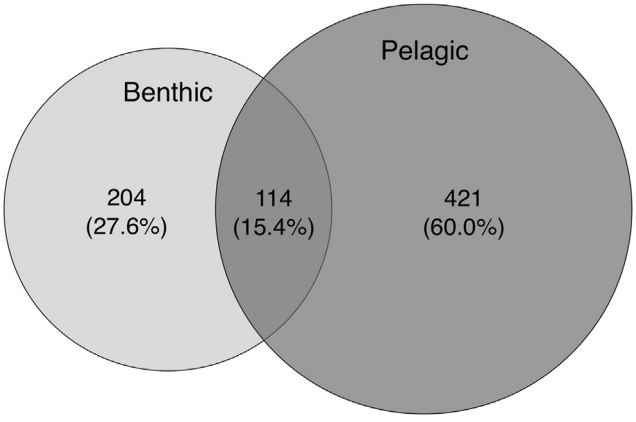

Fig 2 – Venn diagram of the number of OTUs shared between the pelagic and benthic realms.

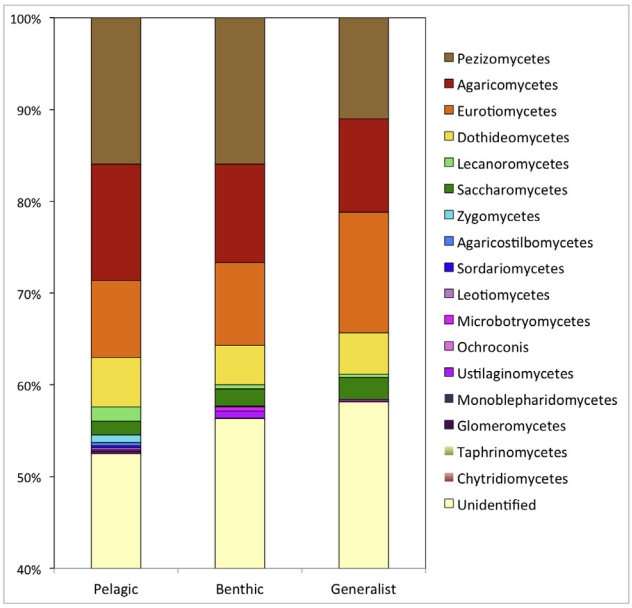

Fig 3 – Relative frequencies of marine fungal classes identified from the pelagic realm, the benthic realm, and the generalist taxa that occurred in both pelagic and benthic realms.

73% of the total explainable variance, as opposed to 18% by geographic location (Table 1). The pure effects of these environmental variables (i.e. testing the correlation of environmental variables while keeping other variables constant) were highly significant (RDA, $p < 0.001$), but not the pure effects of geographic locations (RDA, $p = 0.151$). Assessing the marginal effects of each explanatory variable indicated that depth was the best explanatory variable, explaining 24.3% of the total explainable variance ($p = 0.001$; Table 2). Dissolved oxygen and nitrate were ranked second (23.5%) and the third (22.9%), respectively, for total explained variance.

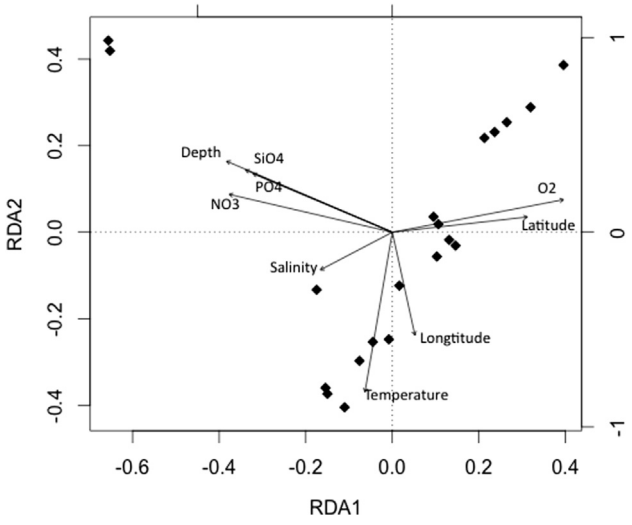

Fig 4 – Redundancy analysis (RDA) ordination diagram of pelagic marine fungal communities, with superimposed vectors representing environmental variables.

Previous studies have also found strong environmental correlation with marine fungal community composition. Subsurface fungal composition, for example, correlated strongly with carbon content and sulfide (Orsi et al., 2013b). Globally, Shearer et al. (2006) found that taxonomic occurrence, as determined from literature reviews, showed that temperature and salinity were the greatest determinates of species distributions. Although the methods, measured variables, and geographic extent of these studies are not directly comparable with ours, they all agree on the strong environmental filter imposed on marine fungal communities by abiotic variables.

Despite differences in measured environmental variables, lifestyles and likely differences in dispersal mechanisms, biogeographic patterns of marine fungi are qualitatively similar to other marine and aquatic microbes, as well as terrestrial fungi. Duarte et al. (2016) found in their extensive literature review that abiotic factors such as temperature, pH, conductivity and presence of various pollutants strongly influenced the community structure of aquatic hyphomycete morphospecies at a regional scale. Whereas their study also found that the community dissimilarity increased as a function of difference in latitude, geographic distance described slightly more variance. Although the authors did not attempt to quantify the relative contributions of geographic distance vs. environment to community dissimilarity patterns, ordination visualization of their data clearly demonstrates that communities cluster by climate. Over broad spatial scales, terrestrial environmental determinates strongly shape fungal communities as well. According to a global study of fungal communities in soil (Tedersoo et al., 2014), potential evapotranspiration and soil pH combined described 3.9% of compositional variance. Similarly, a global study of fungi detected in indoor dust found that rainfall and temperature of sample locations better described community similarity among samples than did geographic proximity (Amend et al., 2010). In a meta-analysis of microbial biogeography studies (mostly bacteria) environmental factors generally predicted a threefold higher amount of community compositional variance compared to geographic distance alone (Hanson et al., 2012).

Species richness and completeness of sampling

The observed singleton OTUs comprised approximately 66.7% of all OTUs in our datasets. Removing singletons resulted in species accumulation curves that approached an asymptote for the sediment, water column and combined samples curves (Fig. 5A, B), suggesting that a reasonably high percentage of abundant OTUs were detected.

OTU richness estimators Chao1 and ACE estimators were generally in agreement, with ACE producing slighter higher values (Table 3). These estimators were calculated for each sample independently (i.e. α diversity), and compared between the benthic and pelagic samples (Table 4). Neither estimator, Chao1 nor ACE, differed significantly between the pelagic samples and the benthic samples (t-test, Chao1: d.f. = 26, p-value > 0.50, ACE: d.f. = 26, p-value > 0.58), indicating that the within-site diversity levels were comparable between the two realms.

Table 1 – Summary of partial RDA analysis results, showing the partitioning of the total explainable variance by environmental factors versus geography for the marine fungal community structure in the pelagic realm.

	Variance	Explained variance	F Value	P Value
Total explainable	0.5024			
Pure environmental factors	0.3667	72.99%	1.4912	0.001
Pure geography	0.091	18.12%	1.2955	0.151
Joint	0.0447	8.89%		

Table 2 – Marginal effects of explanatory variables for the marine fungal community structure in the pelagic realm. Values of the eigenvalues (variance) using only one explanatory variable in RDA, and the variance as percentage of the sum of all eigenvalues of all explainable variables are shown.

Explanatory Variables	Eigenvalue using only one explanatory variables	Eigenvalue as % of total	P Value
Depth	0.0962	24.31%	0.001
O2	0.09312	23.53%	0.001
Nitrate	0.09016	22.78%	0.001
Phosphate	0.08703	21.99%	0.001
Silicate	0.08278	20.92%	0.006
Temp	0.08211	20.75%	0.001
Latitude	0.07508	18.97%	0.001
Longitude	0.05991	15.14%	0.065
Salinity	0.05605	14.16%	0.081

The total OTU richness of the pooled pelagic samples (i.e. γ diversity) was 1.7 (ACE) to 1.9 (Chao1) times higher than the total species richness of the pooled benthic samples, consistent with the larger number of sample sites.

Many studies have reported significant contributions of singletons in estimating the total number of OTUs and species richness from the next generation sequencing (NGS) datasets (e.g. Zinger et al., 2011; Rämä et al., 2014; Lie et al., 2014). Our results also confirmed this trend, singleton OTUs comprising approximately 66.7% of the total OTUs identified in the datasets. There has been an ongoing debate as to whether or not singletons are artifacts of datasets generated by NGS. However, recent comparative studies addressing this question report the majority of the rare taxa are likely to be real, rather than sequencing artifacts (Zinger et al., 2011; Zhan et al., 2013; Lie et al., 2014). The ICoMM dataset used in our study also supports the presence of an underexplored "rare biosphere," comprised of large number of highly diverse, low-abundance OTUs in marine fungal communities, as in other microbial communities (Sogin et al., 2006).

Conclusion

Despite the ubiquity and abundance of marine fungi, almost nothing is known of its biogeographic structure, or the forces that shape it. Using one of the most extensive datasets yet collected, we examined the global distribution of marine fungal communities. We found that fungal communities residing in the sediment differed significantly from those in the water column. Whereas geographic location influenced fungal community composition, environmental factors such as O_2 and water depth were much stronger predictors.

There are several limitations to our study that might be addressed in future studies of marine fungi. First, the depth of

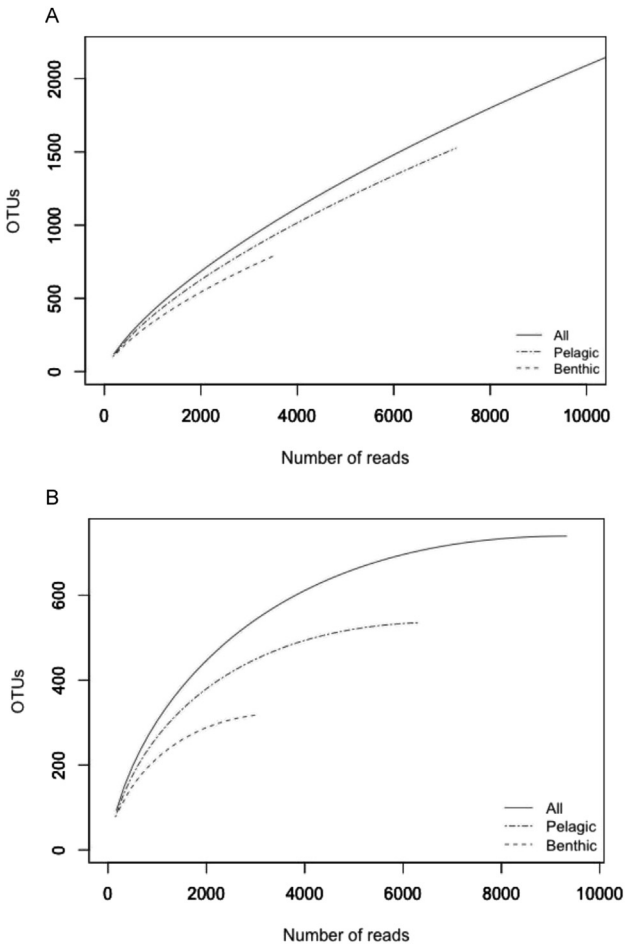

Fig 5 – Species accumulation curve based on fungal OTUs at a dissimilarity level of 3%: (A) based on the entire dataset, and (B) the dataset excluding singletons.

Table 3 – Non-parametric estimators of species richness for the global marine fungi dataset.

Dataset	Chao1		ACE	
	Estimate	Se	Estimate	Se
All	5192	270	5563	49
Pelagic	3553	118	3753	40
Benthic	1832	145	2122	31

Table 4 – Statistical comparison of non-parametric estimators of species richness between the pelagic and the benthic samples (a two sample t-test). Each sample was adjusted to an equal sequencing depth before calculating the indices.

Dataset	n	Chao1		ACE	
		Average of samples	T-test P-value	Average of samples	T-test P-value
Pelagic	20	126.22	0.50	142.02	0.59
Benthic	11	112.30		133.10	

our sequencing effort, hampered by both non-target amplification and the comparatively dated technology employed, was insufficient for capturing many of the sparse and rare OTUs comprising the long tail of our species abundance distribution. Whereas shallow sampling is unlikely to significantly impact conclusions drawn about patterns of community similarity and divergence, it has likely contributed to higher variance and lower analytical power of alpha diversity analyses. Second, sampling locations were unevenly distributed. Although we accounted for spatial autocorrelation, explicitly, in our statistical models, clumped and patchy samples likely reduced our analytical power and hampered our ability to draw conclusions about patterns such as range size or environmental tolerance of individual OTUs. Last, the relatively short read length and conserved sequence of the SSU v9 region are not ideal for resolution of lower taxonomic ranks. In future studies, these shortcomings may be partially overcome by using primers with a higher affinity and specificity for fungal taxa.

Recent estimates predict as many as 5.1 million species of fungi exist on Earth (Blackwell, 2011), and understanding the processes that shape that biota are fundamental to fungal ecology. Whereas there is much to be discovered about the evolution, identity and life strategy of marine fungi, our study shows that marine fungi follow similar biogeographic patterns as other marine (and terrestrial) microbes at global scales. Little, as yet, is known about connectivity with host-associated assemblages or temporal patterns, leaving much to be discovered about these little-studied communities in the oceans covering the majority of our planet.

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Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.funeco.2015.09.003.

REFERENCES

Amaral-Zettler, L., Artigas, L.F., Baross, J., Bharathi, L., Boetius, A., Chandramohan, D., Herndl, G., Kogure, K., Neal, P., Pedros-Alio, C., Ramette, A., Schouten, S., Stal, L., Thessen, A., de Leeuw, J., Sogin, M., 2010. A global census of marine microbe. In: McIntyre, A. (Ed.), Life in the World's Oceans: Diversity, Distribution and Abundance. Blackwell Publishing Ltd., Oxford, pp. 223–245.

Amaral-Zettler, L.A., McCliment, E.A., Ducklow, H.W., Huse, S.M., 2009. A method for studying protistan diversity using massively parallel sequencing of V9 hypervariable regions of small-subunit ribosomal RNA genes. *PLoS One* 4, e6372.

Amend, A.S., 2014. From dandruff to deep-sea vents: Malassezia-like fungi are ecologically hyper-diverse. *PLoS Pathog.* 10, e1004277.

Amend, A.S., Seifert, K.A., Samson, R., Bruns, T.D., 2010. Indoor fungal composition is geographically patterned and more diverse in temperate zones than in the tropics. *Proc. Natl. Acad. Sci.* 31, 13748–13753.

Amend, A.S., Barshis, D.J., Oliver, T.A., 2012. Coral-associated marine fungi form novel lineages and heterogeneous assemblages. *ISME J.* 6, 1291–1301.

Amend, A.S., Oliver, T.A., Amaral-Zettler, L.A., Boetius, A., Fuhrman, J.A., Horner-Devine, M.C., Huse, S.M., Welch, D.B.M., Martiny, A.C., Ramette, A., 2013. Macroecological patterns of marine bacteria on a global scale. *J. Biogeogr.* 40, 800–811.

Barberán, A., Casamayor, E.O., 2010. Global phylogenetic community structure and β-diversity patterns in surface bacterioplankton metacommunities. *Aquat. Microb. Ecol.* 59, 1–10.

Bass, D., Howe, A., Brown, N., Barton, H., Demidova, M., Michelle, H., Li, L., Sanders, H., Watkinson, S.C., Willcock, S., Richards, T.A., 2007. Yeast forms dominate fungal diversity in the deep oceans. *Proc. R. Soc. B Biol. Sci.* 274, 3069–3077.

Blackwell, M., 2011. The Fungi: 1, 2, 3... 5.1 million species? *Am. J. Bot.* 98, 426–438.

Booth, T., Kenkel, N., 1986. Ecological studies of lignicolous marine fungi: a distribution model based on ordination and classification. In: Moss, S.T. (Ed.), The Biology of Marine Fungi. Cambridge University Press, Cambridge, pp. 297–310.

DeLong, E.F., Preston, C.M., Mincer, T., Rich, V., Hallam, S.J., Frigaard, N.-U., Martinez, A., Sullivan, M.B., Edwards, R., Brito, B.R., Chisholm, S.W., Karl, D.M., 2006. Community genomics among stratified microbial assemblages in the ocean's interior. *Science* 311, 496–503.

Duarte, S., Bärlocher, F., Pascoal, C., Cássio, F., 2016. Biogeography of aquatic hyphomycetes: current knowledge and future perspectives. *Fungal Ecol.* 19, 169–181.

Edgcomb, V.P., Beaudoin, D., Gast, R., Biddle, J.F., Teske, A., 2011. Marine subsurface eukaryotes: the fungal majority. *Environ. Microbiol.* 13, 172–183.

Gao, Z., Johnson, Z.I., Wang, G., 2010. Molecular characterization of the spatial diversity and novel lineages of mycoplankton in Hawaiian coastal waters. *ISME J.* 4, 111–120.

Giovannoni, S.J., Stingl, U., 2005. Molecular diversity and ecology of microbial plankton. *Nature* 437, 343–348.
Guo, X., Zhang, Q., Zhang, X., Zhang, J., Gong, J., 2015. Marine fungal communities in water and surface sediment of a sea cucumber farming system: habitat-differentiated distribution and nutrients driving succession. *Fungal Ecol.* 14, 87–98.
Gutierrez, M.H., Pantoja, S., Tejos, E., Quinones, R.A., 2011. The role of fungi in processing marine organic matter in the upwelling ecosystem off Chile. *Mar. Biol.* 158, 205–219.
Hartmann, M., Howes, C.G., Abarenkov, K., Mohn, W.W., Nilsson, R.H., 2010. V-Xtractor: an open-source, high-throughput software tool to identify and extract hypervariable regions of small subunit (16 S/18 S) ribosomal RNA gene sequences. *J. Microbiol. Methods* 83, 250–253.
Hanson, C.A., Fuhrman, J.A., Horner-Devine, M.C., Martiny, J.B., 2012. Beyond biogeographic patterns: processes shaping the microbial landscape. *Nat. Rev. Microbiol.* 10, 497–506.
Hughes, G.C., 1986. Biogeography and the Marine fungi. In: Moss, S.T. (Ed.), The Biology of Marine Fungi. Cambridge University Press, Cambridge, pp. 275–295.
Jebaraj, C.S., Raghukumar, C., Behnke, A., Stoeck, T., 2010. Fungal diversity in oxygen-depleted regions of the Arabian Sea revealed by targeted environmental sequencing combined with cultivation. *FEMS Microbiol. Ecol.* 71, 399–412.
Jones, E.B.G., Pang, K.-L., 2011. Tropical aquatic fungi. *Biodivers. Conserv.* 21, 2403–2423.
Kagami, M., de Bruin, A., Ibelings, B.W., Van Donk, E., 2007. Parasitic chytrids: their effects on phytoplankton communities and food-web dynamics. *Hydrobiologia* 578, 113–129.
Le Calvez, T., Burgaud, G., Mahé, S., Barbier, G., Vandenkoornhuyse, P., 2009. Fungal Diversity in Deep-Sea Hydrothermal Ecosystems. *Appl. Environ. Microbiol.* 75, 6415–6421.
Lie, A.A.Y., Liu, Z., Hu, S.K., Jones, A.C., Kim, D.Y., Countway, P.D., Amaral-Zettler, L.A., Cary, S.C., Sherr, E.B., Sherr, B.F., Gast, R.J., Caron, D.A., 2014. Investigating microbial eukaryotic diversity from a global census: insights from a comparison of pyrotag and full-length sequences of 18S rRNA genes. *Appl. Environ. Microbiol.* 80, 4363–4373.
Mohamed, D.J., Martiny, J.B.H., 2011. Patterns of fungal diversity and composition along a salinity gradient. *ISME J.* 5, 379–388.
Nagahama, T., Hamamoto, M., Nakase, T., Horikoshi, K., 2003. *Rhodotorula benthica sp. nov.* and *Rhodotorula calyptogenae sp. nov.*, novel yeast species from animals collected from the deep-sea floor, and *Rhodotorula lysiniphila sp. nov.*, which is related phylogenetically. *Int. J. Syst. Evol. Microbiol.* 53, 897–903.
Okansen, J., Blanchet, F.G., Kindt, R., Legendre, P., Minchin, P.R., O'Hara, R.B., Simpson, G.L., Solymos, P., Henry, M., Stevens, H., Wagner, H., 2015. Vegan: Community Ecology Package. R Package Version 2.3-0. http://CRAN.R-project.org/package=vegan.
O'Rorke, R., Lavery, S.D., Wang, M., Nodder, S.D., Jeffs, A.G., 2013. Determining the diet of larvae of the red rock lobster (Jasus edwardsii) using high-throughput DNA sequencing techniques. *Mar. Biol.* 161, 551–563.
Orsi, W.D., Edgcomb, V.P., Christman, G.D., Biddle, J.F., 2013. Gene expression in the deep biosphere. *Nature* 499, 205–208.
Orsi, W., Biddle, J.F., Edgcomb, V., 2013. Deep sequencing of subseafloor eukaryotic rRNA reveals active Fungi across marine subsurface provinces. *PLoS One* 8, e56335.
Pang, K.L., Mitchell, J.I., 2005. Molecular approaches for assessing fungal diversity in marine substrata. *Bot. Mar.* 48, 332–347.
Quast, C., Pruesse, E., Yilmaz, P., Gerken, J., Schweer, T., Yarza, P., Peplies, J., Glöckner, F.O., 2012. The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. *Nucleic Acids Res.* 41, D590–D596.
Raghukumar, S., 2004. The role of fungi in Marine detrital processes. In: Ramaiah, N. (Ed.), Marine Microbiology: Facets and Opportunities. National Institute of Oceanography, Goa, India, pp. 91–101.
Rämä, T., Nordén, J., Davey, M.L., Mathiassen, G.H., 2014. Fungi ahoy! Diversity on marine wooden substrata in the high North. *Fungal Ecol.* 8, 46–58.
Richards, T.A., Jones, M.D.M., Leonard, G., Bass, D., 2012. Marine fungi: their ecology and molecular diversity. *Annu. Rev. Mar. Sci.* 4, 495–522.
Sarma, V.V., 2012. Diversity and distribution of Marine fungi on *Rhizophora spp.* In mangroves. In: Raghukumar, C. (Ed.), Biology of Marine Fungi, Progress in Molecular and Subcellular Biology, pp. 243–275.
Schlitzer, R., 2014. Ocean Data View. http://odv.awi.de.
Schoch, C.L., Seifert, K.A., Huhndorf, S., Robert, V., Spouge, J.L., Levesque, C.A., Chen, W., Fungal-Barcoding-Consortium, 2012. Nuclear ribosomal internal transcribed spacer (ITS) region as a universal DNA barcode marker for Fungi. *Proc. Natl. Acad. Sci. U. S. A.* 109, 6241–6246.
Shearer, C.A., Descals, E., Kohlmeyer, B., Kohlmeyer, J., Marvanová, L., Padgett, D., Porter, D., Raja, H.A., Schmit, J.P., Thorton, H.A., Voglymayr, H., 2006. Fungal biodiversity in aquatic habitats. *Biodivers. Conserv.* 16, 49–67.
Schmit, J.P., Shearer, C.A., 2003. A checklist of mangrove-associated fungi, their geographical distribution and known host plants. *Mycotaxon* 85, 423–477.
Singh, P., Raghukumar, C., Verma, P., Shouche, Y., 2011. Fungal community analysis in the deep-sea sediments of the Central indian Basin by culture-independent approach. *Microb. Ecol.* 61, 507–517.
Sogin, M.L., Morrison, H.G., Huber, J.A., Mark Welch, D., Huse, S.M., Neal, P.R., Arrieta, J.M., Herndl, G.J., 2006. Microbial diversity in the deep sea and the underexplored 'rare biosphere'. *Proc. Natl. Acad. Sci. U. S. A.* 103, 12115–12120.
Sul, W.J., Oliver, T.A., Ducklow, H.W., Amaral-Zettler, L.A., Sogin, M.L., 2013. Marine bacteria exhibit a bipolar distribution. *Proc. Natl. Acad. Sci. U. S. A.* 110, 2342–2347.
Tedersoo, L., Bahram, M., Põlme, S., Kõljalg, U., Yorou, N.S., Wijesundera, R., Villarreal Ruiz, L., Vasco-Palacios, A.M., Thu, P.Q., Suija, A., et al., 2014. Fungal biogeography. Global diversity and geography of soil fungi. *Science* 346, 1256688.
Yarden, O., 2014. Fungal association with sessile marine invertebrates. *Front. Microbiol.* 5, 228.
Zhan, A., Hulak, M., Sylvester, F., Huang, X., Adebayo, A.A., Abbot, C.L., Adamowicz, S.J., Heath, D.D., Cristescu, M.E., Maclsaac, H.J., 2013. High sensitivity of 454 pyrosequencing for detection of rare species in aquatic communities. *Methods Ecol. Evol.* 4, 558e565.
Zhang, X.Y., Tang, G.L., Xu, X.Y., Nong, X.H., Qi, S.H., 2014. Insights into deep-sea sediment fungal communities from the east indian ocean using targeted environmental sequencing combined with traditional cultivation. *PLoS One* 9, e109118.
Zinger, L., Amaral-Zettler, L.A., Fuhrman, J.A., Horner-Devine, M.C., Huse, S.M., Welch, D.B.M., Martiny, J.B.H., Sogin, M., Boetius, A., Ramette, A., 2011. Global patterns of bacterial beta-diversity in seafloor and seawater ecosystems. *PLoS One* 6, e24570.
Zinger, L., Boetius, A., Ramette, A., 2014. Bacterial taxa-area and distance-decay relationships in marine environments. *Mol. Ecol.* 23, 954–964.
Zuccaro, A., Schoch, C.L., Spatafora, J.W., Kohlmeyer, J., Draeger, S., Mitchell, J.I., 2008. Detection and identification of fungi intimately associated with the brown seaweed *Fucus serratus*. *Appl. Environ. Microbiol.* 74, 931–941.
Zuur, A.F., Leno, E.N., Smith, G.M., 2007. Analysing Ecological Data. Springer, New York, USA.