

Fungal diversity in oxygen-depleted regions of the Arabian Sea revealed by targeted environmental sequencing combined with cultivation

Cathrine S Jebaraj^{1,2}, Chandralata Raghukumar¹, Anke Behnke², Thorsten Stoeck²

¹National Institute of Oceanography, (Council of Scientific and Industrial Research),
Dona Paula, Goa 403 004, India;

²School of Biology, University of Kaiserslautern, Kaiserslautern, Germany.

Correspondence:

Thorsten Stoeck, School of Biology, University of Kaiserslautern,
Erwin Schrödinger Str. 14, D-67663 Kaiserslautern, Germany.
Tel.: +49 631-2052502; fax: +49 631-2052502; e-mail: stoeck@rhrk.uni-kl.de

Running title: fungal diversity in the Arabian Sea

Summary

By phylogenetic analyses of ca. 1500 small subunit ribosomal RNA gene (18S rDNA) sequences obtained from fungal isolates and environmental samples of the Arabian Sea we demonstrate that fungal diversity in anoxic marine habitats is severely underestimated. Three different primer sets amplified fungal sequences that could be assigned to the Dikarya and the zygomycetes. Only few environmental sequences are closely (>97% similarity) related to previously deposited sequences of described fungi. But a substantial number is closely related to environmental sequences from a variety of other anoxic marine habitats. Community similarity analyses suggest distinctively different structures of fungal communities from permanently oxic sites, seasonally anoxic sites and permanently anoxic sites. This difference increased when comparing these fungal communities to one from a normoxic sampling site, suggesting different adaptation strategies of fungal communities in large to prevailing oxygen conditions. Each of the individual putatively fungal specific primer sets used in this study amplified a substantial number of non-fungal DNA templates as well as different subsets of a fungal community. Most of the 26 cultures obtained in this study were closely related to well-described Dikarya, indicating that standard cultivation mainly produces more of what is already known. However, two of these cultures were highly divergent to previously sequenced organisms and seem to represent novel fungal groups on high taxonomic levels. Furthermore, it appears that none of the cultured isolates is identical to any of the environmental sequences obtained. Our study demonstrates the importance of a multiple primer approach in combination with cultivation to obtain deeper insights into the true fungal diversity in any environmental sample and to enable adequate inter-sample comparisons of fungal communities.

Keywords: fungal diversity; Arabian Sea; cultivation; SSU rDNA,; oxygen minimum zone; PCR primers

Introduction

Fungi are primarily aerobic heterotrophs that play an essential role as decomposers of organic matter in a variety of environments. In marine ecosystems fungi are the major decomposers of woody and herbaceous substrates and their importance lies in their ability to aggressively degrade lignocellulose (Newell, 1996). Marine fungi also contribute to the degradation of dead animals and animal parts (Kohlmeyer & Kohlmeyer, 1979), and are important pathogens of animals and plants or partners in mutualistic symbioses (Raghukumar, 1986, Alsumard *et al.*, 1995; Pivkin, 2000).

Fungi were long thought to play only a minor role in ecosystem processes of anoxic environments (Dighton, 2003). However, many fungal taxa were recently shown to possess metabolic adaptations to utilize nitrate and (or) nitrite as an alternative for oxygen (Shoun *et al.*, 1992). This testifies to their potential to participate in anaerobic denitrification processes. In a recent study, Jebaraj & Raghukumar (2009) showed that fungi isolated from anoxic marine waters of the Arabian Sea are capable of growth under oxygen deficient conditions while performing anaerobic denitrification.

These findings suggest that the abundance and ecological role of fungi in anoxic marine systems is currently underestimated. Support for this assumption comes from analyses of the small subunit ribosomal RNA locus (18S rDNA) amplified from genomic DNA isolated from environmental samples. While usually rare in open ocean surface waters, the majority of environmental 18S rDNA diversity surveys conducted in oxygen-depleted aquatic environments report large proportions of fungal 18S rDNA sequences (review in Epstein & López-García, 2007). In many studies, fungal sequences from anoxic habitats nest within known and well-described taxa (Dawson & Pace, 2002; Stoeck & Epstein 2003; Brad *et al.*, 2008). In contrast, numerous environmental sequences are unique and highly divergent from GenBank accessions of known fungi (e.g. Stoeck *et al.*, 2007; Takishita *et al.*, 2005, 2007a, b; López-García *et al.*, 2007; Laurin *et al.*, 2008). This indicates the potential of such environments as one major source of the 1.3 million undescribed fungal species estimated by Hawksworth (2001) present in natural ecosystems.

Previous studies have detected unknown fungal lineages in anoxic aquatic systems despite using universal 18S rDNA eukaryote primers. Such primers target most eukaryotic organisms across all major clades. Because of this non-targeted approach, many fungi may not have been amplified and the full extent of fungal diversity may be orders of magnitude higher than these studies have shown. Indeed, PCR primers with specificity for fungal DNA, while reducing co-amplification of similar target-DNA from non-fungal sources, were applied successfully for estimating the extent of fungal diversity in soil (e.g. Anderson *et al.*, 2003; Borneman & Hartin, 2000; Malosso *et al.*, 2006). This targeted strategy has gone untested for aquatic samples in general, and anoxic habitats in specific.

Using previously published primers designed for the specific amplification of fungal 18S rDNA from mixed-origin genomic DNA (Vainio & Hantula, 2000; Gomes *et al.*, 2003; Kappe *et al.*, 1996; Maiwald *et al.*, 1994), we analyzed fungal diversity in samples from the oxygen minimum zone (OMZ) of the Arabian Sea. This sea is known to be a biogeochemically active environment for microbial mediated denitrification processes (Naqvi *et al.*, 2006). In order to assess the proportion of fungal sequences that may have been missed by previously published universal eukaryote primer-based clone libraries from anoxic marine sites, we compared clone-libraries constructed with fungal-specific primers to clone libraries constructed from standard universal eukaryote PCR primers (Medlin *et al.*, 1988). Finally, we used cultivation approaches to complement the molecular diversity surveys. The fungal diversity observed at seasonally and perennially oxygen depleted sites was compared to the fungal community in a permanently oxygenated coral reef ecosystem.

Materials and methods

Sampling sites

Samples were collected from three sites in the Arabian Sea (Table 1). (i) Sampling of sediments at the coastal station (15° 29.951' N, 73° 40.827' E) off Goa at 25m depth was carried out in October 2005 during the anoxic season (25_ANS), with no dissolved oxygen (DO) detectable and in January 2006 during the oxic season (25_OXS) when oxygen saturation was restored. (ii) Sampling in the perennial OMZ off Goa (15° 20.10' N, 72° 54.11' E) in a depth of 200m was carried out during May 2007, when we collected anoxic sediment

(200_ANS) and anoxic near bottom water (200_ANW). (iii) As a comparison we sampled the permanently oxygenated sediment from a shallow 3m-depth site (03_CRS) in the coral reef region off Kavaratti (10° 34.588' N, 72° 38.019' E) in February 2007. Coral sand samples were collected by divers in sterile containers. All other sediment samples were collected using a ~ 60 cm long gravity corer while water was collected in 5-L Niskin bottles. All samples were divided into aliquots for the isolation of fungi for cultivation and for total genomic DNA extraction. Samples for isolation were stored at 5° C and processed within 24 hours. Water samples for molecular analyses (5 – 8 L of water) were drawn on Durapore filters (0.45 µm, Millipore (India) Pvt Ltd, Bangalore) under the exclusion of oxygen (Stoeck *et al.*, 2003), and ~ 5-10 g of sediment were frozen immediately in liquid nitrogen prior to storage at -80 °C until further processing. The dissolved oxygen (DO) of the near bottom water was determined by Winkler's titration method or spectrophotometrically (Pai *et al.*, 1993).

Isolation and identification of culturable fungi

Culturable fungi were isolated using the particle-plating technique of Bills & Polishook (1994) with slight modifications. In short, approximately 1g of sediment slurry was serially sieved through a 200-µm and a 100-µm mesh. The particles that passed through the 200-µm mesh but were retained on the 100-µm mesh were spread-plated on different fungal media such as malt extract agar, corn meal agar and Czapek Dox agar (HiMedia Pvt. Ltd., India) prepared in seawater and fortified with streptomycin (0.1 g in 100 ml medium) and penicillin (40,000 Units in 100 ml medium) to inhibit bacterial growth. Incubation was carried out under oxic conditions. The cultures isolated from the different sampling stations were identified based on their morphology and partial (~ 1600 bp) 18S rDNA gene sequence. Genomic DNA was extracted from the freeze-dried mycelial mats of each culture grown aerobically for 5 days at room temperature in 50 ml of malt extract broth (HiMedia Pvt. Ltd., India) in 250-ml Erlenmeyer flask. Total genomic DNA was extracted from the freeze-dried mycelial mats of each culture using a high salt concentration extraction buffer (100 mM Tris HCl (pH 8), 100 mM sodium phosphate buffer (pH 8), 1.5 M NaCl, 100 mM EDTA (pH 8.) and 1 % CTAB). One ml of buffer was added to approximately 500 mg of mycelia and the total genomic DNA was extracted using chloroform-phenol extraction and isopropanol precipitation as described previously (Stoeck & Epstein, 2003). The 18S region of the rDNA was amplified using fungal specific primers NS1

and FR1 (Table 2). The 50- μ l PCR reaction included 0.5 U HotStart Taq DNA polymerase (Qiagen, Valencia, CA) in 1x HotStart Taq buffer (Qiagen, Hildesheim, Germany), 200 μ M of each dNTP, and 0.5 μ M of each oligonucleotide primer. PCR was performed using initial denaturation at 94°C for 5 min, followed by 35 cycles of 94°C for 45 sec, 50°C for 45 sec, and 72°C for 2 min, with a final extension at 72°C for 7 min. The PCR products were cloned separately for each fungal culture using the TA cloning kit (Invitrogen, Carlsbad, CA) according to the manufacturer's instructions. Plasmids were isolated from positive overnight cultures using the Fast Plasmid Mini Prep kit (Eppendorf, Hamburg, Germany). One representative clone of each culture was sequenced bidirectionally (M13 sequencing primers) by MWG-Biotech on an Applied Biosystems (ABI) 3730 DNA Stretch Sequencer, with the XL Upgrade and the ABI Prism BigDye Terminator version 3.1 Cycle Sequencing Ready Reaction Kit. In order to evaluate intra-strain variability of the 18S rDNA, we for five strains (FCAS35, FCAS36, FCAS41, FCAS89, FCAS125) have sequenced four amplicons. Sequences were included in phylogenetic analyses as described below. GenBank accession numbers of sequences from cultured isolates are GQ120154- GQ120179 and xxx for duplicate amplicons.

PCR primer selection, environmental 18S rDNA clone libraries construction and OTU calling

Total DNA from the five environmental samples (25_ANS, 25_OXS, 200_ANS, 200_ANW, 03_CRS) was extracted using a high salt concentration extraction buffer, followed by chloroform-phenol extraction and precipitation with isopropanol as described previously (Stoeck & Epstein, 2003). Amplification of 18S rDNA was carried out using three different primer sets (Table 2). The primer sets were chosen based on their specificity and adequate length of amplified fragments in order to carry out robust phylogenetic analyses.

The first primer set (Fung1) consisted of the fungal specific NS1 and FR1 primers resulting in ~ 1650 bp-fragments. According to homology searches this primer set has only moderate specificity and co-amplifies a range of non-fungal eukaryote genes like Metazoa, Cercozoa, Viridiplantae, Alveolata, Centrohelioczoa and Bangiophyceae (Pang & Mitchell, 2005). The second primer set (Fung2) included the fungal specific UF1 and S3 primers amplifying ~ 900 bp-fragments. This primer set has relatively high fungal specificity and

previously amplified only few non-target taxa (green algae and *Pseudomonas*) from maritime Antarctic soil samples (Malosso *et al.*, 2006). We also used the universal eukaryote primer pair EukA and EukB, routinely used in environmental eukaryote diversity surveys (Euringer & Lueders, 2008; Stoeck *et al.*, 2006; Massana *et al.*, 2004) that amplifies nearly the full-length of the 18S rDNA (Table 2) of a wide range of higher eukaryote taxon groups (Medlin *et al.*, 1988).

Amplicons were ligated into pGEM-T vector and transformed into *E. coli* cells (TOP 10 strain) using Invitrogen's TA-cloning kit as described above. For each library and primer set we selected nearly 100 positively screened colonies (blue-white screening) for overnight growth and plasmid extraction using the 96 well Directprep Kit (Qiagen, Valencia, CA). The presence of 18S rDNA inserts was confirmed by a standard M13-PCR amplification of extracted plasmids. In sum, 1440 positively screened plasmids were subjected to restriction fragment length polymorphism (RFLP) analyses. Therefore, between 200 and 400 ng of amplification products with expected sizes were digested with 7.5 U of the restriction endonuclease *HaeIII* (New England Biolabs, Beverly, Mass.) for 60 min at 37°C, followed by an inactivation step for 20 min at 80°C. The resulting bands were separated by electrophoresis in a 2.5% low-melting-point agarose gel at 80 V for 2 to 3 h. At least one representative clone of each RFLP pattern (n=549) was partially sequenced (ca. 600 nucleotides) at MWG-Biotech as described above.

Because partial fragments could only be adequately aligned within each primer set (sequence overlap was too small for partial fragments to be aligned between sequences obtained by different primer sets), sequences of each individual primer set were grouped separately into operational taxonomic units (OTUs) based on 99.0% sequence similarity using three independent (one for each primer-set derived sequences) DOTUR analyses (Schloss & Handelsman, 2005). One representative of each OTU that was identified as a fungal sequence using a BLASTn search in the GenBank nr-database (Altschul *et al.*, 1997) was chosen for full-fragment sequencing (n= 100). The obtained sequences were checked for chimeras using the Bellerophon Chimera Check program and the Check_Chimera utility (Ribosomal Database Project (Cole *et al.*, 2003), as well as partial treeing analysis (Robison-Cox *et al.*, 1995). Low quality reads (9 sequences) were removed prior to subsequent sequence analyses. This left us with 91 full-fragment sequences that were analyzed together in a final DOTUR analysis. Similarities between two sequences were calculated using a custom program (PairAlign)

provided by M. Nebel (University of Kaiserslautern), which employs IUB matrix-based pairwise alignments. In total, 549 partial and 91 full-fragment environmental sequences have been deposited to the GenBank database under accession numbers GQ120105- GQ120153.

Phylogenetic analyses

Environmental 18S rDNA gene sequences initially were compared to those in GenBank using BLAST analysis to determine their approximate phylogenetic affiliation. Sequences of environmental clones together with their closest GenBank cultured and uncultured matches were aligned using the ARB FastAligner utility. Alignments were manually refined using phylogenetically conserved secondary structures. The conserved and unambiguously aligned positions were used in subsequent phylogenetic analyses. Bayesian analyses were carried out using MrBayes v3.2.1 (Huelsenbeck & Ronquist, 2001) with posterior probability support values calculated using four chains/two runs and running 10 million generations for each alignment. Trees were sampled every 1000th generation. The first 25% of sampled trees were considered 'burn-in' and were discarded. A 50% majority rule consensus of the remaining trees was used to calculate posterior probability values. The AIC in Modeltest (Posada & Crandall, 2001) was used to estimate the best fitting evolutionary model (GTR+I+G), which was chosen for phylogenetic analysis.

Community comparisons

The program package SPADE (Chao & Shen, 2003-2005) was used to calculate the Jaccard index as a measure of similarity between two communities based on abundance of environmental fungal OTUs ($J_{abundance}$). An unweighted pair group mean average (UPGMA) cluster analyses based on $J_{abundance}$ data was performed using the Cluster analysis module of Statistica v. 7 (StatSoft, Tulsa OK). A Venn diagram was constructed to display the overlap in OTU composition between the different primer sets using VENNY (Oliveros, 2007).

Species accumulation curves and Abundance-based Coverage Estimator (ACE) of species richness for all the clone libraries were calculated in EstimateS (Colwell, 2005).

Results and discussion

We subjected ca. 1440 environmental clones obtained from five individual sampling events in the Arabian Sea (Table 1) to restriction fragment length polymorphism (RFLP) analyses. The clones grouped into 549 distinct RFLP patterns, which were subjected to 18S rDNA sequence analyses. Surprisingly, GenBank BLASTn analyses of one representative sequence from each RFLP pattern identified only 268 unique RFLP patterns as members of the kingdom Fungi. Considering the replicates of these unique patterns, we in total could putatively identify 455 out of the initial 1440 clones as taxonomically affiliation to the kingdom fungi. The remaining RFLP patterns (n=281) represented sequences falling into a number of different taxonomic groups (Table 3). The partial sequences (n=268) obtained from representatives of each unique RFLP pattern grouped into 91 distinct operational taxonomic units (OTUs) called at 99% sequence similarity: 25 OTUs for primer set Fung1, 54 OTUs for primer set Fung2, and 12 OTUs for primer set EukAB. After we obtained one complete fragment from at least one representative of each individual OTU, we were able to analyze all sequences in one individual DOTUR run, which resulted in 48 distinct OTUs, indicating that a number of OTUs obtained by the individual primer-set analyses were shared among two or three primer sets.

Selectivity of fungal specific primers

A total of 158 fungal sequences (grouping into 19 OTUs) were obtained with the Fung1 primer set, 179 sequences (34 OTUs) with the Fung2 primer set, and the universal eukaryotic primer set retrieved 118 fungal sequences (9 OTUs). Nearly two-third of the clone's analyzed (932 clones) using BLASTn emerged as non-fungal sequences. As expected, numerous (n = 345) such non-fungal sequences were retrieved with the universal eukaryote primer set EukAB. But also putatively fungal specific primer sets retrieved 311 (Fung1) and 276 (Fung2) non-fungal amplicons (Table 3). These findings met our expectations considering the different degrees of fungal-specificity of the different primer sets used (see Methods section).

Specific PCR primers for environmental studies aim to amplify all the members of the fungal community without bias, while excluding the co-amplification of other eukaryote sequences. As noted earlier (Anderson *et al.*, 2003) this is particularly difficult using the highly conserved 18S rDNA region (Page & Holmes, 1998). Several authors claim that they have

designed or applied truly specific fungal 18S rDNA primer pairs (Smit *et al.*, 1999, Borneman & Hartin, 2000). But there is conflicting evidence for this specificity as other authors reported amplification of non-fungal templates using the same primer pairs (Anderson *et al.*, 2003, Borneman & Hartin, 2000). It seems most, if not all, fungal 18S rDNA primers are prone to observable cross-kingdom amplifications. Further evidence for this comes from BLAST homology analyses for fungal 18S rDNA primers indicating a relatively low success rate for most primer pairs in recovering fungal sequences but co-amplification of non-fungal targets (Pang & Mitchell, 2005). The degree to which cross-kingdom amplification occurs largely depends on the choice of specific primers, PCR conditions, but also the abundance of specific fungal taxon groups in relation to the co-occurrence of eukaryote non-target organisms in the environmental samples under study. Such systematic biases can distort diversity assessments. Furthermore, different fungal specific primer sets may amplify different subsets of the fungal community (Fig. 1 and Anderson *et al.*, 2003). This severely affects comparability of different fungal 18S rDNA data sets obtained from PCR amplification with different primers. A solution may come from the application of a multiple-primer approach as suggested for general microbial eukaryote diversity surveys (Stoeck *et al.*, 2006).

Diversity patterns of fungal communities in the Arabian Sea

An UPGMA cluster analysis of J_{abund} index (Fig. 2) reveals that the fungal communities from the different sampling sites (Table 1) are distinctly different from each other. Of all comparisons, the two coastal sites sampled during anoxic and oxic conditions (25_ANS, 25_OXS) are most similar in their fungal community membership ($J_{abund} = 0.48$). The sediment- (200_ANS) and water column-derived (200_ANW) fungal communities from the OMZ offshore site cluster together with a J_{abund} of 0.35. The fungal community from the coral reef reference site (03_CRS) showed highest dissimilarity to all other samples ($J_{abund} = 0.06-0.1$) The Indian Ocean has a characteristic seasonal anoxic condition that develops along the western margin during October to January (Naqvi *et al.*, 2006). In this process, the coastal sites are subjected to seasonal oxygen fluctuations. Our analysis suggests a clear separation of fungal communities adapted to permanently oxic conditions, temporal anoxia and the ones adapted to permanent anoxia. Recent studies have shown that numerous fungi can adapt to alternate modes of respiration depending on oxygen availability (Daiber *et al.*, 2005). It is then

reasonable to assume that the fungal community of the coastal site (25_ANS, 25_OXS), considered as an entity, may be capable of physiological adaptation when oxygen concentration decreases. This adaptation is neither a prerequisite for survival in oxygenated coral reef sediment (03_CRS) nor for life under permanently anoxic conditions (200_ANS, 200_ANW). Apart from a few exceptions (e.g. some chytridiomycetes) no strictly anaerobic fungi have been described. This, however, contrasts with molecular diversity surveys in anaerobic aquatic environments, where fungal sequences sometimes account for a significant fraction of the clones (Dawson & Pace, 2002; Edgcomb *et al.*, 2002; Luo *et al.*, 2005; Stoeck & Epstein, 2003; Stoeck *et al.*, 2006, 2007). Some recent studies in permanently anoxic habitats have found novel fungal clades restricted to these environments (Luo *et al.*, 2005; Brad *et al.*, 2008; Laurin *et al.*, 2008). Support for the adaptation of fungi to anoxia comes from reports of some ascomycetes (Dumitru *et al.*, 2004; Sonderegger *et al.*, 2004) and some basidiomycetes (Fell *et al.*, 2001) that are capable of fermentation and anaerobic growth. Some of such fungi have even been isolated from anaerobic deep-sea environments (Nagahama *et al.*, 2003).

Considering these wide ecological and physiological adaptations of different fungi, it is not unexpected that that fungal communities thriving under oxygenated conditions (25_OXS) are distinctively different from fungal communities living under anoxic conditions (25_ANS). The same applies to those from anoxic sediments (200_ANS) and anoxic water samples (200_ANW).. However, the actual degree of dissimilarity is difficult to assess with our data due to severe undersampling (Fig. 3).

Phylogeny of environmental fungal rDNA sequences

The analyses of intra-strain variability of the 18S rDNA of five strains obtained in this study revealed a maximum divergence of 0.77% (FCAS35: 0.18-0.36%; FCAS 36: 0.17-0.5%; FCAS41: 0.25-0.77%; FCAS89: 0-0.14% FCAS125: 0%). Thus, we are confident that 1% divergence between two different OTUs is sufficient to represent distinct genotypes. The environmental OTUs identified in this study are designated as “Fungal OTUs from the Arabian Sea”: FAS_1 to FAS_49. The OTUs from the five sample sites were assigned to the Dikarya (Ascomycota: 27 OTUs; Basidiomycota: 20 OTUs), and one OTU branched within the subphylum Mucoromycotina (phylum Zygomycota).

OTUs belonging to the Ascomycota (Fig. 4) grouped within the subphyla Saccharomycotina and Pezizomycotina. FAS_2 is the most frequently represented OTU with close similarity to the yeast *Kodamaea* sp. (99.67%) belonging to Saccharomycetes. Saccharomycete yeasts are able to cope with anoxia while producing ethanol (Prior *et al.*, 1989). A large number of OTUs (FAS_6, FAS_7, FAS_8, FAS_9, FAS_10, FAS_11 and FAS_12) belong to Sordariomycetes. Their closest known relatives belong to the genus *Fusarium*, which serves as a model to study fungal denitrification under low-oxygen conditions (Takaya *et al.*, 1999; Daiber *et al.*, 2005). Six OTUs from the Arabian Sea (Fig. 4) branch within the Eurotiomycetes. These sequences are affiliated with a number of environmental sequences obtained from a hypersaline anoxic Mediterranean deep-sea basin (Alexander *et al.*, 2009), anoxic meromictic lake sediments (Takishita *et al.*, 2007a), anoxic coastal sediments (Dawson & Pace 2002), acidic mine drainages (Baker *et al.*, 2004), hydrothermal vent habitats (López-García *et al.*, 2007), boiling springs lake sediment (Wilson *et al.*, 2008). This strongly indicates that the respective phyloclade includes extremophile fungi that are capable to thrive in the most extreme aquatic environments known to date. Described representatives in this clade are *Penicillium* and *Aspergillus*, versatile ubiquitously distributed species that are capable of anaerobic denitrification (Takasaki *et al.*, 2004). This agrees with our observation that under laboratory conditions isolate FCAS31a (99.45% similarity with *Penicillium namyslowskii*) grows anaerobically while reducing nitrate (Jebaraj & Raghukumar, 2009). The widespread distribution of ascomycete fungi in oxygen-depleted and extreme habitats is also indicated by the close affiliation of a number of further OTUs from the Arabian Sea to environmental clones obtained from other anoxic habitats and extreme environments. For example, FAS_5 has 99.88% similar to P1_3m2 obtained from anoxic aquifer sediment (Brad *et al.*, 2008), and the dothidiomycetes OTU FAS_24 is 98.92% similar to an environmental clone from Mid-Atlantic hydrothermal vent sediment (López-García *et al.*, 2007).

Within the phylum Basidiomycota (Fig. 5) we identified sequences branching in the subphyla Pucciniomycotina, Agaricomycotina, and Ustilagomycotina. Only few are closely (> 97% sequence similarity) related to previously deposited sequences of described basidiomycetes. For example, FAS_47 is 99.46% similar to the wood-decaying saprophyte *Globulicium hiemale*, and FAS_28 and FAS_29 are similar to the nitrogenous compounds scavenger *Rhodotorula cassicola* (99.83 % and 98.60 % sequence similarity, respectively).

Most OTUs are divergent from sequences of described taxa such as the ustilagomycetes FAS_38 and FAS_39 exhibiting only 93 % sequence similarity to the plant pathogen *Thecaphora spilanthis*. Many basidiomycete OTUs from the Arabian Sea branch in clades that include other environmental fungal sequences from a variety of oxygen-depleted habitats. Examples are three exobasidiomycete OTUs (FAS_30, FAS_31 and FAS_32), which are related to sequences from an anoxic Norwegian Fjord (direct submission), an anoxic Mediterranean deep-sea basin (Alexander *et al.*, 2009) and an anoxic basin in the central Baltic Sea (Stock *et al.*, 2009). The closest described relative of this sequence clade is the plant pathogen *Exobasidium gracile* with at least 37.3 % sequence divergence. The OTUs FAS_40 and FAS_41 branch together with sequences from hydrothermal vent ecosystems (López-García *et al.*, 2007; Le Calvez T, Mahe S & Vandenkoornhuysen P (direct submission), anoxic intertidal sediment (Dawson & Pace, 2002), an anoxic Mediterranean deep-sea basin sequence (Alexander *et al.*, 2009) and deep ocean fungal sequences (Bass *et al.*, 2007). This cluster belonging to Ustilagomycotina is within a clade that has been recognized as “hydrothermal and/or anaerobic fungal group” (López-García *et al.*, 2007). The closest described species branching in this clade is the rare skin pathogen *Malassezia restricta*, with 0.39% and 2.36% sequence divergence to the OMZ OTUs FAS_40 and FAS_41. Finding a sequence related to a human skin-pathogen in anoxic marine environments seems unusual at first glance. However, sequences closely related to *M. restricta* were also reported from hydrate-rich deep-sea sediments of South China Sea (Lai *et al.*, 2007), indicating a wide ecological diversity of taxa falling in this sequence clade. Interestingly OTUs obtained exclusively from oxic regions of our study grouped together within the agaricomycetes along with culture sequences and environmental clones obtained from anaerobic aquifers, hydrothermal sediment and agricultural soil (Euringer & Lueders, 2008; Le Calvez T, Mahe S & Vandenkoornhuysen P, direct submission; Lesaulnier *et al.*, 2008).

One OTU (FAS_49) obtained from our study (Fig 6) originates from a fungus other than Dikarya. This clone together with clone CCW35 from anoxic salt marsh water (Stoeck & Epstein, 2003, sequence similarity 90.45 %) branches among zygomycetes. The closest named species was *Mortierella wolfii*, subphylum Mucoromycotina, with only 90.02 % sequence similarity. There are contrasting reports on the capability of zygomycetes to grow anaerobically (Kurakov *et al.*, 2008; Schmidt *et al.*, 2008). Even though we have a poor

representation of zygomycetes in our study, they are reported to be a major component of fungal communities in oxygen-depleted environments (Slapeta *et al.*, 2005; Brad *et al.*, 2008; Takishita *et al.*, 2007b). Similarly, we did not discover chytridiomycetes in our clone libraries. This was unexpected, because the latter taxon group includes fermentative anaerobes (Orpin, 1977) and was found in previous 18S rDNA diversity surveys in anoxic habitats (Stoeck *et al.*, 2007; Takishita *et al.*, 2007a,b). This poor representation of fungal groups other than ascomycetes and basidiomycetes may be due to undersampling (Fig. 3) combined with a higher efficiency of the primers to amplify genes from Ascomycota and Basidiomycota (Anderson *et al.*, 2003; Malosso *et al.*, 2006). Indeed, also previous studies also failed to obtain for example chytridiomycete sequences using Fung1 (Gomes *et al.*, 2003) and Fung2 primers (Malosso *et al.*, 2006).

Even though we are not able to distinguish native from transitory fungi (both, spores and mycelium contain rDNA, Osharov *et al.*, (2002)), it is reasonable to assume that the majority of fungi detected in this study are indigenous. The reasoning for this assumption is their close relation to a number of fungal sequences detected (some exclusively) in oxygen-deficient environments. Furthermore, we conducted a laboratory experiment to largely exclude the possibility of PCR-amplification of DNA from non-indigenous fungi. Wind or water currents are vectors to disperse fungal spores. Such transitory material could theoretically be sources of genomic DNA despite the corresponding organisms are not active members of the fungal communities under study. Therefore, we harvested spores from two cultured isolates originating from the sampling sites (the basidiomycete isolate FCAS11 and the ascomycete isolate FCAS21). The protocol that we used to extract nucleic acids from the environmental samples under study failed to extract DNA from these spores. This largely (even though certainly not entirely) excludes the possibility that the clone libraries constructed from the Arabian Sea samples include significant proportions of non-indigenous transitory fungi (spores) (results not shown, but available from the authors upon request).

Cultured fungi

Traditionally, fungal diversity studies in environmental samples are based on cultivation approaches. Limitations of this strategy—like the inability to separate biomass from particulate

material and lack of growth media and cultivation conditions suitable for all members of the community—are held responsible for our underestimation of total fungal diversity (Anderson *et al.*, 2003). Molecular phylogenetic analyses of clone libraries constructed from environmental samples have become the gold-standard in fungal diversity research (Pang & Mitchell, 2005). But this strategy is no panacea, as it is afflicted with bias such as PCR-primer (un)specificities as discussed above. Combining cultivation-based and cultivation-independent methods may allow for a more complete picture of fungal diversity as each of the methodological strategies may compensate for the biases of the other.

We obtained 26 cultures from all sampling locations, the taxonomic breadth of the isolated strains is restricted: five isolates (FCAS11, FCAS87, FCAS88, FCAS89 and FCAS 90) could be assigned to Basidiomycota, predominantly Pucciniomycotina (Fig. 5); and twenty-one of them belonged to Pezizomycotina of Ascomycota (Fig. 4). Both divisions of fungi have a high representation of cultured taxa, indicating the accessibility of these groups to cultivation. With the exception of two ascomycetes (FCAS31 and FCAS129) and three basidiomycetes (FCAS87, FCAS90, FCAS11) all cultures have >99% sequence similarities to previously cultured and well-described fungi. This indicates that cultivation using standard techniques generally produces more isolates of already known taxa. We note that cultivation under anoxic conditions may have produced a different set of fungi being more divergent to known taxa. Current efforts are in order to stimulate growth under anoxic conditions using a variety of different media. Thus far, we were able to obtain one of the isolates (isolate FCAS11) discussed in this study under anoxic cultivation conditions. This supports the hypotheses that at least some fungi from the OMZ region, subjected to changing oxygen conditions, can readily adapt to anoxia. We also obtained few isolates that show only low similarity to previously described and sequenced fungi. These are for example the isolates belonging to Basidiomycota FCAS90 exhibiting 97.10% sequences similarity to *Graphiola cylindrica*, isolate FCAS87 with only 62.5% similarity to *Sporisorium reilianum* and isolate FCAS11 with 92.1% sequence similarity to *Rhodotorula aurantiaca*. These examples reveal another decisive advantage of cultivation over clone library analysis. Having access to (putatively) novel cultures in living condition paves way for an in-depth analysis of their phylogeny, morphology and ecology. A current multi-gene analysis of isolate FCAS11 along with ultrastructural analyses points to the discovery of a novel fungal taxon on class-level with the capability of

anaerobic denitrification (Jebaraj CS, Boekhaut T, Muller W, Kauff F, Stoeck T, unpublished). Detailed analysis of the enzymes involved in the nitrate reducing pathways of cultured fungi and their expression profiles *in situ* can help us to understand their role in the OMZ of the Arabian Sea and other anoxic aquatic systems in global biogeochemical cycles.

Acknowledgments

We are grateful to the Captain and colleagues on board CRV Sagar Sukti for their help during the sampling. This study was funded by grants STO4141/2-3 and STO414/3-1 by the Deutsche Forschungsgemeinschaft to TS. CS would like to thank the University of Kaiserslautern for an international fellowship and Dr. SR Shetye, Director, NIO for the travel support and encouragement provided for carrying out the work. We are thankful to Dr Seshagiri Raghukumar for his critical review of the manuscript and for helping us in the identification of the fungi. Furthermore, we acknowledge the comments of two anonymous reviewers, who helped to improve our manuscript. This is NIO's contribution number xxx.

References

- Alexander E, Stock A, Breiner HW, Behnke A, Bunge J, Yakimov MM & Stoeck T (2009) Microbial eukaryotes in the hypersaline anoxic L'Atalante deep-sea basin. *Environ Microbiol* **11**: 360–381.
- Altschul S F, Madden T L, Schaffer AA, Zhang J, Zhang Z, Miller W & Lipman D J (1997) Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucleic Acids Res.* **25**: 3389–3402.
- Alsumard LT, Golubic S & Priess K (1995) Fungi in corals: symbiosis or disease? Interactions between and fungi cause pearl-like skeleton biomineralization. *Mar Ecol Prog Ser* **117**: 137–147.
- Anderson IC, Campbell CD & Prosser JI (2003) Potential bias of fungal 18S rDNA and internal transcribed spacer polymerase chain reaction primers for estimating fungal biodiversity in soil. *Environ Microbiol* **5**: 36-47.
- Baker BJ, Lutz MA, Dawson SC, Bond PL & Banfield JF (2004) Metabolically active eukaryotic communities in extremely acidic mine drainage. *Appl Environ Microbiol* . **70**: 6264–6271.
- Bass D, Howe A, Brown N, Barton H, Demidova M, Michelle H, Li L, Sanders H, Watkinson CV, Willcock S & Richards TA (2007) Yeast forms dominate fungal diversity in the deep oceans. *Proc R Soc B* **274**: 3069–3077.
- Bills GF & Polishook JD (1994) Abundance and diversity of microfungi in leaf litter of a lowland rain forest in Costa Rica. *Mycologia* **86**: 187-198.
- Borneman J & Hartin RJ (2000) PCR primers that amplify fungal rRNA genes from environmental samples. *Appl Environ Microbiol* **66**: 4356–4360
- Brad T, Braster M, van Breukelen BM, van Straalen NM & Roling WFM (2008) Eukaryotic diversity in an anaerobic aquifer polluted with land fill leachate. *Appl Environ Microbiol* **74**: 3959-3968.
- Chao A & Shen TJ (2003–2005) *ProgramSPADE(Species Prediction and Diversity Estimates)* [WWW document]. <http://chao.stat.nthu.edu.tw>.
- Cole JR, Chai B, Marsh TL *et al.*, (2003) The Ribosomal Database Project (RDP-II): previewing a new autoaligner that allows regular updates and the new prokaryotic taxonomy. *Nucleic Acids Res* **31**: 442–443.
- Colwell RK (2005) EstimateS: Statistical estimation of species richness and shared species from samples. Version 7.5. User's Guide and application published at: <http://purl.oclc.org/estimates>.
- Daiber A, Shoun H & Ullrich V (2005) Nitric oxide reductase (P450nor) from *Fusarium oxysporum*. *J Inorg Biochem* **99**: 85–193.

- Dawson SC & Pace NR (2002) Novel kingdom-level eukaryotic diversity in anoxic environments. *Proc Natl Acad Sci USA* **99**: 8324–8329
- Dighton J (2003) *Fungi in ecosystem processes*. Marcel Dekker Inc, New York.
- Dumitru R, Hornby JM, Nickerson KW (2004) Defined anaerobic growth medium for studying *Candida albicans* basic biology and resistance to eight antifungal drugs. *Antimicrob Agents Chemother* **48**: 2350–2354.
- Edgcomb VP, Kysela DT, Teske A, de Vera Gomez A & Sogin ML (2002) Benthic eukaryotic diversity in the Guaymas Basin hydrothermal vent environment. *Proc Natl Acad Sci USA* **99**: 7658–7662.
- Epstein S & López-García P (2007) ‘Missing’ protists: a molecular prospective. *Biodivers Conserv* **17**: 261–276.
- Euringer K & Lueders T (2008) An optimised PCR/T-RFLP fingerprinting approach for the investigation of protistan communities in groundwater environments. *J Microbiol Methods* **75**: 262-268.
- Fell JW, Boekhout T, Fonseca A & Sampaio JP (2001) The Mycota VII. Systematics and Evolution. Part B. (McLaughlin DJ, McLaughlin EG & Lemke PA eds), pp. 1-36. Springer-Verlag, Berlin.
- Gomes NCM, Fagbola O, Costa R, Rumjanek NG, Buchner A, Mendona-Hagler L & Smalla K (2003) Dynamics of fungal communities in bulk and maize rhizosphere soil in the tropics. *Appl Environ Microbiol* **69**: 3758–3766.
- Hawksworth DL (2001) The magnitude of fungal diversity: the 1.5 million species estimate revisited. *Mycol Res* **105**: 1422-1432.
- Huelsenbeck JP & Ronquist F (2001) MRBAYES: Bayesian inference of phylogenetic trees. *Bioinformatics* **17**: 754–755.
- Jebaraj CS & Raghukumar C (2009) Anaerobic denitrification in fungi from the coastal marine sediments off Goa, India. *Mycol Res* **113**: 100-109.
- Kappe R, Fauser C, Okeke CN, Maiwald M (1996) Universal fungus-specific primer systems and group specific hybridization oligonucleotides for 18S rDNA. *Mycoses* **39**: 25–30.
- Kohlmeyer J & Kohlmeyer E (1979). *Marine Mycology. The Higher Fungi*. Academic Press, New York.
- Kurakov AV, Lavrent’ev RB, Nechitailo TY, Golyshin PN & Zvyagintsev DG (2008) Diversity of facultatively anaerobic microscopic mycelial fungi in soils. *Microbiology* **77**: 90–98.
- Lai X, Cao L, Tan H, Fang S, Huang Y & Zhou S (2007) Fungal communities from methane hydrate-bearing deep-sea marine sediments in South China Sea. *The ISME Journal* **1**: 756–762

- Laurin V, Labbé N, Parent S, Juteau P & Villemur R (2008) Microeukaryote diversity in a marine methanol-fed fluidized denitrification system. *Microb Ecol* **56**: 637–648.
- Lesaulnier C, Papamichail D, McCorkle S, Ollivier B, Skiena S, Taghavi S, Zak D & van der Lelie D (2008) Elevated atmospheric CO₂ affects soil microbial diversity associated with trembling aspen. *Environ Microbiol* **10** : 926–941.
- López-García P, Vereshchaka A & Moreira D (2007) Eukaryotic diversity associated with carbonates and fluid–seawater interface in Lost City hydrothermal field. *Environ Microbiol* **9**: 546–554.
- Luo Q, Krumholz LR, Najar FZ, Peacock AD, Roe BA, White DC & Elshahed MS (2005) Diversity of the microeukaryotic community insulfide-rich Zodletone Spring (Oklahoma). *Appl Environ Microbiol* **71**: 6175–6184.
- Maiwald M, Kappe R & Sonntag HG (1994) Rapid presumptive identification of medically relevant yeasts to the species level by polymerase chain reaction and restriction enzyme analysis. *J Med Vet Mycol* **32**: 115-122.
- Malosso E, Waite IS, English L, Hopkins DW & O'Donnell AG (2006) Fungal diversity in maritime Antarctic soils determined using a combination of culture isolation, molecular fingerprinting and cloning techniques. *Polar Biol* **29**: 552- 561.
- Massana R, Balagué V, Guillou L & Pedrós-Alió C (2004) Picoeukaryotic diversity in an oligotrophic coastal site studied by molecular and culturing approaches. *FEMS Microbiol Ecol* **50**: 231-243.
- Medlin L, Elwood HJ, Stickel S & Sogin ML (1988) The characterization of enzymatically amplified eukaryotic 16Slike rRNA-coding regions. *Gene* **71**: 491–499.
- Nagahama T, Hamamoto M, Nakase T, Takaki Y, Horikoshi K. (2003) *Cryptococcus surugaensis* sp. nov., a novel yeast species from sediment collected on the deep-sea floor of Suruga Bay. *Int J Syst Evol Microbiol* **53**: 2095–2098.
- Naqvi SWA, Naik H, Pratihary A, DeSouza W, Narvekar PV, Jayakumar DA, Devol AH, Yoshinari T, Saino T (2006) Coastal versus open-ocean denitrification in the Arabian Sea. *Biogeosciences* **3**: 621-633.
- Newell SY (1996) Established and potential impacts of eukaryotic mycelial decomposers in marine/terrestrial ecotones. *J Exp Mar Biol Ecol* **200**: 187– 206.
- Oliveros JC (2007) VENNY. An interactive tool for comparing lists with Venn Diagrams. <http://bioinfogp.cnb.csic.es/tools/venny/index.html>.
- Orpin CG (1977). Invasion of plant tissue in the rumen by the flagellate *Neocallimastix frontalis*. *J Gen Microbiol* **98**: 423-430.

- Osherov, N., J. Mathew, A. Romans and G.S. (2002) Identification of conidial-enriched transcripts in *Aspergillus nidulans* using suppression subtractive hybridisation. *Fungal Genet Biol* **37**: 197–204.
- Page RDM & Holmes EC (1998) *Molecular Evolution: A Phylogenetic Approach*. Blackwell Science, Oxford.
- Pai SC, Gong GC & Liu KK (1993) Determination of dissolved oxygen in seawater by direct spectrophotometry of total iodine. *Mar Chem* **41**: 343-351.
- Pang KL & Mitchell JI (2005) Molecular approaches for assessing fungal diversity in marine substrata. *Bot Marina* **48**: 332-347.
- Pivkin MV (2000) Filamentous fungi associated with holothurians from the Sea of Japan, off the Primorye Coast of Russia. *Biol Bull* **198**: 101-109.
- Posada D & Crandall KA (2001) Selecting the best-fit model of nucleotide substitution. *Syst Biol* **50**: 580–601.
- Prior BA, Kilian SG, & du Preez JC (1989) Fermentation of D-xylose by the yeasts *Candida shehatae* and *Pichia stipitis*. *Process Biochem* **24**:21–32.
- Raghukumar C (1986) Fungal parasites of the marine green algae, *Cladophora* and *Rhizoclonium*. *Bot Mar* **29**: 289-297.
- Robison-Cox JF, Bateson MM & Ward DM (1995) Evaluation of nearest-neighbour methods for detection of chimeric small-subunit rRNA sequences. *Appl Environ Microbiol* **61**: 1240-1245.
- Schloss PD & Handelsman J (2005) Introducing DOTUR, a computer program for defining operational taxonomic units and estimating species richness. *Appl Environ Microbiol* **71**: 1501–1506.
- Schmidt SK, Wilson KL, Meyer AF, Gebauer MM & King AJ (2008) Phylogeny and ecophysiology of opportunistic “Snow Molds” from a subalpine forest ecosystem. *Microb Ecol* **56**: 681–687.
- Shoun H, Kim DH, Uchiyama H & Sugiyama J (1992) Denitrification by fungi. *FEMS Microbiol Let* **94**: 277-282.
- Slapeta J, Moreira D & López-García P (2005) The extent of protist diversity: insights from molecular ecology of freshwater eukaryotes. *Proc R Soc B* (2005) **272**: 2073–2081.
- Smit E, Leeflang P, Glandorf B, van Elsas JD & Wernars K (1999) Analysis of fungal diversity in the wheat rhizosphere by sequencing of cloned PCR-amplified genes encoding 18S rRNA and temperature gradient gel electrophoresis. *Appl Environ Microbiol* **65**: 2614–2621.
- Sonderegger M, Jeppsson M, Larsson C, Gorwa-Grauslund MF, Boles E, Olsson L, Spencer-Martin I, Hahn-Hagerdal B, Sauen U (2004) Fermentation performance of engineered and evolved xylose-fermenting *Saccharomyces cerevisiae* strains. *Biotechnol Bioeng* **87**: 90-98.

- Stock A, Bunge J, Jurgens K, Stoeck T (2009) Protistan diversity in the suboxic and anoxic waters of the Gotland Deep (Baltic Sea) as revealed by 18S rRNA clone libraries. *Aquat Microb Ecol* **55**: 267-284.
- Stoeck T & Epstein S (2003) Novel eukaryotic lineages inferred from small-subunit rRNA analyses of oxygen depleted marine environments. *Appl Environ Microbiol* **69**: 2657–2663.
- Stoeck T, Hayward B, Taylor GT, Varela R & Epstein SS (2006) A multiple PCR-primer approach to access the microeukaryotic diversity in environmental samples. *Protist* **157**: 31-43.
- Stoeck T, Kasper J, Bunge J, Leslin C, Ilyin V & Epstein S (2007) Protistan diversity in the arctic: a case of paleoclimate shaping modern biodiversity? *PLoS ONE* **2**: e728.
- Stoeck T, Taylor GT & Epstein SS (2003) Novel eukaryotes from the permanently anoxic Cariaco Basin (Caribbean Sea). *Appl Environ Microbiol* **69**: 5656– 5663.
- Takasaki K, Shoun H, Nakamura A, Hoshinu T & Takaya N (2004) Unusal transcription regulation of *niaD* gene under anaerobic conditions supporting fungal ammonia fermentation. *Biosci Biotechnol Biochem* **68**: 978-980.
- Takaya N, Suzuki S, Kuwazaki S, Shoun H, Maruo F, Yamaguchi M, Takoe K (1999) Cytochrome P450_{nor}, a novel class of mitochondrial cytochrome P450 involved in nitrate respiration in the fungus *Fusarium oxysporum*. *Arch Biochem Biophys* **372**: 340- 346.
- Takishita K, Miyake H, Kawato M & Maruyama T (2005) Genetic diversity of microbial eukaryotes in anoxic sediment around fumaroles on a submarine caldera floor based on the small-subunit rDNA phylogeny. *Extremophiles* **9**: 185–196.
- Takishita K, Tsuchiyaa M, Kawatoa M, Ogurib K, Kitazatob H & Maruyamaa T (2007a) Diversity of microbial eukaryotes in anoxic sediment of the saline meromictic lake Namako-ike (Japan): on the detection of anaerobic or anoxic-tolerant lineages of eukaryotes. *Protist* **158**: 51- 64.
- Takishita K, Yubuki N, Kakizoe N, Inagaki Y & Maruyama T (2007b) Diversity of microbial eukaryotes in sediment at a deep-sea methane cold seep: surveys of ribosomal DNA libraries from raw sediment samples and two enrichment cultures. *Extremophiles* **11**: 563–576.
- Vainio EJ & Hantula J (2000) Direct analysis of wood-inhabiting fungi using denaturing gradient gel electrophoresis of amplified ribosomal DNA. *Mycol Res* **104**: 927-936.
- Wilson MS, Siering PL, White CL, Hauser ME & Bartles AN (2008) Novel archaea and bacteria dominate stable microbial communities in North America's largest hot spring. *Microb Ecol* **56**:292.305.

Figure Legends:

Fig. 1. Venn diagram showing the number of OTUs that were recovered simultaneously by two or three of the primer sets used in this study (Fung1, Fung2, EukAB, see Table 2) and number of OTUs recovered exclusively with a single primer set. All OTUs, which were retrieved by the universal eukaryote primer set (EukAB), were also detected with the two fungal specific primer sets (Fung1, Fung2).

Fig. 2. Dendrogram resulting from calculated Jaccard index based on incidence ($J_{\text{incidence}}$) of unique operational taxonomic units (OTUs), as a measure of community similarity between the samples under study. For information on sampling sites, see Table 1. Similarity values were transformed into a distance matrix and subsequent cluster analysis was performed using the unweighted pair group mean average (UPGMA) algorithm.

Fig. 3 Collectors curves of fungal OTUs. Species accumulation curve (Sobs) gives the number of species expected in the pooled samples for the given empirical data. Abundance-based Coverage Estimator (ACE) is an estimator of species richness (mean among runs) for the clone libraries.

Fig. 4. Bayesian tree of the 18S rDNA sequences of phylum Ascomycota showing the position of environmental OTUs obtained from the Arabian Sea. The tree was constructed by using a GTR+I+G evolutionary model with the variable-site gamma distribution shape parameter at 0.6817 and the proportion of invariable sites at 0.282 as suggested by Modeltest, based on 925 unambiguously aligned positions. Significant support from Bayesian MCMC analysis (>95) is indicated by a black filled dot at the respective node. Other support values are only displayed when >50 . Numbers in parenthesis following environmental OTU names indicate the number of sequences of this OTU that were found in the individual libraries. Color-coding for libraries is given in Table 1.

Fig. 5. Bayesian tree of the 18S rDNA sequences of phylum Basidiomycota showing the position of environmental OTUs obtained from the Arabian Sea. The tree was constructed by using a GTR+I+G evolutionary model with the variable-site gamma distribution shape parameter at 0.5470 and the proportion of invariable sites at 0.3147 as suggested by Modeltest, based on 785 unambiguously aligned positions. Further legend as in Fig. 4.

Fig. 6. Bayesian tree of the 18S rDNA sequences of phylum Zygomycota, Glomeromycota and Chytridiomycota showing the position of environmental OTUs obtained from the Arabian Sea. The tree was constructed by using a GTR+I+G evolutionary model with the variable-site gamma distribution shape parameter at 0.5710 and the proportion of invariable sites at 0.3903 as suggested by Modeltest, based on 1436 unambiguously aligned positions. Significant support from Bayesian MCMC analysis (>95) is indicated by a black filled dot at the respective node. Other support values are only displayed when >50 . Numbers in parenthesis following environmental OTU names indicate the number of sequences of this OTU that were found in the individual libraries. Color-coding for libraries is given in Table 1.

Figures

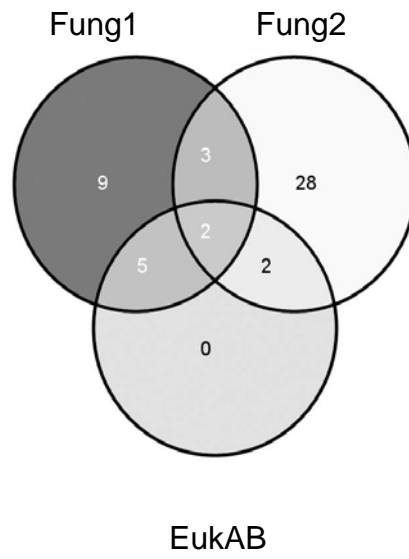


Fig. 1.

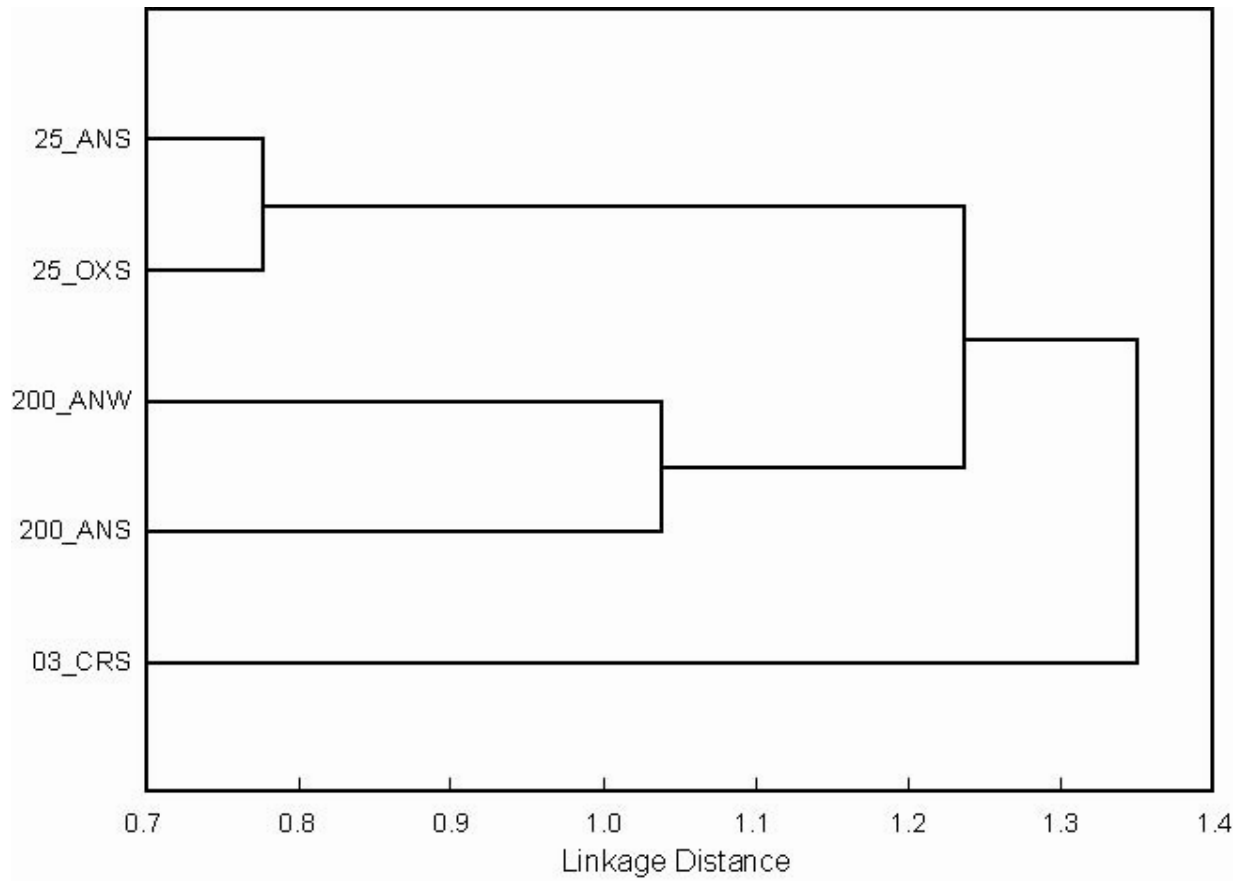


Fig. 2.

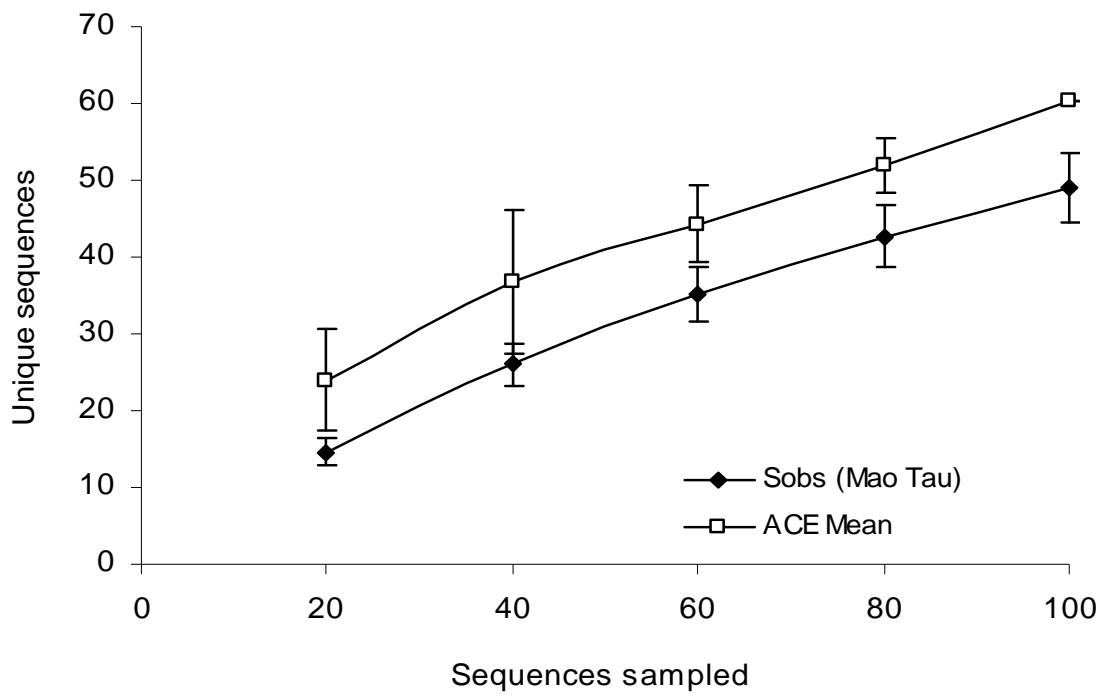


Fig. 3

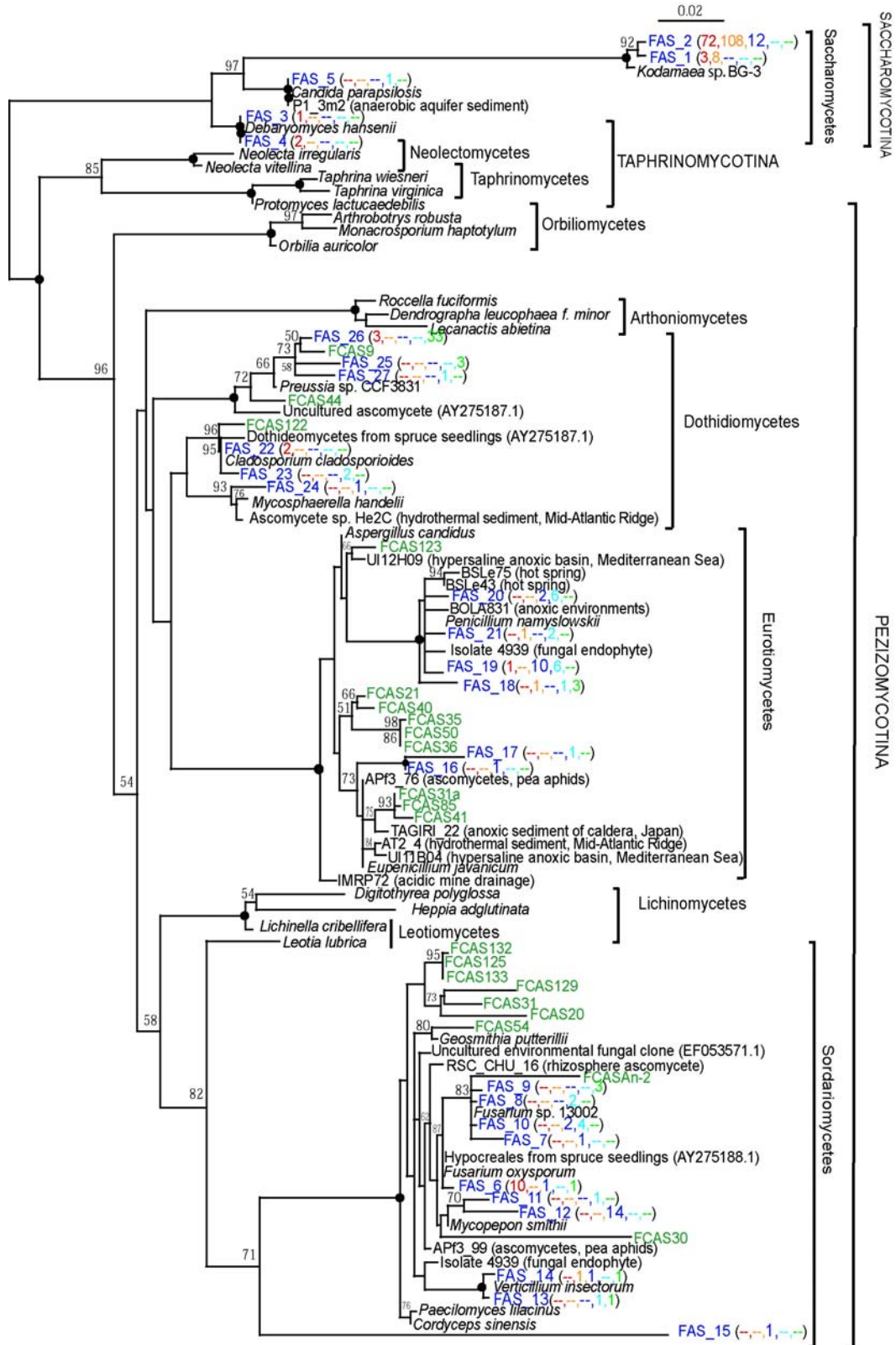


Fig. 4

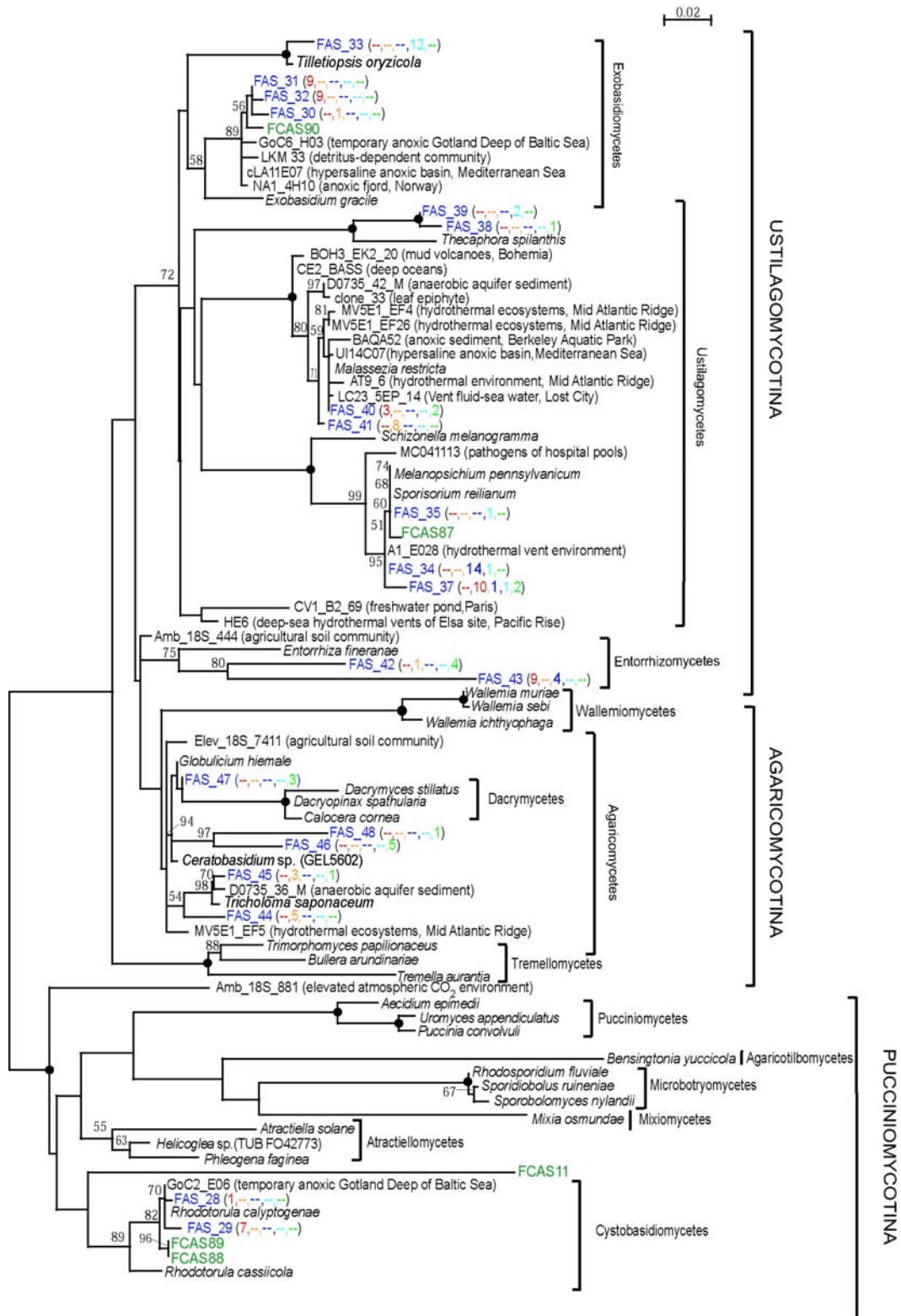


Fig. 5

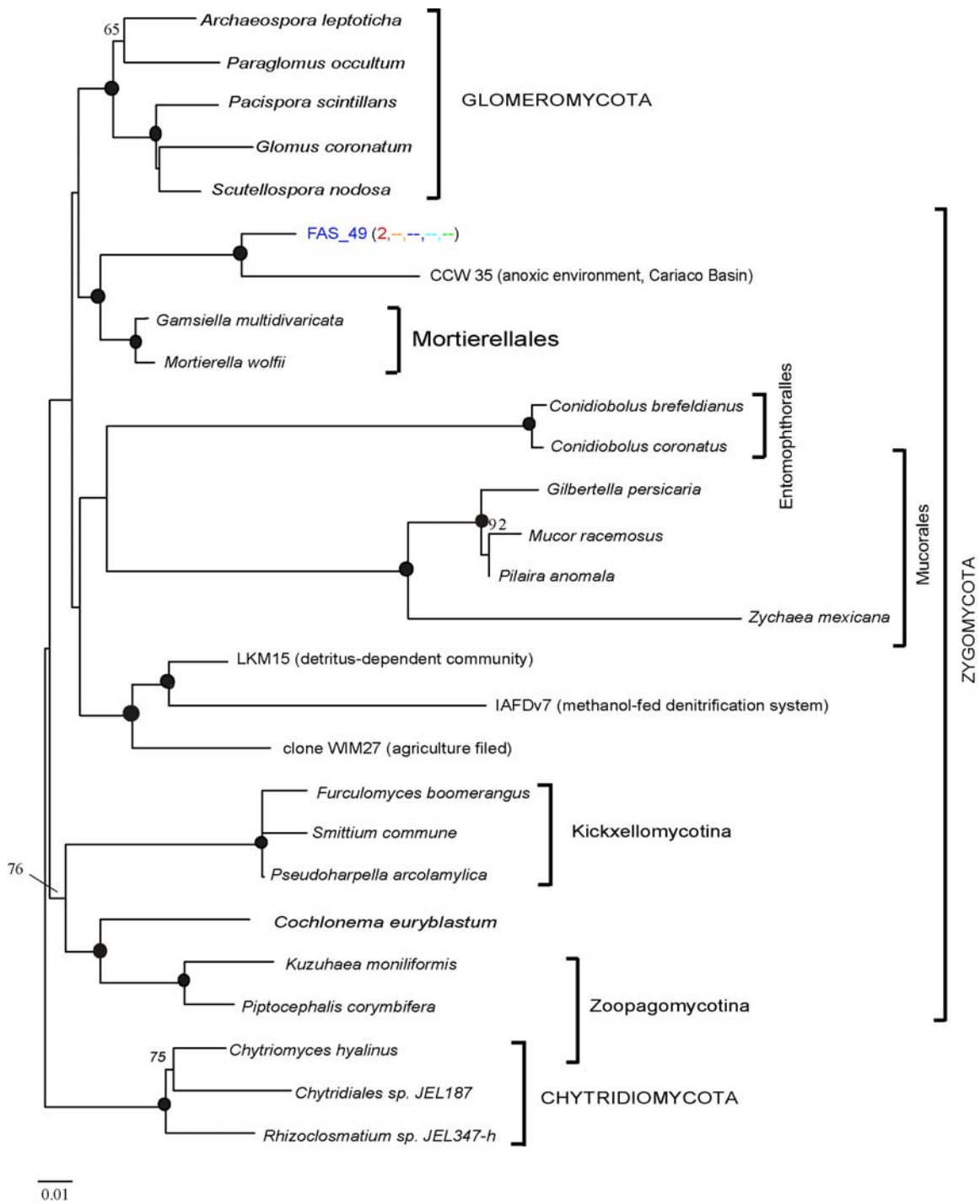


Fig. 6

Table 3: Taxonomic distribution of the non-target clones retrieved by each primer set (Supplementary table).

Name of Clone	Closest BLAST hit	Acc. No.	Max Ident (%)
Primer set: Fung1			
G511E10	<i>Nephtys hombergii</i>	AY527054.1	97
G511E6	<i>Chaetonotus</i> sp.	AJ001735.1	95
G511H8	<i>Eucalanus inermis</i>	AY335857.1	98
G531C9	<i>Salmo trutta</i>	DQ009482.1	99
GPS1A1	<i>Exumella mediterranea</i>	AY629259.1	97
GPS1A10	<i>Pandea</i> sp.	AY920765.1	99
GPS1A11	<i>Exumella mediterranea</i>	AY629259.1	97
GPS1A2	<i>Calanus pacificus</i>	L81939.1	96
GPS1A3	<i>Exumella mediterranea</i>	AY629259.1	97
GPS1A4	<i>Neocalanus cristatus</i>	AF514344.1	95
GPS1A5	<i>Neocalanus cristatus</i>	AF514344.1	95
GPS1A9	<i>Exumella mediterranea</i>	AY629259.1	97
GPS1B1	<i>Amoebophrya</i> sp. ex <i>Prorocentrum micans</i>	AY208893.1	90
GPS1B10	<i>Oikopleura</i> sp. cf. <i>dioica</i>	AY116613.1	98
GPS1B12	<i>Exumella mediterranea</i>	AY629259.1	96
GPS1B2	<i>Exumella mediterranea</i>	AY629259.1	97
GPS1B3	<i>Amoebophrya</i> sp. ex <i>Prorocentrum micans</i>	AY208893.1	90
GPS1B5	<i>Exumella mediterranea</i>	AY629259.1	96
GPS1B6	<i>Calanus pacificus</i>	L81939.1	96
GPS1B8	<i>Eucalanus inermis</i>	AY335857.1	98
GPS1C10	<i>Neocalanus cristatus</i>	AF514344.1	96
GPS1C12	<i>Amoebophrya</i> sp. ex <i>Karlodinium micrum</i>	AF472553.1	84
GPS1D8	<i>Gilia reticulata</i>	DQ080013.1	99
GPS1D9	<i>Exumella mediterranea</i>	AY629259.1	97
GPS1E10	<i>Velamen parallelum</i>	AF293693.1	99
GPS1E7	<i>Corallochytrium limacisporum</i>	L42528.1	99
GPS1E8	<i>Paranaitis kosteriensis</i>	AY996078.1	93
GPS1E9	<i>Protaspis</i> sp.	FJ824125.1	93
GPS1F7	<i>Paranaitis kosteriensis</i>	AY996078.1	94
GPS1G4	<i>Exumella mediterranea</i>	AY629259.1	97
GPS1G5	<i>Neocalanus cristatus</i>	AF514344.1	96
GPS1H12	<i>Monosiga brevicollis</i>	AC198536.1	100
GPS1H2	<i>Neocalanus cristatus</i>	AF514344.1	96
GPS1H3	<i>Calanus pacificus</i>	L81939.1	95
GPS1H4	<i>Temora longicornis</i>	AY446900.1	97
GPS1H5	<i>Gilia reticulata</i>	DQ080013.1	99
GPW1A1	<i>Exumella mediterranea</i>	AY629259.1	97
GPW1A10	<i>Exumella mediterranea</i>	AY629259.1	97
GPW1A11	<i>Physophora hydrostatica</i>	AF358072.1	99
GPW1A12	<i>Corallochytrium limacisporum</i>	L42528.1	99

GPW1A2	<i>Exumella mediterranea</i>	AY629259.1	97
GPW1A3	<i>Exumella mediterranea</i>	AY629259.1	97
GPW1A9	<i>Corallochytrium limacisporum</i>	L42528.1	99
GPW1B1	<i>Exumella mediterranea</i>	AY629259.1	97
GPW1B3	<i>Gilia reticulata</i>	DQ080013.1	99
GPW1B4	<i>Exumella mediterranea</i>	AY629259.1	97
GPW1B8	<i>Gilia reticulata</i>	DQ080013.1	100
GPW1B9	<i>Nanomia bijuga</i>	AY937324.1	99
GPW1C1	<i>Exumella mediterranea</i>	AY629259.1	97
GPW1C12	<i>Paranaitis kosteriensis</i>	AY996078.1	95
GPW1C2	<i>Exumella mediterranea</i>	AY629259.1	97
GPW1C3	<i>Gilia reticulata</i>	DQ080013.1	99
GPW1C4	<i>Exumella mediterranea</i>	AY629259.1	97
GPW1C5	<i>Gilia reticulata</i>	DQ080013.1	99
GPW1C7	<i>Exumella mediterranea</i>	AY629259.1	97
GPW1C8	<i>Nanomia bijuga</i>	AY937324.1	99
GPW1D1	<i>Gaussia princeps</i>	GQ325591.1	99
GPW1D12	<i>Exumella mediterranea</i>	AY629259.1	97
GPW1D2	<i>Exumella mediterranea</i>	AY629259.1	97
GPW1D4	<i>Exumella mediterranea</i>	AY629259.1	97
GPW1D7	<i>Gaussia princeps</i>	GQ325591.1	99
GPW1D9	<i>Nanomia bijuga</i>	AY937324.1	99
GPW1E1	<i>Exumella mediterranea</i>	AY629259.1	97
GPW1E10	<i>Hippopodius hippopus</i>	AF358069.1	98
GPW1E2	<i>Exumella mediterranea</i>	AY629259.1	97
GPW1E4	<i>Corallochytrium limacisporum</i>	L42528.1	99
GPW1F12	<i>Corallochytrium limacisporum</i>	L42528.1	99
GPW1F3	<i>Exumella mediterranea</i>	AY629259.1	97
GPW1F4	<i>Exumella mediterranea</i>	AY629259.1	97
GPW1F5	<i>Exumella mediterranea</i>	AY629259.1	97
GPW1F9	<i>Corallochytrium</i> sp. LM578	EF060864.1	97
GPW1G1	<i>Exumella mediterranea</i>	AY629259.1	97
GPW1G12	<i>Corallochytrium limacisporum</i>	L42528.1	99
GPW1G2	<i>Nanomia bijuga</i>	AY937324.1	99
GPW1G6	<i>Oikopleura</i> sp.	AB013015.1	97
GPW1G8	<i>Exumella mediterranea</i>	AY629259.1	97
GPW1H1	<i>Exumella mediterranea</i>	AY629259.1	97
GPW1H10	<i>Exumella mediterranea</i>	AY629259.1	97
GPW1H6	<i>Corallochytrium limacisporum</i>	L42528.1	99
GPW1H8	<i>Oikopleura dioica</i>	AB013014.1	97
PS11A11	<i>Bryocamptus pygmaeus</i>	AY627015.1	94
PS11A3	<i>Xestoleberis hanaii</i>	AB076633.1	98
PS11A4	<i>Xestoleberis hanaii</i>	AB076633.1	98
PS11A6	<i>Xestoleberis hanaii</i>	AB076633.1	98
PS11C11	<i>Bryocamptus pygmaeus</i>	AY627015.1	94
PS11D2	<i>Xestoleberis</i> sp.	AY191450.2	96

PS11D7	<i>Xestoleberis</i> sp.	AY191450.2	97
PS11E1	<i>Aporcelaimellus</i> sp.	AY284813.1	91
PS11G1	<i>Xestoleberis hanaii</i>	AB076633.1	98
PS11G2	<i>Kroyeria</i> sp.	DQ538499.1	93
PS11G8	<i>Xestoleberis hanaii</i>	AB076633.1	98
PS11H1	<i>Xestoleberis hanaii</i>	AB076633.1	98
PS11H12	<i>Xestoleberis hanaii</i>	AB076633.1	98
Primer set: Fung2			
G512A1	<i>Chaetonotus</i> sp.	AJ001735.1	96
G512A2	<i>Nannochloris</i> sp.	AB183585.1	99
G512A4	<i>Cryothecomonas longipes</i>	AF290540.1	93
G512B12	<i>Protaspis</i> sp.	FJ824125.1	93
G512C1	<i>Ebria tripartita</i>	DQ303923.1	87
G512C2	<i>Cryothecomonas longipes</i>	AF290540.1	92
G512C3	<i>Nannochloris</i> sp.	AB183585.1	99
G512C5	<i>Nannochloris</i> sp.	AB183585.1	99
G512C9	<i>Protaspis</i> sp.	FJ824125.1	93
G512D1	<i>Cryothecomonas longipes</i>	AF290540.1	93
G512D2	<i>Exuviaella pusilla</i>	DQ388459.1	95
G512D6	<i>Thraustochytriidae</i> sp.	DQ367048.1	90
G512E9	<i>Burkholderia</i> sp.	CP000152.1	75
G512F8	<i>Protaspis</i> sp.	FJ824125.1	93
G512G9	<i>Nannochloris</i> sp.	AB183585.1	99
G512H1	<i>Ebria tripartita</i>	DQ303923.1	87
G512H8	<i>Massisteria marina</i>	AF174372.1	91
G532C2	<i>Agrobacterium tumefaciens</i>	AE009008.1	88
G532C8	<i>Pelobacter carbinolicus</i>	CP000142.2	89
G532E8	<i>Thelypteris navarrensis</i>	DQ629429.1	100
GPS2A1	<i>Corallochytrium limacisporum</i>	L42528.1	99
GPS2A10	<i>Corallochytrium limacisporum</i>	L42528.1	99
GPS2A11	<i>Corallochytrium limacisporum</i>	L42528.1	99
GPS2A9	<i>Liriope tetraphylla</i>	AY428814.1	99
GPS2B10	<i>Corallochytrium limacisporum</i>	L42528.1	99
GPS2B2	<i>Liriope tetraphylla</i>	AY428814.1	99
GPS2C5	<i>Corallochytrium limacisporum</i>	L42528.1	99
GPS2D12	<i>Corallochytrium limacisporum</i>	L42528.1	99
GPS2D7	<i>Corallochytrium limacisporum</i>	L42528.1	98
GPS2G10	<i>Neocalanus cristatus</i>	AF514344.1	95
GPS2H8	<i>Corallochytrium limacisporum</i>	L42528.1	99
GPW2A11	<i>Corallochytrium limacisporum</i>	L42528.1	99
GPW2A5	<i>Corallochytrium limacisporum</i>	L42528.1	99
GPW2A7	<i>Corallochytrium limacisporum</i>	L42528.1	99
GPW2B1	<i>Gaussia princeps</i>	GQ325591.1	99
GPW2B11	<i>Neocalanus cristatus</i>	AF514344.1	95
GPW2B4	<i>Corallochytrium limacisporum</i>	L42528.1	99
GPW2B6	<i>Corallochytrium limacisporum</i>	L42528.1	99

GPW2B7	<i>Neocalanus cristatus</i>	AF514344.1	95
GPW2B8	<i>Corallochytrium limacisporum</i>	L42528.1	99
GPW2C2	<i>Corallochytrium limacisporum</i>	L42528.1	99
GPW2C3	<i>Neocalanus cristatus</i>	AF514344.1	95
GPW2C5	<i>Gaussia princeps</i>	GQ325591.1	99
GPW2C7	<i>Neocalanus cristatus</i>	AF514344.1	95
GPW2C8	<i>Eucyrtidium hexastichum</i>	AB246681.1	99
GPW2D1	<i>Corallochytrium limacisporum</i>	L42528.1	99
GPW2D5	<i>Corallochytrium limacisporum</i>	L42528.1	99
GPW2D6	<i>Gaussia princeps</i>	GQ325591.1	99
GPW2D7	<i>Corallochytrium limacisporum</i>	L42528.1	99
GPW2D9	<i>Corallochytrium limacisporum</i>	L42528.1	99
GPW2E1	<i>Lagenidium thermophilum</i>	AB284572.1	88
GPW2E7	<i>Eucyrtidium hexastichum</i>	AB246681.1	99
GPW2F1	<i>Corallochytrium limacisporum</i>	L42528.1	99
GPW2F10	<i>Larcopyle butschlii</i>	AB246693.1	96
GPW2F3	<i>Eucyrtidium hexastichum</i>	AB246681.1	99
GPW2F4	<i>Aidanosagitta neglecta</i>	DQ351882.1	97
GPW2F5	<i>Gaussia princeps</i>	GQ325591.1	99
GPW2F7	<i>Corallochytrium limacisporum</i>	L42528.1	99
GPW2G3	<i>Gaussia princeps</i>	GQ325591.1	99
GPW2G4	<i>Liriope tetraphylla</i>	AY428814.1	99
GPW2G5	<i>Corallochytrium limacisporum</i>	L42528.1	99
GPW2G6	<i>Corallochytrium limacisporum</i>	L42528.1	99
GPW2H10	<i>Corallochytrium limacisporum</i>	L42528.1	99
GPW2H3	<i>Corallochytrium limacisporum</i>	L42528.1	99
GPW2H4	<i>Corallochytrium limacisporum</i>	L42528.1	99
GPW2H5	<i>Corallochytrium limacisporum</i>	L42528.1	99
PS12E4	<i>Prasinophyte sp.</i>	AY425302.1	99
PS12H9	<i>Corallochytrium limacisporum</i>	L42528.1	99
Primer set: EukAB			
G513A1	<i>Hirschmanniella sp.</i>	AY284615.1	100
G513A12	<i>Cryothecomonas longipes</i>	AF290540.1	90
G513A8	<i>Nanomia bijuga</i>	AF358071.1	100
G513A9	<i>Pycnogonum litorale</i>	AY446922.1	100
G513B1	<i>Physophora hydrostatica</i>	AF358072.1	100
G513B10	Uncultured archaeon clone SBAK-mid-28	DQ522924.1	93
G513B12	<i>Eucalanus inermis</i>	AY335857.1	98
G513B2	<i>Axonolaimus helgolandicus</i>	AY854232.1	93
G513B8	<i>Cryothecomonas longipes</i>	AF290540.1	90
G513B9	<i>Ichthyodinium sp.</i>	AB276368.1	90
G513C2	Uncultured marine euryarchaeote VIM1-28	AY380603.1	97
G513C3	<i>Cryptosporidium muris</i>	X64343.1	85
G513C8	<i>Physophora hydrostatica</i>	AF358072.1	100
G513C9	Cercomonadida environmental sample	EU647174.1	88
G513D5	<i>Ebria tripartita</i>	DQ303923.1	90

G513D8	Uncultured archaeon clone SBAK-shallow-09	DQ522931.1	95
G513E10	<i>Eucalanus inermis</i>	AY335857.1	97
G513E11	<i>Cryptosporidium muris</i>	X64343.1	85
G513E12	<i>Metopion fluens</i>	AF411285.1	92
G513E3	Uncultured marine euryarchaeote VIM1-28	AY380603.1	98
G513E7	Uncultured marine euryarchaeote VIM1-28	AY380603.1	96
G513F1	<i>Metopion fluens</i>	AF411285.1	93
G513F11	<i>Temora longicornis</i>	AY446900.1	98
G513F3	<i>Petalomonas cantuscygni</i>	AF386635.1	100
G513F7	<i>Rhodopseudomonas palustris</i>	CP000283.1	80
G5336B	<i>Desmolaimus sp.</i>	EF591333.1	95
G5337G	<i>Corydalus armatus</i>	EU815238.1	92
G533A8	<i>Corydalus armatus</i>	EU815238.1	92
G533B3	<i>Mydas sp.</i>	AM992462.1	99
G533C1	Stramenopile sp. MAST-12	EF219381.1	89
G533E11	<i>Corydalus armatus</i>	EU815238.1	92
G533E5	<i>Mydas sp.</i>	AM992462.1	99
G533G5	<i>Mydas sp.</i>	AM992462.1	100
G533H11	<i>Corydalus armatus</i>	EU815238.1	92
G533H8	<i>Corydalus armatus</i>	EU815238.1	98
GPS3A1	<i>Mesodinium pulex</i>	DQ845294.1	100
GPS3A6	<i>Monosiga brevicollis</i>	AC198536.1	100
GPS3B12	<i>Philocelis brueggemanni</i>	AY078378.1	74
GPS3B7	<i>Dictyostelium aureostipes var. helvetium</i>	AM168095.1	89
GPS3C11	<i>Calomicrolaimus sp.</i>	AY854219.1	98
GPS3C4	Uncultured archaeon clone	DQ522931.1	96
GPS3D4	<i>Laxus oneistus</i>	Y16919.1	94
GPS3E1	<i>Desmolaimus sp.</i>	EF591333.1	97
GPS3E3	<i>Pseudaphanostoma smithrii</i>	AY078375.1	95
GPS3E6	<i>Laxus oneistus</i>	Y16919.1	94
GPS3F1	<i>Calomicrolaimus sp.</i>	AY854219.1	98
GPS3F3	<i>Stramenopile sp.</i>	EF219381.1	100
GPS3F9	<i>Dictyostelium intermedium</i>	AM168044.1	100
GPS3G12	<i>Simplicomorpha gigantorhabditis</i>	AF102894.1	91
GPS3G2	<i>Cryothecomonas longipes</i>	AF290540.1	90
GPS3G3	Uncultured archaeon clone MD2902-A25	EU048605.1	96
GPS3G7	<i>Arxula adenivorans</i>	Z50840.2	86
GPS3H1	<i>Sarcocystis tarandi</i>	EF056017.1	84
GPS3H12	<i>Sarcocystis tarandivulpes</i>	EF056012.1	76
GPW3A1	<i>Exumella mediterranea</i>	AY629259.1	97
GPW3A2	<i>Neocalanus cristatus</i>	AF514344.1	93
GPW3A3	<i>Neocalanus cristatus</i>	AF514344.1	93
GPW3A4	<i>Neocalanus cristatus</i>	AF514344.1	93
GPW3A7	<i>Exumella mediterranea</i>	AY629259.1	96
GPW3B1	<i>Neocalanus cristatus</i>	AF514344.1	93

GPW3B3	<i>Neocalanus cristatus</i>	AF514344.1	92
GPW3B4	<i>Exumella mediterranea</i>	AY629259.1	97
GPW3C1	<i>Metridia gerlachei</i>	AY118076.1	96
GPW3C10	<i>Exumella mediterranea</i>	AY629259.1	96
GPW3C2	<i>Neocalanus cristatus</i>	AF514344.1	93
GPW3C3	<i>Dictyocoryne profunda</i>	AB101540.1	92
GPW3D1	<i>Neocalanus cristatus</i>	AF514344.1	93
GPW3D5	<i>Neocalanus cristatus</i>	AF514344.1	93
GPW3E1	<i>Neocalanus cristatus</i>	AF514344.1	93
GPW3F1	<i>Neocalanus cristatus</i>	AF514344.1	93
GPW3F11	<i>Exumella mediterranea</i>	AY629259.1	96
GPW3G11	<i>Exumella mediterranea</i>	AY629259.1	96
GPW3G2	<i>Monosiga brevicollis</i>	AC198536.1	99
GPW3G5	<i>Neocalanus cristatus</i>	AF514344.1	93
GPW3G7	<i>Didymocyrtilis tetrathalamus</i>	AB193605.1	92
GPW3H1	<i>Neocalanus cristatus</i>	AF514344.1	93
GPW3H10	<i>Exumella mediterranea</i>	AY629259.1	97
GPW3H11	<i>Neocalanus cristatus</i>	AF514344.1	93
GPW3H2	<i>Eucalanus inermis</i>	AY335857.1	98
GPW3H4	<i>Neocalanus cristatus</i>	AF514344.1	93
GPW3H5	<i>Exumella mediterranea</i>	AY629259.1	96
GPW3H6	<i>Exumella mediterranea</i>	AY629259.1	96
PS13A10	<i>Colpodella</i> sp.	DQ174732.1	99
PS13A11	<i>Lithocystis</i> sp.	DQ093795.1	82
PS13A12	<i>Bacillus</i> sp.	EF536723.1	100
PS13A2	<i>Marinobacter aquaeolei</i>	CP000514.1	76
PS13A4	<i>Xestoleberis hanaii</i>	AB076633.1	97
PS13A6	<i>Stramenopile</i> sp. MAST-12	EF527137.1	100
PS13A8	<i>Varistrombidium</i> sp. Kielum	DQ811090.1	94
PS13A9	<i>Acmostomum dioicum</i>	AF503516.1	82
PS13B11	<i>Ebria tripartita</i>	AY179972.1	83
PS13B12	<i>Xestoleberis hanaii</i>	AB076633.1	97
PS13B3	<i>Acmostomum dioicum</i>	AF503516.1	78
PS13B7	<i>Acmostomum dioicum</i>	AF503516.1	83
PS13B8	<i>Petalomonas cantuscygni</i>	AY821956.1	85
PS13B9	<i>Escherichia coli</i>	CP000800.1	93
PS13C10	<i>Varistrombidium</i> sp. kielum	DQ811090.1	94
PS13C11	<i>Cryptosporidium muris</i>	EF100289.1	88
PS13C4	<i>Acmostomum dioicum</i>	AF503516.1	82
PS13C5	<i>Porphyromonas gingivalis</i>	AE015924.1	81
PS13C9	<i>Colpodella</i> sp. RM11	DQ174732.1	89
PS13D10	<i>Escherichia coli</i> E24377A	CP000800.1	93
PS13D11	<i>Acmostomum dioicum</i>	AF503516.1	78
PS13D9	<i>Colpodella</i> sp.	DQ174732.1	93
PS13E11	<i>Acmostomum dioicum</i>	AF503516.1	78
PS13E6	<i>Prasinophyte symbiont</i>	EF527133.1	97

PS13E8	<i>Lithocystis</i> sp.	DQ093795.1	87
PS13F11	<i>Cryptosporidium muris</i>	EF100289.1	88
PS13F3	<i>Calomicrolaimus</i> sp.	AY854219.1	96
PS13F7	<i>Aplysina lacunosa</i>	AY591802.1	80
PS13F9	<i>Sphaerosyllis hystrix</i>	AF474288.1	95
PS13G11	<i>Acmostomum dioicum</i>	AF503516.1	82
PS13G4	<i>Rana sphenoccephala</i>	EF196788.1	82
PS13G7	<i>Strombidium</i> sp.	AY143564.1	97
PS13G8	<i>Bacillus</i> sp.	EF536723.1	100
PS13G9	<i>Acmostomum dioicum</i>	AF503516.1	78
PS13H10	<i>Lithocystis</i> sp.	AB191437.1	84
PS13H6	<i>Acmostomum dioicum</i>	AF503516.1	78
PS13H7	<i>Cryptosporidium muris</i>	EF100289.1	88
PS13H8	<i>Cryptosporidium muris</i>	EF100289.1	88

Table 1. Sampling sites and characteristics. DO = dissolved oxygen, Temp = temperature

Sampling site	Designation*	Sampling Date	DO (μM)	Temp ($^{\circ}\text{C}$)	Salinity (PSU)	Sampling material	Vol of sample used
Near shore off Goa (25m)	25_ANS (brown)	Oct 2005	~ 0	22.5	35.4	Sediment	~ 500 mg
Near shore off Goa (25m)	25_OXS (orange)	Jan 2006	118.4	26.9	34.7	sediment	~ 500 mg
OMZ off Goa (200m)	200_ANS (blue)	May 2007	4.28	14.8	35.6	sediment	~ 500 mg
OMZ off Goa (200m)	200_ANW (turquoise)	May 2007	4.28	14.8	35.6	water	~ 5 - 8 l
Coral reef off Kavaratti (3m)	03_CRS (green)	Feb 2007	93.74	28.7	34.0	sediment	~ 500 mg

*color-coding in Fig. 4, 5 and 6.

Table 2. Primer sets used in this study to amplify 18S rDNA sequences from genomic environmental DNA.

Primer set	Primers	Primer Sequence (5' -3')	Reference
Fung1	NS1	GTA GTC ATA TGC TTG TCT C	Vainio & Hantula,2000
	FR1	AIC CAT TCA ATC GGT AIT	Gomes <i>et al.</i> , 2003
Fung2	UF1	CGA ATC GCA TGG CCT TG	Kappe <i>et al.</i> , 1996
	S3	AGT CAA ATT AAG CCG CAG	Maiwald <i>et al.</i> , 1994
EukAB	EukA	AAC CTG GTT GAT CCT GCC AGT	Medlin <i>et al.</i> , 1988
	EukB	TGA TCC TTC TGC AGG TTC ACC TAC	Medlin <i>et al.</i> , 1988