

Fungal diversity from various marine habitats deduced through culture-independent studies

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Keywords: marine habitats, molecular diversity, fungal phylotypes, environmental sequences

Running title: un-cultured fungal diversity from marine habitats

Abstract

Studies on the molecular diversity of the micro-eukaryotic community have shown that fungi occupy a central position in a large number of marine habitats. Environmental surveys using molecular tools have shown the presence of fungi from a large number of marine habitats such as deep-sea habitats, pelagic waters, coastal regions, hydrothermal vent ecosystem, anoxic habitats and ice-cold regions. This is of interest to a variety of research disciplines like ecology, evolution, biogeochemistry and biotechnology. In this review we have summarized how molecular tools, have helped to broaden our understanding of the fungal diversity in various marine habitats. Majority of the environmental phylotypes could be grouped as novel clades within Ascomycota, Basidiomycota, Chytridiomycota or as basal fungal lineages. Deep-branching novel environmental clusters could be grouped within Ascomycota as the Pezizomycotina clone group, deep-sea fungal group I and soil clone group I, within Basidiomycota as the hydrothermal and/or anaerobic fungal group group and as Cryptomycota or the Rozella clade within Chytridiomycota. However, a basal true marine environmental cluster is still to be identified as most of the clusters include representatives from terrestrial regions. The challenge for future research is to explore the true marine fungi using molecular techniques.

Introduction

Molecular description of the microbial diversity from a number of natural habitats has revealed hitherto unknown microbial wealth (Nelson *et al.*, 2010). The marine ecosystem is no exemption and the diversity estimates using molecular tools have brought to light, rich microbial diversity (Treusch *et al.*, 2010). Surveys have been carried out in the marine habitats to study the diversity and distribution of prokaryotic and micro-eukaryotic kingdoms (Richards & Bass, 2005; Epstein & López-García *et al.*, 2007; López-García & Moreira, 2008, Jones & Pang 2012). Molecular studies on micro-eukaryotic diversity have revealed that fungal sequences could be retrieved from a number of marine habitats. Several of these environmental fungal sequences are highly divergent from the well-described fungal taxa and have phylogenetic importance (López-García *et al.*, 2007; Le Calvez *et al.*, 2009; Jones *et al.*, 2011). A few studies have exclusively studied the fungal diversity from marine habitats. They have also added to the list of environmental fungal sequences that are divergent from the known fungal taxa (Bass *et al.*, 2007; Gao *et al.*, 2008; Gao *et al.*, 2010; Jebaraj *et al.*, 2010; Singh *et al.*, 2011; 2012a; b; Arfi *et al.*, 2012; Thaler *et al.*, 2012).

Understanding the fungal diversity in the marine ecosystem is important to study the fungal evolutionary history as early fungal divergence is known to have taken place in the marine realm (López-García & Moreira, 2008; Le Calvez *et al.*, 2009). There are increasing evidences to show the active participation of fungi in the marine habitats and their involvement in the biogeochemical processes (Stoeck *et al.*, 2007; Stock *et al.* 2009; Alexander *et al.*, 2009; Edgcomb *et al.*, 2011). Molecular diversity estimates have shown that fungi occupy an important ecological niche in the marine environment. But still the extent of fungal distribution in the marine habitats is debatable (Richards *et al.*, 2012). Here we have summarized the fungal diversity unraveled from the marine habitats, especially the unique fungal sequences and clusters identified from these regions. An evaluation of these studies will be of immense help for further explorations of the marine environment and aid future research to retrieve true marine fungal phylotypes and isolates.

Marine habitats

Marine habitats can be broadly classified into coastal and deep-sea regions and the molecular diversity of fungi has been reported from both these regions. However studies from the coastal regions (Table 1)

are very few in comparison with the studies from the deep-sea regions (Table 2). Each of these major marine habitats harbors a number of unique, self-contained niches such as the coral reef, the mangrove and hydrothermal vent systems. Two of these niches, the hydrothermal vent and oxygen-depleted habitats have been studied extensively. The molecular diversity understood from both these biogeochemically active regions are listed individually (Table 3 and 4).

Coastal regions

Coastal regions are characterized by eutrophication from terrestrial run off and high primary production (Danovaro & Pusceddu, 2007). This leads to large availability of organic matter to consumers as detritus. Prokaryotic heterotrophs are known to play a huge role as primary degraders in the coastal waters, followed by eukaryotic heterotrophs (Strom, 2008). Though planktonic forms of fungi have been reported from coastal waters, only a few studies have been carried out on fungi as they are generally not known to be free-living. Diversity of fungi has been studied only from a few selected coastal locations along the coasts off Brazil (Cury *et al.*, 2011), coral reef regions off Hawaii (Gao *et al.*, 2008, Gao *et al.*, 2010) and mangrove areas (Arfi *et al.*, 2012). These molecular studies have retrieved fungal sequences belonging to Ascomycota, Basidiomycota and Chytridiomycota and some of them group into novel environmental clusters (Table 1).

The coral reef ecosystem in the coastal waters of the tropical regions is a dynamic, self-sustained system with vast biodiversity and fungi were largely considered to be pathogens in this system (Kim *et al.*, 2006; Yarden *et al.*, 2007; Vega Thurber *et al.*, 2009). However metagenomic analysis and the functional diversity of coral associated microbes show that fungi are a dominant community and they are involved in the nitrogen cycling within the coral reef ecosystem (Wegley *et al.*, 2007). The macroorganisms such as sponges, oysters, sea anemones also are important members within the coral reef ecosystem. These organisms are micro-habitats for the microbial communities. Highly diverse environmental sequences have been identified from these regions (Gao *et al.*, 2008; 2010; Singh *et al.*, 2012b). Studies on the culturable fungal diversity and their biotechnological potential have shown that many novel fungi are associated with it. The secondary metabolites produced by them are also very varied and have wide applications (Zhou *et al.*, 2011; Raghukumar & Ravindran, 2012). The mangrove ecosystems situated along some coastal marine environments are another important niche for identification of marine fungi and many true obligate marine species have been isolated from these

regions (Suetrong *et al.*, 2009). A large number of true marine fungi were also described from mangrove regions, but their phylogenetic significance has not been understood clearly (Sarma & Hyde, 2001; Fryar *et al.*, 2004; Jones *et al.*, 2009). These barriers have been removed by the application of molecular techniques as the taxonomic affiliation of fungal cultures and environmental sequences are assigned based on the phylogenetic analysis of the marker gene sequence (Hyde *et al.*, 2011). A recent study by Arfi *et al.* (2012) from the coastal mangrove regions using pyrosequencing approach has shown a rich fungal diversity and Agaricomycetes are shown to be the dominant groups. This study is the first and only known report on the molecular diversity of mangrove regions. However, studies on the molecular diversity of fungi from the coastal regions are just a hand full and these regions need to be further explored.

Deep-sea regions

Low temperatures, high hydrostatic pressure, absence of light and very low biological diversity are the salient features of the deep-sea regions. Species counts in the deep-sea sediments are very low in comparison to coastal sediments and majority of the culturable fungi isolated are common terrestrial forms (Damare *et al.*, 2008, Singh *et al.*, 2010). The majority of the fungi obtained from deep-sea habitats belonged to Ascomycota, Basidiomycota with a large representation of yeasts belonging to both the phyla (Bass *et al.*, 2007, Lai *et al.*, 2007). Molecular studies have revealed the presence of many deep-branching novel sequences belonging to the major fungal phyla Ascomycota, Basidiomycota, Chytridiomycota and Zygomycota (Table 2). There has been a large interest in the deep-sea habitats, because these regions are known to be the cradle of molecular evolution. Unexpectedly large diverse types of micro-eukaryotes, novel marine alveolates, straminopiles, heterokonts and dinoflagellates were retrieved from the deep-sea regions (López-García *et al.*, 2001, Edgcomb *et al.*, 2002, López-García *et al.*, 2003, López-García *et al.*, 2007).

The molecular divergence of fungi is hypothesized to have taken place in the marine realm. And unique niches such as the thermal vents and the oxygen-depleted regions were studied specifically to understand the fungal evolution (Table 3 and 4). These regions are the hub of taxonomic diversity and are of special importance for evolutionary studies, as they could harbor a large number of deep-branching ancient fungal phylotypes (Heckman *et al.*, 2001). True to this hypothesis, novel fungal phylotypes were retrieved from this region using universal micro-eukaryotic primers (Edgcomb *et al.*,

2002; López-García *et al.*, 2003; López-García *et al.*, 2007). A large number of novel fungal cultures were isolated and unsuspected species richness was brought to light through studies using fungal specific primers for metagenomic analysis (Le Calvez *et al.*, 2009). Though rich knowledge of the fungal diversity from the vent region has been obtained using molecular tools, the information is rather incomplete. Novel fungal clades could be identified only within Ascomycota, Basidiomycota and Chytridiomycota and no representatives are reported from the basal fungal groups (Table 3). Diversity estimates using specific primer sets for each phylum with special focus on the basal groups can help us to further characterise the fungal distribution in this region.

Vast diversity of novel environmental phylotypes was also found through molecular investigations of marine oxygen-depleted environments (listed in Jebaraj *et al.*, 2012), and a few oxygen-depleted freshwater environments (Dawson & Pace, 2002; Luo *et al.*, 2005; Slapeta *et al.*, 2005). Most of the studies focused on the molecular ecology using universal eukaryotic primers and revealed a large diversity of novel Ciliates, Cercomonads, Stramenopiles, Radiolarians and Ophisthokonts from this region (Dawson & Pace, 2002; Stoeck & Epstein, 2003; Stoeck *et al.*, 2003; Takishita *et al.*, 2005; Lou *et al.*, 2005; Behnke *et al.*, 2006; Alexander *et al.*, 2009). Fungal sequences have been a major component from these studies and some of the sequences obtained were restricted to known and well described taxa (Table 4). Wide distribution of fungi from anoxic habitats shows their adaptability to a variety of environmental conditions (Jebaraj *et al.*, 2012). Representative phylotypes belonging to the major known environmental clusters and also novel environmental clusters were isolated from the vent regions and ODEs.

The number of novel phylotypes obtained from the deep-sea habitats is higher compared to the other marine habitats (Fig. 1). RNA based libraries could also be used to recover novel sequences and it was possible to show that these sequences were metabolically active and have a role in the deep-sea ecosystem (Edgcomb *et al.*, 2011). This emphasizes the fact that fungi play an active ecological role in the deep-sea habitats. Development of probes and combination of genomic and culture based techniques can help us to understand the significance of these clusters in marine habitats. This is of great interest to the mycologist as the early fungal divergence is known to have taken place in the marine realm and molecular studies such as these could give us an insight into the fungal evolution.

Highly diverse and novel clusters of environmental fungal sequences identified from marine habitats

Environmental fungal sequences obtained from the marine habitats clustered within the major fungal phyla Ascomycota, Basidiomycota, Chytridiomycota or grouped as basal fungal clades. A very few representation of fungal sequences belonging to Zygomycota were retrieved, and phyla such as Glomeromycota, Blastocladiomycota or Neocallimastigomycota had no representation in any of the molecular studies. The taxonomic position of the fungal sequences that clustered close to known fungal taxa could be clearly identified. However, many of the environmental sequences clustered away from the known fungal taxa and grouped to form environmental clades. Analysis of these environmental sequences shows that a few distinct environmental clusters can be identified within Ascomycota, Basidiomycota, Chytridiomycota and Zygomycota (Fig. 1).

Ascomycota

Environmental sequences retrieved from marine habitats grouped into three major environmental groups within Ascomycota (Fig. 1). A major cluster of sequences identified within Saccharomycotina is the deep-sea fungal group (DSF Group-I) with *Candida* sp. as its closest described taxa (Nagano *et al.*, 2010; Thaler *et al.*, 2012). This group was described by Nagano *et al.* (2010) with fungal sequences from the deep-sea regions off Japan based on ITS and LSU rRNA clone library analysis (Table 2). A similar cluster can also be delineated based on the phylogenetic analysis of SSU rRNA sequences obtained from other deep-sea regions (Nagahama *et al.*, 2011; Bass *et al.*, 2007) and oxygen-depleted environments (Takishita *et al.*, 2007b). This was evident from the phylogenetic analysis of the environmental sequences from deep-sea regions which included environmental phylotypes (CYSGM-19, KD10 BASS, S04H05) from these studies (Nagahama *et al.*, 2011). Studies from the South China Sea and the Arabian Sea have also retrieved environmental sequences which cluster close to *Candida* sp. (Lai *et al.*, 2007, Jebaraj *et al.*, 2010). But these studies were not useful to determine their taxonomic affiliation to this cluster because representative environmental sequences belonging to this cluster were not included during phylogenetic analysis. Inclusion of representative sequences from major environmental clusters can be of great help for taxonomic characterization.

Another set of highly diverse fungal sequence clusters within Pezizomycotina as an environmental clone group (PCG). It branches close to well-known fungal cultures *Penicillium* sp. *Eupenicillium* sp. and *Aspergillus* sp. and clusters around single fungal phylotypes (DH148-5-EKD21, BOLA831, AT2-4) obtained from the pioneering molecular studies of the marine regions (López-García *et al.*, 2001; Dawson & Pace, 2002, López-García *et al.*, 2003). This PCG can be observed distinctly when representative environmental phylotypes (BOLA831, AT2-4, TAGIRI 22, UI1104B, UI12H09 from marine habitats such as the anoxic coastal sediments (Dawson & Pace, 2002), anoxic fumaroles (Takishita *et al.*, 2005), and hypersaline anoxic Mediterranean deep-sea basin (Alexander *et al.*, 2009) were included during analysis (Takishita *et al.*, 2005, Jebaraj *et al.*, 2010)., *Penicillium* sp. and *Aspergillus* sp. are ubiquitously distributed species that are capable of anaerobic denitrification (Takasaki *et al.*, 2004, Jebaraj & Raghukumar, 2009). This cluster like the DSF Group-I can also be identified through the ITS and LSU rRNA sequence analysis (Gao *et al.*, 2008; Nagano *et al.*, 2010).

A third soil clone group (SCGI), clusters between Taphrinomycotina and Saccharomycotina as a monophyletic clade (Nagahama *et al.*, 2011). It has representative fungal sequences from soils of terrestrial origin and is a well-supported clade within Ascomycota. It is placed in a high taxonomic order, and is almost equivalent to a subphylum (Porter *et al.*, 2008). The phylotype S08fH11 is the first representative from marine habitats in the SCGI cluster and is distinctly diverse to the known SCGI terrestrial sequences. Targeted studies to specifically understand the occurrence of this novel cluster in marine habitats could give us new insights and widen our perception of the marine fungal diversity (Nagahama *et al.*, 2011).

Basidiomycota

Novel environmental sequences grouped together into the hydrothermal and/or anaerobic fungal group (Hy-An Group) within Basidiomycota, Ustilagomycotina (López-García *et al.*, 2007). This cluster is so called because it was first described during the survey at the marine, anoxic vent habitat; however studies have added a number of environmental sequences belonging to this group from all the major marine habitats (Fig. 1). It has representative sequences obtained from all the molecular surveys from the deep-sea hydrothermal vent regions (Edgcomb *et al.*, 2002; López-García *et al.*, 2003, López-García *et al.*, 2007; Le Calvez *et al.*, 2009; Edgcomb *et al.*, 2011). Environmental sequences obtained from the coastal (Gao *et al.*, 2008, 2010), deep-sea (Bass *et al.*, 2007, Lai *et al.*, 2007), oxygen-depleted

environments (Dawson & Pace, 2002, Alexander *et al.*, 2009; Jebaraj *et al.*, 2010) also cluster within this group. Their distribution is rather ubiquitous from marine and are reported from fresh water (Euringer & Lueders, 2008; Lesaulnier *et al.*, 2008) and terrestrial habitats (Moon-van der Stay *et al.*, 2006). The first known representative to this group was obtained during the molecular survey of the Guaymas Basin vent region (Edgcomb *et al.*, 2002). This phylotype (A1_E024) was not included during the phylogenetic analysis and was probably removed as it was highly divergent. But its availability at the public database and inclusion during later analyses, made it possible to define its phylogenetic affiliation and define the Hy-An Group. Interestingly *Malassezia* sp. is the closest known fungal taxa to this group, which is a well known pathogenic fungus. Attempts to cultivate this fungus from marine habitats have not been very successful because of its special growth requirements (Nagahama *et al.*, 2011). This is a big challenge for the culture based fungal diversity studies, as the laboratory conditions may not be always conducive for isolation of fungal cultures. It also calls for immediate attention to improve our growth media and laboratory set-up as we aim to cultivate the uncultured from the marine habitats. Environmental sequences belonging to this cluster were also identified from RNA-based clone libraries constructed from hydrothermal vent regions (Edgcomb *et al.*, 2011). It is highly probable that the fungi belonging to this cluster are having an important ecological role that is worth investigating.

Chytridiomycota and basal fungal lineages

The largest number of novel environmental sequences belonging to Kingdom Fungi is recovered within Chytridiomycota. A large clade of environmental sequences almost contributing to about more than half of the known Chytrid population has been identified. This cluster, Cryptomycota has the largest number of representatives from a vast range of ecosystems including the freshwater, marine and agriculture systems (Jones *et al.*, 2011). A combination of molecular techniques has helped in the characterization of the morphology and life-cycle of this novel group of organisms which cluster close to the Chytrid *Rozella* sp. This clade has been identified in a large number of marine environments and was identified as *Rozella* and LKM 11 cluster (Bass *et al.*, 2007; Takishita *et al.*, 2007a; Stock *et al.*, 2009; Nagahama *et al.*, 2011). This can be regarded as one of the most important contributions of molecular taxonomy to Kingdom Fungi. Such a basal clade from the marine habitats with ancient fungal forms could be very interesting and may hold the key for understanding the animal-fungal evolutionary history. Many other clusters could also be identified from marine habitats such as the basal clone group (BCGI). Phylotypes belonging to this clade have so far been isolated from the oxygen-depleted habitats, arctic regions and

deep-sea habitats (Takishita *et al.*, 2007b; Bass *et al.*, 2007; Tian *et al.*, 2009; Nagahama *et al.*, 2011). Other independent novel clusters also group within the Chytridiomycota which are well supported and highly divergent from known forms (LeCalvez *et al.*, 2009). Phyla Chytridiomycota and Zygomycota are polyphyletic and a large number of environmental sequences within these phyla are only categorized as basal fungal lineages. Occurrences and study of Zygomycota from marine habitats has been scanty (Stoeck & Epstein, 2003; Stoeck *et al.* 2006; Jebaraj *et al.*, 2010). The environmental phylotypes (M1_18C05, CCW24, CCW 35 and FAS_49) obtained within this group have to be characterized further through phylogenetic analysis. Additional environmental sequences from this cluster may be required to prove the taxonomic position of these clone groups.

Conclusion

Application of molecular tools has added a new dimension to fungal diversity by bringing to light the presence of novel environmental phylotypes from a variety of marine habitats. Majority of these fungal phylotypes were obtained as a sub-section from the metagenomic analysis of the micro-eukaryotes (Table 1 - 4). In many instances, the wide taxonomic diversity of these sequences has over-shadowed the fungal sequences retrieved along with it. The studies of the micro-eukaryotes also used universal primer sets for a large number of samplings and this has helped in the identification of a number of novel clades such as the uncultured marine alveolate group-I, II and Uncultured Marine Stramenopile clade repeatedly. However, there are a large number of primer sets available for studying fungal diversity (Pang & Mitchell, 2005). Molecular surveys across the oceanic systems using chosen universal fungal primer sets, and sampling up to saturation could aid in the identification of novel marine clusters. Development of probes for the clades identified from the various studies can be of great importance in characterizing undescribed and uncultured marine fungi.

Phylogenetic analysis of the environmental sequences generated from molecular diversity surveys is crucial to understand their taxonomic assessment. The sequences that cluster close to known cultures and well-described taxa can be assigned their proper phylogenetic place with ease. However, appropriate selection of molecular data available from databases and careful construction of various models is essential to understand the phylogenetic relationship of environmental phylotypes that are divergent from known taxa. It has been a general practice to include representative sequences from all major groups of interest for analysis. Thus the divergent sequences fit as novel clades within the major

taxonomic ranks. Environmental phylotypes during analysis from other studies along with its taxonomic representatives has aided in the identification of major environmental clusters (López-García *et al.*, 2007; Nagahama *et al.*, 2011; Nagano *et al.*, 2010; Thaler *et al.*, 2012). But some phylotypes which belong to these environmental clusters could not be assigned their rightful place within these clades because representatives of these clusters were not included during analysis (Lai *et al.*, 2007; Jebaraj *et al.*, 2010). Increasing the availability of molecular data for each taxonomic group and diligent implementation of phylogenetic analysis are promising approaches to unravel the yet to be described marine fungal diversity.

Acknowledgements

The authors have no conflict of interest on this publication. This is NIOs contribution number xxx (to be filled in later).

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Figure Legend

Fig. 1 Schematic representation of the fungal tree of life: major environmental clades identified are marked in red and their occurrence in the marine habitats is denoted by initials (C: coastal, D: deep-sea, H: hydrothermal vent, O: oxygen-depleted region) and the topology shown is derived from Hibbett *et al.*, 2007.

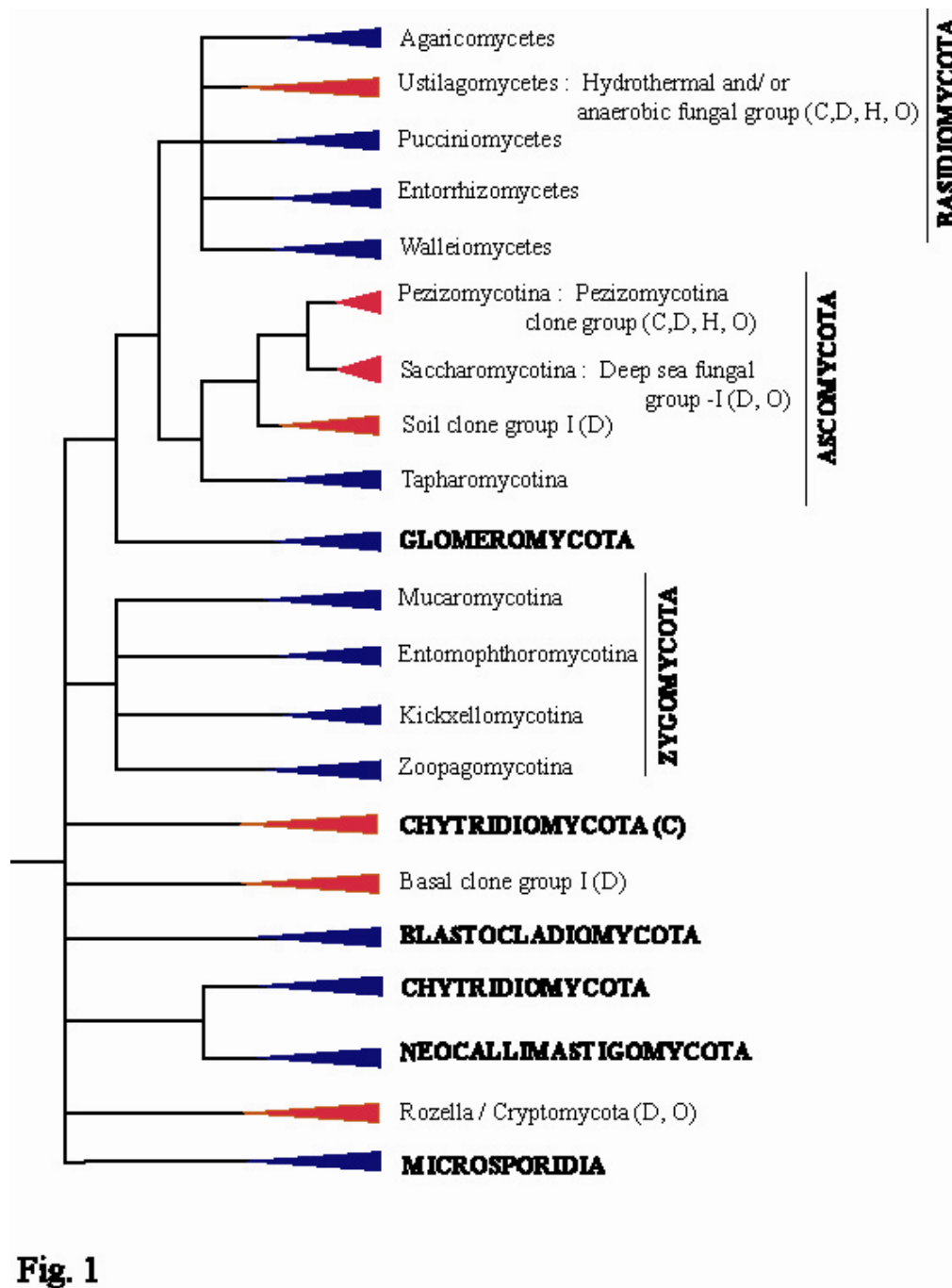


Fig. 1

Table 1. Phylogenetic affiliation of environmental fungal sequences from coastal regions:

Si. No.	Sampling area (collection depth)	Molecular methodology applied	Taxonomic affiliation : novel environmental clusters identified	Reference
1.	Bocas del Toro, Panama	based clone library analysis	Ascomycota : Novel forms Basidiomycota, Chytridiomycota	Wegley <i>et al.</i> , 2007
2.	Marine sponges from Hawaiian Islands (1-3 m)	DGGE analysis of ITS or SSU rRNA sequence.	Ascomycota : Marine fungal clades, PCG Basidiomycota : Hy-An Group	Gao <i>et al.</i> , 2008 [†]
3.	Off Hawaii (5- 200 m)	DGGE and SSU rRNA based clone library analysis	Ascomycota, Basidiomycota : novel environmental and Marine fungal clades	Gao <i>et al.</i> , 2010 [†]
4.	Off Brazil (0.5 – 50 m)	DGGE and SSU rRNA based clone library analysis	Ascomycota, Basidiomycota, Chytridiomycota, basal fungal lineages	Cury <i>et al.</i> , 2011
5.	Saint Vincent Bay (mangrove sediment)	454 pyrosequencing of ITS region	Ascomycota, Basidiomycota	Arfi <i>et al.</i> , 2012

[†] Study used fungal specific primers, PCG : Pezizomycotina clone group, Hy-An Group : hydrothermal and/or anaerobic fungal group

Table 2. Phylogenetic affiliation of environmental fungal sequences from deep-sea regions:

Si. No.	Sampling area (collection depth)	Molecular methodology applied	Taxonomic affiliation : novel environmental clusters identified	Reference
1.	Drake passage of the Antarctic polar front (250 - 3000 m)	SSU rRNA based clone library analysis	Ascomycota : PCG	López-García <i>et al.</i> , 2001
2.	South China Sea (350 - 3011 m)	ITS based clone library analysis	Ascomycota, Basidiomycota : Hy-An Group	Lai <i>et al.</i> , 2007 [†]
3.	Eleven different deep-sea regions (250 – 4000 m)	SSU rRNA based clone library analysis	Ascomycota: DSF Group I Basidiomycota : Hy-An Group Chytridiomycota : Cryptomycota, BCGI, novel environmental clades,	Bass <i>et al.</i> , 2007 [†]
4.	Off Japanese Islands (1200 -10000 m)	ITS and LSU rRNA based clone library analysis	Ascomycota : PCG, DSF Group I Basidiomycota, Chytridiomycota : Cryptomycota	Nagano <i>et al.</i> , 2010 [†]
5.	Sagami Bay (1080 m)	SSU rRNA based clone library analysis	Ascomycota : SCGI, DSF Group I Basidiomycota: Hy-An Group, Chytridiomycota : Cryptomycota, BCGI	Nagahama <i>et al.</i> , 2011 [†]

6.	Central Indian basin (~ 5000 m)	SSU rRNA and ITS based clone library analysis	Ascomycota, Basidiomycota	Singh <i>et al.</i> , 2011 [‡]
7.	Central Indian basin (~ 5000 m)	SSU rRNA and ITS based clone library analysis	Ascomycota, Basidiomycota	Singh <i>et al.</i> , 2012 a [‡]
8.	Central Indian basin (~ 5100 m)	SSU rRNA and ITS based clone library analysis	Ascomycota, Basidiomycota	Singh <i>et al.</i> , 2012 b [‡]
9.	Gulf of Mexico (2400 m)	based clone library analysis	Ascomycota : DSF Group -I	Thaler <i>et al.</i> , 2012 [‡]

[†] Study used fungal specific primers, [‡] Study used universal eukaryotic and fungal specific primers, PCG : Pezizomycotina clone group, Hy-An Group : hydrothermal and/or anaerobic fungal group, DSF Group-I : deep sea fungal group, SCGI : soil clone group.

Table 3. Phylogenetic affiliation of environmental fungal sequences from hydrothermal vent regions:

Si. No.	Sampling area (collection depth)	Molecular methodology applied	Taxonomic affiliation : novel environmental clusters identified	Reference
1.	Guyamas vent field	SSU rRNA based clone library analysis	Ascomycota: Hy-An Group, Basidiomycota	Edgcomb <i>et al.</i> , 2002
2.	Mid-Atlantic Ridge hydrothermal area (2264 m)	SSU rRNA based clone library analysis	Ascomycota: PCG, Basidiomycota : Hy-An Group	López-García <i>et al.</i> , 2003
3.	Lost-city hydrothermal vent (750 - 900 m)	SSU rRNA based clone library analysis	Ascomycota, Basidiomycota : Hy-An Group	López-García <i>et al.</i> , 2007
4.	Mid-Atlantic Ridge system (860 - 2630 m)	SSU rRNA based clone library analysis	Ascomycota : novel clusters Basidiomycota : novel clusters, Hy-An Group, Chytridiomycota : novel clusters	Le Calvez <i>et al.</i> , 2009 [†]
5.	Peru margin and trench (252 – 5086 m)	SSU rRNA clone library constructed from environmental DNA and cDNA	Ascomycota, Basidiomycota : Hy-An Group	Edgcomb <i>et al.</i> , 2011

[†] Study used fungal specific primers, SSU rRNA : Small subunit ribosomal RNA gene, Hy-An Group : hydrothermal and/or anaerobic fungal group

Table 4. Phylogenetic affiliation of environmental fungal sequences from oxygen-depleted environments:

Si. No.	Sampling area (collection depth)	Molecular methodology applied	Taxonomic affiliation : novel environmental clusters identified	Reference
1.	Berkeley aquatic park and Bolinas tidal flat (coastal anoxic sediment)	SSU rRNA based clone library analysis	Ascomycota : PCG, Basidiomycota: Hy-An Group, Chytridiomycota : Cryptomycota	Dawson & Pace 2002
2.	Sippewisset salt marsh (1.5 m)	SSU rRNA based clone library analysis	Ascomycota, basal fungal groups	Stoeck & Epstein 2003
3.	Cariaco basin (270 – 900 m)	SSU rRNA based clone library analysis	Ascomycota	Stoeck <i>et al.</i> , 2003
4.	Kagoshima bay (204 m)	SSU rRNA based clone library analysis	Ascomycota : PCG, Chytridiomycota: BCGI, Cyptomycota	Takishita <i>et al.</i> , 2005
5.	Norwegian fjord (180 m)	SSU rRNA based clone library analysis	Fungi	Behnke <i>et al.</i> , 2006
6.	Cariaco basin (340 m)	SSU rRNA based clone library analysis	Ascomycota : PCG, Chytridiomycota : novel environmental clades	Stoeck <i>et al.</i> , 2006

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|-----|--|--|---|------------------------------------|
| 7. | Mariager Fjord
(10 – 30 m) | SSU rRNA clone library
constructed from environmental
DNA and cDNA | Fungi | Stoeck <i>et al.</i> , 2007 |
| 8. | Kamikoshiki Island
(22 m) | SSU rRNA based clone library
analysis | Ascomycota,
Chytridiomycota :
Cryptomycota | Takishita <i>et al.</i> ,
2007a |
| 9. | Sagami Bay
(1174 -1178 m) | SSU rRNA based clone library
analysis | Ascomycota : DSF Group I,
Basidiomycota,
Chytridiomycota: Cryptomycota,
BCGI | Takishita <i>et al.</i> ,
2007b |
| 10. | Gotland deep, Baltic
Sea (200 – 240 m) | SSU rRNA clone library
constructed from environmental
DNA and cDNA | Ascomycota, Basidiomycota,
Chytridiomycota: Cryptomycota | Stock <i>et al.</i> , 2009 |
| 11. | Cariaco basin (300 –
320 m) & Framvaren
fjord (36 m) | 454 sequencing of SSU rRNA
gene V9 region | Fungi | Stoeck <i>et al.</i> , 2009 |
| 12. | L'Atalante basin
(3501 m) | SSU rRNA based cDNA clone
library analysis | Ascomycota: PCG Basidiomycota
: Hy-An Group | Alexander <i>et al.</i> ,
2009 |

13.	Arabian Sea (25 – 200 m)	SSU rRNA based clone library analysis	Ascomycota : PCG Basidiomycota : Hy-An Group, Zygomycota : Novel clusters	Jebaraj <i>et al.</i> , 2010 [‡]
14.	Norwegian Fjord (18 – 180 m)	SSU rRNA based clone library analysis	Fungi	Behnke <i>et al.</i> , 2010
15.	Norwegian fjord (20 m)	454 sequencing of SSU rRNA region	Fungi	Stoeck <i>et al.</i> , 2010

[‡] Study used universal eukaryotic and fungal specific primers, PCG: Pezizomycotina clone group, Hy-An Group: hydrothermal and/or anaerobic fungal group, DSF Group-I: deep sea fungal group, BCGI: basal clone group