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Marine Fungi: Their Ecology and Molecular Diversity

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Annu. Rev. Mar. Sci. 2012. 4:495–522

First published online as a Review in Advance on September 19, 2011

The *Annual Review of Marine Science* is online at marine.annualreviews.org

This article's doi:
10.1146/annurev-marine-120710-100802

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1941-1405/12/0115-0495$20.00

Keywords

saprotroph, osmotroph, parasite, pseudofungi, chytrid, small subunit ribosomal DNA

Abstract

Fungi appear to be rare in marine environments. There are relatively few marine isolates in culture, and fungal small subunit ribosomal DNA (SSU rDNA) sequences are rarely recovered in marine clone library experiments (i.e., culture-independent sequence surveys of eukaryotic microbial diversity from environmental DNA samples). To explore the diversity of marine fungi, we took a broad selection of SSU rDNA data sets and calculated a summary phylogeny. Bringing these data together identified a diverse collection of marine fungi, including sequences branching close to chytrids (flagellated fungi), filamentous hypha-forming fungi, and multicellular fungi. However, the majority of the sequences branched with ascomycete and basidiomycete yeasts. We discuss evidence for 36 novel marine lineages, the majority and most divergent of which branch with the chytrids. We then investigate what these data mean for the evolutionary history of the Fungi and specifically marine-terrestrial transitions. Finally, we discuss the roles of fungi in marine ecosystems.

INTRODUCTION

Fungi are key players in terrestrial environments (Gargas et al. 1995, James et al. 2006a, Wang & Qiu 2006) and perform vital functions as decomposers, driving nutrient cycles in detritus environments, and as parasites and symbionts (Webster & Weber 2007). Fungi represent a significant proportion of the microbial diversity on Earth (Hawksworth 2001, Hibbett et al. 2007, Mueller et al. 2007, O'Brien et al. 2005). The total diversity of the Fungi has been estimated to be 1.5–1.6 million species (Hawksworth 1991, 2001). However, much of our current understanding of the ecology and evolutionary complexity of fungi is derived from the study of cultured fungal isolates in the most part from terrestrial environments. Furthermore, this diversity estimate is based on the study of fungal communities in and around plant ecosystems sampled from Western Europe and then extrapolated to a global figure using data on global plant diversity (Hawksworth 1991, 2001). This is controversial because it assumes that plant species diversity and fungal species diversity are correlated (May 1991, 1994; Mueller & Schmit 2007) and is likely to be too low because it is based on counts derived from morphological observations and therefore does not account for cryptic species diversity (Hawksworth 2001). Perhaps even more importantly, the estimate focuses only on fungi in a limited set of habitats and therefore does not account for diverse and abundant fungi that are not associated with plant and soil environments, such as animal hosts, sediment, freshwater, or marine ecosystems.

Our understanding of fungal evolutionary complexity is expanding as researchers use increasingly powerful molecular methods to investigate environmental diversity (e.g., Buée et al. 2009, Jumpponen & Jones 2009, O'Brien et al. 2005). For example, work using sequencing of internal transcribed spacer (ITS) markers from soil DNA samples has suggested a revision of global fungal diversity from Hawksworth's estimate of 1.5 million species to 3.5–5.1 million (O'Brien et al. 2005). Yet current culture collections of fungi number only ~75,000 isolates (Hawksworth 2001, Kis-Papo 2005) with updated counts suggesting 64,000 ascomycete isolates and 32,000 basidiomycete isolates (Kirk et al. 2008), not all of them representing different species. Independent of the accuracy of these estimates, it is likely that less than 5% of the diversity of fungal species is currently described and maintained in culture. Furthermore, the use of environmental DNA (eDNA) methods is increasingly expanding our understanding of the diversity of fungi at the highest taxonomic levels with a number of discoveries of previously undescribed phylogroups (Jones et al. 2011, Lara et al. 2010, Porter et al. 2008, Schadt et al. 2003), suggesting that these estimates are conservative and that culture collections are in no way representative of natural diversity.

Of the cultured species (Hawksworth 2001, Kis-Papo 2005), one count suggests there are only 467 isolates belonging to 244 genera retrieved from marine environments (Kis-Papo 2005). This might imply that only ~0.6% of studied fungi are derived from the marine environment, which would be surprising as marine habitats account for 70% of the surface of the globe and have been shown to harbor fungi from the air-surface interface to depths of kilometers. These results have been interpreted to suggest that fungi are both nondiverse and low in abundance in marine environments (Burgaud et al. 2009, Kis-Papo 2005, Le Calvez et al. 2009). In contrast, fungi are thought to be a major contributor to the decomposition of woody and herbaceous substrates and animal remains in coastal and surface marine environments (Kohlmeyer & Kohlmeyer 1979, Mann 1988, Newell 1996). This raises questions about the significance of fungal communities in marine environments: Are we overlooking a large diversity of fungi?

The purpose of this review is to bring together the growing body of molecular data, focusing primarily on environmental small subunit ribosomal DNA (SSU rDNA) sequence data to summarize our current knowledge of the evolutionary diversity of marine fungi. Using these data, we can begin to investigate a number of theories and observations relating to the ecological role of fungi

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in marine ecosystems. We hope this will form an important foundation for better understanding of the diversity and function of fungi in marine environments as metagenomic and large-scale diversity sequencing projects gather pace.

MOLECULAR DIVERSITY OF MARINE FUNGI

The kingdom Fungi was traditionally loosely classified as four major groups: (*a*) Ascomycota, (*b*) Basidiomycota (which together form the subkingdom Dikarya and have been the major focus of experimental research and genome-sequencing initiatives), (*c*) the zygomycetes, and (*d*) the chytrids (Hibbett et al. 2007, Jones et al. 2011, Webster & Weber 2007). This early model of fungal taxonomy has been revised at a number of levels, including the placement of the microsporidia with (Hirt et al. 1997, 1999) and potentially within the Fungi (Adl et al. 2005, James et al. 2006a, Keeling 2003) and the division of the chytrids and zygomycetes into multiple interbranching paraphyletic clades, followed by subsequent taxonomic reclassifications (see Hibbett et al. 2007; James et al. 2006a,b; Liu et al. 2009). There still remains much uncertainty relating to the major divisions of the Fungi below the Dikarya.

Progress in drawing the fungal tree of life has been made by studying fungi that have been isolated and cultured mainly from terrestrial environments (Hibbett et al. 2007; James et al. 2006a,b; Liu et al. 2009). These approaches are limited because they preferentially sample microbes that are easily cultured or that possess larger body sizes and/or distinctive morphologies. Furthermore, these approaches bring their own specific limitations for studying fungi in marine environments:

1. The culturing of fungal isolates from marine samples has often led to the recovery of non-fungal microbes, which are ecologically, morphologically, and trophically similar to fungi but are not true fungi (discussed below).
2. The ecological preferences of most fungi suggest that those in marine ecosystems are likely to reside on or in host organisms or in benthic environments, including deep-sea sediments. These habitats are difficult to examine by microscopy and in some cases pose severe sampling difficulties.
3. The majority of fungi harbor very high levels of cryptic diversity that is indistinguishable using microscopy of environmental samples and/or culturing. Further complications arise because similar fungal morphotypes such as yeasts and flagellated zoospores branch in distant and paraphyletic positions on the fungal tree of life (James et al. 2006a, Liu et al. 2009), making classifications based on observations of general morphological characters difficult and often misleading.

Molecular methods—specifically the polymerase chain reaction (PCR) amplification of taxonomically informative gene markers from eDNA samples combined with clone library construction, sequencing, and phylogenetic analyses—have demonstrated that microbial diversity is much more complicated than previously thought (Giovannoni et al. 1990, López-García et al. 2001, Moon-van der Staay et al. 2001, Olsen et al. 1986, Pace 1997). The environmental DNA, PCR, and clone library approach has been used for both prokaryotes and eukaryotes to place many previously unrecognized branches on the tree of life, in many cases redefining our understanding of the evolutionary complexity of the eukaryotes (Bass & Cavalier-Smith 2004, Dawson & Pace 2002, Edgcomb et al. 2002, López-García et al. 2001, Moon-van der Staay et al. 2001, Pace 1997, Rappé & Giovannoni 2003, Richards & Bass 2005, Richards et al. 2005, Stoeck et al. 2006), although these results have been the subject of much debate and revision (e.g., Berney et al. 2004, Cavalier-Smith 2004). Molecular approaches have also demonstrated that poorly recognized groups are important ecosystem components (Bass & Cavalier-Smith 2004, Chambouvet et al. 2008, Massana et al. 2004b, Moreira & López-Garcia 2002). Yet most molecular surveys of microbial eukaryotic

Ascomycota: filamentous or yeast forms that reproduce sexually with internal spore maturation within a sac-shaped cell called an ascus; include the laboratory model organisms *Saccharomyces cerevisiae* and *Neurospora crassa*

Basidiomycota: filamentous or yeast forms that reproduce sexually with external spore maturation on a basidium

Zygomycete: multinucleated cell that produces filaments and lacks a complex fruiting body

Chytrid: informal name given to a fungus that produces motile flagellated spores (zoospores) during its life cycle

True fungi: large and highly diverse group of eukaryotes (mainly microbes, largely osmotrophic and frequently with chitin-rich cell walls) that form a large clade in the opisthokont supergroup

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diversity are recovered using only one primer set and sample less than 500 clones, with the result that no eukaryote-wide study has reached sampling saturation (e.g., Edgcomb et al. 2002, Stoeck et al. 2006). This has led to the suggestion that further sampling from the very same environments would reveal more diversity and demonstrate that our understanding of the complexity of the tree of life is still very incomplete (Curtis et al. 2002, Moreira & López-Garcia 2002, Sogin et al. 2006, Stoeck et al. 2006).

Small subunit ribosomal DNA (SSU rDNA) clone library: experimental process involving the extraction of DNA from environmental samples, PCR-targeted amplification of SSU rRNA (marker) genes, cloning of amplicons, clone sequencing, and phylogenetic analysis

Environmental clone library analyses specifically targeting fungi have generally sampled regions within the ribosomal RNA (rRNA) gene array using a range of approaches and sequence targets, with some researchers focusing on the SSU rDNA sequence and others on the ITS regions. The two ITS regions are sections of DNA located between the SSU (18S) and large subunit (LSU) (28S) rRNA genes, separated by the 5.8S rRNA gene. The variable nature of the ITS regions relative to the flanking rRNA genes enables increased resolution and accuracy when assigning sequences to genus- and species-level classifications within well-sampled groups (Bruns & Gardes 1993, Gardes & Bruns 1993, Horton & Bruns 2001). This process is facilitated by increasingly well-sampled sequence databases (Buchan et al. 2002, James et al. 2006a, O'Brien et al. 2005). However, many database sequence classifications may be erroneous (Vilgalys 2003), and variation in rates of ITS divergence between taxonomic groups can hinder classifications using these sequences (Nilsson et al. 2008, 2006; Vilgalys 2003). The ITS approach is useful for determining species diversity and can be used for ecosystem comparisons when targeting well-defined taxonomic groups, but it is of limited use for inferring higher-level phylogenetic relationships and identifying novel groups, as ITS phylogenies demonstrate weak resolution among deeper branching relationships in the Fungi (Horton & Bruns 2001).

Some researchers have focused on sampling the SSU rRNA gene to investigate novel fungal diversity among higher taxonomic groups (Anderson et al. 2003, Bass et al. 2007, Jebaraj et al. 2009, Porter et al. 2008, Schadt et al. 2003, Vandenkoornhuyse et al. 2002), although this gene cannot discriminate between closely related fungal species and is generally less intensively sampled than the ITS regions. Therefore, others have called for the combination of the SSU and ITS approaches (O'Brien et al. 2005), enabling multigene phylogenetic analyses encompassing the SSU, 5.8S, and LSU sequences, which can lead to improved phylogenetic support among both lower and higher phylogenetic nodes (e.g., Jones et al. 2011, Porter et al. 2008) and help to identify the phylogenetic placement of many orphan environmental ITS sequences currently accumulating in sequence databases.

Molecular Sampling in Marine Environments

There is now a growing trend in the use of molecular techniques to investigate microbial diversity from marine environments. These data have given a mixed impression of the relative importance of fungal lineages in marine environments. For example, in 2005 we conducted a meta-analysis of 13 SSU rDNA clone library studies. This analysis brought together 49 SSU rDNA environmental clone libraries, with a total of 1,077 sequences from soils, freshwater, and marine samples. Of these sequences, 124 (11.5%) clustered within, or close to, known fungal sequences (Richards & Bass 2005). This analysis also suggested that although fungi are present in aquatic sediments, low-oxygen aquatic environments, freshwater, and soils, there appeared to be very few sequences recovered from the upper column and surface marine waters. This pattern was confirmed by a separate, specifically marine analysis, which sampled 23 coastal water libraries (1,349 clones) and 12 open ocean libraries (826 clones) and recovered a total of only 16 fungal clones, equivalent to 0.8% of the marine SSU rDNA sequences sampled (Massana & Pedrós-Alió 2008). Consequently, both molecular analyses and culture-based inventories (Kis-Papo 2005) have suggested that fungi are relatively nondiverse and in low abundance in upper and surface marine ecosystems.

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Sequencing of eukaryotic SSU rDNA V9 and V4 diversity tags from marine coastal waters using 454 methods has hinted that such environments contain more fungal diversity than previously detected using clone library methods (Stoeck et al. 2010). These diversity sequencing methods still suggest that, compared with other eukaryotic groups, fungi appear relatively nondiverse, constituting less than 5% of the operational taxonomic units recovered from marine environments. However, these methods hint at an increased diversity in comparison with clone library methods (Pawlowski et al. 2011, Stoeck et al. 2010). Furthermore, fungal-specific clone library analyses have identified new fungal diversity in deep waters, anoxic marine waters, hydrothermal vent environments, and deep-sea marine sediments (Bass et al. 2007, Burgaud et al. 2009, Edgcomb et al. 2011, Jebaraj et al. 2009, Le Calvez et al. 2009). The results of this summary and other fungal sequences detected in marine environments are summarized in **Figures 1–5**. This work has made use of a range of eDNA samples and sampling techniques and has therefore made it feasible to sample previously inaccessible microbial communities, for example, specifically targeting communities in deep-sea environments.

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Deep-Sea and Hydrothermal Vent Environments

Using SSU rDNA clone library methods, Bass and coauthors (2007) investigated the composition of fungal communities in deep-sea sediments and water column samples of depths of 500 m to 4,200 m, including several hydrothermal vent samples. The sequences recovered showed deep-sea fungal communities to be dominated by ascomycete and basidiomycete forms but generally revealed a low diversity of fungi. The authors also noted that the vast majority of these sequences branched closest to taxonomic groups known to have a yeast morphotype, suggesting that (*a*) these taxa were easier to recover or (*b*) yeast forms dominate these environments. Many of these phylotypes were also shown to branch close to known pathogens, suggesting the presence of fungal pathogens of deep-sea animals (discussed in detail below). The phylogeny reported by Bass et al. (2007) included additional marine environmental SSU rDNA sequences recovered from general eukaryotic PCR experiments and showed seven clusters of highly unique sequences, six of which branched specifically within the fungal radiation, indicating the presence of unknown fungal forms in marine environments.

A study by Le Calvez et al. (2009) using similar eDNA methods and targeting deep-sea hydrothermal vent ecosystems also recovered several novel fungal lineages. These lineages included three unknown phylotypes branching within the basidiomycete radiation and two unknown phylotypes branching close to chytrid sequences (Le Calvez et al. 2009). The primer set used in this study was different from that chosen by Bass et al. (2007), but both studies demonstrate the identification of novel fungal lineages in deep-sea marine environments.

Edgcomb et al. (2011) again used similar approaches to identify eukaryotic microbial communities in deep-sea sediment cores but this time targeted both DNA- and RNA-based diversity profiles. Although the authors specifically targeted a wide diversity of eukaryotic microbes, they consistently recovered a high frequency of basidiomycete yeast sequences branching closely to known *Cryptococcus* and *Malassezia* species (Edgcomb et al. 2011): 42% of the sequences recovered from RNA-derived libraries branched with *Cryptococcus* sequences. This result is consistent with another molecular analysis of eukaryotic diversity in marine sediments showing that *Cryptococcus curvatus* yeasts, with a closely related genotype to those recovered by Edgcomb et al. (2011), can dominate deep-sea microbial eukaryotic communities (Takishita et al. 2006), whereas culture sampling has recovered a related *Cryptococcus* species (*Cryptococcus surugensis*) from deep-sea sediments (Nagahama et al. 2003b) (shown in **Figure 3**). The recovery of these taxa in RNA-derived libraries suggests that they are metabolically active in sedimentary ecosystems (Edgcomb et al. 2011).

Interestingly, the vast majority of the fungi sampled from deep-sea environments branch close to, or within, clades of known terrestrial fungi. This suggests that in many cases fungi residing in terrestrial or marine surface environments are capable of relatively easily making the transition to deep-sea habitats and is consistent with evidence that some fungi are capable of altering their membrane composition to tolerate high hydrostatic pressure under short-term experimental conditions (Simonato et al. 2006). Taken together, these data imply that for some fungi, colonization of deep-sea habitats by surface-dwelling strains is a viable ecological transition.

Saprotrophy: process of acquiring nutrients from the digestion of dead organic matter, usually from plants or animals

Anoxic Marine Environments

A large fraction of the marine biosphere is anoxic or partially anoxic. In terrestrial environments, fungi are regularly found in saprotrophic and detritus habitats that are often low in oxygen. Fungi have been shown to possess a range of cellular and genomic adaptations to life in anoxic environments (e.g., Embley 2006, Gojkovic et al. 2004, Hall et al. 2005). Furthermore, many fungi have been shown to play a role in anaerobic denitrification (Shoun et al. 1992), e.g., *Fusarium oxyporum* (Takaya et al. 1999, Uchimura et al. 2002). SSU rDNA sequences closely related to *F. oxyporum* have been recovered from marine anaerobic environments (**Figure 1**; Jebaraj et al. 2009), and four marine isolates, including a *Fusarium* species, have been demonstrated to grow in suboxic conditions, utilizing nitrate for respiration and accumulating nitrite, and are therefore theoretically capable of participating in anaerobic denitrification in marine environments (Jebaraj & Raghukumar 2009).

A study by Jebaraj et al. (2009) examined the diversity of fungi in oxygen-depleted regions of the Arabian Sea using clone library methods. These researchers used multiple fungal-specific SSU rDNA primer sets and one general eukaryotic-specific primer set to amplify SSU sequences (Jebaraj et al. 2009). Each primer set revealed an overlapping subset of fungal diversity, with the fungal-specific primers showing a greater diversity of fungi per sampling effort than clone libraries constructed using universal eukaryotic primers. This result demonstrates the importance of using different primers to control for PCR biases and suggests that a significant portion of fungal diversity is missed when using universal primer sets (Jebaraj et al. 2009, Stoeck et al. 2006).

The phylogeny of Jebaraj et al. (2009) identified 48 distinct fungal phylotypes (clustered at 99% sequence similarity): 27 branching within the ascomycete radiation, 20 branching within the basidiomycetes, and only 1 unique phylotype branching among the lower fungi. Several Dikarya sequences formed highly novel branching positions in the phylogeny and clustered with additional environmental sequences recovered from oxygen-depleted habitats (Jebaraj et al. 2009). Indeed, many sequences branched within a clade previously termed the hydrothermal and/or anaerobic fungal group, which branches with the *Malassezia* yeasts also identified from deep-sea eukaryotic environmental clone libraries (Bass et al. 2007, Edgcomb et al. 2011, López-Garcia et al. 2007)—this interesting group is discussed further below. No chytrid-like sequences were identified from this study, suggesting the possibility that these taxonomic groups have a low diversity in the marine

Figure 1

Subsection of a phylogenetic analysis showing the diversity of small subunit ribosomal DNA sequences recovered from marine environmental DNA analyses. This section of the tree focuses on Pezizomycotina. The tree was calculated using PhyML (parameters $\Gamma = 0.722$ and $I = 0.117$) with both PhyML and LogDet distance bootstraps. Marine phylotypes are highlighted in yellow. Novel marine phylogenetic groups are marked NMFA (*red*, novel marine fungi ascomycetes). Gray dashed lines are false branch extensions. Ecological information is annotated on the tree.

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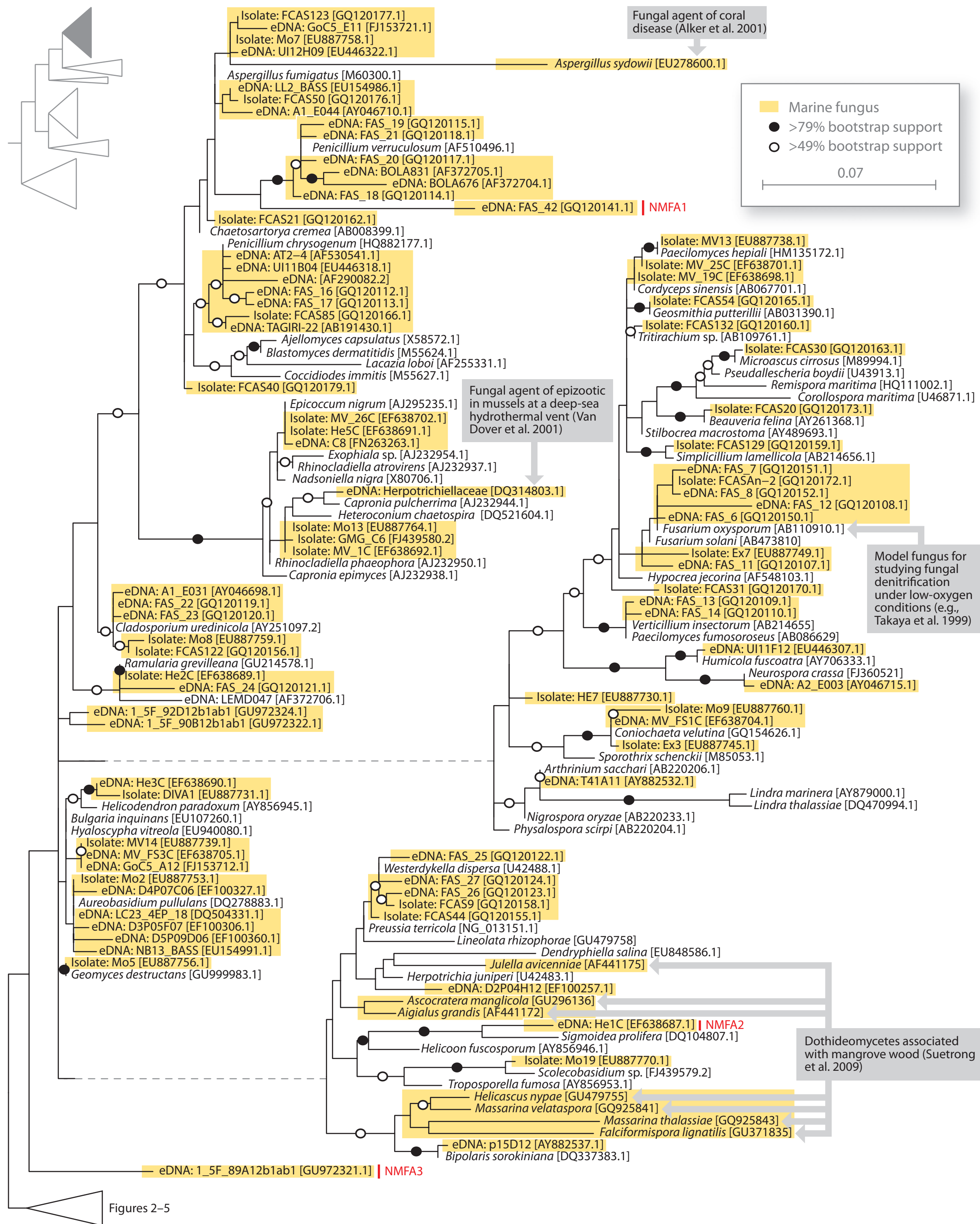

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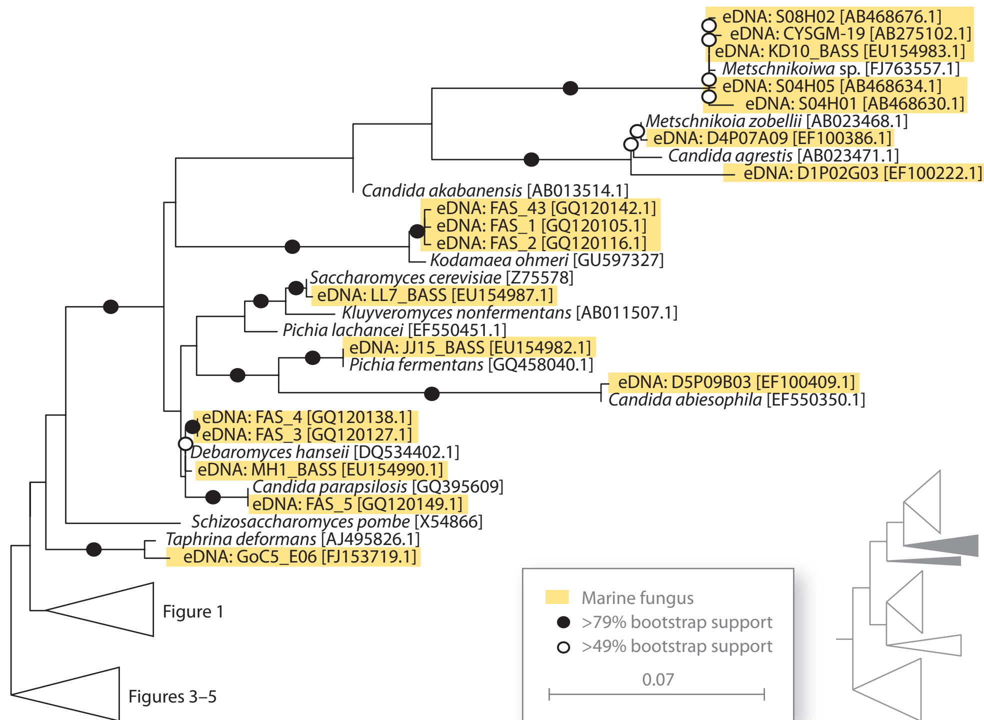


Figure 2

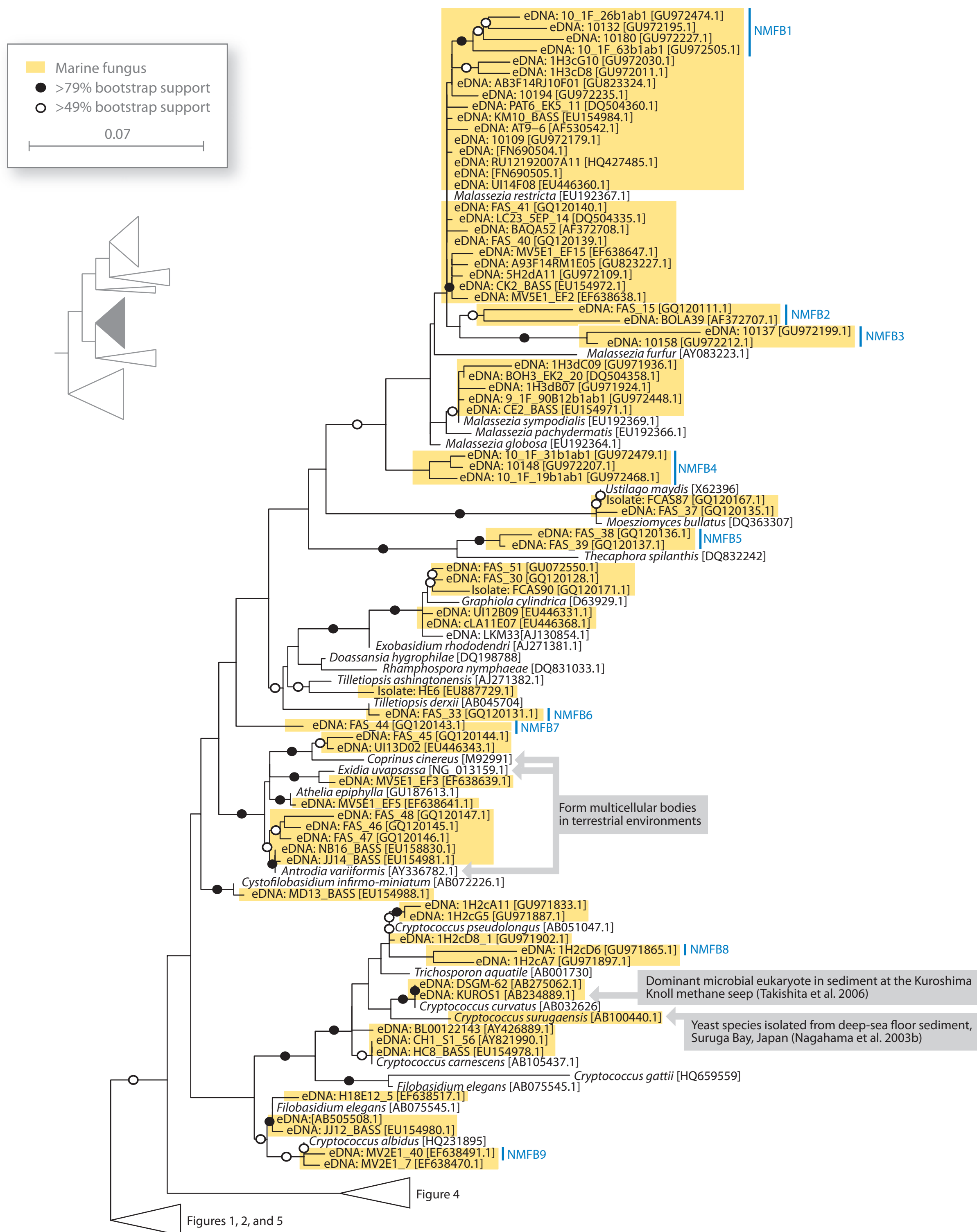
Subsection of a phylogenetic analysis showing the diversity of small subunit ribosomal DNA sequences recovered from marine environmental DNA analyses. This section of the tree mainly focuses on Saccharomycotina. The tree was calculated using PhyML (parameters $\Gamma = 0.722$ and $I = 0.117$) with both PhyML and LogDet distance bootstraps. Marine phylotypes are highlighted in yellow.

environments investigated or alternatively that the PCR primers or DNA sampling methodologies used for this study were collectively biased toward the Dikarya.

Fungal-specific clone library analyses of deeper water column samples, sediments, and anoxic environments (Bass et al. 2007, Jebaraj et al. 2009, Le Calvez et al. 2009) have significantly increased the diversity of the fungi recovered from marine studies, especially when compared with surface water samples (Massana & Pedrós-Alió 2008). Two of these studies (Jebaraj et al. 2009, Le Calvez et al. 2009) combined eDNA analysis with isolation and culture experiments, demonstrating little overlap between the sequences recovered from isolated cultures and eDNA and suggesting the possible presence of a more complex fungal community than identified either by eDNA or culture-based analyses alone. Nonetheless, marine fungal-specific clone library analyses (Bass et al. 2007, Edgcomb et al. 2011, Jebaraj et al. 2009, Le Calvez et al. 2009) reveal a simple fungal community with relatively few phylotypes in total. This is a stark contrast to terrestrial environments, where

Figure 3

Subsection of a phylogenetic analysis showing the diversity of small subunit ribosomal DNA sequences recovered from marine environmental DNA analyses. This section of the tree mainly focuses on part of the basidiomycete radiation (see **Figure 4** for the remaining basidiomycete branches). The tree was calculated using PhyML (parameters $\Gamma = 0.722$ and $I = 0.117$) with both PhyML and LogDet distance bootstraps. Marine phylotypes are highlighted in yellow. Novel marine phylogenetic groups are marked NMFB (*blue*, novel marine fungi basidiomycetes). Ecological information is annotated on the tree.



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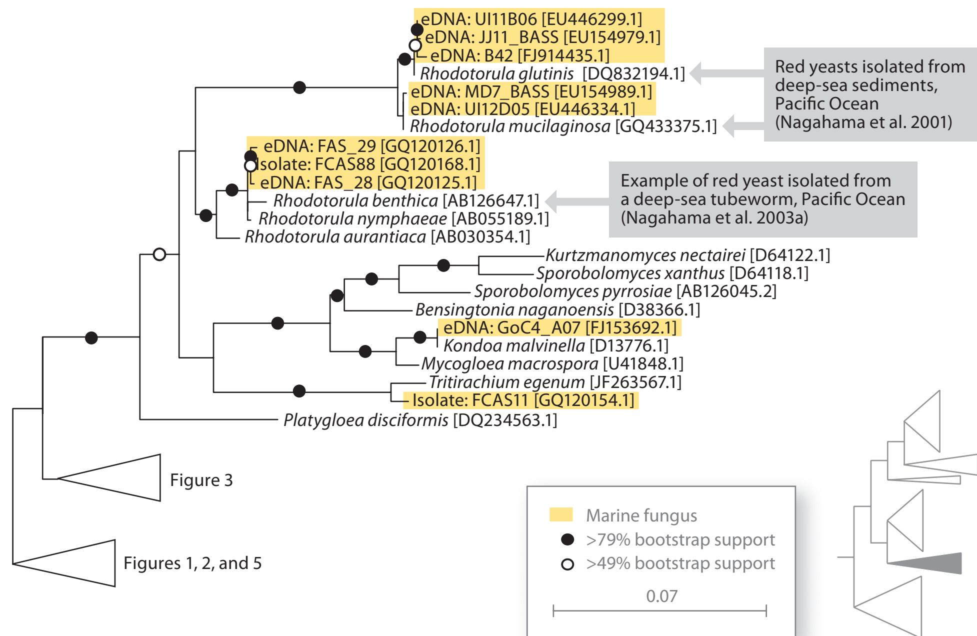

Figure 4

Subsection of a phylogenetic analysis demonstrating the diversity of small subunit ribosomal (SSU) DNA sequences recovered from marine environmental DNA analyses. This section of the tree focuses on the other subsection of the basidiomycete phylogeny (see **Figure 3** for the remaining basidiomycete branches). The tree was calculated using PhyML (parameters $\Gamma = 0.722$ and $I = 0.117$) with both PhyML and LogDet distance bootstraps. Marine phylotypes are highlighted in yellow. Ecological information is annotated on the tree. Three *Rhodotorula* sequences are not highlighted in yellow, because actual SSU sequences were not recovered from a marine environment; marine provenance here is based on comparison of internal transcribed spacer (ITS) sequences in the papers referenced.

fungal communities appear to be much more complex (e.g., Buée et al. 2009, Jumpponen & Jones 2009, O'Brien et al. 2005), clearly demonstrated by the comparison of species accumulation curves from marine environments (Bass et al. 2007, Jebaraj et al. 2009) with the results of clone library analyses of nonmarine habitats. Based on currently available data, it is difficult to describe the difference in the diversity and relative abundance of fungal forms between terrestrial and marine environments. Yet the molecular data seem to be consistent with the results of culturing efforts, although different diversity profiles are revealed by these two methods, and suggest that marine fungi are relatively nondiverse and lower in abundance. Therefore, some fundamental questions remain to be addressed: (*a*) Is this apparent difference in diversity and abundance due to an asymmetric sampling effort between marine and terrestrial environments? (*b*) Is there a

Figure 5

Subsection of a phylogenetic analysis showing the diversity of small subunit ribosomal DNA sequences recovered from marine environmental DNA analyses. This section of the tree mainly focuses on the lower fungi (i.e., chytrids and zygomycetes). The tree was calculated using PhyML (parameters $\Gamma = 0.722$ and $I = 0.117$) with both PhyML and LogDet distance bootstraps. Marine phylotypes are highlighted in yellow. Novel marine phylogenetic groups are marked NMF (*orange*, novel marine fungi). Ecological information is annotated on the tree.

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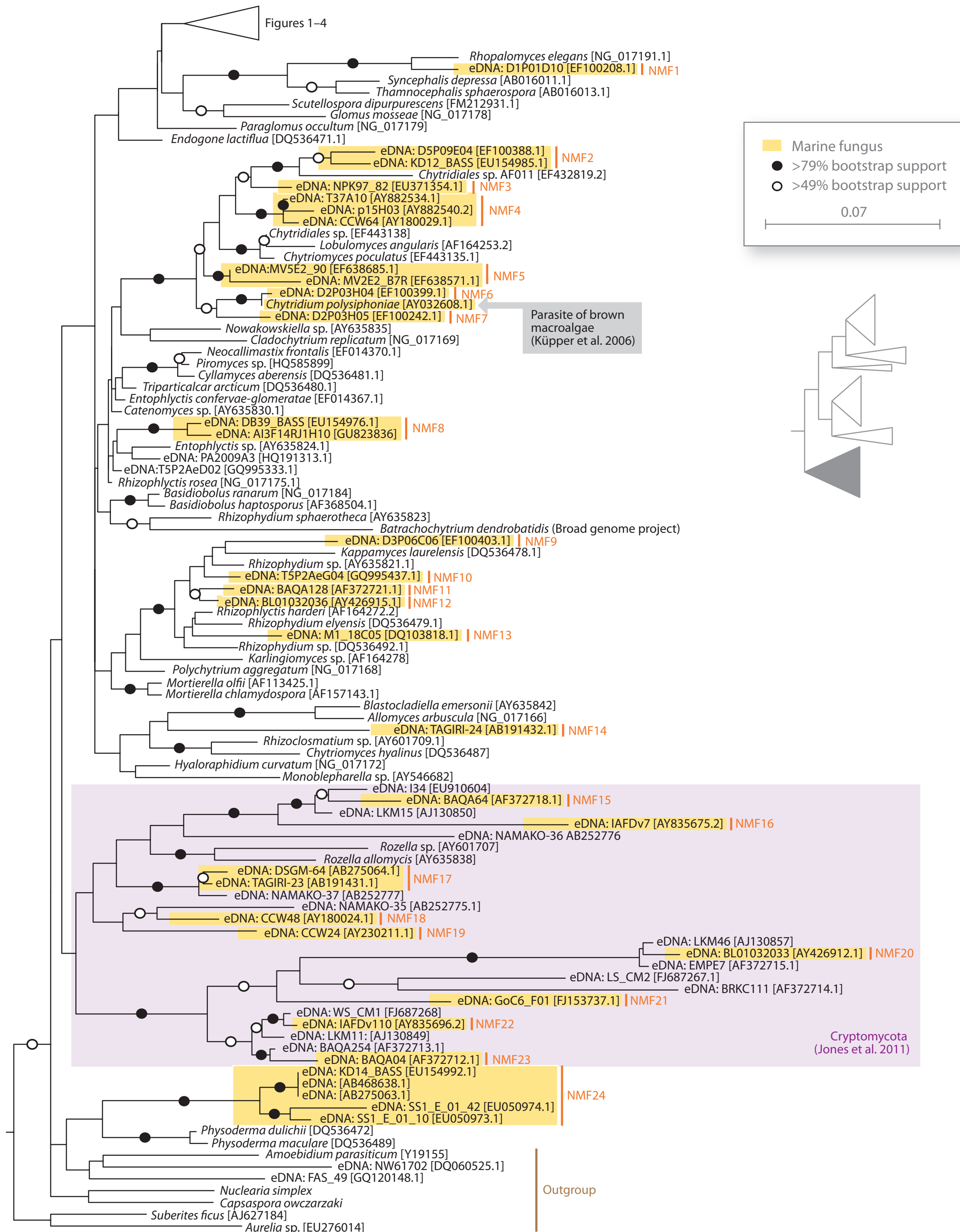

real ecological barrier that has prevented marine fungi from diversifying to the same degree as terrestrial fungi? (*c*) Is the broad success and evolutionary complexity of the fungal radiation so intimately linked with the radiation of land plants and the colonization of terrestrial environments that these forms dominate the diversity of the fungal kingdom? (*d*) Are marine niches dominated by a different set of microbes performing the equivalent roles in the ecosystem as fungi in terrestrial environments, so as to outcompete fungi in marine habitats? To begin to address these questions, it is important to investigate which fungi have been detected in marine environments.

PHYLOGENETIC SUMMARY OF THE SMALL SUBUNIT RIBOSOMAL DNA DIVERSITY OF MARINE FUNGI

To bring together the growing body of data investigating the molecular diversity of fungi in marine environments, we have calculated a summary phylogenetic tree of all unique fungal SSU rDNA sequences detected from marine eDNA samples. Sampling was conducted by a literature review followed by phylogenetic tree construction to obtain a preliminary census of the molecular diversity of fungal SSU sequences. Using this preliminary tree, we conducted BLASTn searches (Altschul et al. 1990) of the NCBI nonredundant DNA database (GenBank) to identify additional marine fungal eDNA SSU sequences. We focused on SSU, rather than ITS, sequences to generate as robust a phylogeny as possible, allowing us to construct a comprehensive picture of the diversity and identify any novel or distinct phylogenetic groups. Therefore, many marine culture-derived sequences are not included in our analysis, as ITS rDNA is often the marker of choice in culture-based studies (Horton & Bruns 2001).

Our tree (**Figures 1–5**) was constructed in an iterative fashion with many highly similar sequences from the same environment sample and/or publication removed and thus represents a summary of the diversity of marine fungal SSU sequences available in GenBank. Therefore, this phylogenetic tree should be viewed not as an exhaustive census, but rather as a summary of our understanding of the molecular diversity of marine fungi.

We also note that the nucleic acid extraction methods used in many of the published environmental clone library studies often do not include chemical/physical preparations specifically tailored to the lysis of fungal cells. It is therefore possible that DNA from cells enclosed within robust chitin-rich cell walls typical of many fungi may have been missed by these studies. Furthermore, the PCR primers used convey differential PCR bias toward or against particular fungal groups. Therefore, our summary tree is likely to be incomplete and is affected by systematic biases in the sampling methods used.

The sequences in **Figures 1–5** are derived from a range of methods, including both fungal-specific clone library analyses and general eukaryotic analyses, and the sequences used were generated by a number of different primer combinations. Therefore, the number of DNA positions that could be reliably sampled across all the data sets analyzed was limited. Nonetheless, we were able to sample a masked alignment region of 656 characters surrounding the variable SSU V4 region (Wuyts et al. 2000). We then conducted preliminary phylogenetic analysis to remove redundant and highly similar SSU sequences. The alignment was calculated using MUSCLE (Edgar 2004), extensively corrected by eye, and was masked using SEAVIEW (Galtier et al. 1996). Phylogenetic analysis was conducted using PHYML (Guindon & Gascuel 2003) with 100 PHYML bootstraps and 1,000 LOG-DET distance bootstraps (Lockhart et al. 1994).

The Dikarya Majority on Land and at Sea

Our phylogeny (**Figures 1–5**) shows a diverse collection of environmental marine fungal SSU rDNA sequences, with the vast majority branching within the basidiomycetes and ascomycetes

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(**Figures 1–4**). This dominance of Dikarya in marine habitats is consistent with previous analyses (Bass et al. 2007, Edgcomb et al. 2011, Jebaraj et al. 2009, Le Calvez et al. 2009). Many Dikarya sequences sampled during our preliminary phylogenetic analysis were highly similar to each other; much of this redundancy was removed during the tree processing steps described above and is not shown in **Figures 1–4**. Consequently, this phylogenetic analysis actually underestimates the relative dominance of Dikarya (**Figures 1–4**) compared to lower fungi (**Figure 5**).

Our phylogenetic analysis suggests that filamentous fungi are more diverse in marine habitats than previous studies have suggested (Bass et al. 2007). Examples include lineages within the Pezizomycotina ascomycete fungi, *Neurospora*, *Aspergillus*, *Cordyceps*, and *Fusarium* (**Figure 1**), and some basidiomycetes, e.g., *Ustilago*-like lineages (**Figure 3**). The phylogeny also identified several marine sequences branching close to taxa that form multicellular structures in terrestrial environments, e.g., *Coprinus*, *Antrodia*, and *Exidia* (**Figure 3**). We had previously suggested that yeast forms dominate fungal diversity in the deep water column and marine sediment environments (Bass et al. 2007). The summary phylogeny partially supports this analysis, with a large proportion of the sequence diversity recovered clustering with known yeast taxa [e.g., *Candida*, *Metschnikowia*, *Pichia*, *Taphrina*, *Malassezia*, *Cryptococcus*, and *Rhodotorula* (**Figures 2–4**)], but suggests that marine fungal communities comprise a wider diversity of fungal phenotypes.

We also note that a large proportion of the filamentous Pezizomycotina diversity shown in **Figure 1** was recovered from cultured isolates (Burgaud et al. 2009, Jebaraj et al. 2009, Le Calvez et al. 2009), suggesting that eDNA studies miss these usually filamentous fungi. This may be explained by primer bias or by the DNA extraction process failing to recover DNA from these microbes, owing to their robust cell walls reducing DNA recovery from these groups. Alternatively, these Pezizomycotina microbes may be at a very low level of abundance, or present as cysts, and are consequently often missed during eDNA sampling. Yet filamentous forms seem to be readily recovered during culturing experiments, perhaps because this sampling method can preferentially select for such fungal forms (e.g., Le Calvez et al. 2009). In conclusion, our phylogenetic results suggest that Dikarya are the most readily recovered marine fungal lineages, and the majority of sequences recovered from eDNA analyses cluster on the phylogeny with known yeast groups, although culturing efforts suggest the presence of a higher proportion of filamentous forms.

Novel Marine Fungal Phylotypes and the Marine-Terrestrial Transition

Environmental clone library sequencing has identified a number of novel and phylogenetically distinct clades that branch with the fungi but seem to represent highly unique groups (Jones et al. 2011, Lara et al. 2010, Porter et al. 2008, Schadt et al. 2003). Our summary phylogeny shows 36 such groups (**Figures 1**, **3**, and **5**). We defined these novel groups on the basis of a minimum of 3% nucleotide character difference, identified using BLASTn analysis, to all sequences in the GenBank nonredundant database that were not marine eDNA sequences. Interestingly, although the majority of marine fungal sequences recovered for this analysis branched within the Dikarya (**Figures 1–4**), 24 of the 36 novel groups branched among the lower fungi (**Figure 5**). **Figure 6** plots the percentage difference of all 36 novel fungal groups to cultured and described fungi versus the percentage difference of the novel groups to all fungal SSU sequences recovered from terrestrial environments (including both eDNA and cultured isolates). This graph clearly demonstrates that the novel marine lower fungi generally comprise more divergent SSU types compared to novel groups within the Dikarya. Furthermore, the novel marine lower fungi seem to be particularly distantly related to cultured and described fungi. Taken together, these data suggest that marine environments host a significant number of highly novel groups, with the majority of these novel groups branching below the Dikarya radiation, close to chytrid branches, implying that they may

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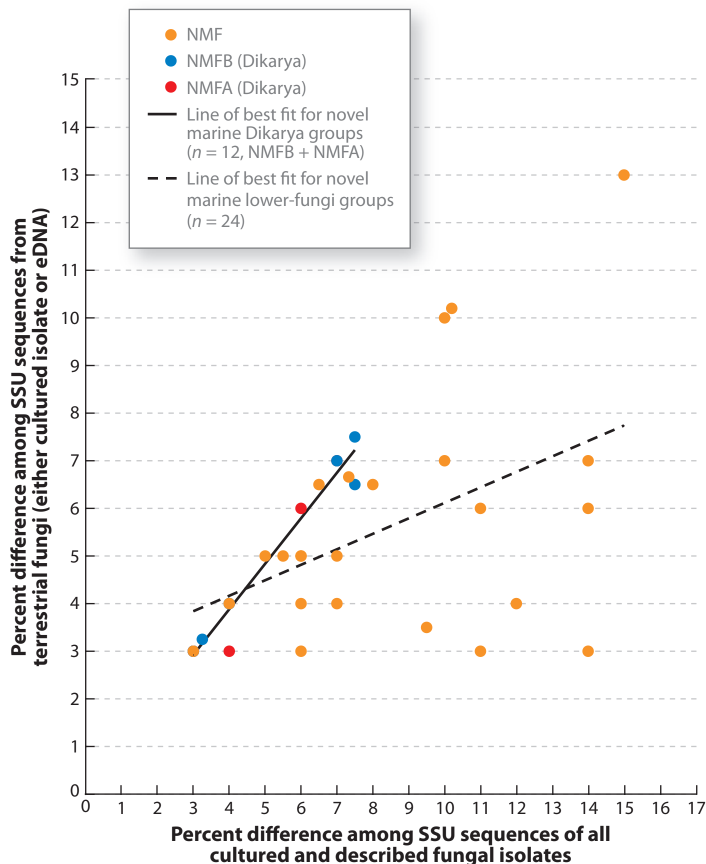

Figure 6

Graph comparing the percentage difference of novel marine fungi to terrestrial fungi verses the percentage difference of the novel fungi to all isolated and described fungi (either terrestrial or marine). This graph demonstrates that among the marine fungi, the novel marine groups branch mainly among the lower fungi (**Figure 5**) and that these novel lower fungal branches (shown in *orange*) have higher levels of small subunit (SSU) ribosomal DNA sequence difference to cultured and described fungi (*x axis*) than fungi detected in terrestrial environments (*y axis*). This demonstrates that novel marine fungi (NMF) tend to have closer relatives detected by sequencing of terrestrial environments than they have represented in culture collections, suggesting that isolation and description of marine fungi are lagging behind. Abbreviations: eDNA, environmental DNA; *n*, number of novel phylotypes identified in the tree analysis (see **Figures 1–5**); NMFA, novel marine fungi ascomycetes; NMFB, novel marine fungi basidiomycetes.

form flagellated zoospores (**Figure 5**). These results are consistent with the conclusion of Le Calvez et al. (2009), who suggest that the marine environment hosts numerous, unclassified deep-branching fungal forms, reflecting an ancient transition from marine environments to terrestrial environments. The lower fungi may be significantly more diverse and numerous in marine habitats than **Figure 5** suggests if the PCR process (including primer choice/design) used to generate most marine clone libraries (whether targeting fungi or microbial eukaryotes in general) is biased toward Dikarya fungi.

The majority of fungal environmental SSU rDNA sequences and isolated cultures are from terrestrial environments, leading to the hypothesis that fungi are a mainly terrestrial group (e.g.,

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Le Calvez et al. 2009). However, all terrestrial life-forms, including freshwater forms, must trace their ancestry back to marine-dwelling ancestors. It has therefore been inferred that fungi arose in the oceans and then colonized terrestrial environments (Le Calvez et al. 2009). The transition from marine to terrestrial environments involves a radical change in biological capacities, and therefore it is widely suggested that symbioses between fungi and photosynthetic microbes facilitated multiple terrestrial colonization events, including the phototrophic microbial ancestor of the land plants (Pirozynski & Malloch 1975, Selosse & Le Tacon 1998, Simon et al. 1993). Fossil evidence has suggested that several fungal subgroups [e.g., vesicular-arbuscular (VA) mycorrhizae, ascomycetes, blastocladiomycetes] were established in the Devonian 400 Mya, suggesting the fungal lineage is much older than this (Berbee & Taylor 2010) and therefore that the fungal kingdom is much older than the colonization of land by both plants and fungi. These results imply a long phase of fungal evolution in aquatic/marine environments before fungi colonized the land (i.e., an ancient marine fungal divergence) (Le Calvez et al. 2009). Evidence of divergent deep-branching marine fungal SSU sequences (e.g., **Figures 5** and **6**) has been used to argue that these lower fungal marine groups are derived from an ancient phase of fungal evolution in marine environments prior to the colonization of terrestrial environments (Le Calvez et al. 2009). However, environmental sequencing of marine DNA samples also shows that marine lineages frequently form short branches interdispersed between terrestrial lineages (**Figures 1–5**). One interpretation is that the evolutionary diversification of the Fungi has involved multiple marine-terrestrial transitions in both directions.

In contradiction to the ancient marine fungal divergence theory, studies of freshwater environments have recovered novel deep-branching fungal sequences at a much higher frequency than in the marine environment. For example, freshwater studies frequently report that a high proportion of all the clones recovered from general eukaryotic clone libraries are fungal sequences [e.g., 19% (Lefèvre et al. 2008), 23% (Lefèvre et al. 2007), 33% (Berney et al. 2004), or 25% (Lepère et al. 2006)]. Furthermore, in contrast to the majority of fungal sequences recovered from marine environments, which are Dikarya (e.g., **Figures 1–5**; Bass et al. 2007, Edgcomb et al. 2011, Jebaraj et al. 2009, Le Calvez et al. 2009), a much larger proportion of the freshwater fungal sequences branch among the lower fungi (**Figure 7**). These results point to a large and underexplored diversity of lower fungal groups in freshwater (Lefèvre et al. 2007, 2008; Lefranc et al. 2005; Slapeta et al. 2005) compared to marine environments. With freshwater environments being part of the terrestrial biome, this emerging pattern therefore seems to be inconsistent with the idea that divergent deep-branching novel fungal forms are derived directly from marine ancestors, separate from the terrestrial branch(es) (Le Calvez et al. 2009). A more realistic model might be a terrestrial origin or very early primary terrestrial radiation of fungi followed by multiple marine-terrestrial transitions in both directions. Indeed, phylogenetic analysis of marine ascomycetes has identified multiple terrestrial-to-marine transitions (Spatafora et al. 1998, Suetrong et al. 2009), whereas data supporting multiple losses of flagella within the fungal radiation (James et al. 2006a,b; Liu et al. 2009) show a similarly complex evolutionary pattern involving multiple aquatic-to-nonaquatic transitions.

Cryptomycota: A New Basal Branch in the Fungi?

Interestingly, freshwater studies have also identified a large and complex clade of environmental sequences that form one of the deepest branches in the Fungi (e.g., Berney et al. 2004; Lefèvre et al. 2007, 2008; van Hannen et al. 1999). Van Hannen and coauthors (1999) first identified this group from sequencing DNA recovered from a freshwater-derived experimental detritus system, and it has now been recovered from numerous environment types (Jones et al. 2011, Lara et al. 2010). Of the four fungal-like sequences recovered by van Hannen et al. (1999), three formed this

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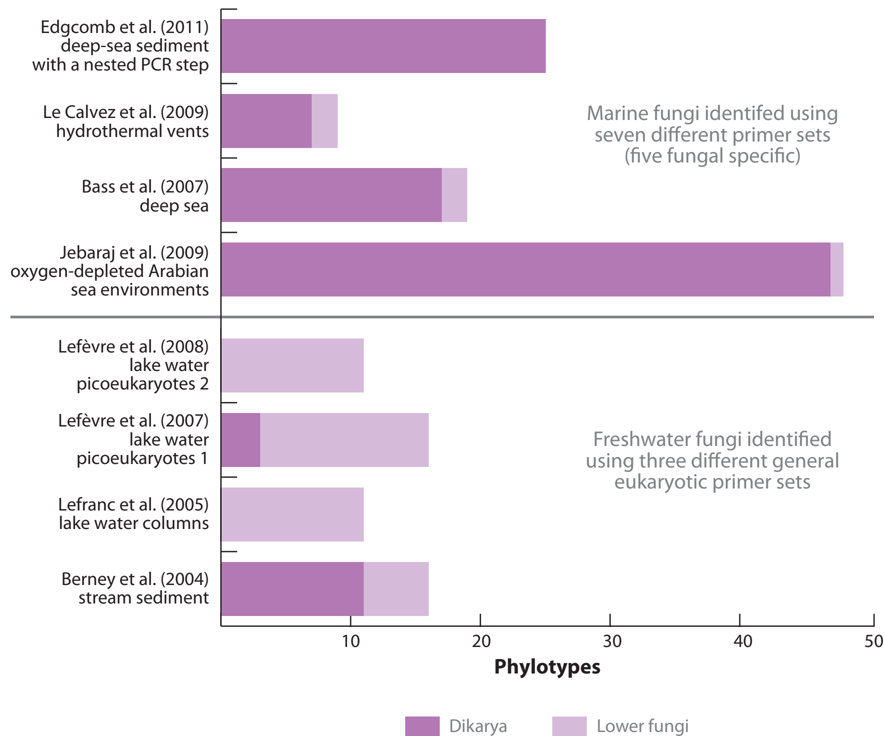

Figure 7

Comparison of small subunit ribosomal clone library analyses from marine and freshwater environmental DNAs, demonstrating that freshwater sampling identifies many more lower fungi phylotypes than similar marine analyses, whereas marine analyses tend to detect Dikarya fungi. Note that the freshwater studies summarized used general eukaryotic primers, whereas the marine studies summarized used both general eukaryotic primers and fungal-specific primers.

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deep-branching clade. Subsequent clone library experiments have considerably expanded the diversity of this clade in soils and freshwater environments (e.g., Jones et al. 2011; Lefèvre et al. 2007, 2008; Lefranc et al. 2005); sequencing of marine libraries has also recovered representatives of this group (**Figure 5**; also see Massana et al. 2004a; Takishita et al. 2005, 2007). Using terminal restriction fragment length polymorphism analyses, Lepère et al. (2006) found that representatives of this group are highly abundant in freshwater environments and that population abundance is linked to an abundance of algal populations.

Phagotrophy: process of acquiring nutrients by engulfing large particles or prey cells in a phagosome (cell vacuole), followed by digestion and intracellular absorption of nutrients

Osmotrophy: mode of nutrition involving secretion of enzymes to break down complex biological polymers followed by uptake of simplified molecules

Further phylogenetic analysis with additional environmental rDNA sampling suggests that this group branches with the intracellular parasitic genus *Rozella*, thought to be the first branch in the fungal radiation (James et al. 2006a, Jones et al. 2011, Lara et al. 2010). This is an interesting relationship because the genus *Rozella* comprises intracellular parasites that do not possess a chitin/cellulose cell wall for the stages of their life cycle identified and furthermore appear to be capable of phagotrophy (Held 1981). These characteristics represent distinct differences from the fundamental fungal phenotype: a rigid chitin wall, the use of osmotrophy, and an inability to perform phagotrophy. Thus it is questionable whether the *Rozella* genus and this wider clade of environmental sequences should be classified within the Fungi or whether they represent an intermediate form (Lara et al. 2010, Jones et al. 2011).

Jones et al. (2011) used fluorescence in situ hybridization to investigate the ecology and cell biology of this novel group and showed that it possesses at least three distinct life-cycle phases: (*a*) a flagellated zoospore, (*b*) single cells attached to other (often algal) cells, and (*c*) cysts or resting cells. Using a combination of cell wall markers, the authors could not identify a fungal chitin/cellulose cell wall, suggesting that similar to *Rozella*, these microbes live without this cellular trait for much of their life cycle. In anticipation of formal classification, and because this clade groups with true fungi, Jones et al. (2011) named this group cryptomycota (hidden fungi).

Cryptomycota: highly diverse group of microbes, mainly known from environmental DNA techniques; includes *Rozella*

Interestingly, both the cryptomycota and chytrid-like sequences recovered from freshwater environments branch most closely to fungi known to parasitize algae, other fungi, or protists (Held 1981; Lefèvre et al. 2007, 2008; Lefranc et al. 2005). These observations have led some authors to re-evaluate the functional role of fungal parasites in freshwater environments, suggesting that they play a significant role in the microbial loop of freshwater ecosystems (Lefèvre et al. 2008).

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ECOLOGICAL ROLE OF FUNGI IN MARINE ENVIRONMENTS

Fungi are considered as saprotrophs, parasites, or symbionts, partly because of the bias of research interests, but mainly as a consequence of the evolution of fungal cell biology and feeding strategies. In contrast to the example of the cryptomycota discussed above (Jones et al. 2011), fungi generally possess robust chitin-rich cell walls and obtain nutrients exclusively by feeding osmotrophically. This process involves the secretion of depolymerizing enzymes followed by the transportation of nutrients, usually as digested monomers, back into the cell.

These traits underpin the ecological success of the Fungi and are mechanistically linked; the chitin/cellulose reinforces the fungal cell and enables it to resist (*a*) the substantial osmotic pressure produced during osmotrophic feeding, (*b*) structural strains during growth (usually as polarized cells in the form of hyphae or rhizoids), and (*c*) the diverse and heterogeneous environments within which fungal filaments grow. These adaptations drive the high metabolic rate, fast growth, and ecological success of fungi (Bartnicki-Garcia 1987). However, as a consequence of this lifestyle, many fungi have lost the ability to perform phagocytosis and therefore cannot engulf and digest prey cells in the same way as many other eukaryotes. This reliance on osmotrophy determines the ecology of fungi: They thrive in nutritionally rich environments such as plant and animal host organisms, soils, sediments, and detritus environments, where they can attach to substrates, secrete enzymes, break down complex biological polymers, and take up nutrients.

These ecological characteristics in part may explain why fungi have been considered both nondiverse and of low abundance in many upper and surface marine water column samples (Kis-Papo 2005, Massana & Pedrós-Alió 2008, Richards & Bass 2005). Many pelagic and surface water environments are often low in nutrients, and the microbial eukaryotic component of the planktonic food chain is predominately that of free-floating or swimming single-celled organisms performing primary production and/or phagotrophic grazing. Such environments are unlikely to favor organisms that feed primarily by attachment to larger physical substrates and osmotrophy, partly because secreted enzymes and target nutrients are likely to be lost by rapid diffusion in the liquid environment, but also because of key differences in the relationship between photosynthesis and biomass accumulation on land and at sea. Fixed carbon on land is largely invested in the construction of large and complex plant tissues rich in energy and nutrients. These complex structures are difficult to digest, driving the evolution of the osmotrophic lifestyle, specialized plant/fungi associations, and the subsequent diversification of fungi. Yet in the open ocean, primary producers do not extend much beyond the scale of small single-celled organisms (~3 μm in diameter) and do not make complex energy- and nutrient-rich structures like land plants.

As such, the very niches that favor fungal diversification and osmotrophic feeding on land are largely absent in the open ocean. Therefore, this trophic relationship is essentially closed off to the microbial community, with only single-celled primary producers and phagotrophic grazers supported in the upper oceanic water column.

There are, of course, exceptions to this model: (*a*) In the lower water column, below the photozone, as particulate matter descends and enters the sediment, logically there must be increased niche availability for saprotrophs (potentially fungi) driving the principal steps in food webs, which at these lower depths are born out of detritus processing. Observations that fungi are the dominant active eukaryotic microbes in these environments are consistent with this hypothesis (Edgcomb et al. 2011, Takishita et al. 2006). Detrital processing represents a largely understudied area of marine ecology but represents an important gap in our understanding of how nutrients, including carbon, are processed. (*b*) Coastal systems harboring large multicellular algae or mangroves must provide numerous niches for saprotrophs and endophytes, again providing potential ecosystems for marine fungi (discussed below). (*c*) A large fraction of marine fungi must also reside as parasitic forms in marine animals.

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Fungi as Parasitic Agents in the Marine Environment

It is striking that many fungi that cause or are associated with disease in marine habitats are closely related in phylogenetic analyses to well-characterized nonmarine taxa. **Figures 1–3** show several examples, with marine sequences branching closely to terrestrial parasitic fungi, e.g., *Cordyceps/Paecilomyces* (**Figure 1**), *Geomyces* (**Figure 1**), *Taphrina* (**Figure 2**), *Candida* (**Figure 2**), *Malassezia* (**Figure 3**), and *Ustilago* (**Figure 3**). Furthermore, fungi are often isolated from marine animals, suggesting a range of additional and unstudied parasitic associations. For example, new species of the basidiomycete yeast *Rhodotorula* have been isolated from deep-sea tubeworms and clams by Nagahama et al. (2003a) and have also been recovered from a range of eDNA and culturing studies derived from deep-sea samples (Nagahama et al. 2001, 2003a) (**Figure 4**). Although they have not been directly shown to be parasitic agents, they appear to be closely associated with certain marine invertebrates (Nagahama et al. 2003a). Similarly, black yeast (Herpotrichiellaceae of the order Chaetothyriales) has been proposed as a causal agent in disease of the deep-sea mussel *Bathymodiolus brevior* in the Fiji Basin (Van Dover et al. 2007). Other known fungal pathogens closely related to terrestrial fungi are particularly prevalent in marine mammals, including *Aspergillus* (aspergillosis), *Blastomyces* (blastomycosis), *Coccidioides* (coccidioidomycosis), *Cryptococcus* (cryptococcosis), *Candida* (candidiasis), *Fusarium* (fusariomycosis), *Histoplasma*, *Sporothrix*, dermatitis caused by *Malassezia*, and a diverse group of disease-causing zygomycetes (Higgins 2000). Many of these groups are frequently recovered by eDNA analysis (e.g., Bass et al. 2007, Edgcomb et al. 2002, Jebaraj et al. 2009), confirming their widespread presence in marine environments (**Figures 1–4**).

Fungi generally have been shown to associate with coral and are suggested to represent opportunistic pathogens of corals weakened by environmental stress (Le Campion-Alsumard et al. 1995). For example, *Aspergillus sydowii* (shown in **Figure 1**) is closely related to terrestrial *Aspergillus* species and has been identified as a pathogen of sea fan corals (Alker et al. 2001, Shinn et al. 2000). Such corals are known to release defensive chemicals during infection by endoliths such as *A. sydowii* (Alker et al. 2001).

Cawthorn (2011) identified the ascomycete fungus *Fusarium* as a disease-causing agent in American lobsters (*Fusarium* is also represented in multiple marine eDNA analyses; see **Figure 1**), noting its opportunistic nature and propensity to invade through damaged or dead tissues (Cawthorn 2011). In a review of emerging diseases and their links to climate and anthropogenic

factors, Harvell and coauthors (1999) found that three of 34 mass mortalities in marine environments are attributed to fungi.

Wang & Johnson (2009) pointed out that studies of fungal pathogens of marine algae mostly focus on seaweed or macroalgae and also rely on cultivation-based methods, which can give a false impression of natural diversity. This means that culture-independent molecular probing of potentially infected tissue could reveal important new information about organismal interactions of marine fungi. Several dozen ascomycete fungi are already known to be pathogens of marine algae (Kohlmeyer & Kohlmeyer 1979), while chytrids (e.g., *Chytridium polysiphoniae*) are also known to parasitize macroalgae (Küpper et al. 2006). Seaweeds have been shown to respond to fungal attack, by the production of antifungal lobophorolide and tolytoxin by the brown alga *Lobophora variegata* against the parasitic ascomycetes *Lindra thalassiae* and *Dendryphiella salina* (Kubanek et al. 2003). Many ascomycetes appear to be specific to red, green, or brown algae, although some are generalists. Some cause galls on seaweeds, whereas others are involved in symbioses with seaweeds, outside of which neither partner appears able to grow. For example, the ascomycete *Mycophycias ascophylli* is involved in a three-way symbiosis with the phaeophyte *Ascophyllum nodosum* and its red algal epiphyte *Polysiphonia lanosa*. The fungus interacts with both, possibly transferring nutrients between them (Spooner & Roberts 2005).

Another relatively well-studied set of interactions is between sponges and fungi. A culture-based study by Li & Wang (2009) revealed a large diversity of fungi associated with sponge species (e.g., Mycosphearellales, Eurotialies, Dothideales, Hypocreales, Diapothales, Xylariales, Pleosporales, Aphyllophorales, and Saccharomycetales). They were able to classify their isolates into three categories: those found in all sponge species, those in more than one but not all, and specialists found only in a single sponge species. *Cladosporium*, *Penicillium*, *Aspergillus*, and *Eupenicillium* were recovered from the majority of sponges investigated, suggesting that fungi from these groups are regularly associated with sponges. Again, these phylogenetic groups are consistently recovered in marine eDNA analyses, and many of the lineages are closely related to nonmarine species (**Figure 1**).

The isolation-independent denaturing gradient gel electrophoresis approach used by Gao et al. (2008) also showed a high diversity of fungi associated with sponges, and demonstrated that fungal communities differed between sponge species and between sponges and the surrounding seawater. They found a high incidence and microdiversity of *Malassezia* lineages. *Malassezia* are ubiquitous lipophilic yeasts and are found in soils, sediments, and deep-sea habitats and on terrestrial metazoa. Gao et al. (2008) suggested that their analysis of sponges had detected the highest diversity to date of *Malassezia* lineages from a single host. It is currently unclear whether the diversity of sponge-associated fungi represents symbionts and/or parasites.

Our phylogenetic analysis also demonstrated a large number of *Malassezia*-like sequences recovered from a number of marine environments, including deep-sea water column and sediment samples. These sequences formed a large clade displaying complex microvariation, suggesting a radiation of marine *Malassezia* lineages and suggesting that this represents an important and diverse group of fungi in marine environments (**Figure 3**; also see Bass et al. 2007, Edgcomb et al. 2011, Jebaraj et al. 2009, López-Garcia et al. 2007).

Fungi as Saprotrophic Agents in the Marine Environment

In terrestrial ecosystems, fungi perform critical roles as saprotrophs in detrital environments, breaking down complex biopolymers and recycling nutrients. This process is important because it underpins the wider ecosystem, but it is much less clear which organisms perform equivalent roles in marine environments. Marine yeasts are generally associated with nutrient concentrations [e.g.,

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pollution, plankton blooms, and macroalgae (Kohlmeyer & Kohlmeyer 1979)], suggesting that fungi are also important saprotrophs in marine environments. Furthermore, saprotrophy and life in detritus are often associated with anoxic or partially anoxic conditions, and fungi have been shown to possess a range of cellular and genomic adaptations to anoxic environments (discussed briefly above). Therefore, although marine fungal diversity appears to be limited compared to terrestrial environments (Kis-Papo 2005, Massana & Pedrós-Alió 2008, Richards & Bass 2005), fungi may still play a critical role in detritus processing in marine ecosystems (Mann 1988, Raghukumar 2004), thus providing essential nutrients to the wider food web, such as the amino acids lysine and methionine, various vitamins, polyunsaturated fatty acids, and sterols (an important precursor for the manufacture of cholesterols in marine animals) (Phillips 1984). These pathways, through which organic matter re-enters the food web, are vital for the survival of detritivorous animals, which are unable to synthesize such compounds by themselves (Raghukumar 2004). For example, Crustacea require the polyunsaturated fatty acid docosahexaenoic acid for growth (Harrison 1990), which is provided to benthic food webs by detrital microbes (Raghukumar 2004), although it is not clear whether true fungi mediate this process. In addition, fungi are thought to play a role in the degradation of tucinin, an animal cellulose, which occurs in the test of tunicates (Kohlmeyer & Kohlmeyer 1979). Indeed, phylogenetic analysis of environmental marine fungal sequences shows a number of sequences branching closely to known saprotrophic fungi, including, for example, *Aspergillus* (**Figure 1**), *Fusarium* (**Figure 1**), *Coprinus* (**Figure 3**), and *Exidia* (**Figure 3**).

One relatively strong research focus regarding marine fungal interactions has been the breakdown of calcareous structures by fungi, whether of living or dead organisms. Marine fungi are known to degrade structures such as mollusc shells, burrow linings, and barnacle shells (Hyde et al. 1998). For example, the ascomycete genera *Arenariomyces*, *Corollospora*, and *Lindra* degrade foraminiferan tests and, with related genera including *Remispora*, attack other calcareous structures, for example, those of barnacles and shipworms (Spooner & Roberts 2005). Other endolithic fungi (the general term for those living inside rocks and boring into calcareous substances) associated with many coral genera include basidiomycetes, many ascomycetes (e.g., *A. sydowii*), and the chytrids *Dodgella priscus* and *Conchyliastrum enderi*. *Ostracoblabe* and *Lithopythium* are often cited as endolithic fungi (Golubic et al. 2005, Kendrick et al. 1982), although sequence data for all four of these genera are currently unavailable in GenBank.

Fungi may also be important in degrading lignocellulose in marine environments, as they are in terrestrial ones. They are able to tolerate reduced oxygen concentrations (discussed above) and could potentially be the main degrader of these compounds in low-oxygen and anoxic marine sediments (Hyde et al. 1998) and of plant material in the oceans in general, especially in mangrove ecosystems. Evidence for lignocellulose degradation by marine fungi (through the production of endoglucanase enzymes, allowing growth on a carboxymethylcellulose substrate) has been found in over 30 strains of phylogenetically diverse fungi isolated from marine environments (Hyde et al. 1998). Mangrove leaves are rich in lignocellulosic structural polymers, soluble organics, phenolics, and tannins, making them highly resistant to degradation from many microbes. However, fungi such as *Pestalotiopsis* and *Cladosporium*—common primary saprotrophs—are often recovered from mangrove leaves (Raghukumar 2004). The initial production of degrading enzymes by fungal colonizers therefore likely plays a vital role in the breakdown of robust plant materials.

Fungal strains isolated from leaves of *Spartina alterniflora* have proven capable of cellulose, cellobiose, pectin, lipid, tannic acid, starch, and xylan degradation (Gessner 1980). Suetrong et al. (2009) reported a diversity of intertidal marine Dothideomycetes mainly associated with mangrove habitats in tropical/subtropical environments, noting that these species are well adapted to marine environments with active discharge of ascospores and with a mucilaginous sheath. A representative

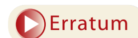
diversity of species associated with mangrove plants is summarized in **Figure 1**, showing the wide diversity of Dothideomycetes fungi associated with this marine environment (Suetrong et al. 2009). Dothideomycetes are frequently found as saprobes on decaying woody material in marine environments. They are also parasites or symbionts of seagrasses or marine algae. Suetrong et al. (2009) therefore posed the question of whether this pattern represents a radiation, or multiple radiations, of Dothideomycetes specifically in mangrove marine habitats.

Pseudofungi: group of stramenopiles (heterokonts) unrelated to true fungi but that feed and grow using analogous methods to true fungi

Labyrinthulids and thraustochytrids: stramenopile (heterokont) protists with partially similar morphologies and analogous life cycles to chytrid fungi; produce a network of filaments for feeding and movement

Endomyxa: a diverse group of filose amoebae, parasites, and uncharacterized environmental sequences currently included within the phylum Cercozoa (supergroup Rhizaria), comprising the sister group to core Cercozoa

Marine snow: organic detritus falling from the upper water column layers, often comprising mucus secreted by phytoplankton, most prolifically diatoms (which form aggregations in the water column)



Filling the Fungal Niche in the Marine Ecosystems: Fungal Analogs

The relative contribution of fungi to disease load and saprotrophic operations within marine ecosystems has been obscured by the high incidence of and taxonomic confusion caused by organisms that have fungal-like characteristics but are not true fungi (Cavalier-Smith 1987, Cavalier-Smith & Chao 2006), i.e., those that do not branch within the fungal clade within the opisthokont supergroup (Adl et al. 2005, Simpson & Roger 2004). These fungi-like organisms also feed by osmotrophy, in many cases forming polarized cells analogous to hyphae and rhizoids of true fungi, and grow on standard fungal growth media; hence they are often recovered during culture-based analyses of environmental diversity and have historically been considered to be fungi. However, these fungal-like organisms have evolved this lifestyle independently and possess cellular characteristics that separate them from true fungi. For example, true fungi generally form a robust exoskeleton containing chitin during part of their life cycle however, the exoskeleton of the non-fungal analogs does not contain chitin (Bartnicki-Garcia 1987).

Fungal analogs include oomycetes; *Pirsonia*; hyphochytriomycetes [collectively forming the pseudofungi (Cavalier-Smith & Chao 2006)]; labyrinthulids and thraustochytrids (which are stramenopiles); ichthyosporeans (e.g., *Ichthyophonus*), which also branch within the opisthokonts but separately from true fungi; and endomyxan Cercozoa [ascetosporeans (e.g., *Paradinium* and *Haplosporidium*) and phytomyxids (plasmodiophorids and phagomyxids)]. All these groups have at some stage been classified as true fungi. Ascetosporea are well known for causing MSX disease of oysters and other diseases of marine invertebrates; plasmodiophorids and their sister group phagomyxids are important marine parasites of seagrasses, diatoms, and phaeophycean algae (e.g., Parodi et al. 2010).

Indeed, general surveys of marine fungi, both recent and from earlier in the twentieth century, report more fungal analogs than true fungi, thereby implying that true fungi are relatively unimportant in marine habitats. For example, a comprehensive review of marine fungi from Woods Hole by Sparrow (1936) described mostly oomycetes, along with labyrinthulids, a thraustochytrid, *Protomyxa* (an unclassified large branched amoeba, probably endomyxan), and the chytrid *Chytridium*—the only true fungus listed (**Figure 5**; also see Sparrow 1936). A similarly comprehensive review of zoosporic fungal parasites of marine biota (Raghukumar 1996) described many pseudofungal groups, including 11 genera of oomycetes infecting diatoms, green, red, and brown algae, and crustaceans, and also hyphochytriomycetes, including members of the genus *Anisolpidium*, parasitizing filamentous brown algae, and *Hyphochytridium peniliae*, causing mycosis of the planktonic cladoceran *Penilia avirostris*.

Labyrinthulids and thraustochytrids (e.g., *Schizochytrium*, *Ullkenia*, *Labyrinthula*, *Labyrinthuloides*) are generally considered exclusively marine, although more recently labyrinthulids have been found in nonmarine habitats (Douhan et al. 2009). These stramenopiles parasitize a wide range of marine organisms, including diatoms, octopus, squid, seagrasses, shellfish, and fish. Thraustochytrids have been detected on marine substrates such as salp fecal pellets and marine snow. They reproduce through the production of motile zoospores, which swim to a food source and colonize it (in an analogous manner to fungal chytrids). There is also evidence of saprotrophic

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function among fungal analogs, and studies within mangrove swamps reveal the colonization of fallen leaves by the oomycete *Halophytophthora* sp. of red mangrove plants within two hours of submergence (Raghukumar 2004).

SUMMARY POINTS

1. Currently known fungal diversity in marine environments represents a tiny fraction of that from terrestrial environments.
2. Fungal sequences detected in marine environments span a large diversity of forms and lineages, including chytrids, filamentous hyphal forms, and multicellular forms.
3. Dikarya yeast forms appear to dominate the known diversity of marine fungi.
4. Marine environments, like freshwater environments, harbor a number of highly divergent, deep-branching, and uncultured fungi, of which future study will greatly improve our understanding of fungal cell diversity and evolution.
5. There appears to have been frequent marine-terrestrial and terrestrial-marine colonization events during the radiation of the Fungi.
6. Fungi play diverse ecological roles in marine ecosystems and have frequently been associated with parasitism of marine animals, plants, and algae.
7. Many ecological niches inhabited by true fungi in terrestrial and freshwater habitats are occupied by diverse fungal analogs in marine habitats.
8. Fungal-specific molecular studies in marine environments are so far relatively few, yet many reveal a marine fungal diversity that is significantly higher than other methods suggest but much less diverse than terrestrial environments.

FUTURE ISSUES

1. What are the main drivers of detrital and saprotrophic processes in marine environments?
2. What is the diversity of true fungal parasites in marine environments, and what dangers do they represent for marine conservation?
3. What is the true diversity of lower fungi in marine environments, and how do they relate to terrestrial taxa and the fungal tree of life?
4. What are the evolutionary and physiological changes between truly marine fungi and their close terrestrial relatives?
5. Do fungal analogs perform the majority of saprotrophic functions in marine environments?
6. Do fungal analogs actually exclude true fungi from colonizing apparently suitable marine habitats?
7. By what evolutionary mechanisms have fungal analogs been able to occupy fungal-like niches in marine habitats?

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DISCLOSURE STATEMENT

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

ACKNOWLEDGMENTS

This work was primarily supported by the European Funding Agencies from the ERA-net program BiodivERsA, under the BioMarKs project. The authors are supported by research grants from the Biotechnology and Biological Sciences Research Council (BBSRC), Department for Environment, Food and Rural Affairs (DEFRA), and Natural Environment Research Council (NERC).

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Erratum

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Annual Review of Marine Science

Volume 4, 2012

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Errata

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