Marine microbial eukaryotic diversity, with particular reference to fungi: Lessons from prokaryotes

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Novel molecular, analytical and culturing techniques have resulted in dramatic changes in our approaches towards marine eukaryotic diversity in recent years. This article reviews marine fungal diversity in the light of current knowledge, citing examples of how progress in understanding marine prokaryotes has often contributed to this new approach. Both ‘true fungi’ (termed mycenaean fungi in this review) and straminipilan fungi are considered. Molecular phylogenetic studies of prokaryotes has resulted in their redefinition as belonging to the Kingdoms Bacteria and Archaea. Likewise, major refinements have taken place in the phylogenetic classification of eukaryotes. In the case of fungi, it has now been realized that they are polyphyletic, belonging to the Kingdom Mycenae (Fungi), as well as the Kingdom Straminipila or Chromista. Although the total number of fungi on earth is estimated to be about 1.5 million, only a meagre number of obligate marine fungi, about 450 mycenaean and 50 straminipilan fungi have been described so far. It is likely that most of the true marine fungi have not yet been discovered. These are likely to have evolved between 1,500 million years ago (Ma) when fungi probably evolved in the sea and 900 Ma when they conquered land together with green plants. It now appears that most of the true marine fungi have not been cultured so far, similar to the ‘great plate count anomaly’ of bacteria. Thraustochytrids, which are abundant in the water column, but not easily culturable from that source is an example. Intelligent and novel culture methods might bring forth unusual and new marine fungi, as happened in the case of *Pelagibacter ubique* belonging to the SAR 11 group of bacteria. Molecular techniques might bring to light novel marine fungi, as is happening with bacteria. Such fungi may defy our conventional wisdom regarding these organisms in terms of morphology. Thus, several recent studies using 18S rRNA gene community profiles have discovered picoplanktonic marine fungi in the water column. Studies such as those on molecular diversity of eukaryotes in permanently anoxic habitats have also indicated that fungi may be abundant in exotic habitats and possess unusual physiology. A search for fungi in biodiversity-rich habitats, such as the coral reefs and the deep-sea, using a combination of molecular and novel culture methods is likely to reveal a fascinating diversity of marine fungi.

[Key words: Microbes, eukaryotes, fungi, prokaryotes, diversity]

Introduction

The last two decades have seen unprecedented changes in our approaches towards various aspects of marine microbial eukaryotic diversity, encompassing taxonomy, phylogeny and discovery. Many of these gains were preceded by, and even resulted from the insights that were obtained regarding marine prokaryotes, both photo- and heterotrophic. This gain in knowledge may be attributed to two approaches (Fig. 1). One was the development of novel tools, including molecular and analytical ones. The second was the search for organisms in novel habitats that either had been neglected before or were not easily accessible owing to technical reasons. Thus, the application of epifluorescence microscopy method for direct counts of bacteria in the water column during 1970s resulted in the discovery that bacterial numbers often amount to a staggering $1 \times 10^6$ cells/ml of seawater, most of which could not be cultivated. Bacterial biomass probably comprises the largest amount of living organic carbon in the water column. The application of new approaches has not only led to the discovery of unique marine organisms, but also to redefining our concepts on taxonomy and phylogeny of entire kingdoms of living organisms. The effect has been felt on every group of eukaryotic microbes, including protozoans, stramenopiles, alveolates and fungi. Specialists in each of these groups continue to benefit in their own unique ways by modern tools and ideas. Among the various marine microbial eukaryotes, the fungi are ecologically closest to heterotrophic bacteria, being similar in their mode of nutrition. Ecosystem analysts have always been interested in the relative roles of bacteria and fungi as
remineralizers\(^2\). The taxonomic and functional diversity of marine fungi and their biomass in the ecosystem are fundamental questions that need to be addressed in order to improve our knowledge on their ecological role. This article briefly reviews modern developments in our approaches to marine prokaryote diversity, and discusses examples on their effect in the way we address marine fungal diversity.

I will address fungi in their broad sense as considered by Barr\(^3\). Thus, fungi will be defined as a polyphyletic group based on their nutritional and ecological characteristics. All eukaryotic organisms with an absorptive and osmoheterotrophic mode of nutrition will be classified as fungi. Thus, fungi would not only include ‘true fungi’ placed under the Kingdom Fungi but also the ‘pseudofungi’ or ‘straminipilan fungi’ comprising the Oomycetes and Labyrinthulomycetes of the Kingdom Straminipila sensu Dick\(^4\). Since, in the above context, it is contradictory to distinguish between ‘true’ and ‘pseudo’ fungi, I shall avoid the term ‘Kingdom Fungi’ and use instead the term ‘Kingdom Mycenae’ as given by Agrios\(^5\). However, unlike Agrios’ circumscription, the Kingdom Mycenae will not include the ‘stramenipilan fungi’, but shall only encompass the chytridiomycetes, the zygomycetes, the ascomycetes and the basidiomycetes. Hence, the present discussion concerns organisms belonging to the Kingdom Mycenae as well as the Kingdom Straminipila.

Hawksworth’s seminal paper on the census of fungal diversity puts the total number of fungi at about 1.5 million species, the number of species discovered so far amounting only to about 70,000\(^{\text{(ref. 6)}}\). Despite numerous debates on Hawksworth’s estimates regarding the actual diversity of 389

The approach

Phylogeny

Novel methods

Novel habitats

Diversity

The impact

Table 1—The known and unknown of microbial diversity (from Bull & Stach\(^7\))

<table>
<thead>
<tr>
<th>Taxonomic group</th>
<th>Known species</th>
<th>Estimated species</th>
<th>% Known</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacteria</td>
<td>4,900</td>
<td>1,000,000</td>
<td>0.49</td>
</tr>
<tr>
<td>Fungi</td>
<td>75,000</td>
<td>1,500,000</td>
<td>5</td>
</tr>
<tr>
<td>Viruses</td>
<td>4,000</td>
<td>400,000</td>
<td>1</td>
</tr>
<tr>
<td>Protozoa</td>
<td>40,000</td>
<td>200,000</td>
<td>20</td>
</tr>
</tbody>
</table>

species, it is generally regarded that fungi probably constitute the most diverse group of microorganisms on earth\(^7\) (Table 1), followed by bacteria, viruses and protozoans.

Recent revolutions in prokaryote and eukaryote phylogeny

Whittaker’s 5 Kingdom classification recognized the Kingdoms Monera, Protista, Fungi, Plantae and Animalia\(^8\). This classification, drawing inspiration from many earlier evolutionary biologists, was by itself considered a major step in our understanding of the evolution of life, drastically different from earlier simplistic two Kingdom classification of Plantae and Animalia. Major discoveries in molecular biology during 1970’s and 1980’s led to yet another major revolution, this time related to the phylogeny of prokaryotes. Carl Woese\(^9\), based on 16S rRNA genes of bacteria, showed that the extremophilic bacteria, comprising the methanogens, hyperthermophiles and halophiles actually constituted a group very distinct from other bacteria. These findings have completely revolutionized the way we have looked at bacteria. Woese’s\(^9\) work has shown that what we conventionally understood as ‘bacteria’ have been evolutionarily much more complex and actually comprised two distinct groups, enough to justify classifying them under two separate Kingdoms, the Archaea and the Bacteria\(^9\). The 1980s also began to witness exciting new discoveries regarding the phylogeny of eukaryotes, with contributions from several evolutionary biologists\(^10\).

One of the major advancements was with regard to the Kingdom Protista or Prototista, which was in vogue even till 1990, albeit as a conglomeration of diverse, unrelated organisms\(^11\). Studies on the 18S rRNA gene, as well as many other functional genes with respect to phylogeny and evolution of this group revealed that several protistan groups were evolutionarily distinct, even justifying the erection of
new kingdoms to accommodate them. The last 15 years have seen much improvement with regard to our understanding of this group, resulting in numerous debates and continual transformation in classifying these organisms. Two of the most recent classifications of eukaryotes\textsuperscript{10, 12} are given in Table 2. Fungi, as defined above, occur within the Kingdom Mycenae (Kingdom Fungi) and the Kingdom Chromista (Kingdom Straminipila \textit{sensu} Dick\textsuperscript{4}). Following Adl \textit{et al.}\textsuperscript{12}, fungi are found in the Supergroup Opisthokonta, First Rank Fungi (Mycenae), as well as in the Supergroup Chromalveolata, First Rank Stramenopiles (Hyphochytriales, Labyrinthulomycetes and Peronosporomycetes).

### Marine fungal diversity: the basis

It is important to consider that fungi evolved in the oceans in order to assess the diversity of marine fungi. While opinions differ as to when exactly fungi evolved from their ancestors, it is now generally accepted that the event took place at least a billion years before present. According to Heckman \textit{et al.}\textsuperscript{13}, this event probably took place around 1500 million years ago (Ma) (Fig. 2). The earliest plants, the chlorophycean algae appear to have emerged 1100 Ma. It is also believed that plants conquered land much earlier than thought before, roughly around 1000 Ma, with the help of symbiotic fungi. Even if the exact dates of fungal origin are debated, fungi doubtlessly originated in the sea. This would mean that the lineages of early true marine fungi, which evolved in the sea prior to their conquest of land together with plants, probably continue to exist in the sea. As on A.D. 2000, about 450 species of obligate mycenaean marine fungi have been described\textsuperscript{14} (Table 3). Not many species have been described since then. Among the truly obligate marine fungi of the Kingdom Mycenae known at present, those belonging to the Ascomycotina and mitosporic fungi are the most common. Basidiomycetes and the earliest of true fungi, the chytrids, are few in number, the Zygomycotina being almost absent. When compared to nearly 70,000 species of fungi known so far on earth (out of 1.5 million estimated total number, a factor of ca. 20), the number of approximately 700 species of marine, mycenaean fungi is meagre. Even if the total number of obligately marine mycenaean fungi is estimated to be 14,000 (20 times that of 700), this represents a disappointingly poor diversity of fungi in the sea compared to land, casting even serious doubts about their importance in the marine ecosystem. The straminipilan fungi belonging to Labyrinthulomycetes, which are truly marine, are more frequent in the oceans than the mycenaean fungi, but even here the known number of species\textsuperscript{13} does not exceed about 50. The most ‘primitive’ of mycenaean fungi, the chytridiomycetes are also extremely rare in the marine environment. In this article, I would like to present arguments to support that our knowledge of the true diversity of marine fungi in the sea is very meagre and that most of the true marine species have not yet been discovered. My arguments are built around the following premises.

- **Mycenaean, as well as straminipilan fungi evolved in the sea.**
- **No mycenaean fungus which evolved in the sea has been described so far.** The so-called ‘obligate marine fungi’ that have been described are not truly marine. They were originally terrestrial fungi that subsequently migrated to the sea and became adapted to their marine habitat, and did not originate in

### Table 2—Two modern views of eukaryote classification. Groups containing fungi are shown in bold.

<table>
<thead>
<tr>
<th>Cavalier-Smith\textsuperscript{10}</th>
<th>Adl \textit{et al.}\textsuperscript{12}</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kingdom Protozoa</td>
<td>Supergroup Amoebozoa</td>
</tr>
<tr>
<td>Kingdom Fungi (Kingdom Mycenae)</td>
<td>Supergroup Rhizaria</td>
</tr>
<tr>
<td>First Rank Fungi (Mycenae in this paper)</td>
<td>Supergroup Excavata</td>
</tr>
<tr>
<td>Kingdom Animalia</td>
<td>Supergroup Opisthokonta</td>
</tr>
<tr>
<td>Kingdom Protista</td>
<td>First Rank Fungi</td>
</tr>
<tr>
<td>Kingdom Chromista</td>
<td>(Mycenae in this paper)</td>
</tr>
<tr>
<td>Subkingdom Cryptista</td>
<td>First Rank</td>
</tr>
<tr>
<td>Subkingdom Chromobiota</td>
<td>Mesomycetozoa</td>
</tr>
<tr>
<td>Infra kingdom Haptista</td>
<td>First Rank</td>
</tr>
<tr>
<td>Supergroup Archaeplastida</td>
<td>Choanomonoda</td>
</tr>
<tr>
<td>Kingdom Plantae</td>
<td>First Rank Metazoa</td>
</tr>
<tr>
<td>Kingdom Chromista</td>
<td>Supergroup Chromalveolata</td>
</tr>
<tr>
<td>Subkingdom Cryptista</td>
<td>Rank Cryptophyceae</td>
</tr>
<tr>
<td>Subkingdom Chromobiota</td>
<td>First Rank Haptophyta</td>
</tr>
<tr>
<td>Infra kingdom Heterokonta</td>
<td>First Rank Stramenopiles</td>
</tr>
<tr>
<td>(containing the</td>
<td>(containing the fungal groups</td>
</tr>
<tr>
<td>Hyphochytriales,</td>
<td>Haptophyta</td>
</tr>
<tr>
<td>Labyrinthulomycetes and</td>
<td>First Rank</td>
</tr>
<tr>
<td>Peronosporomycetes)</td>
<td>Alveolata</td>
</tr>
</tbody>
</table>

\text{\textit{Note: This table shows the classification of eukaryotes with an emphasis on fungi.}}


**\textsuperscript{14}** Table 3—Species diversity of marine fungi.

<table>
<thead>
<tr>
<th>Kingdom</th>
<th>Number of Species</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mycenae</td>
<td>450</td>
</tr>
<tr>
<td>Stramenopiles</td>
<td>70</td>
</tr>
<tr>
<td>Labyrinthulomycetes</td>
<td>50</td>
</tr>
<tr>
<td>Peronosporomycetes</td>
<td>20</td>
</tr>
</tbody>
</table>

**\text{\textit{Note: This table shows the species diversity of marine fungi as per different classifications.}}**
The extremely high biodiversity of terrestrial fungi known so far, as compared to marine fungi is the likely result of our culture methodology and our notions of fungal morphology (predominantly mycelial).

All original marine mycenean fungi (those that evolved in the sea) may not have become extinct. Such truly marine fungi await discovery.

The stramenipilan fungi are likely to be extremely diverse in the sea.

Many of our conventional notions on marine fungal diversity have begun to crumble in the light of recent discoveries. In the rest of this article, I shall try to substantiate the above arguments using the following points.

1. Isolation methods based on conventional culturing may not reveal the true diversity of marine fungi.
2. Many marine fungi may not fulfil the morphological criteria that we routinely use to define fungi.
3. Marine fungi may be found in extreme environments.
4. Molecular tools will reveal a much greater diversity of marine fungi than so far.

The stramenipilan fungi are likely to be extremely diverse in the sea. Many of our conventional notions on marine fungal diversity have begun to crumble in the light of recent discoveries. In the rest of this article, I shall try to substantiate the above arguments using the following points.

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2. Many marine fungi may not fulfil the morphological criteria that we routinely use to define fungi.
3. Marine fungi may be found in extreme environments.
4. Molecular tools will reveal a much greater diversity of marine fungi than so far.

I shall examine these possibilities in the light of discoveries made regarding prokaryotes.

### Uncultured diversity of bacteria and fungi

It is now a well-recognized fact that ‘unculturable’ bacteria, a term that I would rather substitute with ‘uncultured bacteria’, in the sea are much more abundant than those that have been cultured. This ‘great plate count anomaly’ has been recognized for more than two decades now, following the employment of epifluorescence direct detection methods to count the bacteria (Fig. 3). Total bacterial numbers in...
seawater amount to a few billion cells per ml in coastal waters and a few million cells per ml in oceanic waters\textsuperscript{17}. Culturable numbers, on the other hand are in the range of a few hundred cells per ml. In other words, only about 0.01% of bacteria that are actually present come into culture, while the rest of > 99 % do not grow in culture. Yet this does not necessarily imply that > 99 % of the bacteria are unknown in terms of species diversity. Of the total number of bacteria present in the water column, only a few may be active, some others active but finicky when it comes to culturing and the others ‘sleepy’, refusing to come out of dormancy. In a study on bacteria in coastal waters, Ramaiah \textit{et al.}\textsuperscript{18} examined the total counts of bacteria using the acridine orange direct count method, culturable bacteria using nutrient agar plates and active, but uncultured bacteria using the nalidixic acid method. Their results (Table 4) showed that while only 23 to 41 % of the total bacteria were physiologically active and viable, an even smaller portion of 0.04 – 0.007 % of bacteria could be brought into culture. The discrepancy between cultured and uncultured bacteria certainly signifies that we require more than culture tools to assess the true diversity of marine bacteria. Molecular methods have come in handy for this task.

A similar example as for bacteria may be cited for the stramenipilan fungi, the thraustochytrids, aplanomyctids and labyrinthulids belonging to the Labyrinthulomycetes in the water column. Thraustochytrids are unicellular, produce plasma membrane extensions, the ectoplasmic net elements, and reproduce by means of zoospores. As is typical of the Kingdom Straminipila, these zoospores possess two unequal (heterokont) flagella, the longer anterior flagellum bearing tripartite hairs\textsuperscript{5}. They are also characterized by mitochondria with tubular cristae. Aplanochytrids may be unicellular or colonial and reproduce by spores that glide using ectoplasmic net elements, or by zoospores as above. Labyrinthulids are colonial and move within ectoplasmic net elements. Despite the fact that the first thraustochytrid was described by the great mycologist F.K. Sparrow\textsuperscript{19} nearly 70 years ago and that labyrinthulids have been known for a long time, only about 50 species of Labyrinthulomycetes are known so far. These fungi can be detected using the acriflavine direct detection (AfDD) method\textsuperscript{20} (Fig. 4) and cultured from natural samples using simple organic nutrient media. Several studies in recent years have demonstrated high abundance of Labyrinthulomycetes in the water column, using the AfDD method. We enumerated

<table>
<thead>
<tr>
<th>Location and season</th>
<th>Total counts</th>
<th>Direct viable counts</th>
<th>Culturable numbers</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positra, February 1997</td>
<td>$2.42 \times 10^6$ ml$^{-1}$</td>
<td>$1.01 \times 10^6$ ml$^{-1}$</td>
<td>170 ml$^{-1}$</td>
</tr>
<tr>
<td>Padubidri, May 1997</td>
<td>$3.14 \times 10^6$ ml$^{-1}$</td>
<td>$0.72 \times 10^6$ ml$^{-1}$</td>
<td>160 ml$^{-1}$</td>
</tr>
<tr>
<td>Kulai, May 1997</td>
<td>$2.21 \times 10^6$ ml$^{-1}$</td>
<td>$0.66 \times 10^6$ ml$^{-1}$</td>
<td>1010 ml$^{-1}$</td>
</tr>
<tr>
<td>Ennore, April 1997</td>
<td>$2.53 \times 10^6$ ml$^{-1}$</td>
<td>$0.38 \times 10^6$ ml$^{-1}$</td>
<td>350 ml$^{-1}$</td>
</tr>
</tbody>
</table>

Fig. 3—A) Epifluorescence photomicrography of acridine-orange stained bacterial cells in a seawater sample. x 1500. 3-B) Epifluorescence photomicrography of acriflavine stained thraustochytrid cells (arrows) in seawater sample x 1000. Bar in both photographs represents 10 µm.
Labyrinthulomycetes in the water column of the Arabian Sea, using both the AfDD method, as well as culture methods\textsuperscript{21} (Table 5). Cultured thraustochytrids amounted to only 0.06 to 3.65 \% of the total thraustochytrids. In general, we have met with very little success in culturing thraustochytrids from oceanic waters.

As with uncultured bacteria, then, the following questions need to be addressed.

1. Will novel culture methods reveal greater thraustochytrid diversity?
2. Has thraustochytrid diversity been underestimated because many of them have not been cultured?
3. Can a clue be obtained using molecular diversity?

**Culturing the uncultured**

Studies on 16S rRNA gene sequences from natural samples in the 1990s indicated that the hitherto uncultured group of bacteria termed the SAR-11 group was actually dominant in the water column. In other words, nearly 50 years of marine bacteriology was not aware of this important group of bacteria. One of the major achievements in marine bacterial diversity in recent years was the culturing of a member of the ubiquitous SAR-11 group of pelagic bacteria, *Pelagibacter ubique* by Rappé et al.\textsuperscript{22} (see also Giovannoni & Stingl\textsuperscript{23}). Connan & Giovanni\textsuperscript{24} cultured diverse new marine microorganisms using high-throughput methods in low-nutrient media. A scheme of this method is given in Fig. 5. Such methods need to be applied also to marine fungi to discover hitherto uncultured species. This is particularly true of the thraustochytrids, which are conventionally isolated by baiting with pine pollen or brine shrimp larvae, or plated on organically rich nutrient media\textsuperscript{25}. It is obvious that such methods will be highly selective to species that require high amounts of nutrients.

Marine mycelial fungi are generally isolated using two methods. They are either isolated on to nutrient media from their spores on natural materials, particularly of lignocellulosic nature, after sporulation following moisture-chamber incubation, or by plating various substrata on nutrient media (see Kohlmeyer & Kohlmeyer\textsuperscript{26}). Yet, terrestrial mycologists have always used a wide variety of methods with the aim of isolating a high diversity of species in culture, a practice not often followed by their marine counterparts. Such methods include the particle

Table 5—Total and cultured numbers of thraustochytrids in the water column at a few locations in the Arabian Sea (based on Raghukumar et al.\textsuperscript{21}).

<table>
<thead>
<tr>
<th>Location</th>
<th>Numbers based on culturing</th>
<th>Total numbers based on acriflavine direct detection method (AfDD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>19ºN,69º50’E</td>
<td>81 L\textsuperscript{−1}</td>
<td>130 × 10\textsuperscript{3} L\textsuperscript{−1}</td>
</tr>
<tr>
<td>15ºN,64ºE</td>
<td>146 L\textsuperscript{−1}</td>
<td>40 × 10\textsuperscript{3} L\textsuperscript{−1}</td>
</tr>
<tr>
<td>21ºN,64ºE</td>
<td>53 L\textsuperscript{−1}</td>
<td>55 × 10\textsuperscript{3} L\textsuperscript{−1}</td>
</tr>
<tr>
<td>21ºN,67º50’E</td>
<td>0 L\textsuperscript{−1}</td>
<td>3 × 10\textsuperscript{3} L\textsuperscript{−1}</td>
</tr>
</tbody>
</table>
using the ‘dilution to extinction’ method discussed above. It is likely that many true marine fungi are extremely fastidious to growth and the culture media that are used to isolate terrestrial fungi may not be useful for marine fungi. There are no clear directions yet as to how we should approach to culture unknown marine fungal diversity. This is of no surprise. It is only now that marine bacteriologists who vastly outnumber marine mycologists are learning to cope with the problem. It is to be hoped that marine mycologists will discover their own unique methods to culture the true marine fungi.

**Culture-independent diversity, using molecular tools**

The molecular revolution of marine bacterial diversity can be said to have started with the discovery of the ‘great plate count anomaly’, emphasized by Jannasch & Jones in 1959 and Staley & Konopka in 1985 (see ref 1) and subsequently confirmed innumerable times by marine microbiologists. This stimulated Norman Pace and his group to develop ideas for identifying microbes from natural systems using gene cloning. Giovannoni & Stingel made pioneering studies on marine bacteria using gene cloning. A general methodology for studying diversity of microorganisms by these methods is schematically presented in Fig. 5. Central to this methodology is the extraction of the total community nucleic acids from environmental samples and preparing a database of rDNA gene sequences. These sequences can then be analysed for the diversity of a given group of organisms, and also to prepare organism-specific probes which can be used further to examine natural samples for the presence and abundance of specific organisms. Using these methods, microbial diversity of a natural sample can be assessed independent of culturing methods, provided there is a reliable database of the rDNA gene sequences of the group of interest. Fortunately, such databases are now available for most groups of organisms, including fungi and the databases are getting continually improved. In the case of bacteria, such studies have revealed the following facts:

- The most abundant bacterioplankton have never been cultured.
- The major marine prokaryotic groups appear to have cosmopolitan distributions.
- A relatively small number of 9 uncultured marine bacterioplankton clades account for 80% of 16S rRNA gene clones.
- Marine Archaea are abundant and fall within two groups.
- Much genetic diversity occurs within the major prokaryotic plankton.
- Particle-associated and freely suspended marine prokaryotes are different.
- Stratification of bacterioplankton populations is typical of the ocean surface layer.

We are yet to make such advances in the case of eukaryotic microbes in the oceanic water column.

**Do true marine fungi look radically different from terrestrial fungi?**

Two extremely interesting publications appeared in 2001 on the molecular eukaryotic diversity in the oceans. Lopez-Garcia et al. examined the molecular diversity of eukaryotes within the range of 0.2 – 5 µm from the aphotic zone of 250 – 3000 m of the Atlantic polar front. Moon-vander Staay et al., likewise, studied picoplankton below the size fraction of 3 µm at a depth of 75 m in the equatorial Pacific. Their studies, using 18S rRNA signatures, as given above, revealed the startling fact that fungal signatures were...
The discovery of picoplanktonic, photoautotrophic cyanobacteria was discovered more than 50 years ago by Butcher in 1952 and was further confirmed by studies using microscopy and pigment composition. Further studies on the presence and importance of these phytoplankton of bacterial size, belonging to species of *Synechococcus* in 1979 and the discovery of species of *Prochlorococcus* in 1988 heralded research on picoplanktonic eukaryotes. Earlier studies on prokaryotic, autotrophic picoplankton also revealed a complex assemblage of phototrophic eukaryotes of < 3 \( \mu m \). Some of the small eukaryotic photosynthesizers were subsequently described as members of a new order, Parmales (see Moreira & Lopez-Garcia). The smallest eukaryote ever found, *Ostreococcus tauri*, is a member of the Prasinophyceae and has a cell size of 0.8–1.1 \( \mu m \times 0.5–0.7 \mu m \). It contains a compact, tightly packed cellular organization comprising the nucleus, one mitochondrion and one chloroplast tightly. Such a cellular organization also appears in other photosynthetic picoeukaryotes such as the Bolidophyceae.

It was not till the two publications given above that heterotrophic eukaryotes of the picoplankton size were discovered. Lopez-Garcia et al. found signatures of picoplanktonic true fungi at a depth of 3000 m in the Antarctic waters, picoplanktonic thaustochytrids at 2000 m and a new lineage of heterokonts, close to Oomycetes, at 250 m. Moon van der Staay et al. discovered molecular signatures of eukaryotic Oomycetes and an early heterotrophic divergence of stramenopiles in the Pacific waters. Significantly, the signatures observed in these waters revealed what appeared to be novel picoplanktonic organisms belonging not only to fungi, but also to the alveolates (ciliates and dinoflagellates) and heterokonts (stramenipilan organisms, including stramenipilan fungi). Among fungi, these authors discovered a novel group of sequences at the base of fungi and among the stramenipilan fungi, three phylotypes distantly related to thaustochytrids and labyrinthulids. Scanning electron micrographs of picoeukaryotes from 200 m deep Mediterranean waters showed cells in the range of 1 \( \mu m \) or less, one of these with heterokont flagellae, as in the stramenopiles.

It now appears possible that picoplanktoic single-celled fungi may be prevalent in the sea. If so, a fresh look at our conventional notions of how a fungus looks may need revision. It is also possible that marine single-celled mycenaean fungi have so far been placed wrongly as protozoans. Several members of choanozoans are a case in point. Choanozoans occupy a crucial position in understanding the origin of animals and fungi from a common ancestor. It has recently been shown that at least some of the members of choanozoans may actually lie at the base of fungal evolution. Thus, the single-celled nucleariids have been shown to be a sister clade to mycenaean fungi. The single-celled fish parasites, the ichthyosporeans too are probably similarly related. Sumathi et al. have recently shown that the single-celled choanozoan protist, *Corallochytrium limacinisporum* Raghukumar, actually possesses the fungal signatures ergosterol and the \( \alpha \)-aminoreductase gene of the lysis synthesis pathway, thus displaying a relationship to fungi.

The mycelial form of fungi is suited to penetrate particulate organic matter and traverse space filled with air. Such a form is unsuitable for organisms living planktonically in the water column, a habitat that favours single-celled forms more. Therefore, as surmised in the beginning of this article, it may well turn out that fungi at the base of the clade of the Kingdom Mycenae are single-celled and not mycelial and are typical inhabitants of the sea. A search for such organisms in the future may actually enhance our knowledge on the diversity of marine fungi. **Fungi in extreme marine environments**

Fungi have never been considered to be truly anaerobic in respiration. The only obligate fungal anaerobes known so far belong to the Neocallimasticales belonging to chytrids. No other such fungi have been discovered so far. Could anaerobic fungi be prevalent in the sea? The work of Dawson & Pace using 18S rDNA gene diversity in intertidal, black, anoxic sediments has revealed the extensive presence of eukaryotes in these habitats. These authors discovered numerous novel branches among the stramenopiles, including the fungal groups of oomycetes, hypochytriomycetes and labyrinthulomyctes, as well as among protozoans. They further suggested that anaerobic eukaryotes may be the phylogenetically most diverse organisms in the sea. Likewise, Stoeck & Epstein, studied the diversity of micro-eukaryotes in the suboxic waters and anoxic sediments of salt marshes. Most of the sequences represented deep, novel branches within green plants, cercozoans, and alveolates, as well as within...
mycenaen and stramenopilan fungi. Further work of Stoeck et al.\textsuperscript{38} on the permanently anoxic waters of the Cariaco Basin in the Caribbean Sea revealed organisms belonging to deep branches within the above groups and three novel lineages branching at the base of the eukaryotic evolutionary tree. These preceded, were contemporary with, or immediately followed the earliest eukaryotic branches. A significant number of these appeared to be new organisms. They\textsuperscript{38} suggested that these newly discovered protists might retain traits reminiscent of an early eukaryotic ancestor(s), at the base of the evolutionary trees. These exciting papers\textsuperscript{28-30, 34, 36-39} have a tremendous implication to marine fungal diversity.

Two of the exciting locations for investigations on anaerobic fungi in the Arabian Sea are the permanent oxygen minimum zone (OMZ) of the northern and central Arabian Sea and the seasonal OMZ of the west coast of India\textsuperscript{39}. It has been well known that the former is characterized by high densities of denitrifying bacteria. Jayakumar \textit{et al}.\textsuperscript{40} examined the diversity in the Arabian Sea OMZ of the prokaryotic \textit{nirS} gene sequences involved in the conversion of nitrite to nitric oxide. These authors found 12 major clusters, most of which did not show a high level of identity with other \textit{nirS} sequences reported earlier. The dominant type in one of the surface samples was close to \textit{Pseudomonas aeruginosa}. Highest diversity was found in samples with high nitrite. It is likely that fungi characterized by anaerobic denitrification also occur in such regions. Thraustochytrids are present in high numbers at these OMZ depths and their physiology is likely to provide us some surprise. Indeed, recent reports found several terrestrial fungi capable of converting nitrate to ammonia through denitrifying processes\textsuperscript{41}. Preliminary work carried out on such fungi in the coastal waters of Goa (Sumathi, personal communication) has already yielded several fungi, albeit belonging to known species, which are capable of denitrification.

**Exotic habitats of marine fungal diversity**

Several exotic marine ecosystems are yet to be studied intensively for fungal and other microbial diversity. Coral reefs, the deep sea and hydrothermal vents are some of the examples. Few obligate marine fungi have been reported from these habitats. For example, the Kohlmeyers described several marine fungi from dead corals\textsuperscript{42}. Surprisingly, not many have been added to this list since then, contrary to that of lignicolous fungi. Lignicolous marine fungi presently comprise about 450 species, new species having been discovered consistently since the seminal paper of Barghoorn \& Linder in 1944 (see Hyde \textit{et al}\textsuperscript{14}).

Bacteria associated with invertebrates, especially in coral reef habitats is an exciting new area, of particular interest in the discovery of bioactive molecules\textsuperscript{43, 44}. Although several fungi associated with sponges and producing interesting new molecules have been reported, all such species belong to terrestrial genera and few obligate marine fungi associated with such animals have been reported so far. This is a fertile area for future research. Similar is the case with deep-sea fungi. Terrestrial species of fungi adapted to deep-sea conditions probably play an important role in the ecology of deep-sea sediments\textsuperscript{45}. Fungal mycelia are easily detected in such sediments and fungi are probably prevalent in these habitats. Kohlmeyer \& Kohlmeyer\textsuperscript{26} described obligate deep-sea fungi from decaying wood and bryozoan shells. Few have been described since then.

The evolutionary history of fungi strongly suggests that obligate marine fungi, unlike those reported so far from the sea are bound to exist. Biodiversity-rich ecosystems such as the coral reefs, the deep-sea and anoxic sediments are bound to harbour a high diversity of obligate marine fungi. Unfortunately few have been described so far. The paucity in our knowledge on the real diversity of obligate marine fungi in the above habitats brings us back to the questions that we asked in the beginning and we may conclude as follows.

1. We might have failed to recognize obligate marine fungi in exotic habitats because they look different from our conventional wisdom of fungi. \textit{Corallochytrium limacisporum} Raghukumar, an inhabitant of coral reefs waters, the nucleariids and the ichthyosporeans discussed earlier are possible examples of atypical fungi in the marine environment.
2. Culture methods for marine fungi have been highly inadequate so far.
3. Molecular methods will provide us greater insights into marine fungal diversity.

**References**

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