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Evolution and Physiology of Amphibious Yeasts

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Abstract

Since the emergence of the first fungi some 700 million years ago, unicellular yeast-like forms have emerged multiple times in independent lineages via convergent evolution. While tens to hundreds of millions of years separate the independent evolution of these unicellular organisms, they share remarkable phenotypic and metabolic similarities, and all have streamlined genomes. Yeasts occur in every aquatic environment yet examined. Many species are aquatic; perhaps most are amphibious. How these species have evolved to thrive in aquatic habitats is fundamental to understanding functions and evolutionary mechanisms in this unique group of fungi. Here we review the state of knowledge of the physiological and ecological diversity of amphibious yeasts and their key evolutionary adaptations enabling survival in aquatic habitats. We emphasize some genera previously thought to be exclusively terrestrial. Finally, we discuss the ability of many yeasts to survive in extreme habitats and how this might lend insight into ecological plasticity, including amphibious lifestyles.

Contents

1. INTRODUCTION

Fungi are a diverse and monophyletic group of eukaryotes found in virtually all environments on our planet. While the majority of described fungal species are terrestrial, increasing evidence suggests that aquatic environments harbor a large and phylogenetically diverse group of fungi as well. Marine species, for instance, account for only 1% of the total number of currently described fungi on Earth (11, 54, 107). This estimate illustrates how far we are from understanding the diversity and functions of fungi in aquatic environments and the difficulties in isolating and culturing those species (3, 35, 46).

Since the divergence of the last common ancestor of fungi over a billion years ago (55, 56, 81, 97), fungi have diversified radically in their morphologies, trophic strategies, metabolic capacities, and habitats. Two notable evolutionary events coincided with fungal emergence onto land and development of the yeast cell. The loss of the flagellum marked a transition from aquatic to land environments, where motility became less critical to fungal life cycles. Concurrently, the development of hyphal structures likely enabled foraging for resources and saprotrophy. Intriguingly, reverse transitions from a filamentous back to a single-celled lifestyle occurred repeatedly and independently throughout the fungal kingdom via convergent evolution to generate characteristic yeast forms (90).

Because of their shape and limited foraging ranges, yeasts maintain close and sustained contact with other microbes, with which they interact physically and chemically at the microscale. Several lines of evidence indicate that yeasts' interactions with viruses and bacteria are an important component of yeast evolution and ecology (1, 61, 80, 110). Recent studies suggest that viruses may play an important role in regulation of yeast populations (6, 12, 99). In oceans, sediments, lakes, and other environments, yeast-bacterial interactions span the mutualist-antagonist continuum. Beneficial interactions include biofilm formation and nutrient exchange as well as gain of function through horizontal gene transfer (1, 53, 61, 67). Antagonistic relationships are also well documented (80).

Because fungal research has long been biased toward terrestrial habitats and hosts, we are likely underestimating the number of aquatic species (3, 46). While many described yeast species are assumed to be principally adapted to terrestrial environments, an increasing number of studies suggest that a large number of these species also thrive in aquatic environments. A large number of these species are found both in aquatic habitats and on land and are potentially amphibious (2, 17) (see the glossary and table 1 in the **Supplemental Material**). Increasing evidence suggests that these fungi possess highly diverse, novel metabolic capacities, mechanisms of cell division and replication, and other adaptations related to expansion into aquatic habitats. However, given the comparative lack of effort to isolate, characterize, and sequence these fungi, we lack information on mechanisms and strategies enabling them to transition from terrestrial to aquatic niches and vice versa. Given the importance of yeasts for our biosphere, it is critical to understand how they evolve and adapt to new environments. In this review we discuss the diversity and key evolutionary adaptations to aquatic habitats (**Figure 1**). We give specific emphasis to some genera previously thought to be exclusively terrestrial, and we highlight key adaptations to aquatic and
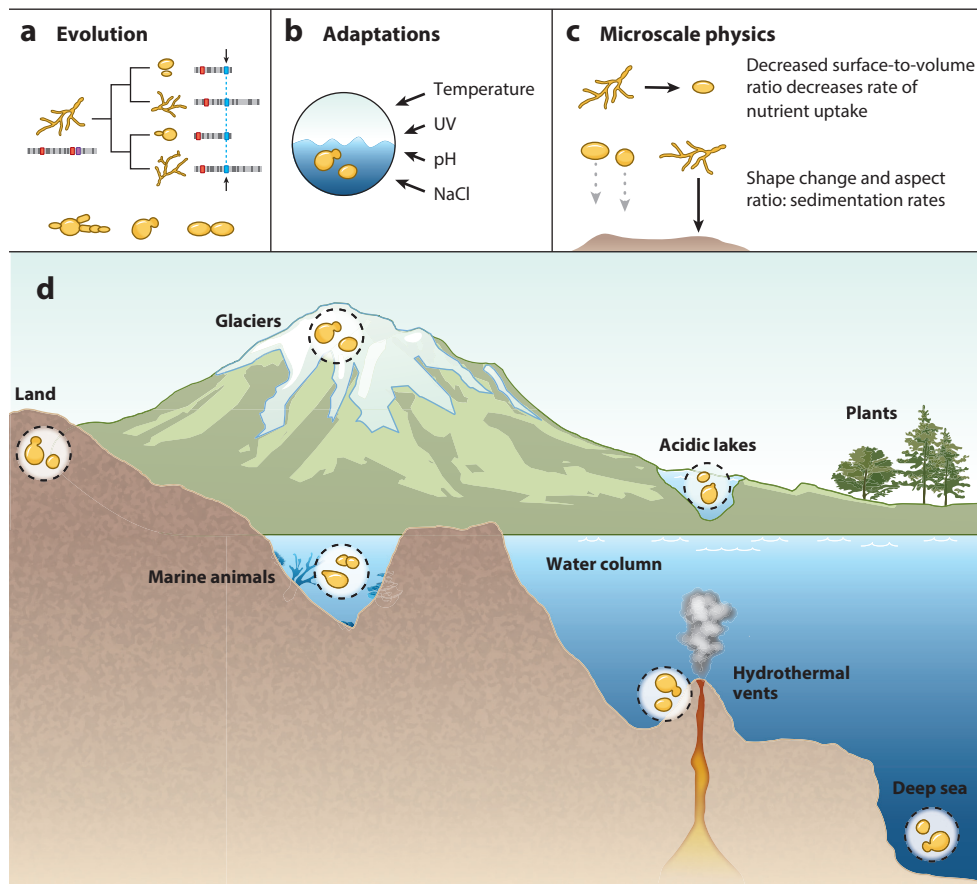
Figure 1

Key ideas discussed in this review. (*a*) Convergent evolution of the yeast lifestyle, with reduced genomes and different modes of reproduction. (*b*) Adaptations of amphibious yeasts to different stressors and physicochemical conditions. (*c*) Surface-to-volume ratio and sedimentation rates change as a consequence of transitions from filamentous to single-celled yeast morphology. (*d*) Distribution of amphibious yeasts in diverse environments.

extreme environments and provide new insights into their evolution and modes of reproduction. We further discuss how the physical forces operating at microscale might shape the evolution and the ecology of amphibious yeasts.

2. ORIGIN AND EVOLUTION OF THE YEAST LIFESTYLE

2.1. Convergent Losses of Multicellularity

We know comparatively little about the early evolution of fungi, due to the lack of sufficient fossil information and conflicting phylogenetic placements (22, 64). For these reasons, we lack reliable inference about the habitat and physiology of the last common ancestor of all fungi (see **Supplemental Material**). However, several lines of evidence point toward a likely aquatic origin for fungi (63) followed by high diversification during land colonization (Section 3.1).

While there are multiple routes by which fungi evolved from unicellular ancestors to multicellular forms (89), transitions to the yeast lifestyle appear to be secondary in the evolution of fungi (**Figures 1**, **2**). Yeast evolution likely accompanied genome reduction in a much more complex multicellular ancestor (90) (**Figure 2**; **Supplemental Material**, table 1). Although the genomic potential for yeast growth appeared once around 770 million years ago (31), evidence suggests that a truly yeast-like phenotype occurred much later. This emergence of the yeast phenotype is attributed to the convergent evolution of regulatory mechanisms appearing throughout the Dikarya (90). The majority of yeasts belong to this subkingdom, which originated concurrently with the appearance of the first land plants some 500 million years ago.
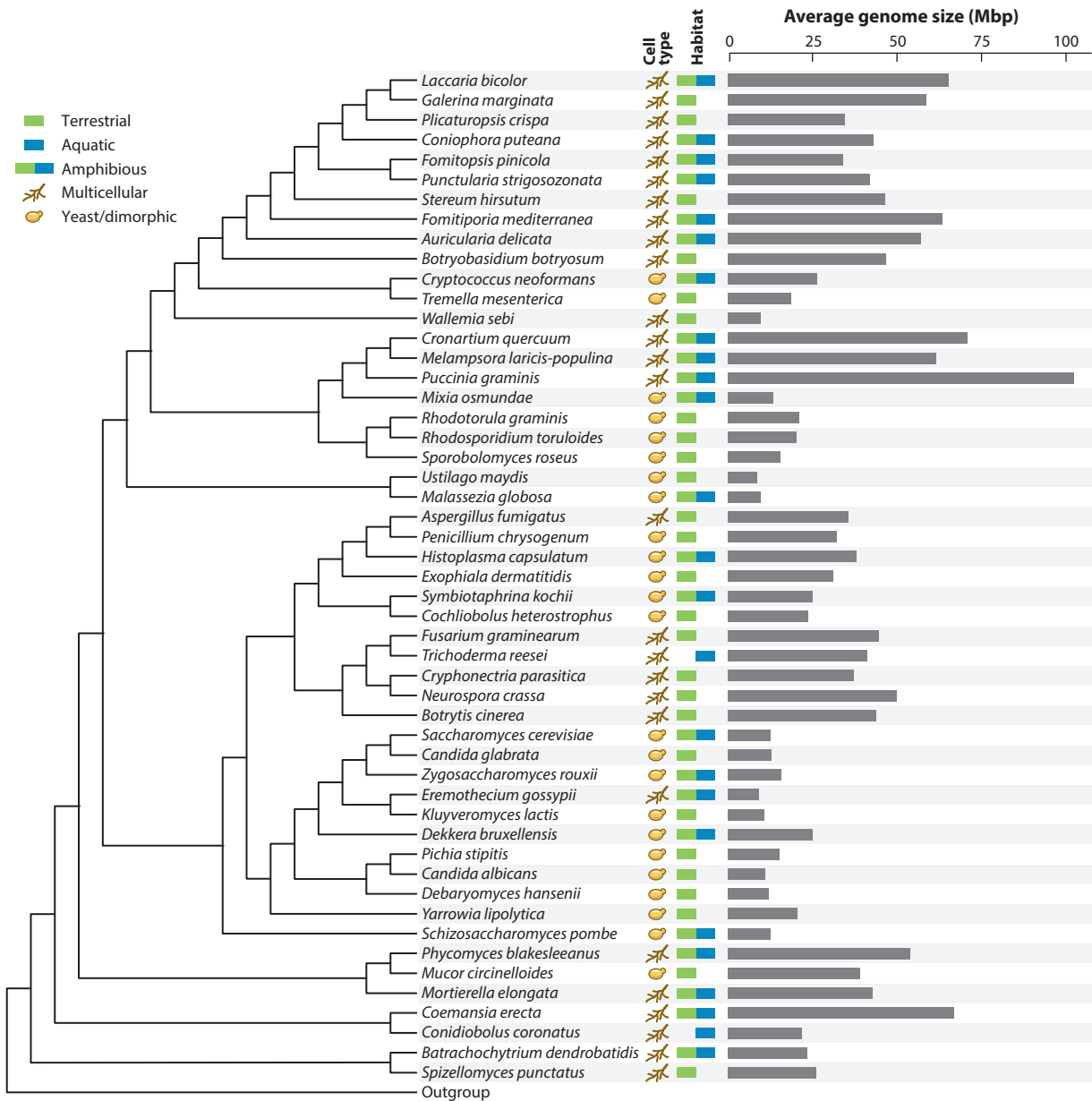
Yeasts are a polyphyletic group of fungi that spend the majority or entirety of their life cycle as unicellular forms (**Figure 3**). Interestingly, many yeast species with a dominant unicellular stage can exhibit filamentous forms, and several primarily filamentous species can transform into yeast-like forms under certain growth conditions (dimorphism), probably highlighting their complex ancestral origin (89). Yeasts exhibit strikingly similar genomic, phenotypic, and physiological characteristics despite their divergence tens to hundreds of millions of years ago. Several molecular mechanisms leading to trait convergence have been proposed (119). The yeast-like phenotype can be explained by both homology, including deep homology, and homoplasy as well as by pleiotropic latent homologies (90). The latter idea implies that similar traits in distant lineages can be generated repeatedly if they can readily be recruited for the same new functionality (96). This reduces the mutational target size for evolution (89), leading to a higher probability of convergence than if genes mutated independently. While homology refers to phenotypic similarities due to shared ancestry in related species, "deep homology" refers to shared complex regulatory wiring, inherited from a common ancestor, that underlies independent phenotypic traits (89, 113), suggesting that shared mechanisms of fruiting body initiation in Ascomycota and Basidiomycota may be an indication of deep or latent homologies underlying their evolutionary history.

Convergent evolution is a fundamental mechanism of adaptations to similar selective pressures, and it appears to be particularly common throughout the evolution of the fungal kingdom (**Figure 2**). The yeast lifestyle has evolved in divergent lineages, with similar phenotypic and physiological characteristics, via independent reduction of complexity in multicellular ancestors.

2.2. Advantages of the Yeast Lifestyle

The yeast lifestyle describes single-celled or dimorphic fungi with a dominant unicellular stage (**Figure 3**). Yeasts typically have compact and streamlined genomes with few introns and intergenic regions, and they have rapid metabolism and fast growth (91). All of these characteristics suggest that yeasts have lost the main innovations gained among terrestrial fungi, such as mycelial growth, secretion of some digestive enzymes, and control of their environment via expression

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Figure 2

Independent evolution of yeast-like forms in diverged clades. Species that have a yeast phase are indicated. The green rectangles represent terrestrial habitats, while the blue ones represent aquatic habitats. The gray rectangles represent the average genome size for a given genus. The phylogeny was adapted from Reference 90, and habitat data were collected in October 2020 from the GlobalFungi database (**https://globalfungi.com**).
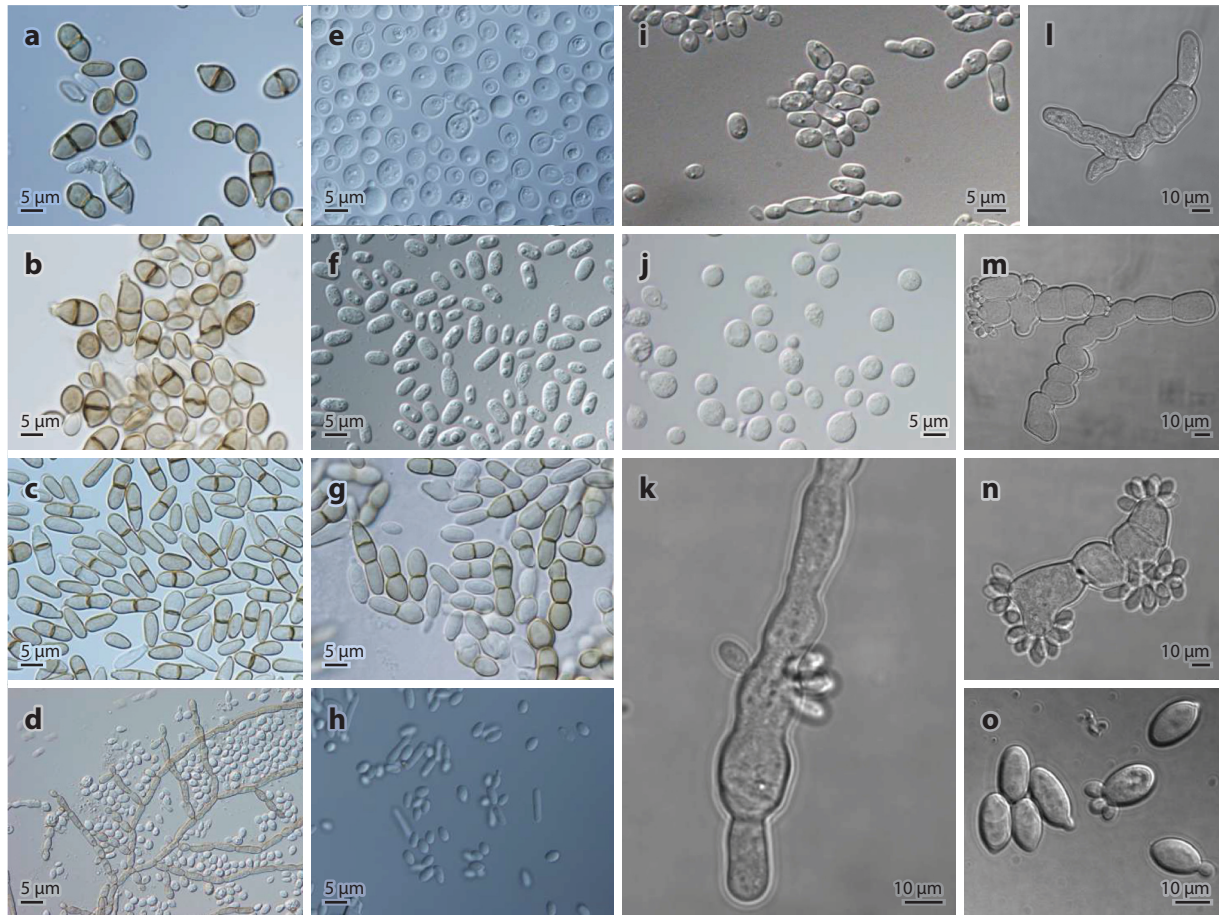
Figure 3

Modes of reproduction and the intra- and interspecies diversity of cell shape and size among some amphibious yeasts. (*a–c,g*) *Hortaea werneckii*. (*d*) *Exophiala phaeomuriformis*. (*e*) *Bullera* sp. (*f*) *Rhodosporidiobolus oreadorum*. (*h*) *Meyerozyma guilliermondii*. (*i*) *Camptobasidium gelus*. (*j*) *Papiliotrema laurentii*. Images *k–o* represent different modes of reproduction and division of unidentified yeasts.

of secondary metabolites. One notable exception to this characterization is that some yeasts can produce ethanol, which provides a competitive advantage over ethanol-intolerant species. Yeasts are estimated to have lost between 3,000 and 5,000 genes as a result of transitions from more complex ancestors (90, 95). This lack of characteristic phenotypic and metabolic fungal traits implies that yeasts must evolve in environments where selection acts on efficient use of resources and rapid processing of available nutrients, a niche generally dominated by certain lineages of bacteria and archaea.

The characteristics of yeast-like forms, with their reduced genomes, slow growth, and small cell size, likely confer considerable advantages for adaptation to oligotrophic and changing environments (i.e., from terrestrial to aquatic environments and vice versa).

2.3. Yeasts' Functional Morphology: The Challenging Life at Microscale

Form and function are generally intimately linked, and studies show that marine microorganisms are no exception (120). Recent advances in microfluidic approaches show that physical properties

that have no importance at macroscale, such as viscosity, Brownian motion, molecular diffusion, and kinematic reversibility, for instance, have profound consequences on most planktonic life. At small scales, these properties can influence processes as diverse as nutrient uptake, sedimentation, motility, cellular interactions, biofilm formation, and dispersal (28, 47, 74, 105, 111, 112, 120). Such studies are largely absent for yeasts, so we can only speculate as to how yeast morphology impacts the aquatic fungal lifestyle (**Figure 1**). Moreover, yeast biology and ecology are typically studied over large spatiotemporal scales, even though the microenvironments of yeasts are likely more informative. To better understand the selective pressures operating at microscale in the microenvironment of individual yeast cells, the yeastosphere (a new term we introduce here; see the glossary in **Supplemental Material**), which is analogous to the rhizosphere of plants and the phycosphere of planktonic cells, should be considered (112, 121).

Microorganisms live in low–Reynolds number (Re) (see the glossary in **Supplemental Material**) environments where viscous forces dominate advective inertial forces. In practical terms, this means that for a microorganism to keep moving it has to constantly exert energy. If, for instance, a 1-μm bacterium stops rotating its flagellum, it will stop moving in less than 0.3 ms and the coasting distance will be less than 1 Å (five times less than the diameter of a hydrogen atom). Consequently turbulence does not occur, as flow simply follows pressure differentials in a pattern known as Stokes flow or creeping flow (28). Another characteristic of physics at the size scale of small yeasts is that water molecules constantly move cells in a random pattern. This is known as Brownian motion. As a consequence, cells cannot maintain a constant orientation. While at large scales (high Re numbers) chemicals are transported via air or water flows, molecules at the microscale are transported by diffusion, which is much faster.

2.3.1. Dispersal. How size and shape influence dispersal of cells that are not actively motile is a fundamental question for the ecology and biology of yeasts. For microscopic organisms the diffusion coefficient (D) of a small particle or cell can be calculated using the Stokes-Einstein equation: $D = k_B T / 6\pi\eta r$ (84) (where k_B is the Boltzmann constant, T is the absolute temperature, η is the dynamic viscosity, and r the radius). Einstein estimated that a particle 1 μm in diameter (in water at 17°C) is displaced by 0.8 μm in 1 s (29). Therefore a small bacterium (~1 μm) such as *Pelagibacter* is displaced by Brownian motion to a new location about every second. However, if the cell's diameter is just 10 times larger, the cell's dispersal will be a thousand times slower (i.e., will take 20 min) (28). If the cell is 100 times larger (in the size range of diatoms or a dinoflagellate, for instance), the time to move to an adjacent location by Brownian motion would be over 30 years (28). Therefore, while Brownian motion might be useful for dispersing small yeasts (~2–5 μm) it is not efficient for yeasts that are slightly larger (5–10 μm) (see the **Supplemental Material** for an extended discussion on spores' dispersal).

2.3.2. Sedimentation. Sedimentation is the process by which a particle sinks under the force of gravity. It plays a major role in the chemical and biological structure of lakes, oceans, and seas.

The tendency of a particle to sink depends mainly on its density. Given that most cells are denser than water, they tend to sink even in highly salty seawater despite their small size. As deduced from Stokes's estimation of sinking velocity given a particle's size (62), a cell 1 μm in diameter would sink about 1 mm per day while a 100-μm cell would sink about 10 m a day (28). Most motile microorganisms can swim faster than they sink. However, nonmotile organisms, such as yeasts, rely on buoyancy mechanisms to counter the force of gravity. Many aquatic unicellular organisms, including yeasts, modulate their density using gas vacuoles, polysaccharide mucilage sheaths, or glycogen. Vij et al. (127) recently reported that some amphibious cryptococcal cells use their polysaccharide capsule to reduce cell density by increasing volume without significantly

increasing cell mass, a mechanism previously reported in bacteria. Aside from cell size, a microorganism can slow its sedimentation rate by changing its shape (**Figure 1**). Many bacteria that live suspended in aquatic environments are rod shaped, elongated cells with a higher aspect ratio and thus an increased frictional resistance (129). Moreover, as shown by the higher sedimentation rates of a snowflake-shaped cluster of yeast that evolved in vitro (106), the unicellular nature of yeasts favors their persistence in the water column. However, because of their spheroid shapes, yeasts have very low frictional resistance and consequently tend to sink faster. While sedimentation might be a disadvantage for organisms relying on light and oxygen, some yeasts might benefit from increased access to nutrients that are on ocean and lake floors.

2.4. Diversity of Modes of Reproduction

Yeasts reproduce asexually by budding and in some species by fission (e.g., *Schizosaccharomyces pombe*, *Penicillium marneffei*). Some budding species, such as *Candida albicans*, can produce attached chains of yeast that form hyphal or pseudohyphal structures (128). These hyphal or pseudohyphal forms of polarized growth are the hallmark of invasion by filamentous fungi. They are essential for anchoring and entering a substrate and are likely associated with substrate attachment in aquatic ecosystems, such as on debris or hosts (**Figure 3**).

Sex is not required for yeast populations to be maintained. Some species are in genera such as *Candida* that have never been known to undergo sexual reproduction (75). Sex is indispensable for the long-term survival of species, and truly aquatic yeasts, such as *Metschnikowia bicuspidata*, regularly undergo sex and produce ascospores adapted for infection of aquatic hosts (76). We do not know whether amphibious yeasts are capable of having sex in aquatic ecosystems, where it is unclear how pheromones can be sufficiently concentrated. Yeast sex involves fusion of isogamous cells and leads to the formation of a diploid cell type. In some Basidiomycota, conjugation of gametes triggers a morphological transition to hyphae with a dikaryotic condition where the gamete nuclei remain unfused and continue dividing conjugately. Yeasts are unusual in that they have a propensity to grow as diploids, in contrast to other members of the Dikarya, which typically undergo meiosis immediately after nuclear fusion of gametes. Sexual sporulation in yeasts leads to a special cell called an ascus in the Ascomycetes (internal division) or a basidium in the Basidiomycetes (external spore production).

2.5. Diversity of Modes of Division

Cell shape is generally intimately tied to adaptive function for a given environment throughout the biosphere. Morphogenesis is a response that integrates chemical and physical cues as well as biological signals from proximal cells. How does the aquatic environment impact cell morphogenesis? In principal, the conditions for growth and division are completely different between terrestrial and aquatic environments. As discussed above, water provides distinct physical challenges for dispersion, buoyancy, oxygen and nutrient availability, diffusion of small molecules, and density-dependent communication within and between species that might lead to specific morphological adaptations and innovations.

Most of what is understood at the molecular level about fungal morphogenesis is derived from study of model, primarily terrestrial, species such as *Saccharomyces cerevisiae* and *Aspergillus nidulans* (10, 24, 114). Fungal cell shapes are relatively limited [buds, hyphae, pseudohyphae, (**Figure 3**)] and emerge from spatially and temporally controlled targeting of membrane- and cell wall–remodeling enzymes (10). The cell wall enzymes transiently weaken and then repair the wall to accommodate additional membrane and thus drive expansion (79). In the case of hyphae, this persists at a site that promotes exploration while cells remain attached and in some

cases connected across many microns with shared cytoplasm. In contrast, transient-growth yeast cells produce buds that generally detach from a mother cell, facilitating dispersal, depending on the physical environment. An additional critical feature of fungal morphogenesis is the degree of coupling between nuclear division and cell division (34). Tight coordination leads to the formation of uninucleate, single yeast cells with each division, whereas syncytial hyphae with tens to hundreds of nuclei sharing a common cytoplasm form where there is little coupling between cell and nuclear division. Thus, while fungal shapes are limited compared to the shapes of animal cells, the variability in nuclear content coupled to the ability to produce small, spherical cells or long hyphae is a highly efficient way to interact with varied environments.

What types of morphologies are adapted to aquatic lifestyles? For exclusively aquatic, early diverging fungi (i.e., chytrids), a flagellum forms from centriole structures and this enables propulsions through water along with actin-rich pseudopods (32, 83). However, many aquatic or amphibious fungi seem to have the same suite of morphologies as terrestrial fungi, at least for vegetative growth (38, 85). A caveat to this conclusion is that there are few documented examples of fungi imaged in the field, directly from a water column or in the sampled water, and this is in part due to the sparsity of individuals or their presumed tight association with a substrate, which makes detecting the fungi through label-free imaging very difficult (3). Thus, it is possible that there are distinct aquatic shapes that have evaded detection.

What is clear is that when amphibious yeasts are brought back to the lab and cultured, they have multimorphic tendencies with different cell shapes coexisting in the same environment (38, 74, 85). This raises the possibility of morphological bet-hedging such that a given condition produces a variable distribution of shapes, any one of which may be optimal for the next environmental fluctuation. These variable distributions of morphologies are highly responsive to cues such as temperature, pH, osmolarity, agitation, and cell density, such that the environment can lead to enrichment in particular shapes. Another feature of the amphibious species is that shape and mode of nuclear division are not restricted to a particular lineage of cells and can often change from cell cycle to cell cycle. This is well documented in *Hortaea werneckii*, a melanin-producing, extremophile black yeast (see **Supplemental Material**) that can routinely shift between budding and fission as well as pseudohyphal and hyphal growth forms (85, 132). An interesting trait of the ubiquitous and amphibious *Aureobasidium pullulans* is the ability of multinucleate yeast mother cells to spawn uninucleate buds, often by producing many buds simultaneously, which is never seen in the conventional, model budding yeasts, such as *S. cerevisiae*, but has been seen in the dimorphic human pathogen *Mucor* (59, 82, 85). The degree of genetic heterogeneity and exchange between nuclei in these syncytial mothers remains to be seen, raising the possibility that there is some heterogeneity in genetic content between buds produced by the same mother (45). Asexual recombination in a multinucleate mother, combined with some level of mutation, potentially enables efficient sampling of different genotypes to adapt to the challenges of aquatic environments, where finding a partner for mating may be especially challenging. Finally, there is strong evidence of selection for spore shapes in aquatic fungi, indicating that at this stage of reproduction there is substantial variation from terrestrial counterparts (21). Thus, amphibious fungi take advantage of the fungal tool kit for cell and nuclear division and exploit multinucleation combined with budding and high variability in shapes within a given population.

Physical and chemical cues along with internal spatial landmarks direct the protein machinery that then orchestrates the directed secretion that sculpts fungal cells. This machinery is in general highly conserved, and so the ability of fungal cells to adapt their shape to an environment is likely dependent on subtle changes to regulation rather than presence and absence of specific regulators (27). Thus, it is not clear simply from genome content what might enable an amphibious lifestyle for any given fungus.

3. AMPHIBIOUS YEASTS: BETWEEN AQUATIC AND TERRESTRIAL ENVIRONMENTS

Yeasts exhibit excellent survival in water (118). Aquatic habitats are extremely diverse in size and physiochemical properties and may well harbor the largest pool of yeast cells (17). Wetlands and phytotelmata, containing high levels of organic materials, enable large autochthonous yeast populations (51). An increasing number of studies demonstrate the large number of yeast species adapted to diverse aquatic environments (**Figure 1**), such as seawater [e.g., genus *Yamadazyma* (15)], deep-sea hydrothermal vents (33), environments associated with macro- and microalgae (36), hypersaline water (42, 49), and glacial and subglacial environments of polar regions and similar habitats (18, 100, 131). Many of these same species of yeasts are also found on land, exemplifying the amphibious habit.

As with other microorganisms, yeasts can also thrive in tap water and other wet, indoor niches [e.g., bathrooms, kitchens (94)]. Recent studies of such niches have revealed a diversity of ascomycetous yeast from the genera *Aureobasidium*, *Candida*, *Debaryomyces*, *Exophiala*, *Meyerozyma*, *Naganishia*, *Pichia*, *Rhodotorula*, *Saccharomyces*, and *Yarrowia* (8, 94). The main characteristics of these yeasts include production of extracellular polysaccharides; degradation of cleaning agents; and tolerance of high temperatures, high salt concentrations, and alkaline pH values (8, 93, 94).

3.1. Adaptations to Aquatic Environments

Yeasts are found in diverse environments, but their presence in aquatic ecosystems may reveal traits or adaptive characteristics that make them more suitable than other fungi for inhabiting such ecosystems. How have the traits of amphibious yeasts, which have presumably evolved in terrestrial ecosystems, predisposed them to thrive in aquatic environments?

Insights into how amphibious yeasts might be preadapted to life in aquatic environments could come from looking at where yeasts are found. Benthic zones, at the bottom of oceans and lakes, differ in many ways from pelagic zones, which range from surface waters to great depths. Moreover, microorganisms live on internal and external surfaces of myriad hosts, not all of which have been investigated for yeasts. Filtered water is the easiest to sample and has been useful for documenting yeast diversity and concentration, which is typically <10 cells/L in marine water and <100 cells/L in freshwater (86). Surveys of pelagic fungi cultured from seawater demonstrate a high diversity of yeasts, with over 200 species found (52). An expanded viewpoint is offered by cultivation-independent surveys relying on barcode sequencing of environmental DNA (eDNA), which has included a diversity of habitats such as open ocean water (107), through deep-sea sediments, including vents and whale falls (78, 88) as well as near-shore sampling of corals or sediments (4, 102). In contrast, studies in freshwater habitats have more typically focused on water exclusively (e.g., 57, 65). In freshwater ecosystems, yeasts are even more often encountered and more diverse, although perhaps a smaller proportion of the fungal community.

The following patterns emerge from the last 15 years of eDNA surveys of fungi in aquatic ecosystems. In marine studies, yeasts are common to dominant among fungi both in surface waters (52, 107) and at greater depths (9, 87). Environmental DNA studies cannot reveal traits of the cells that are the origin of the sequence data, but when the sequences closely match those of organisms whose traits are well known, e.g., Saccharomycetes, a strong inference can be made that the cells have a yeast morphology with diverse metabolism and high salt tolerance. Because of matches of most sequences to described species such as *S. cerevisiae* and *Rhodotorula glutinis* (107), most species detected in such surveys are presumed to be dispersed in from terrestrial habitats or amphibious (73). Saccharomycetes are ubiquitous across eDNA studies, although dominant groups are found to differ, which may reflect biases in the ability of molecular markers to detect

particular lineages. Different studies of similar habitats also vary with regard to which yeasts pre-
dominate, with *Malassezia* prevalent in some deep-sea samples (9) but not others (88, 125). Marine
yeasts have commonly been detected on hosts, including corals, eelgrass, and sponges, among oth-
ers (4, 30, 85, 92), and the density of yeast cells is greater on substrates and sediments than in the
water column (86). The consistent pattern is that the distribution of yeasts is surprisingly patchy,
and this applies to the group as a whole as well as clades of yeasts.

For amphibious yeasts, the main challenges in aquatic and marine ecosystems are low nutri-
ents, lower oxygen, often increased UV exposure, and salinity. Many yeasts are readily able to adapt
to most of these pressures. Regarding oligotrophic nutrient conditions, it appears that yeasts are
more common in nearshore habitats and polluted waters than offshore waters (73). Likely most
yeast growth depends on a proper substrate, such as wood, an animal host or cadaver, or sediments.
Although substrates are likely sparse in aquatic ecosystems, turbulence allows cells to move within
water until they find a suitable substrate. Amphibious yeasts need to increase their probability of
contact with and attachment to substrates. Attachment of spores to their substrates might be facil-
itated by production of sticky or mucilaginous sheaths or spore walls (66). Species like *Rhodotorula
mucilaginosa* and *R. glutinis*, the most common red yeasts in aquatic habitats, produce mucilaginous
colonies that may reflect this adaptation. Mucilaginous extracellular polysaccharides of *A. pullu-
lans*, a common amphibious black yeast, are known to lead to adhesion to substrates (5). Beyond
attachment, extracellular polysaccharides are associated with improved growth in oligotrophic
conditions and nutrient-poor media, potentially because they bind to ionic and nonionic nutri-
ents (37, 69). Yeasts encode lectins, which are carbohydrate-binding, primarily cell wall proteins
that are involved in aggregation and adhesion (116). The presence of lectins may facilitate adap-
tation in aquatic environments by attaching to polysaccharides on hosts or detritus.

Oxygenation of water decreases quickly as depth increases, with sedimentary layers of deep
waters particularly anoxic. However, many yeasts are fermentative. This trait allows amphibious
yeasts to utilize both terrestrial and aquatic ecosystems. A few clades in the Ascomycota are able to
perform fermentation (25), and this is consistent with the Basidiomycota being more prevalent at
the water surface rather than in sediments (86, 98). Nevertheless, some studies have found that the
Basidiomycota make up a large portion of the yeasts from deep-sea waters and sediments (9, 115),
and some taxa, such as *Malassezia*, are able to grow in anoxic conditions if not anaerobically (117).

Yeasts surviving on surface waters face the challenge of high UV irradiation. The main de-
fense against UV irradiation is the production of pigments that absorb the radiation, such as
melanin. Melanized yeasts, including *Exophiala* and *Aureobasidium*, are common in aquatic habi-
tats. Melanized fungi are quite resistant to other stresses such as hydrostatic pressure, because
melanin increases cell strength and rigidity (23). Remarkably, melanin also interacts with a wide
range of electromagnetic radiation frequencies, functioning as a protective and energy-harvesting
pigment in fungi (23).

3.2. Extremophile Microbes: Beyond Prokaryotes

Amphibious yeasts, by merely persisting on land and liquid, have to overcome differentials of
salinity, hydrostatic pressure, and temperature that would be considered extreme by most mea-
sures (**Figure 1**). Many of the most extreme environments on earth are aquatic, and these are
typically enriched in yeasts compared with other fungi. One characteristic of these extremophiles
is their propensity for ecological plasticity and wide niche breadth—by definition a requirement
for amphibious lifestyles.

Bacteria and archaea are considered the hardiest organisms on our planet, and many of them
are extraordinarily adapted to extreme habitats long considered unsuitable for life. However, many
yeast species can thrive in environments such as hydrothermal vents, deep ocean waters, solar

salterns, acidic lakes, polar regions, deserts, and the upper atmosphere (reviewed in 19). Here, yeasts tolerate and even thrive under extreme physical and chemical conditions, including extreme temperatures, radiation, pressure, pH, salinity, and osmotic stress, to cite but a few (19, 108). We presume that many of the characteristics conferring this durability contribute to amphibious yeasts' ability to withstand environmental differentials between liquid and land.

While many yeasts cannot withstand the extreme lower and upper physicochemical limits that some prokaryotes can endure, a number of yeast species exhibit extraordinary adaptations to extreme environments (19, 39), including many black yeasts such as *H. werneckii* that can grow in nearly saturated salt solutions (16, 48). There has been no report yet of yeasts isolated from hyperthermophilic (78) or extremely acidic environments; however, increasing evidence suggests that some basidiomycetous yeasts such as *Malassezia* spp. might live in frozen Antarctic soils and even in hydrothermal vents that can be strongly acidic and have temperatures as high as 400°C (2).

4. EVIDENCE FOR ADAPTATION TO EXTREME ENVIRONMENTS

In terms of physicochemical parameters, such as extreme temperatures, salt concentrations, and pH values, the Earth offers many opportunities for marginalized life (39). Specialized yeasts and yeast-like organisms also inhabit environments with extremely high pressure, toxic environments (17), and environments exposed to radioactive irradiation. Many true yeasts tolerate single or even multiple stresses.

Yeasts that thrive at cold temperatures are numerous, and are mainly known among basidiomycetes. The lowest reported temperature at which psychrophilic yeasts can grow is between −2°C and −7°C, but most psychrophilic yeasts are usually defined by an upper growth limit of 20°C and an optimal growth temperature of 15°C (19). While thermophiles are generally less common among fungi, a few ascomycetous yeast species can thrive at a maximum of 48–50°C, with yeasts with dormant structures having advantages in such environments.

Salinity of oceans (∼0.6 M NaCl) could be a barrier to growth of amphibious fungi; however, almost all terrestrial yeasts can grow well at salt concentrations higher than those of seawater (73, 77). Many yeasts are preadapted to grow in environments of high osmolarity, such as those that are commonly involved in food fermentation or that inhabit nectar or the gut, for example, the ubiquitous *Debaryomyces hansenii*. Extremophilic yeasts can cope with high osmotic pressures, such as high sugar concentrations of 50–60% w/v [water activity (a_w) up to 0.62–0.65 (26)] and up to 25% NaCl w/v [a_w = 0.8 (19)].

Yeasts generally prefer weakly acidic environments (pH of 4.5–5.5), and some can also cope with extremely high acidity (pH = 2.3). While bodies of water rarely reach this level of acidity, the decomposition of organic substances and the release of organic acids, commonly reach these levels at a small spatial scale. Alkaline environments are generally less tolerated, although some yeasts can grow in media with a pH of 10 or even higher (19). Different marginal pH values can also be achieved at high concentrations of metals from industrial or mining effluents, where the toxicity of ions is limited in addition to the pH itself.

Yeasts in extreme environments have developed systems that not only protect them from single stressors but also enable them to cope with multiple combinatorial-stress conditions. For example, *Rhodotorula taiwanensis*, which was discovered in an acid mine drainage, tolerates both extremely low pH, and extremely high radioactivity in what might be the most hostile yeast habitat yet described (60, 122). For this reason yeasts are considered among the best options for bioremediation of acid radioactive waste (122). An example of a combinatorial-stress environment is hydrothermal vents in the deep sea, where low temperature, high hydrostatic pressure, high salinity, and low nutrient availability are highly selective (14). In cases where multiple stresses occur sequentially,

stress cross protection may facilitate survival (13). The term polyextremophile was introduced by Gostinčar et al. in 2011 (40) for those fungi that are able to tolerate a variety of stresses and are therefore able to colonize a variety of environments and tolerate a wide range of ecological conditions. In contrast, monoextremophiles (more abundant) are those organisms that are better adapted and specialized to thrive in an environment with a single stressor. It appears that monoextremophilic species are more common than polyextremophilic ones (which are rare), but this could be misleading because of the anthropocentric testing of survival of the most obvious stress conditions of a given environment. In evolutionary terms, polyextremophily may be a preadaptation of a more specialized monoextremophily. The conditions to which such species are exposed in an environment with multiple stress factors can select for traits that serve as preadaptations for evolution (40).

One of the representatives of the polyextremophiles is *A. pullulans*, a generalist species that can be found almost everywhere—it survives even in unconventional habitats and is a strong competitor. This species is not a classical yeast: In addition to growing as single cells, it has a hyphal stage and can develop resting chlamydospores (43, 130). Its polyextremophile nature and its nutritional versatility offer this organism great adaptability to conquer new environments (40).

Another example is *R. mucilaginosa*, a true yeast that can also be found in a variety of environments, such as the atmosphere, spoiled food, humans (sputum, hair, tuberculosis lung, nails, ulcers), animals (feces, larvae, gut), plant substrates (red pepper, rotten wood), lake water, marsh water, paper mill effluent, soil, polluted river water, acid mine drainage, sea water, and water from hydrothermal vents (75). *R. mucilaginosa* cells can survive for more than 600 days in river water (118).

The closest we have to a model extremophilic amphibious yeast is *H. werneckii* (Ascomycota), an extremely halotolerant species that grows in environments with 0 to 30% (w/v) NaCl (132). Its primary ecological niche is worldwide natural hypersaline environments, but it can colonize various niches, all of which are salty: from seawater and adjacent rocks and beach soil to marine organisms (sponges, corals), salt marsh and mangrove plants, and saltern microbial mats (reviewed in 85, 132).

4.1. Genomic Signatures and Physiological Adaptations for Tolerance to Extreme Conditions

Fluctuations in physiochemical environmental parameters often disturb cellular homeostasis and cause molecular damage, putting the fungal cell under stress. Consequently, the cells must be able to adapt to these dynamic changes if they are to survive, grow, and colonize. This stress adaptation depends on three fundamental abilities: (*a*) detection of environmental signals, (*b*) transduction of these signals to regulate the cellular processes that mediate stress adaptation, and (*c*) adaptive responses that enable cells to survive stress. These adaptive processes are those that counteract or detoxify the initial stress and repair or eliminate the molecular damage caused by this stress (13). Different niches exert different evolutionary pressures, and this has led to a considerable diversity among fungal species with respect to the robustness of specific stress responses. This evolutionary adaptation of stress resistance to the local niche has led to a certain divergence between fungal species in the regulation of the cellular processes that mediate adaptation to specific stresses (13).

4.2. Temperature Extremes

The yeasts that survive and thrive in polar and subpolar regions must be adapted to low temperatures and the freezing of water to ice crystals and the resulting dehydration and low water availability. In addition, they must be adapted to relatively high ion concentrations, oligotrophic conditions and sometimes to high UV irradiation and hypoxia. Studies conducted so far have

shown that some yeast genera are particularly well adapted to such conditions. These yeasts use different strategies to thrive in these cold environments, such as the production of cold-active enzymes, "antifreeze" compounds, and extracellular polymers. They also need to maintain the fluidity and plasticity of their cell membranes down to subzero temperatures, which can be achieved by an increased amount of unsaturated fatty acids and a reduced mean length of fatty acid chains (reviewed in 20). Recently, it has been shown that some psychrotolerant yeasts are very resistant to dehydration (68).

Yeasts living in hot environments have developed some adaptation strategies to maintain cell integrity and functionality. Heat causes an increase in the fluidity of the cell membranes. In response, thermotolerant yeasts adjust the concentrations of saturated fatty acids (which reach 30–40%) present in the lipids and maintain an optimal fluidity level (7). In the thermotolerant ascomycetes species *Kazachstania* (*Candida*) *slooffiae* and *Kazachstania bovina*, phospholipid composition and cytochrome concentration were also found to change in response to a temperature increase (reviewed in 19). In *Cryptococcus* yeasts, temperature stress induced transposon-associated mutations, hypothesized to impact adaptation to the host during infection, and facilitated drug resistance (50).

4.3. Osmotic Stress

In the last two decades, numerous studies have been conducted on the black yeast *H. werneckii*, which has become a model organism for halotolerance studies on eukaryotes (reviewed in 103). It is unique in its adaptability to fluctuations in salt concentration, as it can grow both without NaCl and in the presence of up to 5 M NaCl (103). It is naturally well adapted to environments with low water activity due to several of its properties: composition of the plasma membrane, enzymes involved in fatty acid modifications (44, 72, 123), osmolyte composition and ion accumulation (71, 101), melanization of the cell wall (70, 72), differences in the high-osmolarity-glycerol (HOG) signaling pathway (124), and differential gene expression. In its natural environment, it is constantly exposed not only to low a_w and high concentrations of toxic inorganic ions but also to changing salinity (101, 126).

Survival in hyperosmotic conditions is regulated in some yeasts by the conserved (HOG) signaling pathway, a branched MAPK (mitogen-activated protein kinase) signal transduction system (58). The primary roles of the HOG pathway are to control uptake and balance of the osmolyte glycerol, which is compatible with cellular functions, and to regulate the expression of genes such as those encoding salt efflux pump proteins (109). Diverse ascomycete yeasts appear to have this pattern of uptake of glycerol under high salt conditions (77).

Under hyperosmotic conditions, rapid response to the resulting water loss is achieved by the synthesis of glycerol and some other compatible solutes. The specific architecture of the HOG signaling pathway could be the background for the extreme halotolerance of *H. werneckii*. HwHog1 is phosphorylated only when the extracellular NaCl concentration is ≥3 M (124). HwHog1 promotes the differential induction or repression of osmoresponsive genes as a function of osmolarity and also through physical interaction with chromatin and RNA polymerase II (126). In many natural hypersaline environments, the concentration of toxic Na^+ is much higher than that of K^+, and therefore the mechanisms that maintain the stable and high intracellular K^+/Na^+ ratio are critical for survival in such environments. *H. werneckii* can maintain high K^+/Na^+ ratios over a wide range of Na^+ concentrations in the environment. This homeostasis is maintained by the regulated transport of K^+ and Na^+ across the plasma membrane, as the cation transporters in this fungus are diverse and highly enriched. Exposure to high salt concentrations can lead to an increased production of reactive oxygen species in the cell. In *H. werneckii*, the maximum tolerated salt concentration correlated with the number of genes encoding three major enzymes of the cellular oxidative stress response: superoxide dismutases, catalases, and peroxiredoxins (41).

Debaryomyces hansenii, the most common ascomycetous yeast in marine waters, has different mechanisms to overcome osmotic stress in saline environments. It has a broad salinity tolerance, which is important for its wide distribution in the ocean and also in glacial environments. Its amphibious character is supported by the fact that this species is often found as a member of plant yeast communities and was the most important ascomycetous yeast species found in the fresh frass of various leaf-eating caterpillars (118). It is able to accumulate Na^+ without being intoxicated, even if K^+ is present in the environment in low concentrations. Furthermore, Na^+ improves growth and protects *D. hansenii* in the presence of additional stress factors such as high temperatures and extreme pH values. A number of beneficial factors are said to be involved in the increased halotolerance of *D. hansenii*: Glycerol, the main compatible solute, is retained in the cell by an active glycerol-Na^+ symporter; potassium uptake is not inhibited by sodium; and sodium-protein targets in *D. hansenii* appear to be more resistant. Despite the availability of the genome sequence of *D. hansenii*, no candidate genes responsible for halotolerance have been identified (104).

Exposure to different types of stress can lead to similar reactions via what is known as the core stress response (CSR). CSR genes are commonly up- or downregulated by different types of stress (13). In *S. cerevisiae*, between 200 and 300 genes have been found to be upregulated in response to various stress factors such as heat shock, osmotic stress, oxidative stress, pH increase or decrease, and amino acid starvation. In addition, between 300 and 600 genes were often downregulated after exposure to these various stress treatments. For example, CSR affects about 10–14% of the *S. cerevisiae* genome (13). To our knowledge, no such studies have yet been conducted on the other abovementioned yeast species.

5. CONCLUDING REMARKS AND FUTURE PERSPECTIVES

The ubiquity and abundance of yeasts in terrestrial and aquatic environments suggest that they play fundamental roles in these ecosystems. Yeasts play important roles in carbon cycling and have strong potential for biodegradation and biofuels production. Yeasts also play important roles in human and animal health and are an emerging target for clinical research. It would not be a stretch to claim that yeasts have been essential for the development of human culture and civilization all over the world. Despite their importance, we know comparatively little about their interactions with their environment or other microorganisms, such as bacteria and viruses, at the microscale.

In this review we highlighted key adaptations of yeasts to aquatic and extreme environments and provided new insights into their evolution and modes of reproduction. We also discussed how the physical forces operating at microscale might shape the evolution and the ecology of amphibious yeasts. Future studies of their diversity, distribution, physiology, and reproduction will almost certainly bring new perspectives and insights into how amphibious yeasts exploit nutrients, disperse, speciate, and interact with other microbes at scales relevant to individual cells.

DISCLOSURE STATEMENT

LITERATURE CITED

1. Álvarez-Pérez S, Lievens B, Fukami T. 2019. Yeast–bacterium interactions: the next frontier in nectar research. *Trends Plant Sci*. 24(5):393–401
2. Amend A. 2014. From dandruff to deep-sea vents: *Malassezia*-like fungi are ecologically hyper-diverse. *PLOS Pathog*. 10(8):e1004277

3. Amend A, Burgaud G, Cunliffe M, Edgcomb VP, Ettinger CL, et al. 2019. Fungi in the marine environment: open questions and unsolved problems. *mBio* 10(2):e01189-18

4. Amend AS, Barshis DJ, Oliver TA. 2012. Coral-associated marine fungi form novel lineages and heterogeneous assemblages. *ISME J.* 6(7):1291–301

5. Andrews JH, Harris RF, Spear RN, Lau GW, Nordheim EV. 1994. Morphogenesis and adhesion of *Aureobasidium pullulans*. *Can. J. Microbiol.* 40(1):6–17

6. Applen Clancey S, Ruchti F, LeibundGut-Landmann S, Heitman J, Ianiri G. 2020. A novel mycovirus evokes transcriptional rewiring in the fungus *Malassezia* and stimulates beta interferon production in macrophages. *mBio* 11(5):e01534-20

7. Arthur H, Watson K. 1976. Thermal adaptation in yeast: growth temperatures, membrane lipid, and cytochrome composition of psychrophilic, mesophilic, and thermophilic yeasts. *J. Bacteriol.* 128(1):56–68

8. Babič MN, Zupančič J, Gunde-Cimerman N, Zalar P. 2017. Yeast in anthropogenic and polluted environments. In *Yeasts in Natural Ecosystems: Diversity*, ed. P Buzzini, M-A Lachance, A Yurkov, pp. 145–69. Cham, Switz.: Springer

9. Bass D, Howe A, Brown N, Barton H, Demidova M, et al. 2007. Yeast forms dominate fungal diversity in the deep oceans. *Proc. Biol. Sci.* 274(1629):3069–77

10. Bi E, Park H-O. 2012. Cell polarization and cytokinesis in budding yeast. *Genetics* 191(2):347–87

11. Blackwell M. 2011. The fungi: 1, 2, 3…5.1 million species? *Am. J. Bot.* 98(3):426–38

12. Boynton PJ. 2019. The ecology of killer yeasts: interference competition in natural habitats. *Yeast* 36(8):473–85

13. Brown AJP, Cowen LE, di Pietro A, Quinn J. 2017. Stress adaptation. In *The Fungal Kingdom*, ed. J Heitman, BJ Howlett, PW Crous, EH Stukenbrock, TY James, NAR Gow, pp. 463–85. Washington, DC: ASM

14. Burgaud G, Arzur D, Durand L, Cambon-Bonavita M-A, Barbier G. 2010. Marine culturable yeasts in deep-sea hydrothermal vents: species richness and association with fauna. *FEMS Microbiol. Ecol.* 73(1):121–33

15. Burgaud G, Coton M, Jacques N, Debaets S, Maciel NOP, et al. 2016. *Yamadazyma barbieri* f.a. sp. nov., an ascomycetous anamorphic yeast isolated from a Mid-Atlantic Ridge hydrothermal site (-2300 m) and marine coastal waters. *Int. J. Syst. Evol. Microbiol.* 66(9):3600–6

16. Butinar L, Spencer-Martins I, Gunde-Cimerman N. 2007. Yeasts in high Arctic glaciers: the discovery of a new habitat for eukaryotic microorganisms. *Antonie Van Leeuwenhoek* 91(3):277–89

17. Buzzini P, Lachance M-A, Yurkov A, eds. 2017. *Yeasts in Natural Ecosystems: Diversity*. Cham, Switz.: Springer. 499 pp.

18. Buzzini P, Margesin R, eds. 2013. *Cold-Adapted Yeasts: Biodiversity, Adaptation Strategies and Biotechnological Significance*. Berlin: Springer. 549 pp.

19. Buzzini P, Turchetti B, Yurkov A. 2018. Extremophilic yeasts: the toughest yeasts around? *Yeast* 35(8):487–97

20. Buzzini P, Turk M, Perini L, Turchetti B, Gunde-Cimerman N. 2017. Yeasts in polar and subpolar habitats. In *Yeasts in Natural Ecosystems: Diversity*, ed. P Buzzini, M-A Lachance, A Yurkov, pp. 331–65. Cham, Switz.: Springer

21. Campbell J, Shearer C, Marvanová L. 2006. Evolutionary relationships among aquatic anamorphs and teleomorphs: *Lemonniera*, *Margaritispora*, and *Goniopila*. *Mycol. Res.* 110(Part 9):1025–33

22. Choi J, Kim S-H. 2017. A genome Tree of Life for the Fungi kingdom. *PNAS* 114(35):9391–96

23. Cordero RJ, Casadevall A. 2017. Functions of fungal melanin beyond virulence. *Fungal Biol. Rev.* 31(2):99–112

24. Coudert Y, Harris S, Charrier B. 2019. Design principles of branching morphogenesis in filamentous organisms. *Curr. Biol.* 29(21):R1149–62

25. Dashko S, Zhou N, Compagno C, Piškur J. 2014. Why, when, and how did yeast evolve alcoholic fermentation? *FEMS Yeast Res.* 14(6):826–32

26. Deak T. 2007. *Handbook of Food Spoilage Yeasts*. Boca Raton, FL: CRC. 352 pp.

27. Diepeveen ET, Gehrmann T, Pourquié V, Abeel T, Laan L. 2018. Patterns of conservation and diversification in the fungal polarization network. *Genome Biol. Evol.* 10(7):1765–82

28. Dusenbery DB. 2009. *Living at Micro Scale: The Unexpected Physics of Being Small*. Cambridge, MA: Harvard Univ. Press. 416 pp.

29. Einstein A. 1956. *Investigations on the Theory of the Brownian Movement*. Mineola, NY: Courier Corporation. 119 pp.

30. Ettinger CL, Eisen JA. 2020. Fungi, bacteria and oomycota opportunistically isolated from the seagrass, *Zostera marina*. *PLOS ONE* 15(7):e0236135

31. Floudas D, Binder M, Riley R, Barry K, Blanchette RA, et al. 2012. The Paleozoic origin of enzymatic lignin decomposition reconstructed from 31 fungal genomes. *Science* 336(6089):1715–19

32. Fritz-Laylin LK, Lord SJ, Mullins RD. 2017. WASP and SCAR are evolutionarily conserved in actin-filled pseudopod-based motility. *J. Cell Biol.* 216(6):1673–88

33. Gadanho M, Sampaio JP. 2005. Occurrence and diversity of yeasts in the Mid-Atlantic Ridge hydrothermal fields near the Azores Archipelago. *Microb. Ecol.* 50(3):408–17

34. Gladfelter A, Berman J. 2009. Dancing genomes: fungal nuclear positioning. *Nat. Rev. Microbiol.* 7(12):875–86

35. Gladfelter AS, James TY, Amend AS. 2019. Marine fungi. *Curr. Biol.* 29(6):R191–95

36. Godinho VM, Furbino LE, Santiago IF, Pellizzari FM, Yokoya NS, et al. 2013. Diversity and bioprospecting of fungal communities associated with endemic and cold-adapted macroalgae in Antarctica. *ISME J.* 7(7):1434–51

37. Golubev WI. 1991. Capsules. *The Yeasts* 4:175–98

38. Goshima G. 2021. Growth and division mode plasticity by cell density in marine-derived black yeasts. bioRxiv 2021.05.16.444389. **https://doi.org/10.1101/2021.05.16.444389**

39. Gostinčar C, Grube M, de Hoog S, Zalar P, Gunde-Cimerman N. 2010. Extremotolerance in fungi: evolution on the edge. *FEMS Microbiol. Ecol.* 71(1):2–11

40. Gostinčar C, Grube M, Gunde-Cimerman N. 2011. Evolution of fungal pathogens in domestic environments? *Fungal Biol.* 115(10):1008–18

41. Gostinčar C, Gunde-Cimerman N. 2018. Overview of oxidative stress response genes in selected halophilic fungi. *Genes* 9(3):143

42. Gostinčar C, Lenassi M, Gunde-Cimerman N, Plemenitaš A. 2011. Fungal adaptation to extremely high salt concentrations. *Adv. Appl. Microbiol.* 77:71–96

43. Gostinčar C, Ohm RA, Kogej T, Sonjak S, Turk M, et al. 2014. Genome sequencing of four *Aureobasidium pullulans* varieties: biotechnological potential, stress tolerance, and description of new species. *BMC Genom.* 15:549

44. Gostinčar C, Turk M, Plemenitaš A, Gunde-Cimerman N. 2009. The expressions of Δ^9-, Δ^{12}-desaturases and an elongase by the extremely halotolerant black yeast *Hortaea werneckii* are salt dependent. *FEMS Yeast Res.* 9(2):247–56

45. Gostinčar C, Turk M, Zajc J, Gunde-Cimerman N. 2019. Fifty *Aureobasidium pullulans* genomes reveal a recombining polyextremotolerant generalist. *Environ. Microbiol.* 21(10):3638–52

46. Grossart H-P, Van den Wyngaert S, Kagami M, Wurzbacher C, Cunliffe M, Rojas-Jimenez K. 2019. Fungi in aquatic ecosystems. *Nat. Rev. Microbiol.* 17(6):339–54

47. Guadayol O, Mendonca T, Segura-Noguera M, Wright AJ, Tassieri M, Humphries S. 2021. Microrheology reveals microscale viscosity gradients in planktonic systems. *PNAS* 118(1):e2011389118

48. Gunde-Cimerman N. 2000. Hypersaline waters in salterns—natural ecological niches for halophilic black yeasts. *FEMS Microbiol. Ecol.* 32(3):235–40

49. Gunde-Cimerman N, Zalar P. 2014. Extremely halotolerant and halophilic fungi inhabit brine in solar salterns around the globe. *Food Technol. Biotechnol.* 52(2):170–79

50. Gusa A, Williams JD, Cho J-E, Averette AF, Sun S, et al. 2020. Transposon mobilization in the human fungal pathogen *Cryptococcus* is mutagenic during infection and promotes drug resistance in vitro. *PNAS* 117(18):9973–9980

51. Hagler AN, Mendonça-Hagler LC, Pagnocca FC. 2017. Yeasts in aquatic ecotone habitats. In *Yeasts in Natural Ecosystems: Diversity*, ed. P Buzzini, M-A Lachance, A Yurkov, pp. 63–85. Cham, Switz.: Springer

52. Hassett BT, Vonnahme TR, Peng X, Jones EBG, Heuzé C. 2019. Global diversity and geography of planktonic marine fungi. *Botanica Marina* 63(2):121–39

53. Hatoum R, Labrie S, Fliss I. 2012. Antimicrobial and probiotic properties of yeasts: from fundamental to novel applications. *Front. Microbiol.* 3:421

54. Hawksworth DL, Lücking R. 2017. Fungal diversity revisited: 2.2 to 3.8 million species. In *The Fungal Kingdom*, ed. J Heitman, BJ Howlett, PW Crous, EH Stukenbrock, TY James, NAR Gow, pp. 79–95. Washington, DC: ASM

55. Heckman DS, Geiser DM, Eidell BR, Stauffer RL, Kardos NL, Hedges SB. 2001. Molecular evidence for the early colonization of land by fungi and plants. *Science* 293(5532):1129–33

56. Hedges SB, Blair JE, Venturi ML, Shoe JL. 2004. A molecular timescale of eukaryote evolution and the rise of complex multicellular life. *BMC Evol. Biol.* 4:2

57. Heeger F, Bourne EC, Baschien C, Yurkov A, Bunk B, et al. 2018. Long-read DNA metabarcoding of ribosomal RNA in the analysis of fungi from aquatic environments. *Mol. Ecol. Resour.* 18(6):1500–14

58. Hohmann S. 2009. Control of high osmolarity signalling in the yeast *Saccharomyces cerevisiae*. *FEBS Lett.* 583(24):4025–29

59. Howell AS, Savage NS, Johnson SA, Bose I, Wagner AW, et al. 2009. Singularity in polarization: rewiring yeast cells to make two buds. *Cell* 139(4):731–43

60. Huang C-H, Lee F-L, Tien C-J, Hsieh P-W. 2011. *Rhodotorula taiwanensis* sp. nov., a novel yeast species from a plant in Taiwan. *Antonie Van Leeuwenhoek* 99(2):297–302

61. Ianiri G, Coelho MA, Ruchti F, Sparber F, McMahon TJ, et al. 2020. HGT in the human and skin commensal *Malassezia*: A bacterially derived flavohemoglobin is required for NO resistance and host interaction. *PNAS* 117(27):15884–94

62. Inoue K, Nishimura M, Nayak BB, Kogure K. 2007. Separation of marine bacteria according to buoyant density by use of the density-dependent cell sorting method. *Appl. Environ. Microbiol.* 73(4):1049–53

63. James TY, Kauff F, Schoch CL, Matheny PB, Hofstetter V, et al. 2006. Reconstructing the early evolution of Fungi using a six-gene phylogeny. *Nature* 443(7113):818–22

64. James TY, Stajich JE, Hittinger CT, Rokas A. 2020. Toward a fully resolved fungal tree of life. *Annu. Rev. Microbiol.* 74:291–313

65. Jobard M, Rasconi S, Solinhac L, Cauchie H-M, Sime-Ngando T. 2012. Molecular and morphological diversity of fungi and the associated functions in three European nearby lakes. *Environ. Microbiol.* 14(9):2480–94

66. Jones EBG. 2006. Form and function of fungal spore appendages. *Mycoscience* 47(4):167–83

67. Kawarai T, Furukawa S, Ogihara H, Yamasaki M. 2007. Mixed-species biofilm formation by lactic acid bacteria and rice wine yeasts. *Appl. Environ. Microbiol.* 73(14):4673–76

68. Khroustalyova G, Giovannitti G, Severini D, Scherbaka R, Turchetti B, et al. 2019. Anhydrobiosis in yeasts: Psychrotolerant yeasts are highly resistant to dehydration. *Yeast* 36(5):375–79

69. Kimura Y, Nakano Y, Fujita K, Miyabe S, Imasaka S, et al. 1998. Isolation and characteristics of yeasts able to grow at low concentrations of nutrients. *Yeast* 14(3):233–38

70. Kogej T, Gorbushina AA, Gunde-Cimerman N. 2006. Hypersaline conditions induce changes in cell-wall melanization and colony structure in a halophilic and a xerophilic black yeast species of the genus *Trimmatostroma*. *Mycol. Res.* 110(Part 6):713–24

71. Kogej T, Ramos J, Plemenitas A, Gunde-Cimerman N. 2005. The halophilic fungus *Hortaea werneckii* and the halotolerant fungus *Aureobasidium pullulans* maintain low intracellular cation concentrations in hypersaline environments. *Appl. Environ. Microbiol.* 71(11):6600–5

72. Kogej T, Stein M, Volkmann M, Gorbushina AA, Galinski EA, Gunde-Cimerman N. 2007. Osmotic adaptation of the halophilic fungus *Hortaea werneckii*: role of osmolytes and melanization. *Microbiology* 153(Part 12):4261–73

73. Kohlmeyer J, Kohlmeyer E. 1979. *Marine Mycology: The Higher Fungi*. New York: Academic. 690 pp.

74. Krishnamurthy D, Li H, du Rey FB, Cambournac P, Larson A, Prakash M. 2019. Scale-free vertical tracking microscopy: towards bridging scales in biological oceanography. bioRxiv 610246. **https://doi.org/10.1101/610246**

75. Kurtzman CP, Fell JW, Boekhout T, eds. 2011. *The Yeasts: A Taxonomic Study*. London: Elsevier

76. Lachance M-A. 2016. *Metschnikowia*: half tetrads, a regicide and the fountain of youth. *Yeast* 33(11):563–74

77. Lages F, Silva-Graça M, Lucas C. 1999. Active glycerol uptake is a mechanism underlying halotolerance in yeasts: a study of 42 species. *Microbiology* 145(Part 9):2577–85

78. Le Calvez T, Burgaud G, Mahé S, Barbier G, Vandenkoornhuyse P. 2009. Fungal diversity in deep-sea hydrothermal ecosystems. *Appl. Environ. Microbiol.* 75(20):6415–21

79. Levin DE. 2011. Regulation of cell wall biogenesis in *Saccharomyces cerevisiae*: the cell wall integrity signaling pathway. *Genetics* 189(4):1145–75

80. Li H, Goh BN, Teh WK, Jiang Z, Goh JPZ, et al. 2018. Skin commensal *Malassezia globosa* secreted protease attenuates *Staphylococcus aureus* biofilm formation. *J. Investig. Dermatol.* 138(5):1137–45

81. Loron CC, François C, Rainbird RH, Turner EC, Borensztajn S, Javaux EJ. 2019. Early fungi from the Proterozoic era in Arctic Canada. *Nature* 570(7760):232–35

82. McIntyre M, Breum J, Arnau J, Nielsen J. 2002. Growth physiology and dimorphism of *Mucor circinelloides* (syn. *racemosus*) during submerged batch cultivation. *Appl. Microbiol. Biotechnol.* 58(4):495–502

83. Medina EM, Robinson KA, Bellingham-Johnstun K, Ianiri G, Laplante C, et al. 2020. Genetic transformation of *Spizellomyces punctatus*, a resource for studying chytrid biology and evolutionary cell biology. *eLife* 9:e52741

84. Miller CC, Walker J. 1924. The Stokes-Einstein law for diffusion in solution. *Proc. R. Soc. London A* 106(740):724–49

85. Mitchison-Field LMY, Vargas-Muñiz JM, Stormo BM, Vogt EJD, Van Dierdonck S, et al. 2019. Unconventional cell division cycles from marine-derived yeasts. *Curr. Biol.* 29(20):3439–56.e5

86. Nagahama T. 2006. Yeast biodiversity in freshwater, marine and deep-sea environments. In *Biodiversity and Ecophysiology of Yeasts*, ed. G Péter, C Rosa, pp. 241–62. Berlin: Springer

87. Nagano Y, Miura T, Nishi S, Lima AO, Nakayama C, et al. 2017. Fungal diversity in deep-sea sediments associated with asphalt seeps at the Sao Paulo Plateau. *Deep Sea Res. Part II* 146:59–67

88. Nagano Y, Miura T, Tsubouchi T, Lima AO, Kawato M, et al. 2020. Cryptic fungal diversity revealed in deep-sea sediments associated with whale-fall chemosynthetic ecosystems. *Mycology* 11(3):263–78

89. Nagy LG, Kovács GM, Krizsán K. 2018. Complex multicellularity in fungi: evolutionary convergence, single origin, or both? *Biol. Rev. Camb. Philos. Soc.* 93(4):1778–94

90. Nagy LG, Ohm RA, Kovács GM, Floudas D, Riley R, et al. 2014. Latent homology and convergent regulatory evolution underlies the repeated emergence of yeasts. *Nat. Commun.* 5:4471

91. Naranjo-Ortiz MA, Gabaldón T. 2019. Fungal evolution: major ecological adaptations and evolutionary transitions. *Biol. Rev.* 94(4):1443–76

92. Nguyen MTHD, Thomas T. 2018. Diversity, host-specificity and stability of sponge-associated fungal communities of co-occurring sponges. *PeerJ* 6:e4965

93. Novak Babič M, Gostinčar C, Gunde-Cimerman N. 2020. Microorganisms populating the water-related indoor biome. *Appl. Microbiol. Biotechnol.* 104(15):6443–62

94. Novak Babič M, Zalar P, Ženko B, Džeroski S, Gunde-Cimerman N. 2016. Yeasts and yeast-like fungi in tap water and groundwater, and their transmission to household appliances. *Fungal Ecol.* 20:30–39

95. O'Malley MA, Wideman JG, Ruiz-Trillo I. 2016. Losing complexity: the role of simplification in macroevolution. *Trends Ecol. Evol.* 31(8):608–21

96. Osborn HF. 1902. Homoplasy as a law of latent or potential homology. *Am. Nat.* 36(424):259–71

97. Padovan ACB, Sanson GFO, Brunstein A, Briones MRS. 2005. Fungi evolution revisited: Application of the penalized likelihood method to a Bayesian fungal phylogeny provides a new perspective on phylogenetic relationships and divergence dates of Ascomycota groups. *J. Mol. Evol.* 60(6):726–35

98. Pagnocca FG, Mendonça-Hagler LC, Hagler AN. 1989. Yeasts associated with the white shrimp *Penaeus schmitti*, sediment, and water of Sepetiba Bay, Rio de Janeiro, Brasil. *Yeast* 5:S479–83

99. Park M, Cho Y-J, Kim D, Yang C-S, Lee SM, et al. 2020. A novel virus alters gene expression and vacuolar morphology in *Malassezia* cells and induces a TLR3-mediated inflammatory immune response. *mBio* 11(5):e01521-20

100. Perini L, Gostinčar C, Gunde-Cimerman N. 2019. Fungal and bacterial diversity of Svalbard subglacial ice. *Sci. Rep.* 9(1):20230

101. Petrovič U, Gunde-Cimerman N, Plemenitaš A. 2002. Cellular responses to environmental salinity in the halophilic black yeast *Hortaea werneckii*. *Mol. Microbiol.* 45(3):665–72

102. Picard KT. 2017. Coastal marine habitats harbor novel early-diverging fungal diversity. *Fungal Ecol.* 25:1–13

103. Plemenitaš A, Lenassi M, Konte T, Kejžar A, Zajc J, et al. 2014. Adaptation to high salt concentrations in halotolerant/halophilic fungi: a molecular perspective. *Front. Microbiol.* 5:199

104. Prista C, Loureiro-Dias MC, Montiel V, García R, Ramos J. 2005. Mechanisms underlying the halotolerant way of *Debaryomyces hansenii*. *FEMS Yeast Res.* 5(8):693–701

105. Purcell EM. 1977. Life at low Reynolds number. *Am. J. Phys.* 45(1):3–11

106. Ratcliff WC, Denison RF, Borrello M, Travisano M. 2012. Experimental evolution of multicellularity. *PNAS* 109(5):1595–600

107. Richards TA, Leonard G, Mahé F, Del Campo J, Romac S, et al. 2015. Molecular diversity and distribution of marine fungi across 130 European environmental samples. *Proc. Biol. Sci.* 282(1819):20152243

108. Rothschild LJ, Mancinelli RL. 2001. Life in extreme environments. *Nature* 409(6823):1092–101

109. Ruiz A, Ariño J. 2007. Function and regulation of the *Saccharomyces cerevisiae* ENA sodium ATPase system. *Eukaryot. Cell* 6(12):2175–83

110. Schmitt MJ, Breinig F. 2002. The viral killer system in yeast: from molecular biology to application. *FEMS Microbiol. Rev.* 26(3):257–76

111. Schuech R, Hoehfurtner T, Smith DJ, Humphries S. 2019. Motile curved bacteria are Pareto-optimal. *PNAS* 116(29):14440–47

112. Seymour JR, Amin SA, Raina J-B, Stocker R. 2017. Zooming in on the phycosphere: the ecological interface for phytoplankton-bacteria relationships. *Nat. Microbiol.* 2:17065

113. Shubin N, Tabin C, Carroll S. 2009. Deep homology and the origins of evolutionary novelty. *Nature* 457(7231):818–23

114. Si H, Rittenour WR, Harris SD. 2016. Roles of *Aspergillus nidulans* Cdc42/Rho GTPase regulators in hyphal morphogenesis and development. *Mycologia* 108(3):543–55

115. Singh P, Raghukumar C, Verma P, Shouche Y. 2010. Phylogenetic diversity of culturable fungi from the deep-sea sediments of the Central Indian Basin and their growth characteristics. *Fungal Divers.* 40(1):89–102

116. Singh RS, Bhari R, Kaur HP. 2011. Characteristics of yeast lectins and their role in cell-cell interactions. *Biotechnol. Adv.* 29(6):726–31

117. Spatz M, Richard ML. 2020. Overview of the potential role of *Malassezia* in gut health and disease. *Front. Cell. Infect. Microbiol.* 10:201

118. Starmer WT, Lachance MA. 2011. Yeast ecology. In *The Yeasts*, ed. CP Kurtzman, JW Fell, T Boekhout, pp. 65–83. London: Elsevier

119. Stern DL. 2013. The genetic causes of convergent evolution. *Nat. Rev. Genet.* 14(11):751–64

120. Stocker R. 2012. Marine microbes see a sea of gradients. *Science* 338(6107):628–33

121. Stocker R. 2015. The 100 μm length scale in the microbial ocean. *Aquat. Microb. Ecol.* 76(3):189–94

122. Tkavc R, Matrosova VY, Grichenko OE, Gostinčar C, Volpe RP, et al. 2017. Prospects for fungal bioremediation of acidic radioactive waste sites: characterization and genome sequence of *Rhodotorula taiwanensis* MD1149. *Front. Microbiol.* 8:2528

123. Turk M, Méjanelle L, Sentjurc M, Grimalt JO, Gunde-Cimerman N, Plemenitas A. 2004. Salt-induced changes in lipid composition and membrane fluidity of halophilic yeast-like melanized fungi. *Extremophiles* 8(1):53–61

124. Turk M, Plemenitaš A. 2002. The HOG pathway in the halophilic black yeast *Hortaea werneckii*: isolation of the HOG1 homolog gene and activation of HwHog1p. *FEMS Microbiol. Lett.* 216(2):193–99

125. Vargas-Gastélum L, Chong-Robles J, Lago-Lestón A, Darcy JL, Amend AS, Riquelme M. 2019. Targeted ITS1 sequencing unravels the mycodiversity of deep-sea sediments from the Gulf of Mexico. *Environ. Microbiol.* 21(11):4046–61

126. Vaupotič T, Gunde-Cimerman N, Plemenitaš A. 2007. Novel 3′-phosphoadenosine-5′-phosphatases from extremely halotolerant *Hortaea werneckii* reveal insight into molecular determinants of salt tolerance of black yeasts. *Fungal Genet. Biol.* 44(11):1109–22

127. Vij R, Cordero RJB, Casadevall A. 2018. The buoyancy of *Cryptococcus neoformans* is affected by capsule size. *mSphere* 3(6):e00534-18

128. Whiteway M, Bachewich C. 2007. Morphogenesis in *Candida albicans*. *Annu. Rev. Microbiol.* 61:529–53

129. Young KD. 2006. The selective value of bacterial shape. *Microbiol. Mol. Biol. Rev.* 70(3):660–703

130. Zalar P, Gostincar C, de Hoog GS, Ursic V, Sudhadham M, Gunde-Cimerman N. 2008. Redefinition of *Aureobasidium pullulans* and its varieties. *Stud. Mycol.* 61:21–38

131. Zalar P, Gunde-Cimerman N. 2014. Cold-adapted yeasts in arctic habitats. In *Cold-Adapted Yeasts: Biodiversity, Adaptation Strategies and Biotechnological Significance*, ed. P Buzzini, R Margesin, pp. 49–74. Berlin: Springer

132. Zalar P, Zupančič J, Gostinčar C, Zajc J, de Hoog GS, et al. 2019. The extremely halotolerant black yeast *Hortaea werneckii*—a model for intraspecific hybridization in clonal fungi. *IMA Fungus* 10:10