From Dandruff to Deep-Sea Vents: Malassezia-like Fungi Are Ecologically Hyper-diverse

Anthony Amend*

Department of Botany, University of Hawaii at Manoa, Honolulu, Hawaii, United States of America

Introduction

As the dominant component of the mycobiota on human skin [1] —both healthy and diseased [2] —the genus *Malassezia* has received a fair amount of attention. Since the middle of the 19th century, researchers have linked these fungi with skin maladies such as dandruff and eczema [3], but their difficulty to culture axenically long hampered studies of their systematics and diversity [4]. *Malassezia* is the sole genus within the fungal order Malasseziales, contained within the proposed subphylum Malasseziomycetes (anonymous reviewer; personal communication). Although *Malassezia* is sister to the so-called “smut” plant pathogens, they are markedly divergent in ecological terms. A hallmark of *Malassezia* species is their incomplete fatty acids synthesis metabolic pathway, and reliance, instead, on a suite of extracellular lipases, phospholipases, and acid sphingomyelinases [5]. In fact, only a single species, *M. pachydermatis*, is able to survive in axenic culture lacking lipid amendment [6].

Until recently, it was assumed that *Malassezia* evolved into a specialized and narrow niche associated with the skin of mammalian hosts. However, culture-independent studies of fungi from environmental samples show that *Malassezia* are exceedingly widespread and ecologically diverse [3]. Recent studies in little-characterized marine environments point to extensive diversification of *Malassezia*-like organisms, providing exciting opportunities to explore the ecology, evolution and diversity of this enigmatic group.

What Do We Know about the Diversity and Distribution of Putative *Malassezia* spp. from Environmental Sequences?

Despite being difficult to cultivate, putative *Malassezia* are readily detected in environmental DNA samples using standard fungal “barcoding” approaches. Scanning GenBank and the scientific literature, therefore, is useful for approximating occurrence patterns. DNA sequences identical to *M. globosa* and *M. restricta*, which are both well characterized as human skin associates, appear to be cosmopolitan. *M. restricta* may be particularly widespread, and DNA sequences similar to these species have been detected in habitats as diverse as deep-sea sediments [7], hydrothermal vents [8], stony corals [9], lobster larval guts [10], Japanese Eel (*Anguilla japonica*) gut and muscle tissue [11], Antarctic soils [12,13], on the exoskeleton of soil nematodes [14], and various plant roots including mycoheterotrophic species such as orchids (e.g., [15]). Remarkably, the ribosomal DNA sequences of *Malassezia* in these studies are nearly identical to those of human associates, suggesting either a very recent divergence in habitat or else that these organisms are highly tolerant to some of the planet’s most extreme environments. Unsurprisingly, *Malassezia* sequences are not uncommon in studies of human dwellings [16], where human skin contributes substantially to house dust.

Both putatively familiar and novel *Malassezia*-like organisms are abundant on living marine hosts. Pollock and colleagues [17] report *Malassezia* dermatitis in captive pinnipeds. Two recent studies of marine biotrophic fungi show that *Malassezia*-like organisms can numerically dominate fungal communities on invertebrates. A cultivation-independent study of marine sponges from Hawaii [18] revealed a high diversity of *Malassezia*-like sequences, and indicated that a subset of these differed from the adjacent water column. The authors’ analysis further suggested that some of these putative *Malassezia* taxa are host specific at the species level. In a study of the scleractinian coral *Acropora hyacinthus*, Amend and colleagues [9] found that a phylogenetically diverse suite of *Malassezia*-like DNA sequences comprised the majority of fungi on apparently healthy colonies. A single taxon, most closely resembling *Malassezia globosa*, was significantly more abundant amongst corals located in warmer water.

Are Marine *Malassezia* Related to Terrestrial Species?

The evolutionary origins of marine *Malassezia* and their relatedness to better-characterized terrestrial species is a matter of speculation. A phylogeny compiled from environmental samples and sequenced isolates (Figure 1) demonstrates a tremendous amount of phylogenetic novelty contained within and adjacent to the *Malassezia* lineage. Evidence from both large and small subunit loci of the ribosomal cistron demonstrate well-supported clades from various environments, including a large monophyletic group of marine water column mycoplankton, sequences from separate studies of marine anoxic environments, and combinations of host-associated (coral and coralline algae) *Malassezia* that group with presumably free-living taxa in various marine and terrestrial habitats. The relatively long branch lengths separating some of these isolates from their sister taxa suggest either a particularly rapid diversification, or, alternatively, that intermediate taxa remain to be sampled and sequenced.

Citation: Amend A (2014) From Dandruff to Deep-Sea Vents: Malassezia-like Fungi Are Ecologically Hyper-diverse. PLoS Pathog 10(8): e1004277. doi:10.1371/journal.ppat.1004277

Editor: Joseph Heitman, Duke University Medical Center, United States of America

Published August 21, 2014

Copyright: © 2014 Anthony Amend. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Funding: AA acknowledges funding from NSF award 1255972. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing Interests: The author has declared that no competing interests exist.

* Email: amend@hawaii.edu
A phylogeny of environmental sequences from putative Malassezia shows that marine taxa are interdigitated amongst those from human hosts and other terrestrial substrates and do not form a single monophyletic clade. This topology suggests repeated transitions between marine and terrestrial habitats, a pattern not atypical of marine fungi within other Dikarya lineages, including yeasts [19]. The relative ease with which fungi transition between such different environments may owe to the strength of chitinous cell walls, which withstands increased water and osmotic pressures in deep saline habitats [19].

**What Can We Infer about the Ecology and Trophic Status of Marine Malassezia?**

The tremendous diversity of habitats in which Malassezia-like organisms are found suggests that marine species of this group may incorporate a spectrum of trophic strategies ranging from saprotrophy to biotrophy. Resident Malassezia-like organisms on seemingly healthy coral and sponge hosts may be commensals, latent pathogens awaiting host immunosuppression, or both, depending on host and environmental context. A study of crustose
coralline algae around Palmyra Atoll found that a *Malassezia* phylotype was abundant in banding disease lesions [20]. Incidence of the disease increased by an order of magnitude following an El Niño event. A laboratory manipulation study showed that disease virulence correlated with an interaction between increases in CO2 and temperature. Despite efforts, the authors were unable to cultivate the fungus, and it remains to be tested if *Malassezia* is the cause or merely a symptom of the banding disease. Nevertheless, the study presents the possibility that a putative *Malassezia* may act as a pathogen in nonmammal hosts under certain environmental contexts. The high incidence and virulence of the disease raises the possibility that when combined with environmental perturbations, marine *Malassezia* may even exert bottom-up control on reef community structure.

**How Do We Know that *Malassezia* Detected in Marine Environmental DNA Aren’t Contaminants?**

Given the high incidence of *Malassezia* species on human skin [1,2], a healthy skepticism is warranted since mammalian skin cells from terrestrial sources could potentially accumulate in marine samples, or contamination by lab personnel could result in false positives. Potential for contamination is particularly high when environmental DNA sequences are generated using sensitive, high-throughput methods. Nevertheless, multiple lines of evidence support the position of *Malassezia*-like organisms as true marine residents. Edgcomb and colleagues [21] reported a high proportion of *Malassezia*-like sequences in deep-sea sediments detected by sequencing environmental RNA. Because single stranded RNA degrades quickly in situ, its presence supports the notion of active growth as opposed to DNA “contamination” in this habitat. Furthermore, the RNA sequences were distinct from those of any organism known to associate with mammalian hosts, excluding the possibility of lab contamination. A follow-up study using even more stringent protocols and negative controls to exclude exogenous nucleic acids detected *Malassezia*-like sequences in samples located at depths of 1.6 and 45.1 meters below the sea floor [22]. Fungal community composition overall was highly correlated with site geochemistry, suggesting the environmental selection of a metabolically active assemblage. Similarly, an analysis of actively transcribed genes (mRNA) from a coral habitat identified components of multiple metabolic pathways allied with sequenced *Malassezia* genomes [9] — further evidence that these fungi are alive and metabolically active underwater. The fact that *Malassezia*-like organisms are frequently found in remote marine locations far from humans (e.g., [7,8,21,23–27], and many others) also renders the terrestrial input hypothesis less likely.

**What Are Future Directions for Research into Marine *Malassezia*?**

The remarkable environmental plasticity of *M. restricta* lends itself to population-level studies of adaptation and acclimatization among the Earth’s most extreme environments. How do differences in gene content and transcription correlate with residence in arctic soils versus deep-sea vents? What traits mark the transition from saprobic to pathogenic lifestyles? How many times has a marine (or terrestrial) lifestyle evolved independently? As a model system, the genus *Malassezia* has much to offer: three sequenced genomes, *M. globosa*, *M. restricta*, and *M. sympodialis*, contain fewer than 9 Mb and 3,000 genes [20,29], placing them amongst the smallest free-living genomes in the kingdom Fungi. Furthermore, although the sexual cycle has not been observed in this group, a genomic signature of bipolar mating exists [3,5,29,30]. The genus *Malassezia* contains a rich and potentially novel suite of enzymes and metabolites [2] from a variety of inhospitable and relatively unexplored habitats.

Arguably, the greatest challenge to studying marine *Malassezia* is in obtaining axenic cultures, and no marine isolates, to my knowledge, have been recovered to date. Even among species associated with the better-characterized terrestrial mammalian hosts, lab cultivation can be a hit-or-miss affair, involving media and conditions, that can further complicate cultivation efforts.

Nevertheless, there is much to learn about the basic biology and physiology of host-associated marine *Malassezia*-like organisms independent of culturing. Some of these questions can be addressed using microscopy in conjunction with labeling techniques such as fluorescein in situ hybridization (FISH). Even basic questions about where *Malassezia*-like organisms reside on corals remains to be answered. Are *Malassezia*-like organisms associated with coral mucus, for example, or are they more closely associated with the dinoflagellate symbionts? Does their exclusion affect host fitness? Is there evidence of host specificity or co-evolution?

**Conclusions**

Analysis of environmental sequences demonstrates that putative members of the *Malassezia* lineage likely rank among the most widespread fungi on the planet. They are found in a startling diversity of habitats and locations, from polar regions to deep-sea vents. *Malassezia*-like species appear to dominate certain marine habitats, which should most certainly be the focus of future research into the diversity and distribution of this enigmatic group. Clearly, considering *Malassezia* a mere epidermis-commensal is a definition that is only skin deep.

**Acknowledgments**

Thanks to Kamala Earl for valuable insight and assistance with literature review, to Virginia Edgcomb for access to marine sediment RNA data, and to Gerry Cohian, Jerry Koko, Nicole Hyson, Richard O’Rorke, Leah Tooman, F. Javier Cabañes, Joseph Heitman, and three anonymous referees for critical review of the manuscript.

**References**

1. Findley K, Oh J, Yang J, Coulan S, Deming C, et al. (2013) Topographic diversity of fungal and bacterial communities in human skin. Nature 498: 367–370. doi:10.1038/nature12171
2. Ashbee HR (2007) Update on the genus Malassezia. Med Mycol 45: 287–303. doi:10.1080/13693780701191373
3. Boekhout T, Guinho E, Mayer P, Velegzaki A, editors (2010) Malassezia and the Skin: Science and Clinical Practice. Berlin: Springer. 1 pp. doi:10.1007/978-3-642-03616-3
4. Guinho E, Midgley G, Guillo J (1996) The genus Malassezia with description of four new species. Antonie Van Leeuwenhoek 69: 337–353. doi:10.1007/BF00399623
5. Xu J, Saunders CW, Hu P, Grant RA, Boekhout T, et al. (2007) Dandreiia-associated Malassezia genomes reveal convergent and divergent virulence traits shared with plant and human fungal pathogens. Proc Natl Acad Sci U S A 104: 18730–18735. doi:10.1073/pnas.0706756104
6. Cabanes EJ (2014) Malassezia Yeasts: How many species infect humans and animals? PLoS Pathog 10: e1003892. doi:10.1371/journal.ppat.1003892. e001
7. Lai X, Cao L, Tan H, Fang S, Huang Y, et al. (2007) Fungal communities from methane hydrate-bearing deep-sea marine sediments in South China Sea. ISME J 1: 736–762. doi:10.1038/ismej.2007.31

PLOS Pathogens | www.plospathogens.org
8. Le Calvez T, Burgaud G, Mahe S, Barbier G, Vandenkoornhuyse P (2009) Fungal diversity in deep-sea hydrothermal ecosystems. Appl Environ Microbiol 75: 6415–6421. doi:10.1128/AEM.00635-09

9. Amend AS, Barshis DJ, Oliver TA (2012) Coral-associated marine fungi form novel lineages and heterogeneous assemblages. ISME J 6: 1291–1301. doi:10.1038/ismej.2011.193

10. O’Rorke R, Lavery SD, Wang M, Dodder SD, Jeffs AG (2013) Determining the diet of larvae of the red rock lobster (Jasus edwardsii) using high-throughput DNA sequencing techniques. Mar Biol 161: 551–563. doi:10.1007/s00227-013-2357-7

11. Terahara T, Chow S, Kurogi H, Lee S-H, Tsukamoto K, et al. (2011) Efficiency of peptide nucleic acid-directed PCR clamping and Its application in the investigation of natural diets of the Japanese eel Leptocephali. PLoS ONE 6: e25715. doi:10.1371/journal.pone.0025715

12. Arenz BE, Held BW, Jurgens JA, Farrell RL, Blanchette RA (2006) Fungal diversity in soils and historic wood from the Ross Sea Region of Antarctica. Soil Biol Biochem 38: 3057–3064. doi:10.1016/j.soilbio.2006.01.016

13. Fell JW, Scorzetti G, Connell L, Craig S (2006) Biodiversity of micro-eukaryotes in Antarctic Dry Valley soils with. Soil Biol Biochem 38: 3107–3119. doi:10.1016/j.soilbio.2006.01.014

14. Renuke C, Alphé J, Bucqo F (2003) Soil nematodes associated with the mammal pathogenic fungal genus Malassezia (Basidiomycota: Ustilaginomycetes) in Central European forests. Biol Fertil Soils 37: 70–72. doi:10.1007/s00374-002-0556-3

15. Roy M, Watthana S, Siter A, Richard F, Vessabutr S, et al. (2009) Two mycobiocenoses from Thai river sediments associated with a broad diversity of ectomycorrhizal fungi. BMC Biol 7: 51. doi:10.1186/1471-2180-7-51

16. Pitkaranta M, Meklin T, Hyvarinen A, Paulin L, Auvinen P, et al. (2007) Analysis of fungal flora in indoor dust by ribosomal DNA sequence analysis, quantitative PCR, and culture. Appl Environ Microbiol 74: 233–244. doi:10.1128/AEM.00692-07

17. Pollock CG, Roehnback B, Ramsay EC (2000) Fungal dermatitis in captive primiparous female. J Zoo Wildl Med 31: 377–378. doi:10.1638/1043-7260(2000)031 %253B374:FHCP%2520.CO2

18. Gao Z, Li B, Zheng C, Wang G (2008) Molecular Detection of Fungal Communities in the Hawaiian Marine Sponges Suberites zeteki and Mycale armata. Appl Environ Microbiol 74: 6091–6101. doi:10.1128/AEM.01315-08

19. Richards TA, Jones MDM, Leonard G, Bass D (2012) Marine Fungi: Their Ecology and Molecular Diversity. Annu Rev Marine Sci 4: 495–522. doi:10.1146/annurev-marine-120710-100022

20. Williams GP, Price NN, Usuijima B, Seby GA, Callahan S, et al. (2014) Ocean warming and acidification have complex interactive effects on the dynamics of a marine fungal disease. Proc R Soc Lond B Biol Sci 281: 20133069–20133069. doi:10.1098/rspb.2013.3069

21. Edgcomb VP, Beaudoin D, Gast R, Teske A (2011) Marine subsurface eukaryotes: the fungal majority. Environ Microbiol 13: 172–183. doi:10.1111/j.1462-2920.2010.02518.x

22. Orsi W, Orsi W, Biddle JF, Edgcomb VP, et al. (2013) Deep Sequencing of Subseafloor Eukaryotic rRNA Reveals Active Fungi across Marine Subsurface Provinces. PLoS ONE 9: e56335.

23. Williams NM, Crane EE, Roulston TH, Minckley RL, Packer L, et al. (2010) Biological Conservation. Biol Conserv 143: 2280–2291. doi:10.1016/j.biocon.2010.05.024

24. Singh P, Raghuhamaker C, Verma P, Shouche Y (2010) Fungal Community Analysis in the Deep-Sea Sediments of the Central Indian Basin by Culture-Independent Approach. Microb Ecol 61: 507–517. doi:10.1007/s00248-010-9765-8

25. Jeharaj CS, Raghuhamaker C, Belinke A, Strocek T (2010) Fungal diversity in oxygen-depleted regions of the Arabian Sea revealed by targeted environmental sequencing combined with cultivation. FEMS Microbiol Ecol 71: 399–412. doi:10.1111/j.1574-6941.2009.00894.x

26. Bass D, Howe A, Brown N, Barton H, Demidova M, et al. (2007) Yeast forms dominate fungal diversity in the deep oceans. Proc R Soc Lond B Biol Sci 274: 3069–3077. doi:10.1093/rspb/274.23.4698

27. Gao Z, Johnson ZI, Wang G (2009) Molecular characterization of the spatial diversity and novel lineages of mycoplanckton in Hawaiian coastal waters. ISME J 4: 111–120. doi:10.1038/isme.2009.87

28. Saunders CW, Scheynus A, Heitman J (2012) Malassezia Fungi Are Specialized to Live on Skin and Associated with Dandruff, Eczema, and Other Skin Diseases. PLoS Pathog 8: e1002701. doi:10.1371/journal.ppat.1002701.g001

29. Gioti A, Nystedt B, Li W, Xu J, Andersson A, et al. (2013) Genomic insights into the atopic eczema-associated skin commensal yeast Malassezia sympodialis. MBio 4: e00572–12. doi:10.1128/mBio.00572-12

30. Coelho MA, Sampaio JP, Goncalves P (2013) Living and Thriving on the Skin: Malassezia Genomes Tell the Story. MBio 4: e00117–13–e00117–13. doi:10.1128/mBio.00117-13