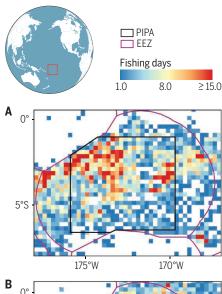
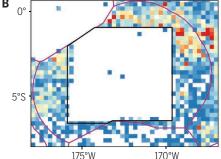
begin enforcing proper use of AIS. Simply having an AIS unit aboard a vessel, but failing to use it properly, can no longer be viewed as legal compliance. As noncompliance becomes better controlled, we also encourage that AIS data be more widely considered as admissible evidence in maritime judicial proceedings.

Evidence suggests that it is possible to equip all commercial fishing vessels in the world with AIS and enforce its use. About 75% of EU fishing vessels complied with 2014 AIS mandates within months (9). We estimated that 71% of large fishing vessels (>24 m) worldwide use AIS, and we observed a 17% increase in global AIS coverage for fishing vessels during 2014 (4). Closing remaining gaps among users resistant to compliance will be difficult but critically important.

Widespread implementation of publicly accessible AIS would effectively bring an end to the era of marine anonymity. There is growing awareness in marine and terrestrial sectors that benefits for human and environmental safety derived from





Observing marine protected areas from space.

Summary of long-line and purse seine fishing as measured using S-AIS data in PIPA during the 6 months before (A) and 6 months after (B) it was closed to commercial fishing by the Kiribati government on 1 Jan 2015 (4).

observation technologies outweigh costs of renegotiating the boundaries of industrial privacy. Recognizing these values, the marine shipping industry has almost universally adopted AIS, as well as supplemental data-sharing systems.

Reforms under way to begin managing the ocean at vastly larger and ecologically meaningful scales will only matter if we can see and act on what is happening in these spaces. Transparency is an extremely important part of this process. Parallel closed-access tracking systems can and should be linked to AIS to improve our view of vessel activity, but closed-access systems allow only part of the picture to be seen by few actors and, consequently, have more limited value to science and transboundary biodiversity management. Unfortunately, current lack of legislative support for AIS has stunted this system into a service that best observes vessels that don't mind being seen. Although the policy shifts we call for require brave revisioning of the primacy of privacy on the oceans, failure to close loopholes will continue to foster illegal activities that steal income and biodiversity from developing nations, promote social injustice at sea, and undermine efforts to cooperatively manage the sustained vitality of our shared marine resources.

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SUPPLEMENTARY MATERIALS

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MICROBIOLOGY

The invisible dimension of fungal diversity

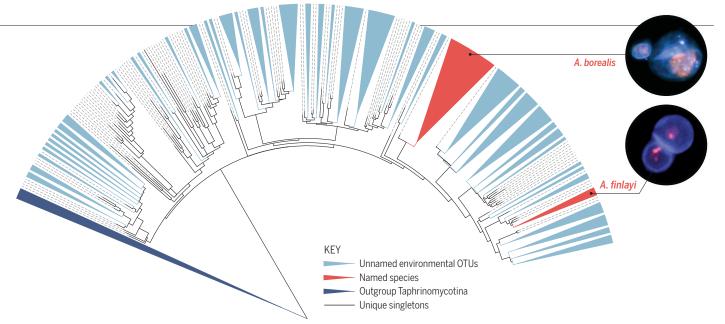
Can microbial taxa be defined from environmental molecular sequences?

Bu David Hibbett

axonomy plays a central role in understanding the diversity of life, translating the products of biological exploration and discovery-specimens and observations-into systems of names that capture the relationships between species. Taxonomic names facilitate communication among scientists and the public and provide conceptual handles for complex phylogenetic hypotheses. However, taxonomy can be challenging, particularly for fungi and other microorganisms, which are morphologically simple and extremely diverse (1). Molecular environmental surveys have revealed previously unknown branches of the fungal tree of life (2-5) and illuminated biogeographic patterns across all groups of fungi (6, 7). Yet the products of this research are not being translated into formal species names, in part because of the very rules designed to facilitate taxonomy.

Two recently recognized groups of fungi, Archaeorhizomycetes and Cryptomycota, illustrate the magnitude of ongoing molecular species discovery. Archaeorhizomycetes are root-associated soil fungi that have been found in more than 100 independent studies. When Menkis et al. (3) pooled environmental sequences of ribosomal internal transcribed spacer (ITS) genes, they found 50 lineages of Archaeorhizomycetes containing at least two independent sequences with 97% similarity, a standard cutoff for recognizing OTUs (operational taxonomic units, often equated with species). They also found 95 unique sequences (singletons). Thus, as many as 145 species of Archaeorhizomycetes have been discovered. But only two have been formally named, Archaeorhizomyces finlayi and A. borealis, based on the only live cultures obtained so far (see the figure).

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Visible and invisible diversity of Archaeorhizomycetes. Phylogenetic analysis of environmental DNA sequences resolves 50 species-level groups of Archaeorhizomycetes (3), but only two have been formally named. The existence of 95 unique singleton sequences suggests that there is much unsampled diversity. Archaeorhizomycetes is the most diverse class in the Taphrinomycotina, which include well-known plant pathogens and the model fission yeast. Tree topology from (3); micrographs by A. Rosling.

The aptly named Cryptomycota have also been detected repeatedly in molecular studies of soil, freshwater, and marine habitats (4, 5). Phylogenetic analyses suggest that Cryptomycota make up an ancient clade of fungi. Cryptomycota cells have been visualized in environmental samples with fluorescent tags, but these observations are not linked to cultures or specimens (4). Lazarus and James analyzed 109 environmental sequences of Cryptomycota and resolved 12 strongly supported groups (5). However, they could only assign three sequences to a formally named taxon, the genus Rozella, which includes endoparasites of protists and other fungi.

Molecular ecological studies have investigated fungal distributions on global (7) and local (8) scales. In one recent global survey, Davison et al. (6) analyzed the distribution of arbuscular mycorrhizal fungi, which form symbioses with about 80% of plants, but do not produce mushrooms and cannot be grown in pure culture. Using 18S ribosomal RNA gene sequences, the authors detected 246 OTUs, of which 93% occurred on multiple continents and 34% on six continents. This level of endemism is surprisingly low for fungi that do not produce airborne spores (6). However, the results provide limited information about distributions of known taxa, because only 41 of the OTUs (17%) include sequences from named species.

In the largest study of fungal diversity to date, Tedersoo et al. (7) analyzed 1.4 million ITS sequences from 365 sites worldwide and recovered ~45,000 OTUs represented by at least two sequences, with a further ~36,000 singletons. Only about one-third of the nonsingleton OTUs matched any sequences in public databases at the 97%

similarity cutoff. In other words, this study detected ~30,000 OTUs that could be new species-about 15 times the number of new fungal species descriptions published in journals and recorded in nomenclatural databases in the same year (1).

Environmental sequences have transformed understanding of the fungal tree of life, from its deepest roots to its finest branches. Why are these discoveries not being formalized in taxonomic names?

First, there are legitimate scientific concerns over the nature of evidence required to delimit species. Mycologists have selected ITS as the official barcode locus (9), but in some groups multiple species may have identical ITS sequences, whereas in other groups there may be multiple forms of ITS in a single genome. Single-cell genomics could provide multiple genes from individuals in environmental samples, but these technically demanding approaches are not yet widely applied in fungal ecology. Until they are, most studies will rely on single markers such as ITS, which can never reveal the patterns of gene flow that provide clues to the limits of reproductively isolated lineages.

Second, species names are no longer needed to test ecological or evolutionary hypotheses. Ecologists once relied on taxonomic resources to identify the species whose interactions they sought to explain; today, they use automated pipelines to cluster OTUs without reference to keys, vouchers, and monographs (10). Ecologists and evolutionary biologists do not need to undertake the tedious work of identification and formal taxon description to address the problems that motivate them. On the other hand, molecular ecological studies are providing unprecedented resources for taxonomists, who could use the new data to describe new species or enhance existing descriptions with expanded geographic ranges and new insights into ecological roles.

Unfortunately, the rules that govern biological nomenclature prohibit formal naming of fungi and other microbial taxa based on environmental sequences. Physical type specimens are required by the botanical and zoological codes of nomenclature, which collectively determine conditions for valid publication of names of fungi and protists, and the bacteriological code requires type cultures. Classical taxonomy is thus cut off, by its own nomenclatural rules, from the major modes of discovery for microbial organisms. Working models for purely sequence-based delimitation of fungal taxa do exist (3, 6, 10, 11), but do not yet confer nomenclatural validity. Community standards for sequence-based taxon definition and revision of the codes of nomenclature are needed to make the products of molecular environmental surveys visible to scientific and lay communities. ■

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Federal barriers to Cannabis research

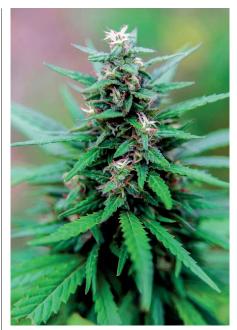
ALTHOUGH THE MAJORITY of the general public (1) and the professional medical community (2) in the United States support the therapeutic use of Cannabis sativa as a pharmacological agent, the U.S. federal government's *Cannabis* research policies have blocked externally valid, randomized clinical trials on the effects of Cannabis. To conduct research on Cannabis, scientists must submit to a lengthy and arduous application process, often lasting for years. The research requires permission from multiple governmental agencies, including some with expressly stated opposition to any therapeutic uses, such as the Drug Enforcement Agency (3).

However, the application process is a mere nuisance compared with the biggest obstacle presented by the federal government: All Cannabis used for research purposes must be purchased through the National Institute on Drug Abuse (NIDA) (4). The tetrahydrocannabinol (THC) potency levels in the Cannabis available through NIDA are much lower than those in Cannabis products used by medical patients. The highest THC level available to researchers is 12.4% (5). The only two clinical studies funded by NIH in 2015 used products with potency levels between 3.5 and 7.0% THC (6, 7). In contrast, the Cannabis sold in Colorado now averages 18.7% THC, with some strains registering as high as 35% THC (8), and no potency limits exist for the concentrates and ingestible products sold in most states where medical Cannabis is legal at the state level.

The scarce research the U.S. government has approved thus offers little insight into the effects actually experienced by patients and recreational users. As long as clinical research on *Cannabis* is controlled by regulators expressly opposed to any increase in its consumption, health care cost reductions may be missed, and intoxication and long-term effects will remain unknown. Most important, many severely ill patients may suffer unnecessarily because no one knows the true risks and benefits of consuming *Cannabis sativa*.

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Cannabis sativa.

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No surprise that comb jellies poop

AS ONE OF the organizers of the recent meeting on comb jellies (ctenophores), I feel obliged to comment on the News In Depth story "Comb jelly 'anus' guts ideas on origin of through-gut" (A. Maxmen, 25 March, p. 1378), published online on

23 March with the title, "Why watching comb jellies poop has stunned evolutionary biologists." I was stunned that videos showing defecation of waste through the anal pores of ctenophores astonished anyone. Those who have looked closely at comb jellies have seen and reported this process for well over a century.

In 1850, Louis Agassiz found that waste products were expelled from comb jellies through sphincter-like anal pores, which open and close during bouts of defecation (1). Thirty years later, the German zoologist Carl Chun used injected dyes and tracking of waste particles to expand on Agassiz's results in great detail (2). Since then, scientists have amply confirmed Agassiz's and Chun's findings and studied how the process of defecation works (3). Nearly every invertebrate textbook in the 20th century shows the anal pores of ctenophores. This literature was omitted or grossly misrepresented in the News story to erroneously claim a novel discovery of a through-gut in comb jellies.

It is now recognized that ctenophores expel waste from both ends. They eject bulky indigestible food fragments, which do not enter the stomach or food canal system, through the mouth. Meanwhile, unused or small waste particles in the food canals are periodically shunted into the stomach and anal canals, where they are expelled through the anal pores (3). In contrast to the implication of the News story, the two exit methods of waste products are not contradictory or mutually exclusive. It should not surprise anyone that comb jellies poop and have a through-gut.

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Digital identifiers for fungal species

SPECIES-LEVEL CLASSIFICATION OF life has been a cornerstone of biology for centuries. Most macro-organisms are described soon after discovery, but species of prokaryotes, micro-eukaryotes, and fungi often lag far behind in formal description because they are small, extremely diverse, and difficult to cultivate and often lack discriminatory morphological characteristics.

D. Hibbett ("The invisible dimension of

fungal diversity," Perspectives, 11 March, p. 1150) recently argued that missing species names (Latin binomials) in the kingdom Fungi hamper communication about formally undescribed species derived from molecular surveys of the environment. He pleaded for changes in the International Code of Nomenclature for Algae, Fungi, and Plants (1). We argue that Latin binomials are not urgently needed for precise communication and delimitation of environmental species known only from DNA barcode sequences.

In the UNITE database for molecular identification of fungi, we have adopted the species hypotheses concept to provide unique digital object identifiers (DOIs) for all fungal species known from sequence data (2, 3). We have, for example, used this concept to analyze the Archaeorhizomycetes species (4) examined by Hibbett. The species hypotheses concept accounts for taxonomic uncertainty through multiple alternative cut-off levels for species delimitation. As in the Linnaean tradition, it relies on molecular keys, reference sequences, and voucher material specified by taxonomists.

Several major microbial identification pipelines, notably QIIME (5), use the species hypotheses identifiers as a community standardization measure. The Barcode of Life Data System similarly assigns barcode index numbers to animal taxa (6). Both systems enable straightforward communication of machine-readable but formally undescribed species across scientific studies. Both serve to facilitate future descriptions of those taxa by aggregating data on, for example, geographical distribution and substrate of collection. When voucher material or cultures become available, a formal species description can draw on the molecular and other data amalgamated in the above databases.

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Response

SPECIES CLASSIFICATION PLATFORMS based on DNA sequences, including the UNITE system, allow ecologists and evolutionary biologists to conduct sophisticated research programs without reference to Latin binomials. However, the entities that they catalog—such as species hypotheses (described by Kõljalg et al.), virtual taxa (1), or barcode index numbers (2)—are obscure concepts. Although useful to specialists, these concepts are unfamiliar to the general public. In contrast, the notion of species is deeply ingrained in human culture, even if evolutionary biologists understand that there is no universal, objective criterion for defining a species (3). Species names in the form of Latin binomials are useful for communicating knowledge of biodiversity not only to nontechnical audiences such as legislators, educators, and members of the media, but also to the vast majority of biologists, who are not microbial ecologists.

Kõljalg *et al.* assert that species classification is a cornerstone of biology and lament the lag between discovery and description of fungal species and other microorganisms. This lag time would be greatly diminished if species could be formally defined based on molecular sequences. However, this is not possible under the International Code of Nomenclature for Algae, Fungi and Plants (4), which requires physical type specimens regardless of their quality or scientific utility. The Code can only be modified once every 7 years by a vote of the Nomenclature Section of the International Botanical Congress. The next opportunity to change the Code will be in 2017. Adoption of sequencebased species description would promote the integration of molecular ecology and traditional taxonomy, which would be facilitated by resources such as UNITE.

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