

Chapter 22

Microbial Endemism and Biogeography

BRIAN P. HEDLUND AND JAMES T. STALEY

THE MICROBIAL BIOGEOGRAPHY DEBATE

The topic of microbial biogeography is almost 100 years old, however, when confronted with questions about the existence and extent of endemism in the microbial world, many microbiologists respond with opinions and theoretical arguments rather than examples of well-conducted studies. We begin this chapter with an overview of this debate as it applies to free-living prokaryotes in part because there are relatively few good microbial biogeography studies. Furthermore, the arguments help to frame microbial biogeography in the larger context of biodiversity in that if endemism is common, then many more species exist.

The Argument for Microbial Endemism

Arguments for endemism among free-living microbes generally draw from examples of endemism of other organisms. First, any international traveler knows that many pathogenic microbes, both viral and bacterial, have distinct biogeographies. For example, dogs and cats entering Australia are quarantined in order to prevent the introduction of the rabies virus onto the continent. And historians are quick to point out one of the great tragedies in human history, the re-peopling of the Americas by Europeans, who brought many new pathogens with them to the new world. This microbial invasion included the variola virus (smallpox), the measles virus, the influenza virus, the yellow fever virus, *Salmonella typhae* (typhoid fever), *Vibrio cholerae* (cholera), and *Yersinia pestis* (plague) and resulted in the infection and death of >95% of the pre-Columbian native population (Diamond, 1999). The fact that some pathogens have distinct biogeographies may suggest that some free-living microbes have biogeographies as well.

Another reason to suppose that endemism exists for free-living microbes is that most plant, animal, and some fungal species are endemic to one continent or another. Indeed, examples of plant and animal families and even orders are known to be endemic to isolated islands (Brown and Lomolino, 1998). Furthermore, a basic tenet of biodiversity is that species diversity increases with decreasing body size (May, 1988, Wilson, 1992). For terrestrial animals, each 10-fold reduction in body length or 1,000-fold reduction in body weight corresponds to roughly a 100-fold increase in species diversity (May, 1988). If such a relationship held for microbes, the number of microbial species on our planet would be astronomical and it would seem that many microbes would have to be endemic to achieve this level of diversity. However, it is important to clarify that there is no theoretical ground for extrapolating a species richness–“body” size correlation to include microbes, and the current lack of a meaningful species definition for most microbes reduces any such relationship to a conceptual level rather than a truly quantitative level. Nevertheless, given that endemism is the norm, why should free-living microbes defy this pattern?

The Argument for Microbial Cosmopolitanism

The cosmopolitan view of the microbial world has a long history (Baas-Becking, 1934) and has withstood the test of time relatively well. Although some pathogens provide fuel for the endemism argument, others are clearly cosmopolitan, consisting of a limited number of globally distributed clonal lineages, including *Escherichia coli*, *Haemophilus influenzae*, *Neisseria meningitidis*, *Staphylococcus aureus*, and *Streptococcus pneumoniae*. Appropriately, Cho and Tiedje (2000) have pointed out that these bacteria are closely associated with humans and their biogeographies are inevitably affected by human activity, but it

could be argued that free-living microbes are also dispersed by human activities, leading to cosmopolitanism, as is also true of many plants and animals.

The cosmopolitan view has recently been staunchly advocated by the eukaryotic microbial ecologist Finlay, among others, who has published frequently in high-profile journals (Finlay, 2002; Finlay and Clarke, 1999; Finlay et al., 1996). The argument states that small body size and immense population size lead to constant dispersion of microbes by a variety of mechanisms (including human-mediated) and, consequently, cosmopolitanism. Some microbes form cysts or spores that are particularly well suited for long-term survival during passive dissemination, and even microbes that do not form specific survival stages are known to survive long periods in metabolically inactive states. These microbial seeds “bloom” when and where conditions favor their growth.

Based on these ideas and corroborating data on the cosmopolitanism of many protist morphospecies, Finlay (2002) has proposed that an ubiquity and biodiversity transition occurs at a body length of between 1 and 10 mm. According to this model, essentially all species with body lengths larger than this transition size have distinct biogeographies, whereas essentially all organisms smaller than the transition size are ubiquitous (i.e., cosmopolitan).

These arguments draw attention to the topic of microbial biogeography and help to set conceptual limits on microbial biogeography and diversity.

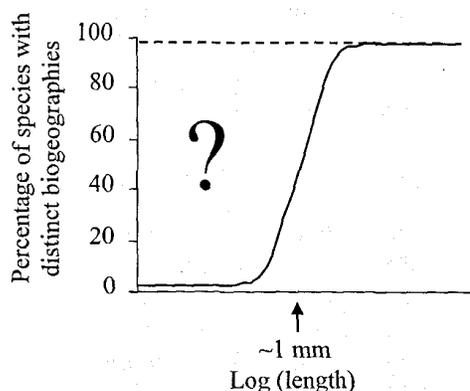


Figure 1. Conceptual figure showing alternative biogeography and body size models. Finlay (2002) has proposed that an ubiquity and biodiversity transition occurs at a body length of 1 to 10 mm, which suggests that many protozoa and nearly all prokaryotes are cosmopolitan (solid line). Alternatively, the apparent ubiquity and biodiversity transition may represent the size at which the morphospecies concept decays, and future molecular studies may show that most microbial species have distinct biogeographies, as do larger organisms (dotted line). It is likely that the percentage of microbes with distinct biogeographical patterns lies somewhere between the two extreme models (question mark).

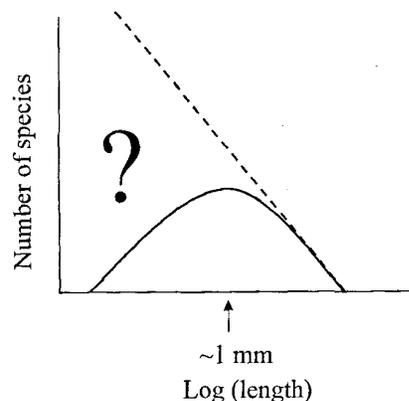


Figure 2. Conceptual figure showing the enormous effect of biogeography and body size models on biodiversity estimates. According to Finlay's model (2002), most of the species diversity on this planet would be in the size range of small insects (solid line). Alternatively, extrapolation of the body size and species diversity relationship (May, 1988) to include microbes would yield a vastly greater number of prokaryotic species (dotted line). In reality microbial diversity may lie between the two extremes (question mark).

They also clarify how little we know. Figure 1 shows Finlay's model (2002) of microbial biogeography (solid line), which invokes that essentially all prokaryotes are cosmopolitan. Although Finlay is dogmatic about this view, we believe that it is far from conclusive and present it here as a conceptual extreme in favor of microbial cosmopolitanism. The opposing extreme (dotted line) is that nearly all organisms have meaningful biogeographies at some taxonomic level, regardless of size. We believe it is important that microbiologists discuss, and eventually work toward resolving, the microbial biogeography debate because it has great implications for our understanding of biodiversity (Fig. 2). If Finlay is correct, then microbes are not particularly diverse (solid line). On the other hand, if microbial endemism is the norm, then the majority of diversity on our planet is microbial (dotted line). In the following sections we discuss some studies on microbial biogeography, which seem to suggest that Finlay's view may be correct on a certain taxonomic level—the protist morphospecies or the prokaryotic genus—but that some microbes have meaningful biogeographies below that level.

ARE MICROBIAL EUKARYOTES REALLY COSMOPOLITAN, OR DO WE NEED A BETTER MAGNIFYING GLASS?

The biogeography of free-living microbial eukaryotes has received a lot of attention; therefore it is

significant that protozoologists seem to agree that most protist morphospecies are cosmopolitan. To illustrate this axiom, Finlay and Clarke (1999) used transmission electron microscopy to survey 25.2 μl of superficial sediment from Priest Pot, a small freshwater pond in the United Kingdom. In this tiny sample they observed siliceous scales representing 32 of the 41 known morphospecies of the ciliate *Paraphysomonas*. In addition, they found that the most numerous *Paraphysomonas* species in Priest Pot were also the most common *Paraphysomonas* species worldwide, as estimated from 73 published surveys of the genus. These data suggest that the more numerous *Paraphysomonas* species are more likely to be cosmopolitan because they produce larger numbers of cysts for dispersal.

More generally, Finlay et al. (1996) have pointed out that intensive surveying of benthic ciliates in several sites in Europe consistently recovered approximately 100 morphospecies, indicating that the diversity of ciliate morphospecies in that habitat is limited and well surveyed. However, it is unclear whether these studies recovered the same 100 morphospecies at all sites or whether some ciliates were particular to one site or another; therefore, it seems that the important details of biogeography were lost in the numbers. Finlay's group has also debunked several candidate endemics (e.g., Esteban et al., 2001), and these studies provide more definitive, if not universally applicable, arguments for the cosmopolitanism of protist morphospecies.

In a study designed to determine a relationship between protist size and cosmopolitanism, Wilkinson (2001) compiled data on polar surveys of testate amoebae. Contrary to the cosmopolitan morphospecies axiom, the survey of 13 Arctic and 13 Antarctic sites revealed that only 29 of the 127 (23%) morphospecies recorded in polar samples were found on both poles. It is important to note, however, that not finding an organism at one pole or another should not be regarded as evidence of its absence, particularly since sampling was limited. The author also noted that each of the 29 cosmopolitan species was small (<135 μm) and that a negative relationship exists between species size and the number of sites at which it was recorded. Based on this data, Wilkinson suggested that the maximum size for cosmopolitan testate amoebae is between 100 and 200 μm . However, an alternative explanation for this pattern would be that the apparent cosmopolitanism of small amoebae is an artifact of practical limitations of the morphospecies concept, as generally alluded to by Foissner (1999). Thus, it is important to ask: Is it species diversity (and therefore endemism) that breaks down with decreasing size? Or is it our ability

to resolve morphologically similar (or identical) species that breaks down with decreasing size? It seems obvious that at some size or morphological complexity limit, the morphodiversity concept should give way to a more sensitive and less subjective molecular phylogenetic diversity concept. Perhaps Wilkinson has described this limitation rather than a true biogeographical pattern. Likewise, Finlay's ubiquity and biodiversity transition may instead be the morphodiversity-molecular phylogenetic diversity transition. In conclusion, it seems that molecular approaches would provide a better magnifying glass than the electron microscope alone for viewing the world of protist diversity and biogeography.

MANY PROKARYOTIC GENERA ARE COSMOPOLITAN

Bacteriologists have also focused on polar communities for studies of biogeography. Staley and Gosink (1999) hypothesized that gas vacuolate sea ice bacteria might be ideal candidates for endemism because they occupy specialized niches on opposite sides of the planet. Furthermore, these bacteria are the most psychrophilic organisms known, as many do not grow at temperatures of $>10^{\circ}\text{C}$; thus, it was hypothesized that this extreme sensitivity may limit transit between the poles. In essence, their approach was to try to disprove their hypothesis by finding a species of gas vacuolate sea ice bacteria that inhabits both poles. Roughly 200 strains were grouped by fatty acid analyses and the unweighted pair group method with arithmetic mean (UPGMA) treeing and the most closely related representatives from each pole, representing three separate genera, were selected for 16S rDNA sequencing and pairwise DNA-DNA hybridization experiments. The end result was that the closest Arctic/Antarctic pairs differed by 11, 18, and 6 16S rDNA nucleotides for *Octadecabacter*, *Polaribacter*, and *Psychromonas* (formerly referred to as "*Iceobacter*"), respectively, and none exhibited more than 42% DNA-DNA hybridization. Thus, although the study recovered very close relatives from both poles, it failed to uncover a species that inhabits both poles ($>70\%$ DNA-DNA hybridization is the currently used criterion for delineating prokaryotic species [Wayne et al., 1987]). As with other studies, however, the absence of finding a cosmopolitan species in this study is not evidence that such species do not exist.

The study by Staley and Gosink (1999) exemplifies the general consensus among microbiologists that most prokaryotic genera are widely distributed in their respective habitats. Further examples of

generic cosmopolitanism come from marine habitats at the opposite end of the thermal spectrum. Shallow hydrothermal fields, deep hot sediments, and hydrothermal vents are habitats for hyperthermophilic archaea and bacteria. A priori, it may seem that these habitats, too, are possible sites for endemic microbes since many are widely separated by oxygenated waters and most hyperthermophiles are extremely oxygen sensitive. However, the same genera of hyperthermophiles often inhabit distant thermal habitats. This pattern was first described in a pair of papers from Stetter's laboratory (Huber et al., 1990; Stetter et al., 1993). Enrichments from hydrothermal vent material from the Macdonald Seamount in Polynesia and from deep sediments from oil wells in the North Sea (off Scotland) and the North Slope of Alaska each showed high levels of DNA-DNA hybridization with probes derived from *Archaeoglobus*, *Thermococcus*, *Pyrococcus*, and *Pyrodictium* isolates from Vulcano, Italy. These studies also helped to decipher the mode of dissemination of marine hyperthermophiles. Using an extinction dilution procedure, Huber et al. (1990) found up to 10^6 viable anaerobic hyperthermophiles per liter of superficial seawater following eruptions of the Macdonald Seamount. Thus, it appears that hyperthermophiles are dispersed into the open ocean where they survive in metabolically inactive states and can be transported by currents.

More recently a very unusual hyperthermophile, "*Nanoarchaeum equitans*," was discovered from samples of hot rocks from the Kolbeinsey Ridge, a shallow hydrothermal system north of Iceland (Huber et al., 2002). "*N. equitans*" is among the smallest organisms known, a coccus with a diameter of 400 nm, and it seems to be an obligate extracellular symbiont of *Ignicoccus*. Phylogenetically, "*N. equitans*" represents a new phylum of *Archaea*, the "*Nanoarchaeota*," which was previously undetected in molecular ecology surveys because it has several base exchanges in the universal primer binding regions of the 16S rDNA. In an initial study of the distribution of the "*Nanoarchaeota*," Hohn et al. (2002) used primers specific for "*Nanoarchaeota*" 16S rRNA genes to amplify genes from DNA purified from a variety of hydrothermal habitats. One gene amplified from deep-sea vent material from the East Pacific Rise, off the west coast of Mexico, was identical to the "*N. equitans*" sequence. Thus, even marine hyperthermophiles that seem to require special symbioses can be cosmopolitan on the level of the 16S rRNA gene. But similarity in rRNA genes often belies differences throughout the rest of the genome and, ultimately, important ecological differences.

NARROWING THE FOCUS: THE GENOME AND ORGANISM SURROUNDING THE 16S RRNA GENE

The small-subunit RNA was chosen by Woese (1987) as the ideal molecule for creating the universal tree of life because it is critical for a major cell function, protein synthesis, and is therefore ubiquitous and highly constrained. The slow rate at which the gene evolves allowed comparison of even the most evolutionarily distant organisms. But it is precisely this slow rate of evolution that limits the gene's utility in subgenus-level biogeography studies. Examples of phenotypically and genetically (<70% DNA-DNA hybridization) distinguishable species of bacteria with zero to two 16S rDNA sequence differences are known (Stackebrandt and Goebel, 1994; Palys et al., 1997, 2000). Furthermore, phylogenetic analyses of protein-encoding genes from members of these closely related species have yielded clearly distinguishable groups with 6 to 10% nucleotide divergence between the species and 0 to 1.5% divergence among members of the same species (Palys et al., 2000).

A recent study of terrestrial thermophiles by Whitaker et al. (2002) is the most elegant example of microbial endemism to date and illustrates why the discovery of similar or identical small-subunit rRNA genes in distant locations is not necessarily evidence that the corresponding organism has no biogeography. Seventy-eight strains of *Sulfolobus "islandicus"* isolated from eight different sites in Iceland, the western United States, and Kamchatka, Russia, were used for the study. A 552-bp fragment of the 16S rRNA gene yielded only 10 polymorphic sites. However, sequencing of fragments of eight protein-encoding genes revealed that all but one was more variable than the 16S rDNA. When the nine gene fragments were concatenated and used in phylogenetic analyses, the Icelandic strains and the North American strains each formed monophyletic clades that were strongly supported. The North American isolates were further divided into groups from Lassen National Park, California, and Yellowstone National Park, Wyoming. Within the North American populations, even isolates from neighboring hot springs, 5.7 km distant, could be divided into monophyletic clades. The Russian strains were more diverse than the North American populations and formed two monophyletic clades that corresponded to the sampling locations from which the strains were isolated, the Mutnovskiy Volcano region and the Uzon Caldera and Geyser Valley region. Furthermore, in agreement with the conclusion that geographic isolation caused the phylogenetic pattern, and not an environmental factor, the authors noted a clear correlation between geographic distance and genetic

distance for pairwise sequence comparisons, and the phylogenetic pattern could not be correlated with any environmental factor. Because *Sulfolobus* "islandicus" is composed of clearly distinguishable populations, this collection of strains is ideal for population ecology studies.

Cho and Tiedje (2000) examined the biogeography of fluorescent *Pseudomonas* strains and uncovered a more complex pattern of endemicity. The fluorescent pseudomonads comprise several closely related species. As a group, they are ubiquitous and typically abundant in soils, freshwater, and coastal marine sediments and water. Thus, superficially, it would seem that they are cosmopolitan bacteria with no meaningful biogeography. To dig deeper, the authors isolated 248 strains of fluorescent pseudomonads from 59 samples from Australia, South America (Chile), South Africa, and North America (California and Saskatchewan) and studied them on several taxonomic levels. On a coarse taxonomic level, the isolates were indeed cosmopolitan. Restriction analysis of PCR-amplified 16S rRNA genes with each of four different 4-bp recognition enzymes (amplified rDNA restriction analysis [ARDRA]) revealed only four types, two of which were found at every sampling region. To examine a finer taxonomic level, the authors amplified 16S-23S intergenic spacer (ITS) fragments and restricted them with each of three 4-bp recognition enzymes (ITS-restriction fragment length polymorphism [RFLP]), resulting in 39 types. Although most of the ITS-RFLP types were unique to a particular sample, suggesting endemicity, three types were found at more than one sampling region, indicating that the ITS-RFLP types are widely distributed. To focus even further, a PCR primer specific for repetitive extragenic palindromes was used in PCRs (BOX-PCR) and products were analyzed on polyacrylamide gels, resulting in 85 unique types. Each BOX-PCR type was unique to one site. Thus, a particular BOX-PCR type could be isolated from adjacent samples, between 5 and 175 m distant; however, it was never isolated from two locations, even sites with similar soil and vegetation within the same region. Similar to the analysis of *Sulfolobus* "islandicus," the genetic distance between these BOX-PCR types correlated with the geographic distance, supporting the hypothesis that geographic separation allowed genetic differentiation. However, the relationships between distant BOX-PCR types were not easy to interpret because BOX-PCR's resolution saturates, preventing meaningful phylogenetic analyses between distantly related clades (Cho and Tiedje, 2000).

Roberts and Cohan (1995) studied the biogeography of desert-dwelling *Bacillus* species using an approach similar to that of Whitaker et al. (2002)

and concluded that two *Bacillus* species are cosmopolitan. Like *Pseudomonas*, *Bacillus* is a ubiquitous, abundant soil organism. Because it forms endospores, it could be thought of as the ideal candidate for cosmopolitanism because its hardiness enhances its survival during dispersion in comparison with other bacteria. Isolates from undisturbed soils in North Africa (Sahara), Asia (Gobi), and North America (Death Valley, Mojave, Sonora) were screened phenotypically, and approximately 100 *B. subtilis* and *B. mojavensis* strains were selected for detailed study. Three protein-encoding genes were PCR amplified from each strain, and nucleotide divergence was calculated from restriction patterns of PCR products digested with 4-bp recognition restriction enzymes. In contrast to the results for *Sulfolobus* "islandicus," phylogenetic analyses of the *Bacillus* data did not produce clear biogeographic clades. Significantly, three genotypes were represented by isolates from separate continents, providing evidence for sub-16S rDNA level microbial cosmopolitanism. In addition, the sequence diversity within each *B. subtilis* local population (each desert) was similar to that of the world population (all deserts pooled), suggesting that each desert contains most or all of the world's diversity of these organisms, similar to the data Finlay and Clarke (1999) presented for *Paraphysomonas*. These data suggest that the rate of migration between the populations is sufficiently high to prohibit any divergence between populations on different continents. However, a closer look at the data suggests that the biogeography pattern is more complex. In particular, two well-separated clades were composed solely of Death Valley isolates (DV4-D-3 and DV2-D-1 groups) and could be regarded as candidate endemics.

CONCLUSIONS AND FUTURE DIRECTIONS

The existence and extent of endemicism among free-living prokaryotes is frequently discussed and has important implications for our understanding of microbial evolution and diversity. Early in the past century it was stated that "everything is everywhere, the environment selects" (Baas-Becking, 1934), and this theory of microbial biogeography endures today in that microbiologists are still unaware of large taxonomic groups (genera, families, orders, etc.) that are restricted due to geographical barriers. However, recent investigations into microbial biogeography using molecular techniques have begun to elucidate a more complex pattern of prokaryotic biogeography below the genus level.

The emerging picture seems to be that some prokaryotes, for example, two species of *Bacillus* (Roberts and Cohan 1995), are cosmopolitan to the extent that isolates from opposite sides of the Earth are indistinguishable from each other by phylogenetic analyses of several protein-encoding genes, whereas others, for example, *S. "islandicus"* (Whitaker et al., 2002), consist of biogeographical groups that show strict endemism. The biogeographical pattern of a particular microbe may be related to its biology and its habitat, which together determine how easily it is dispersed, whether it is likely to remain viable during transport, and whether it is likely to encounter a favorable environment. From what we know of prokaryotic biogeography, cosmopolitanism does not necessarily follow global abundance, as has been suggested for microbial eukaryotes, since endemism has been shown for fluorescent *Pseudomonas* isolates (Cho and Tiedje, 2000). Nevertheless, it seems reasonable to hypothesize that less-abundant microbes are more likely candidates for endemism. Although it is generally agreed that most protist morphospecies are cosmopolitan, protist biogeography has not yet been addressed by molecular approaches; therefore, details of the biogeography of microbial eukaryotes remain unresolved.

The studies by Whitaker et al. (2002) and Cho and Tiedje (2000) resoundingly dispel the myth that no microbes have biogeographies. Thus, future questions of microbial biogeography need not focus on whether microbial endemism exists. Rather, they should focus on how common endemism is and what a given biogeographical pattern means for the population ecology of a given microbe (e.g., migration rates, estimating times of microbial colonization events, whether biogeographic populations have diverged into separate ecotypes). Although we may be moving closer to understanding what a microbial species is (Cohan 2002), we believe that the use of different species definitions by different researchers, and even more so by researchers in different fields of biology, leads to unproductive arguments. For example, depending on what opinion a researcher is predisposed to, he or she might argue that the biogeographic *S. "islandicus"* clades described by Whitaker et al. (2002) are endemic species (an example of endemic species!) or that they are simply endemic populations (there are no endemic microbial species!). So, the challenge is this: can large numbers of closely related microbes be divided into biogeographic clusters? And do the genetic distances between clusters correlate with geographic distance or some obvious geographical demarcation? It seems that the approach of Whitaker et al. (2002), using phylogenetic

analyses of concatenated gene fragments, provides the best model for biogeographical studies.

To get a handle on the extent and degree of endemism in the microbial world, we urge microbiologists to investigate microbes from different habitats and with different lifestyles. Marine plankton, for example, are ecologically important organisms that might be more likely candidates for cosmopolitanism due to constant mixing, yet the biogeography of planktonic bacteria has never been addressed.

REFERENCES

- Baas-Becking, L. G. M. 1934. *Geobiologie of Inleiding Tot de Milieukunde*, p. 263. Van Stockum & Zoon, The Hague: The Netherlands.
- Brown, J. H., and M. V. Lomolino. 1998. *Biogeography*, 2nd ed. Sinauer Associates, Inc., Sunderland, Mass.
- Cho, J.-C., and J. M. Tiedje. 2000. Biogeography and degree of endemism of fluorescent *Pseudomonas* strains in soil. *Appl. Environ. Microbiol.* 66:5448–5456.
- Cohan, F. M. 2002. What are bacterial species? *Annu. Rev. Microbiol.* 56:457–487.
- Diamond, J. 1999. *Guns, Germs, and Steel: the Fates of Human Societies*. W. W. Norton and Co., New York, N.Y.
- Esteban, G. F., B. J. Finlay, N. Charubhun, and B. Charubhun. 2001. On the geographic distribution of *Loxodes rex* (Protozoa, Ciliophora) and other alleged endemic species of ciliates. *J. Zool. Lond.* 255:139–143.
- Finlay, B. J. 2002. Global dispersal of free-living microbial eukaryote species. *Science* 296:1061–1063.
- Finlay, B. J., and K. J. Clarke. 1999. Ubiquitous dispersal of microbial species. *Nature* 400:828.
- Finlay, B. J., G. F. Esteban, and T. Fenchel. 1996. Global diversity and body size. *Nature* 383:132–133.
- Foissner, W. 1999. Protist diversity: estimates of the near-imponderable. *Protist* 150:363–368.
- Hohn, M. J., B. P. Hedlund, and H. Huber. 2002. Detection of 16S rDNA sequences representing the novel phylum "Nanoarchaeota": indication for a broad distribution in high temperature biotopes. *Syst. Appl. Microbiol.* 25:551–554.
- Huber, H., M. J. Hohn, R. Rachel, T. Fuchs, V. C. Wimmer, and K. O. Stetter. 2002. A new phylum of Archaea represented by a nanosized hyperthermophilic symbiont. *Nature* 417:63–67.
- Huber, R., P. Stoffers, J. L. Cheminc, H. H. Richnow, and K. O. Stetter. 1990. Hyperthermophilic archaeobacteria within the crater and open-sea plume of erupting Macdonald Seamount. *Nature* 345:179–181.
- May, R. M. 1988. How many species are there on earth? *Science* 241:1441–1449.
- Palys, T., L. K. Nakamura, and F. M. Cohan. 1997. Discovery and classification of ecological diversity in the bacterial world: the role of DNA sequence data. *Int. J. Syst. Bacteriol.* 47:1145–1156.
- Palys, T., E. Berger, I. Mitrica, L. K. Nakamura, and F. M. Cohan. 2000. Protein-coding genes as molecular markers for ecologically distinct populations. *Int. J. Syst. Environ. Microbiol.* 50: 1021–1028.
- Roberts, M. S., and F. M. Cohan. 1995. Recombination and migration rates in natural populations of *Bacillus subtilis* and *Bacillus mojavensis*. *Evolution* 49:1081–1094.
- Stackebrandt, E., and B. M. Goebel. 1994. Taxonomic note: a

- place for DNA-DNA reassociation and 16S rRNA sequence analysis in the present species definition in bacteriology. *Int. J. Syst. Bacteriol.* 44:846–849.
- Staley, J. T., and J. J. Gosink. 1999. Poles apart: biodiversity and biogeography of sea ice bacteria. *Annu. Rev. Microbiol.* 53:189–215.
- Stetter, K. O., R. Huber, E. Blöchl, M. Kurr, R. D. Eden, M. Fielder, H. Cash, and I. Vance. 1993. Hyperthermophilic archaea are thriving in deep North Sea and Alaskan oil reserves. *Nature* 365:743–745.
- Wayne, L. G., D. J. Brenner, R. R. Colwell, P. A. D. Grimont, O. Kandler, M. I. Krichevsky, L. H. Moore, W. E. C. Moore, E. Stackebrandt, M. P. Starr, and H. G. Trüper. 1987. Report of the Ad Hoc Committee on reconciliation of approaches to bacterial systematics. *Int. J. Syst. Bacteriol.* 37:463–464.
- Whitaker, R., D. Grogan, and J. Taylor. 2002. Biogeographic patterns of divergence between populations of *Sulfolobus* "islandicus." In Abstracts of the 4th International Congress on Extremophiles 2002, Naples, Italy.
- Wilkinson, D. M. 2001. What is the upper size limit for cosmopolitan distribution in free-living microorganisms? *J. Biogeog.* 28:285–291.
- Wilson, E. O. 1992. *The Diversity of Life*. Belknap Press, Cambridge, Mass.
- Woese, C. R. 1987. Bacterial evolution. *Microbiol. Rev.* 51:221–271.