ELSEVIER

Microbial ecology

Spatial scaling of microbial biodiversity

Jessica Green[1] and Brendan J.M. Bohannan[2]

[1] School of Natural Sciences, University of California, PO Box 2039, Merced, CA 95344, USA
[2] Department of Biological Sciences, Stanford University, Stanford, CA 94305, USA

A central goal in ecology is to understand the spatial scaling of biodiversity. Patterns in the spatial distribution of organisms provide important clues about the underlying mechanisms that structure ecological communities and are central to setting conservation priorities. Although microorganisms comprise much of Earth's biodiversity, little is known about their biodiversity scaling relationships relative to that for plants and animals. Here, we discuss current knowledge of microbial diversity at local and global scales. We focus on three spatial patterns: the distance–decay relationship (how community composition changes with geographic distance), the taxa–area relationship, and the local: global taxa richness ratio. Recent empirical analyses of these patterns for microorganisms suggest that there are biodiversity scaling rules common to all forms of life.

Introduction
A central goal of ecology is to understand how biodiversity is generated and maintained. Spatial patterns of species diversity provide information about the mechanisms that regulate biodiversity [1,2] and are important for setting conservation priorities [3,4]. Although spatial patterns have been documented in many studies of plant and animal diversity, such patterns are not as well documented in microbial species (i.e. Bacteria, Archaea, and microscopic Eukarya). This is a serious omission given that microorganisms could comprise much of the biodiversity on Earth [5] and have crucial roles in biogeochemical cycling and ecosystem functioning [6,7].

There are technical and conceptual reasons for our lack of understanding of the scaling of microbial diversity. Technically, it has been challenging to quantify microbial diversity. Most prokaryotic and many eukaryotic microorganisms cannot be identified morphologically and, until recently, could be identified only using traits that require culturing in the laboratory. Culture techniques, however, reveal only a fraction of the diversity of microbial life. Conceptually, it has long been assumed that microbes are different biologically from other forms of life such that their biodiversity scales in a fundamentally different way. It has been assumed that for microorganisms 'everything is everywhere, the environment selects' [8]; that is, that the small size and high abundance of microbes (as well as other aspects of their biology) increase the rate and geographic distance of dispersal to levels where dispersal limitation is nonexistent, resulting in 'cosmopolitan' distributions [9,10].

Because of these technical and conceptual obstacles, there have been few studies of the spatial scaling of microbial biodiversity relative to the number of plant and animal studies. These obstacles have been overcome recently, as evidenced by the growing number of microbial biogeography studies [11]. This is partially a result of the development of molecular approaches enabling a more comprehensive view of microbial diversity [12]. Recent research has challenged the conceptual dogma, providing evidence of microbial endemism [13], and also of a spatial patterning of microbial biodiversity [14–18] that is similar qualitatively to that of plants and animals.

Here, we review our current understanding of the spatial scaling of microbial biodiversity, focusing on free-living bacteria, Archaea and micro-Eukarya. We begin by discussing the differences that are commonly assumed to exist between micro- and macroorganisms that would result in microbial cosmopolitanism. We then review observed patterns of microbial biogeography with a focus on three spatial biodiversity patterns: the distance–decay relationship (how community composition changes with geographic distance), the taxa–area relationship, and the local:global taxa richness ratio. We conclude that the evidence for microbial cosmopolitanism is mixed and often confounded with artifacts resulting from coarse taxonomic resolutions and undersampling, and that there is evidence for universal spatial scaling rules common to all forms of life.

Arguments for microbial cosmopolitanism
The most commonly claimed mechanism underlying a cosmopolitan distribution of microbes is that of large population sizes and short generation times resulting in high dispersal rates [9,19,20]. The probability of chance dispersal (e.g. via an accidental vector such as a bird or mammal) is increased when abundance is high. Microbial communities are very abundant given that a gram of soil can contain 10^9 individual bacteria and perhaps 10^4 ciliates [5,9]. Large abundance at the community-level does not require large population sizes across all species [21]. Variability in population size across species is characterized by the species-abundance curve, which quantifies the relative abundance of the species in a community. The size of a given species population will also depend on how one defines 'species' (see Boxes 1 and 2); broader definitions will result in larger estimated population sizes.

The capacity to disperse over long distances is also necessary for cosmopolitan distributions. The small size of microbes can facilitate long-distance passive dispersal,

Corresponding author: Green, J. (jgreen@ucmerced.edu).

Box 1. Units of microbial biodiversity: taxonomic approaches

'Species' is the most commonly used unit in biodiversity studies, but species are not easy to define owing, in part, to two distinct meanings of the word: taxonomic category and natural unit of life [58]. Microbial taxonomists have focused on the former meaning and devised ways to define microbial taxonomic units [59], although often without reference to evolution and ecology [60]. Recently, there has been increasing interest in understanding microbial species as natural units of life (see Box 2).

Approaches to defining microbial taxa

● *Morphological.* The taxonomy of macroorganisms is often based on morphological traits, which is also the case for microbes (primarily protists) with substantial morphological variation. Below certain body sizes or morphological complexity limits, however, other taxonomic criteria (e.g. genetic similarity) might be more appropriate [55].

● *Phenotypic.* Other phenotypic traits (e.g. metabolic substrate utilization) are used to identify microorganisms exhibiting little morphological differentiation [59]. Such phenotypic traits usually demand that the organism be cultured in the laboratory, which cannot be accomplished presently for the vast majority of prokaryotic (and many eukaryotic) microorganisms [61].

● *Genotypic.* Since the 1970s, the gold standard for genotypic characterization of prokaryotic microbes has been genome hybridization under standard conditions (DNA–DNA hybridization or DDH), which is time-consuming and requires culturing the organisms. Rapid methods of DNA typing that have been developed (e.g. multilocus sequence analysis or MLSA) also require laboratory culture. Ribotyping (sequence analysis of ribosomal genes) is the most commonly used method that does not require laboratory culture, but it has low resolution, is impacted by recombination and horizontal gene exchange, and is too time consuming for routine surveying of microbial diversity. High-throughput variations of ribotyping, including restriction mapping (e.g. TRFLP) and denaturing gel electrophoresis (e.g. DGGE) of ribosomal gene sequences [12], have even lower resolution than ribosomal gene sequence analysis.

Recently, microbial taxa definitions have been explored by means of whole-genome comparisons among cultured bacterial isolates [62]. This technique might soon be possible with uncultured organisms as well. Entire genomes have been sequenced from mixed communities without culturing [63], although this is possible currently only in communities of low diversity [64] or via large-scale DNA sequencing [65]. Whittaker and Banfield [66] describe how population genomic analysis of community genomic data can be applied to resolve independent microbial lineages in the natural environment.

Box 2. Units of microbial biodiversity: species definitions

Microorganisms

The methods described in Box 1 yield hierarchical clusters of organisms. The challenge is to determine the depth of clustering that defines a species. This is especially problematic for prokaryotic microorganisms, for which there is limited information regarding their natural history. A classical approach to define prokaryotic species is the 'Pragmatic' ('arbitrary' or 'anthropocentric') approach [67], which uses characters of interest (e.g. pathogenicity or host range, among others) to produce species delineations with practical applications (e.g. distinguishing pathogens from non-pathogens).

A common definition of species derived from this approach is 'a group of strains that have some degree of phenotypic consistency, exhibit at least 70% DNA–DNA hybridization, and greater than 97% 16S rRNA sequence similarity' [60]. Seventy percent hybridization is chosen because it yields species consistent with the phenotypic (pragmatic) taxonomy, and 97% rRNA similarity yields species consistent with the taxonomy based on hybridization and phenotype that preceded ribotyping. In practice, many studies of prokaryotic diversity abandon species definitions entirely, and define instead 'operational taxonomic units' (OTUs) based on ribosomal gene sequences, ribosomal 'fingerprints' or other techniques that do not require culture.

Attempts to define prokaryotic species from an evolutionary (rather than pragmatic) perspective depend upon assumptions regarding the cohesive force that constrains species, and the rate, extent and specificity of horizontal gene exchange. At one extreme are models assuming that horizontal gene exchange among divergent organisms acts as a cohesive force, much like sexual reproduction for species of macroorganisms [33,68]. Such models are modifications of the Biological Species Concept [69], but can predict 'fuzzy' species boundaries depending on how widely genes are exchanged. At the other extreme are models assuming that cohesion occurs through selective sweeps that purge diversity after a beneficial mutation or horizontal gene exchange event has occurred [34]. These models define species as ecotypes, genetically cohesive and ecologically distinct entities that are maintained by competitive exclusion and that exhibit defined sequence-clustering patterns.

Microorganisms versus macroorganisms

The tension between the pragmatic and evolutionary taxonomies is not unique to microbial taxonomy: most macroorganism species are defined operationally rather than through adherence to a species concept. The resolution of macroorganism species is generally much finer because of their larger body sizes and morphological variation, coupled with less genetic and ecological diversity. For example, the 97% rRNA criterion would join all primates from humans to lemurs in one species [70]. Two-thirds of prokaryotic species share less than 95%, and as little as 65%, of their genes with conspecifics; by contrast, humans and the pufferfish *Fugu rubripes* share more than 75% [62].

and microbes such as *Bacillus* can form dormant life stages that enable them to survive long-distance transport and harsh environmental conditions [22]. It is not known how widespread dispersal adaptations are among microbes [21,23], and few studies have quantified population-level dispersal patterns. Studies have shown some protist [9,24], fungal [25] and bacterial [26–28] taxa with cosmopolitan distributions, suggesting a high capacity for dispersal. However, there is also evidence that some microbial taxa have restricted geographic distributions because of dispersal limitation, implying that not all microorganisms have the capacity to disperse globally [11,29].

Another argument for microbial cosmopolitanism is that their low extinction and speciation rates limit local diversification. The argument for low extinction rates is based on the assumption that microbes have large population sizes, making stochastic extinction events less likely [9]. It has also been argued that microbes develop hardy life

stages (e.g. spores) that can reduce the probability of local extinction following catastrophic environmental conditions. It is not known, however, how widely such traits are distributed among microbial taxa.

The primary argument for lower speciation rates is an apparent lack of dispersal barriers to prevent speciation as a result of geographical isolation (allopatric speciation) [10]. Recent studies have revealed evidence of microbial population isolation, weakening the premise that low allopatric speciation rates are a universal attribute of microorganisms [21]. Another mechanism that could alter speciation rates is horizontal gene transfer [30–32], which can act either as a cohesive force (reducing speciation rates) or as a source of genetic novelty (increasing speciation rates [33]). The short generation times and potentially
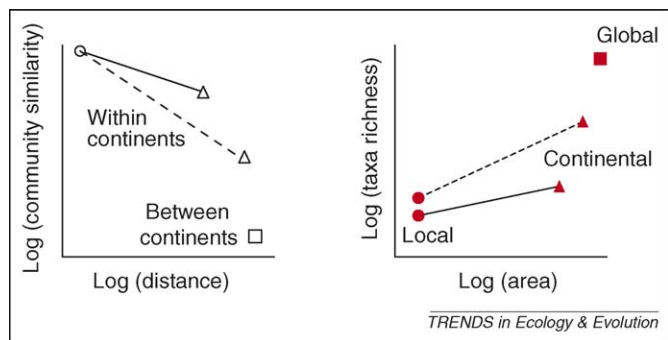
Figure 1. Hypothetical spatial patterns of microbial diversity. **(a)** The distance–decay relationship within two different continents (solid and dashed lines) and the similarity in community composition between those continents (open square). Community similarity is equal for each continent at local scales (open circles) in the limit where replicate samples are completely censused from the same location. **(b)** The taxa–area relationships for two continents. A greater rate in community composition turnover results in a steeper taxa–area relationship slope (dashed line). The local:global richness ratio on a given continent is equal to the taxa richness estimated at the local scale (solid circle) divided by the taxa richness estimated at the global scale (solid square).

large population sizes of microorganisms offers the possibility of rapid rates of evolution relative to that of macroorganisms as a result of the rapid generation of novelty via mutation [34].

Observed spatial patterns of microbial biodiversity
New genetic technologies have enabled detection of large amounts of unculturable microbial diversity [12], prompting a flurry microbial biogeography studies [11]. Here we review a subset of these studies that have focused on three spatial patterns of biodiversity (Figure 1).

Distance–decay relationship
The assumption of global microbial dispersal by a combination of randomizing forces (e.g. wind, water and animal vectors, among others) leads to random primary spatial distributions followed by subsequent population growth in nonrandom spatial niches. According to this cosmopolitan view of the microbial world, spatial patterns of microbial diversity are driven by environmental heterogeneity. Thus, one might expect to find similar microbial communities in similar habitats and differentiated microbial communities along an environmental gradient. One approach for testing this assumption is through an analysis of how similarity in community composition between sites changes with the geographic distance separating the sites, or the 'distance–decay relationship' [35] (Figure 1). When coupled with environmental data, the distance–decay relationship offers a means to assess the relative importance of environmental heterogeneity and dispersal history in controlling the spatial scaling of biodiversity [36]. Although it is accepted widely that macroorganism community composition decays with increasing distance between samples [37], little is known about microbial community turnover rates.

Cho and Tiedje [38] provided one of the first examples of a relationship between the genetic similarity of a free-living bacterial assemblage and geographic distance. They sampled soils from ten sites on four continents to characterize the spatial structure of *Pseudomonas* genotypes (Figure 2) using BOX-PCR, a genomic fingerprinting
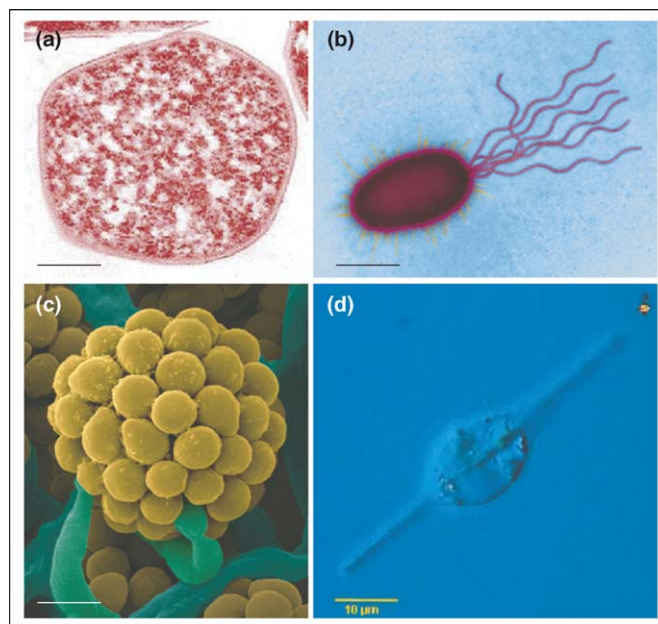
Figure 2. Examples of taxa targeted in microbial biogeography studies. **(a)** Archaea (genus *Sulfolobus*); **(b)** Bacteria (genus *Pseudomonas*); **(c)** Fungi (group Ascomycotes); **(d)** Protozoa (genus *Paraphysomonas*). Scale bars = (a) 0.25 μm; (b) 1.75 μm; (c) 3.95 μm; and (d) 10 μm. Reproduced with permission from (a) Dieter Janckovik and Wolfram Zillig, courtesy of Ken Stedman; (b) and (c) Dennis Kunkel; and (d) David Patterson and Mark Farmer.

technique. The authors found that the genetic similarity of *Pseudomonas* isolates was negatively correlated with geographic distance at regional scales (inter-sample distances ranging from 5 m to 80 km), but not at greater scales (i.e. between continents). Franklin and Mills [39] used AFLP analysis, a molecular fingerprinting method, to document microbial distance–decay patterns at smaller scales (2.5 cm to 11 m). They observed a significant distance–decay relationship with a scale-dependent slope that decreased at larger scales. Hillebrand and colleagues [40] were the first to report a distance–decay relationship for microbial eukaryotes. They gathered morphospecies data on diatoms and ciliates sampled at geographic distances ranging from 1 to 1000 km, and found that community similarity decayed significantly with distance. In none of these studies was the importance of dispersal limitation versus environmental heterogeneity examined.

More recent analyses have explored the effects of environmental heterogeneity and dispersal limitation on microbial biogeography. A global study of *Sulfolobus* strains (Figure 2) isolated from hot-spring habitats found that the decline in genetic similarity with distance was explained by geographic distance, but not by environmental heterogeneity, suggesting that dispersal limitation was driving the relationship [13]. A regional-scale analysis of mountain lakes from the Sierra Nevada (Spain) has shown that the composition of bacterial assemblages was significantly influenced by the geographic distance separating lakes rather than by environmental factors [41]. A regional-scale study of desert ascomycete fungal communities (Figure 2) suggested that geographic distance was a more useful predictor for community turnover than was habitat (as classified by soil and vegetation type) [15].

These studies contradict the hypothesis of microbial cosmopolitanism.

Multiple studies have shown that environmental heterogeneity is the primary factor underlying microbial distance–decay relationships [11]. A recent study of bacterial diversity across North and South America concluded that bacterial turnover was driven primarily by edaphic variables (largely pH) and was independent of geographic distance [42]. A potential confounding factor in this study is the effect of sampling. To characterize community composition at each site ($\sim$100 m^2), samples were drawn from five to ten locations and homogenized into a single bulk sample before TRFLP analysis. Unless every site was spatially homogeneous with respect to diversity, such that the increased sampling effort did not lead to the accumulation of new species, the more thoroughly sampled sites (i.e. those of ten samples) should have yielded a higher observed diversity than the less sampled sites (i.e. those of only five samples), making richness and distance–decay patterns difficult to interpret. Classic indices of compositional similarity are notoriously sensitive to sample size, and future studies of microbial β-diversity (i.e. distance-decay patterns) would benefit from robust statistical approaches [43] to avoid biases posed by unequal sampling effort.

Taxa–area relationship

The relationship between species richness (S) and sampled area (A) (the species–area relationship) is one of the most widely studied patterns in ecology. Although no single species–area relationship generalizes to all habitats, taxonomic groups or spatial scales, a power-law of the form $S \propto A^z$ is commonly assumed (Figure 1). Empirical evidence suggests that z is generally in the range of 0.1 to 0.3 for plants and animals within contiguous habitats and steeper ($0.25 < z < 0.35$) for discrete islands [44].

Advocates of microbial cosmopolitanism have suggested that microbes should be characterized by relatively flat species–area (or, more accurately, taxa–area) curves, with z values lower than those reported for macroorganisms [10]. There are few published studies of taxa–area relationships (TARs) for microbes, rendering comparison with larger organisms difficult. Of these few microbial studies, most report relatively low slopes (i.e. $z < 0.1$), although recent reports indicate higher z values that are consistent with those of macroorganisms (Table 1).

A challenge in TAR studies is estimating the true number of taxa in areas where it is not possible to sample completely. For microbes, detailed distribution maps are unavailable and relying on observed counts of taxa richness might bias z values. For example, reported patterns for marine benthos (diatoms: $z = 0.066$, ciliates: $z = 0.077$) ([45]; Table 1) are based on the cumulative species richness observed in non-contiguous sample points covering large areas. If the true number of species at the largest scales (i.e. synopses of whole seas) is greater than that observed from sampling a small fraction of these large areas, the observed TAR slope z will underestimate the true slope.

One approach to extrapolating microbial taxa richness is to make assumptions about microbial relative-abundance curves and how they vary with spatial scale. This approach has been used to extrapolate ciliate [10,46] and bacterial [47] diversity. Little is known about the spatial scaling of microbial species-abundance curves, making it difficult to assess the validity of projected extrapolations. An alternative approach is to use the slope of the distance–decay relationship to estimate the slope of the TAR [48], which requires only sampling localities spatially in such a way that the decline in similarity with distance can be measured. This method has been applied recently to estimate the spatial scaling of microbial diversity at local [18], regional [15] and global [42] scales.

The TAR slope z should vary with taxonomic resolution, which must be taken into account when comparing the biodiversity scaling of micro- and macroorganisms. Horner-Devine *et al.* [18] found that the slope of the TAR relationship z increased with increasing taxonomic resolution, ranging from $z = 0.019$ for taxa defined as 95% sequence similarity groups to $z = 0.040$ for taxa defined as 99% sequence similarity groups. Their data indicate that spatial biodiversity patterns depend on the defined taxonomic resolution, and that the coarse taxonomic resolutions used commonly for microorganisms (e.g. morphotypes, molecular 'fingerprints' and ribotypes, among others) can result in low z values relative to those of plant and animal species.

The TARs described above were for contiguous areas. Recent 'island' patterns of bacterial biodiversity have been studied by Van der Gast *et al.* [49] in metal-cutting fluids from machines of increasing sump tank size, and Bell *et al.* [16] in water-filled tree holes of varying volume. Both

Table 1. A summary of microbial taxa–area relationships (TARs)

Organism	Characterization	Habitat	TAR type[a]	z	Approx. scale	Refs
Bacteria	16S rRNA sequence	Marsh sediment	Contiguous	0.019–0.040[b]	9×10^{-10}–0.09 km^2	[18]
Bacteria	TRFLP	Soil	Noncontiguous	0.030	400–10^8 km^2	[42]
Ciliates	Morphospecies	Benthos	Noncontiguous	0.043	9×10^{-9}–2×10^6 km^2	[46]
Diatoms	Morphospecies	Benthos	Contiguous	0.066	10^{-4}–10^{12} km^2	[45]
Fungi	ARISA	Desert soil	Contiguous	0.074	4×10^{-11}–10^4 km^2	[15]
Ciliates	Morphospecies	Marine benthos	Contiguous	0.077	10^{-4}–10^{12} km^2	[45]
Bacteria	DGGE	Lakes	Island	0.104	0.1–1.5 km^2	[41]
Phytoplankton	Morphospecies	Aquatic	Island	0.134	4×10^{-9}–10^7 km^2	[17]
Bacteria	DGGE	Sump tanks	Island	0.250–0.295[c]	9–180 liters	[49]
Bacteria	DGGE	Treeholes	Island	0.260	0.05–18 liters	[16]
Bacteria	TRFLP	Forest soil	Contiguous	0.420 and 0.470	10^{-6}–6×10^{-5} km^2	[14]

[a]TAR types are generalizations, and several studies do not fall into strict classifications. Island TARs pertain to studies of discrete areas of increasing size, contiguous TARs estimate the increase in taxa richness for nested areas within a single region, and noncontiguous TARs estimate the increase in taxa richness from local to global scales.
[b]z value scales with taxonomic resolution.
[c]Denotes the range of z values measured at separate time periods.

studies estimated bacterial richness as the number of unique ribotypes (i.e. ribosomal genotypes) detected using DGGE of 16S rDNA, and found that taxa richness increased with volume and that the rate of increase was similar to that reported for plants and animals ($z = 0.245$–0.295 for sump tanks, $z = 0.26$ for tree holes). Reche *et al.* [41] used a similar approach to study bacterial communities in lakes of varying size and reported $z = 0.104$.

These studies differ from many traditional island-biogeography studies of macroorganisms because bacterial taxa richness was quantified in equal volumes sampled from islands that were well-mixed manually [16] or assumed to be so [41,49]. If total bacterial density when communities are well-mixed is invariant across islands, this approach is analogous to sampling an equal number of individuals from every island randomly. By contrast, island z values reported for plants and animals [44] are based commonly on data from exhaustive, multi-year surveys or atlases. The relationship between z estimated from equal-sized random samples per island versus a complete survey of an archipelago will depend on several factors, including non-random spatial or temporal patterns within and between species, the relative abundance of species, and the sampling effort per survey.

Local:global taxa richness ratio

If microorganisms are globally dispersed and cosmopolitan, the species present in local samples will represent a large fraction of the cumulative species pool identified in similar habitats around the world. The most compelling evidence of this pattern comes from research on protist morphospecies. In a study of the flagellate genus *Paraphysomonas* (Figure 2), 80% of the known global species were found in <0.1 cm^2 of sediment collected from Priest Pot, a 1-ha freshwater pond in England [10,20]. Data compiled by Fenchel and Finlay across a wide range of eukaryotic taxonomic groups (e.g. amoebae, diatoms and mollusks, among others) in Priest Pot suggest a more general relationship between body size and global distribution [9]: the local:global species ratio, expressed as a percentage of the global number of freshwater species, decreased consistently with mean body size. A parallel analysis of data collected from Nivå Bay, a 2-ha marine shallow-water habitat in Denmark, revealed the same pattern, indicating that small organisms (<1 mm in length) tend to have a cosmopolitan distribution [9]. Data on polar surveys for testate amoeba assemblages also support this hypothesis [50].

These studies are potentially misleading for two reasons. First, they assume that the magnitude of microbial eukaryote global species richness is known for a given habitat type and taxonomic group. It is accepted widely that the discovery of new animals, plants and microbes is continuing at a rapid pace [51]. Some researchers claim that, for particular groups of microbial eukaryotes such as ciliated protozoa, the number of described species globally is unlikely to increase in the future [46], whereas others claim that a large fraction remain undiscovered [52]. The latter view is supported by the continuous discovery of new 'flagship' protist species [53] that have never been found in other well investigated areas (Figure 3). Second, most protist data rely on morphological
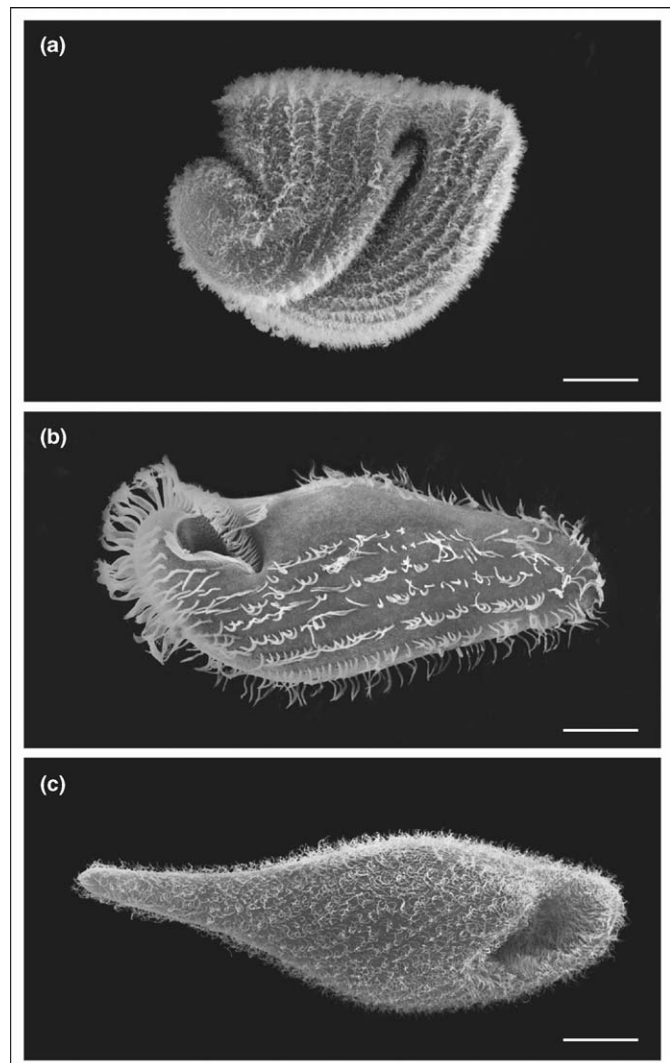

Figure 3. Flagship ciliate species believed to have restricted geographic distribution. **(a)** A still undescribed species from a green riverbed in Botswana, Africa. This species is up to 300 μm long *in vivo*. **(b)** *Saudithrix terricola* has been found only in Saudi Arabian soils to date. It is up to 300 μm long *in vivo*. **(c)** A still undescribed Gondwanan flagship species from the tanks of bromeliads in the Dominican Republic. Specimens are up to 800 μm long *in vivo*. Scale bars = (a) 45 μm; (b) 40 μm; and (c) 35 μm. Reproduced with permission from Wilhelm Foissner and Andreas Zankl.

species definitions. Recent studies indicate that some common flagellate [24] and ciliate [54] morphospecies, when examined using molecular techniques, are composed of several distinct genetic species, suggesting that more sensitive and less subjective taxonomic criteria (e.g. criteria based on genetic similarity) might be more appropriate [55]. Higher resolution taxonomic criteria for microbial eukaryote species would probably lead to increased global species pool estimates and decreased local:global species ratio estimates.

Conclusions and future directions

How biodiversity scales with space is a central question in ecology. It has long been assumed that microorganisms have cosmopolitan distributions, and that this results in fundamentally different biodiversity scaling relationships for microbes relative to those observed for other forms of life. However, recent studies have documented spatial patterns of microbial diversity that are similar qualitatively to those

observed for plants and animals. The quantitative differences in the respective patterns might be a result of the different approaches used to define the taxa of micro- and macroorganisms. Microbial spatial patterns, in particular, can be sensitive to how taxa are defined [18,38,55–57], and thus observations of microbial cosmopolitanism might be the result of taxonomic 'lumping' of microorganisms.

It is also commonly assumed (especially for prokaryotes [5]) that microbial diversity is immense relative to that of plants and animals, at least at local scales. If this is true, then all studies of microbial diversity have undersampled microbial diversity greatly, which could result in a biased picture of the spatial scaling of microbial biodiversity. If the most abundant organisms are also the most widespread (a 'positive range–abundance 'relationship', which has been observed for many plants and animals [2]), undersampling could result in the observation of flat or nonexistent rates of distance–decay and flat taxa–area relationships. Undersampling also increases the importance of sampling effort in describing diversity patterns. For example, if the local sampling effort is greater than the global sampling effort, then artifactually high local:global richness ratios could be observed. Taken together, artifacts of taxonomic lumping, undersampling and unequal sampling could result in the incorrect conclusion that the spatial scaling of microbial biodiversity is different from that of plant and animal diversity.

We suggest, as have others [55,57], that the discussion concerning the spatial scaling of microbial biodiversity be recast. Rather than ask the question 'do microbes have fundamentally different scaling relationships from those of plants and animals?', we suggest that the debate focus instead on the question 'is there a spatial scale, a degree of sampling effort and a level of taxonomic resolution at which microbial biodiversity scaling relationships approach those of macroorganisms?'. This is a tractable question, and one that avoids the task of identifying *a priori* equivalent taxonomic definitions and degrees of sampling for micro- and macroorganisms. To answer this question, microbial ecologists would need to use multiple taxonomic definitions based on a variety of molecular makers (and biochemical and morphological traits, if accessible). Such a polyphasic approach to studies of microbial biogeography is just beginning to be applied. Determining the spatial patterning of microbial diversity will not only increase our understanding of microbial ecology, but will also provide ecologists with a true understanding of the universality of spatial scaling rules.

References
1 Holyoak, M. *et al*. *Metacommunities: Spatial Dynamics and Ecological Communities*, University of Chicago Press (in press)

2 Gaston, K. and Blackburn, T. (2000) *Pattern and Process in Macroecology,* Blackwell Science

3 Ferrier, S. *et al.* (2004) Mapping more of terrestrial biodiversity for global conservation assessment. *BioScience* 54, 1101–1109

4 Stuart, S.N. *et al.* (2004) Status and trends of amphibian declines and extinctions worldwide. *Science* 306, 1783–1786

5 Torsvik, V. *et al.* (2002) Prokaryotic diversity – magnitude, dynamics, and controlling factors. *Science* 296, 1064–1066

6 Bell, T. *et al.* (2005) The contribution of species richness and composition to bacterial services. *Nature* 436, 1157–1160

7 Morin, P.J. and McGrady-Steed, J. (2004) Biodiversity and ecosystem functioning in aquatic microbial systems: a new analysis of temporal variation and species richness-predictability relations. *Oikos* 104, 458–466

8 Bass-Becking, L.G.M. (1934) *Geobiologie of Inleiding to de Milieukunde,* W.P van Stockum & Zoon N.V.

9 Fenchel, T. and Finlay, B.J. (2004) The ubiquity of small species: patterns of local and global diversity. *BioScience* 54, 777–784

10 Finlay, B.J. (2002) Global dispersal of free-living microbial eukaryote species. *Science* 296, 1061–1063

11 Martiny, J.B.H. *et al.* (2006) Microbial biogeography: putting microorganisms on the map. *Nat. Rev. Microbiol.* 4, 102–112

12 Head, I.M. *et al.* (1998) Microbial evolution, diversity, and ecology: a decade of ribosomal RNA analysis of uncultivated organisms. *Microb. Ecol.* 35, 1–21

13 Whitaker, R.J. *et al.* (2003) Geographic barriers isolate endemic populations of hyperthermophilic archaea. *Science* 301, 976–978

14 Noguez, A.M. *et al.* (2005) Microbial macroecology: highly structured prokaryotic soil assemblages in a tropical deciduous forest. *Glob. Ecol. Biogeogr.* 14, 241–248

15 Green, J.L. *et al.* (2004) Spatial scaling of microbial eukaryote diversity. *Nature* 432, 747–750

16 Bell, T. *et al.* (2005) Larger islands house more bacterial taxa. *Science* 308, 1884

17 Smith, V.H. *et al.* (2005) Phytoplankton species richness scales consistently from laboratory microcosms to the world's oceans. *Proc. Natl. Acad. Sci. U. S. A.* 102, 4393–4396

18 Horner-Devine, M. *et al.* (2004) A taxa–area relationship for bacteria. *Nature* 432, 750–753

19 Coleman, A.W. (2002) Microbial eukaryote species. *Science* 297, 337

20 Finlay, B.J. and Clarke, K.J. (1999) Ubiquitous dispersal of microbial species. *Nature* 400, 828

21 Papke, R.T. and Ward, D.M. (2004) The importance of physical isolation to microbial diversification. *FEMS Microbiol. Ecol.* 48, 293

22 Plomp, M. *et al.* (2005) The high-resolution architecture and structural dynamics of *Bacillus* spores. *Biophys. J.* 88, 603–608

23 Staley, J.T. and Gosink, J.J. (1999) Poles apart: biodiversity and biogeography of sea ice bacteria. *Annu. Rev. Microbiol.* 53, 189–215

24 Scheckenbach, F. *et al.* (2005) Molecular identity of strains of heterotrophic flagellates isolated from surface waters and deep-sea sediments of the South Atlantic based on SSU rDNA. *Aquat. Microb. Ecol.* 38, 239–247

25 Pringle, A. *et al.* (2005) Cryptic speciation in the cosmopolitan and clonal human pathogenic fungus *Aspergillus fumigatus*. *Evolution* 59, 1886–1899

26 Glöckner, F.O. *et al.* (2000) Comparative 16S rRNA analysis of lake bacterioplankton reveals globally distributed phylogenetic clusters including an abundant group of Actinobacteria. *Appl. Environ. Microbiol.* 66, 5053–5065

27 Brandao, P.F.B. *et al.* (2002) Discrimination and taxonomy of geographically diverse strains of nitrile-metabolizing actinomycetes using chemometric and molecular sequencing techniques. *Environ. Microbiol.* 4, 262–276

28 Ward, B.B. and O'Mullan, G.D. (2002) Worldwide distribution of *Nitrosococcus oceani*, a marine ammonia-oxidizing γ-Proteobacterium, detected by PCR and sequencing of 16S rRNA and *amoA* genes. *Appl. Environ. Microbiol.* 68, 4153–4157

29 Papke, R.T. *et al.* (2003) Geographical isolation in hot spring cyanobacteria. *Environ. Microbiol.* 5, 650–659

30 Gogarten, J.P. and Townsend, J.P. (2005) Horizontal gene transfer, genome innovation and evolution. *Nat. Rev. Microbiol.* 3, 679–687

31 Sørensen, S.J. *et al.* (2005) Studying plasmid horizontal gene transfer *in situ*: a critical review. *Nat. Rev. Microbiol.* 3, 700–710

32 Thomas, C.M. and Nielsen, K.M. (2005) Mechanisms of, and barriers to, horizontal gene transfer between bacteria. *Nat. Rev. Microbiol.* 3, 711–721

33 Ochman, H. *et al.* (2005) Examining bacterial species under the specter of gene transfer and exchange. *Proc. Natl. Acad. Sci. U. S. A.* 102, 6595–6599

34 Cohan, F.M. (2002) What are bacterial species? *Annu. Rev. Microbiol.* 56, 457–487

35 Nekola, J.C. and White, P.S. (1999) The distance decay of similarity in biogeography and ecology. *J. Biogeog.* 26, 867–878

36 Legendre, P. *et al.* (2005) Analyzing beta diversity: partitioning the spatial variation of community composition data. *Ecol. Monogr.* 75, 435–450

37 Tuomisto, H. *et al.* (2003) Dispersal, environment, and floristic variation of western Amazonian forests. *Science* 999, 241–244

38 Cho, J.-C. and Tiedje, J.M. (2000) Biogeography and degree of endemicity of fluorescent *Pseudomonas* strains in soil. *Appl. Environ. Microbiol.* 66, 5448–5456

39 Franklin, R.B. and Mills, A.L. (2003) Multi-scale variation in spatial heterogeneity for microbial community structure in an eastern Virginia agricultural field. *FEMS Microbiol. Ecol.* 44, 335–346

40 Hillebrand, H. *et al.* (2001) Differences in species richness patterns between unicellular and multicellular organisms. *Oecologia* 126, 114–124

41 Reche, I. *et al.* (2005) Does ecosystem size determine aquatic bacterial richness? *Ecology* 86, 1715–1722

42 Fierer, N. and Jackson, R.B. (2006) The diversity and biogeography of soil bacterial communities. *Proc. Natl. Acad. Sci. U. S. A.* 103, 626–631

43 Chao, A. *et al.* (2005) A new statistical approach for assessing similarity of species composition with incidence and abundance data. *Ecol. Lett.* 8, 148–159

44 Rosenzweig, M.L. (1995) *Species Diversity in Space and Time,* Cambridge University Press

45 Azovksy, A.I. (2002) Size-dependent species–area relationships in benthos: is the world more diverse for microbes? *Ecography* 25, 273–282

46 Finlay, B.J. *et al.* (1998) Protozoan diversity: converging estimates of the global number of free-living ciliate species. *Protist* 149, 29–37

47 Curtis, T.P. *et al.* (2002) Estimating prokaryotic diversity and its limits. *Proc. Natl. Acad. Sci. U. S. A.* 99, 10494–10499

48 Krishnamani, R. *et al.* (2004) Estimating species richness at large spatial scales using data from small discrete plots. *Ecography* 27, 637–642

49 van der Gast, C.J. *et al.* (2005) Island size and bacterial diversity in an archipelago of engineering machines. *Environ. Microbiol.* 7, 1220–1226

50 Wilkinson, D.M. (2001) What is the upper size limit for cosmopolitan distribution in free-living microorganisms? *J. Biogeog.* 28, 285–291

51 Wilson, E.O. (2002) *The Future of Life,* Vintage Books

52 Foissner, W. *et al.* (2005) A huge, undescribed soil ciliate (Protozoa: Ciliophora) diversity in natural forest stands of Central Europe. *Biodiv. Conserv.* 14, 617

53 Foissner, W. (2004) Ubiquity and cosmopolitanism of protists questioned. *Soc. Int. Limnol. News* 6–7

54 Katz, L.A. *et al.* (2005) Reframing the 'Everything is everywhere' debate: evidence for high gene flow and diversity in ciliate morphospecies. *Aquat. Microb. Ecol.* 41, 55–65

55 Hedlund, B.P. and Staley, J.T. (2004) Microbial endemism and biogeography, In *Microbial Biodiversity and Bioprospecting* (Bull, A.T., ed.), pp. 225–231, ASM Press

56 Staley, J.T. *et al.* (1997) *The Microbial World: Foundation of the Biosphere,* American Academy of Microbiology

57 Tiedje, J. (1993) Approaches to the comprehensive evaluation of prokaryotic diversity of a habitat, In *Microbial Diversity and Ecosystem Function* (Allksopp, D. *et al.*, eds), pp. 73–87, CAB International

58 Mayden, R.L. (1997) A hierarchy of species concepts: the denouement in the saga of the species problem, In *Species: the Units of Biodiversity* (Claridge, M.F. *et al.*, eds), pp. 381–425, Chapman and Hall

59 Rosselló-Mora, R. and Amann, R. (2001) The species concept for prokaryotes. *FEMS Microbiol. Rev.* 25, 39–67

60 Gevers, D. *et al.* (2005) Re-evaluating prokaryotic species. *Nat. Rev. Microbiol.* 3, 733–739

61 Amann, R.I. *et al.* (1995) Phylogenetic identification and *in situ* detection of individual microbial-cells without cultivation. *Microbiol. Rev.* 59, 2019–2027

62 Konstantinidis, K.T. and Tiedje, J.M. (2005) Genomic insights that advance the species definition for prokaryotes. *Proc. Natl. Acad. Sci. U. S. A.* 102, 2567–2572

63 Allen, E.E. and Banfield, J.F. (2005) Community genomics in microbial ecology and evolution. *Nat. Rev. Microbiol.* 3, 489–498

64 Tyson, G.W. *et al.* (2004) Community structure and metabolism through reconstruction of microbial genomes from the environment. *Nature* 428, 37–43

65 Venter, J.C. *et al.* (2004) Environmental genome shotgun sequencing of the Sargasso Sea. *Science* 304, 66–74

66 Whitaker, R.J. and Banfield, J.F. (2006) Population genetics in natural microbial communities. *Trends Ecol. Evol.* 21

67 Ward, D.M. *et al.* (1998) A natural view of microbial biodiversity within hot spring cyanobacterial mat communities. *Microbiol. Mol. Biol. Rev.* 62, 1353–1370

68 Lawrence, J.G. (2002) Gene transfer in bacteria: speciation without species. *Theor. Popul. Biol.* 61, 449–460

69 Mayr, E. (1942) *Systematics and Origin of Species,* Columbia University Press

70 Staley, J.T. (1997) Biodiversity: are microbial species threatened? Commentary *Curr. Opin. Biotechnol.* 8, 340–345