

# Microbial diversity and the “lower-limit” problem of biodiversity

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**Abstract** Science is now studying biodiversity on a massive scale. These studies are occurring not just at the scale of larger plants and animals, but also at the scale of minute entities such as bacteria and viruses. This expansion has led to the development of a specific sub-field of “microbial diversity”. In this paper, I investigate how microbial diversity faces two of the classical issues encountered by the concept of “biodiversity”: the issues of defining the *units* of biodiversity and of choosing a *mathematical measure* of diversity. I also show that the extension of the scope of biodiversity to microbial entities such as viruses and many other not-clearly-alive entities raises yet another foundational issue: that of defining a “lower-limit” of biodiversity.

**Keywords** Microbial diversity · Definition of biodiversity · Biodiversity scope · Biodiversity measure · Units of biodiversity · Definition of life

## Introduction

Over the last couple of decades, the discipline of ecology has considerably enlarged its scope of study, growing from small local ecosystems to larger regional ones, and even up to the biosphere as a whole (e.g. Gaston and Spicer 2004; Naeem 2002; Schneider et al. 2004). In parallel, the concept of biodiversity has also witnessed a considerable increase in scope, with more and more species being given its attention, and organismal size going further and further down the scale. Biodiversity science has now begun the massive project of investigating biodiversity at the scales of, for example, bacteria and viruses. In this paper I argue that the extension of the

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concept of “biodiversity” to such microbial entities raises a non-trivial issue: that of defining a “lower-limit” to biodiversity. To make this argument, I first review definitions of biodiversity and analyze the place of microbial diversity within general biodiversity studies (“[Biodiversity, macrobes, microbes and beyond](#)” section). I then argue in favor of fully extending biodiversity to microbes (“[Why microbial diversity?](#)” section), and I revisit two classical issues faced by the concept of biodiversity that microbial diversity also encounters: the issue of defining the *units* of biodiversity and the issue of defining a *mathematical measure* of biodiversity (“[Defining microbial diversity: two classical issues revisited](#)” section). I then focus more specifically on those microbial diversity studies that have come to include entities at the very edge of life—such as viruses—within their scope of investigation (“[Extending microbial diversity at the frontier of life and non-life](#)” section). I argue that such microbial diversity studies, while fully legitimate, make salient a foundational issue facing the concept of biodiversity: that of defining the simplest types of entities that biodiversity ought to be concerned with at the very frontier of life and non-life (“[The “lower-limit problem”](#)” section). This is what I call the “lower-limit problem” of biodiversity. I show that addressing this problem by sticking to the etymology of “biodiversity”, and using “life” as a criterion to delineate the scope of biodiversity, is not as simple as it is assumed to be (“[The “lower-limit problem” and “life”](#)” section). In turn, this raises the question of rethinking the place of the “bio” in the concept of “biodiversity”.

## **Biodiversity, macrobes, microbes and beyond**

The concept of “biodiversity” is now so widespread that it seems to have been around for ages. It is however a fairly recent concept that appeared in the 1980s, with the word being produced from a contraction of the expression “biological diversity”. That longer term was itself coined in the 1950s and is now often used interchangeably with biodiversity (Takacs 1996; Magurran 2004). Of course, biodiversity science tackles questions and uses concepts that existed before the 1980s. These questions and concepts come in a straight line from what used to be called “ecological diversity” in the 1950s (Takacs 1996). One of the likely reasons why the term “biodiversity” was put under the spotlight in the 1980s, and came to replace the expression “ecological diversity”, is that research on ecological diversity at that time appeared to be stuck in a dead-end. This situation came about because ecological diversity studies were incapable of accounting for the relationship between the diversity of species and the stability of ecosystems, from both theoretical and empirical standpoints (Sarkar 2005).<sup>1</sup> Furthermore, specific conservation issues became much more pressing in the 1980s, thereby requiring less abstracted debate and more applied solutions. In short, justifying biodiversity conservation policies became much more central than studying ecological diversity *in abstracto*. Nevertheless, biodiversity studies inherited a large

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<sup>1</sup> It is worth noting that this situation has changed significantly since the beginning of the 1990s. The relationship between diversity and ecosystems properties such as stability, decomposition or primary productivity has become a most fertile area of ecological investigation (e.g. Loreau et al. 2001; Naeem 2002; Petchey and Gaston 2006; Dornelas 2010).

number of concepts, methods and tools that resulted from decades of research in ecological diversity. This is very much so, for instance, when it comes to the definition of mathematical measures of diversity (see, for instance, Whittaker 1960).

One specific way to construe biodiversity is to follow the 1992 United Nations Earth Summit definition. According to this definition, biological diversity is “the variability among living organisms from all sources, including, ‘inter alia’, terrestrial, marine, and other aquatic ecosystems, and the ecological complexes of which they are part: this includes diversity within species, between species and of ecosystems” (Harper and Hawksworth 1995: 6). So construed, biodiversity has one of the broadest scopes one might imagine: it is set to deal with *all* living organisms, and to measure diversity at *all* levels of organization, including in particular diversity among organisms of the same species, among species, and among sets of species or ecosystems. In the very same spirit, Gaston and Spicer mention that “biodiversity is ‘the variety of life’ and refers collectively to variation at all level of biological organization” (Gaston and Spicer 2004: 3). Yet, faced with the demanding issue of assessing biodiversity in practice, and particularly of quantifying its spatial and temporal patterns, others have proposed the adoption of narrower definitions that focus only on *species*. Hubbell, for instance, takes “biodiversity to be synonymous with species richness and relative species abundance in space and time” (Hubbell 2001: 3), and Magurran defines biodiversity simply as “the variety and abundance of species in a defined unit of study” (Magurran 2004: 8).

In any case, independently of which definition one follows, biodiversity appears in no way to be restricted to the most visible living organisms, nor to the most charismatic ones. It is defined to account for the diversity of *all* types of living organisms, big or small. As a matter of record, there is an established tradition of research in diversity studies related to *microbial* entities ever since the 1990s and probably even before (e.g. Brock 1987; Pace 1997). However, debate in the public domain has remained mostly focused on the diversity of larger plants and animals, resulting in a perceived bias of biodiversity studies towards “macrobes” over “microbes” or “microorganisms” (O’Malley and Dupré 2007), that is to say towards those organisms that are easily identifiable by the naked eye as opposed to those that, to be viewed, usually require a microscope of some sort. This is very much so in many related philosophy of science texts that have elaborated on the concept of biodiversity (e.g. Oksanen and Pietarinen 2004; Sarkar 2005; MacLaurin and Sterelny 2008).<sup>2</sup>

This situation may be due to the salience of specific conservation issues in the 1980s–1990s and to the prominence of ecologists—and not microbiologists—in this debate (e.g. Wilson 1992; Rosenzweig 1995; Takacs 1996). It is also most probably due to the fact that general biodiversity and microbial diversity have largely developed—at least up until very recently—as two separate and independent fields of study, the second often falling into the broader scope of microbiology and molecular biology (Øvreås and Curtis 2011). Methodological factors linked to microbial properties—such as size, concentration, distribution—have contributed strongly to this relative isolation of microbial diversity studies. In addition, due to

<sup>2</sup> In this respect, O’Malley and Dupré (2007) and Morgan (2010) are rare exceptions.

their size and their unicellular structure, microbes—unlike macrobes—cannot reliably be taxonomically and metabolically categorized according to directly visible phenotypes: specific molecular and biochemical tools need to be used, even in the case of unicellular eukaryotes. The diversity of microbial organisms can also be astonishingly high at extremely small scales, often micrometers, reaching, for example, some  $10^4$ – $10^6$  taxa per gram of soil (Gans et al. 2005, in regard to bacteria and archaea). Furthermore, species abundance and distribution may significantly vary at such small spatial scales too (Klug and Tiedje 1993; Ranjard and Richaume 2001). Microbial species identification thereby proves to be an extremely delicate task, requiring the use of “microbe-specific” instruments and methods.

Such instruments and methods have usually come from microbiology, and not from ecology. For instance, a traditional approach consists in cultivating microorganisms *in vitro* and in conducting batteries of tests against specific biochemical and physiological traits. Even if this method is highly partial in that it only works for a very limited number of microbial species, typically those for which the habitat and the nutritive environment can easily be reproduced in the laboratory (Brock 1987; Pace 1997), it is still used by researchers and being improved upon (Alain and Querellou 2009). Microbial ecologists also tend to use an increasing number of tools that directly come from the discipline of molecular biology. For instance, a common approach is to use polymerase chain reaction (PCR) to amplify 16S/18S rRNA genes from the sampled community of microorganisms, identify them through classical sequencing techniques, and compare them by alignments to those of other taxa (Amann et al. 1995). Other more organism-focused approaches of “community fingerprinting techniques” have also been developed that provide, for instance through denaturing gradient gel electrophoresis, quantitative information about the most numerically dominant community members (Øvreås and Curtis 2011). On top of these more established methods, “metagenomic” tools have been developed to generate increasingly complex datasets about multilevel microbial systems in a great variety of natural environments (e.g. Handelsman et al. 1998; Tyson et al. 2004; Edwards et al. 2006; Zarraonaindia et al. 2012). All these molecular biology techniques and tools have contributed to a stronger anchoring of microbial diversity studies in microbiology and in molecular biology rather than in general biodiversity research, thereby resulting in a relative isolation of microbial diversity studies from general biodiversity research and ecology. Furthermore, as microbiology was historically highly biased towards specific microorganisms that are pathogenic and that have a direct impact on health and agriculture, its main objectives have been quite different from the more general goals pursued in ecology and biodiversity studies. As a result, microbial diversity studies and the general study of biodiversity have had the strong tendency to develop their fields of research independently from each another, the first one focusing on microorganisms but lacking solid theoretical foundations (Prosser et al. 2007); the second developing tools, methodologies and theories to investigate plant and animal biodiversity, yet largely ignoring the microbial world (Horner-Devine et al. 2004).<sup>3</sup>

<sup>3</sup> This is probably symptomatic of a more general lack of theoretical integration in ecology (see, for instance, Pickett et al. 2007; Scheiner and Willig 2011).

## Why microbial diversity?

Investigating microbial diversity is interesting in its own right as microbes are found everywhere. Because the term “microbe” is typically defined in relationship to size—characterizing organisms that are microscopic and too tiny to see with the naked eye (e.g. Black 2008; Madigan et al. 2010)—it cuts across different phylogenetic groups of organisms. It is usually taken to denote members of the domains Bacteria and Archaea (all of which are unicellular), as well as microscopic members of the domain Eukarya (for instance, unicellular algae, some fungi and protists). Microbes are simply the oldest forms of life. It is likely that they were present on Earth some 3.5 billion years ago (Schopf 2006; see Brasier et al. 2006 for a more critical view), and that for more than 2 billion years, life on Earth—and evolution—was mainly unicellular. One of the most fascinating characteristics of microorganisms is their evolved ubiquity. As is well-known, microorganisms cope extremely well with the usual habitats of large plants and animals in temperate climates and nutrient-rich environments. But microorganisms also thrive in very extreme environmental conditions, as the many discoveries of “extremophiles” show, be they bacteria, archaea or eukaryotes. They colonize not just soil, lakes, oceans, the inside of other larger organisms and man-made buildings (Kembel et al. 2012), but they also colonize otherwise deadly environments. Some have been identified in hot springs, in submarine hydrothermal vents, in the Antarctic ice, and even buried in the ground several kilometers below the surface. Some “hyperthermophiles” develop at temperatures above 100 °C; some “psychrophiles” thrive below the freezing point of water; some “halophiles” grow in extremely salty environments; still other unicellular organisms colonize the deepest layers of the ocean and withstand amazing pressures, or stand up to very acidic environments and to radioactivity (see López-García 2003 for a review). The study of microbial diversity is therefore key to understanding the very ubiquity of life.

Microorganisms are also very interesting to study from a purely quantitative point of view: they are simply the most numerous living entities on Earth. Every cubic centimeter of soil alone may include over  $10^{10}$  bacteria (Torsvik et al. 2002). In total, bacteria and archaea would number over  $10^{29}$ – $10^{30}$  individuals on Earth (Whitman et al. 1998; Kallmeyer et al. 2012).<sup>4</sup> Eukaryotic microbes are also estimated to be extremely numerous, accounting for 10–30 % of cell counts in the deep ocean (López-García et al. 2001). The diversity of microorganisms is also fascinating. Estimates of microbial species run several orders of magnitude higher than those of macrobial ones. Bacteria alone may form as many as  $10^7$ – $10^{12}$  species (Dykhuizen 1998), whereas the number of animal and plant species is estimated to be closer to  $10^6$  (Staley and Gosink 1999). The diversity of archaea and of microbial eukaryotic species is also estimated to be very high (in the case of archaea, see Lipp et al. 2008; in the case of microbial eukaryotes, see Moon-van der Staay et al. 2001; Epstein and López-García 2008).

<sup>4</sup> These estimates should be taken with caution and are disputed (see Whitman et al. 1998; Lipp et al. 2008; Kallmeyer et al. 2012). In particular, estimates of the total biomass that these unicellular organisms represent differ significantly. Nevertheless, estimates of the total number of unicellular organisms on Earth are consistent in terms of orders of magnitude.

Microorganisms also play an extremely significant chemical transformation role. They are active in numerous chemical processes that regulate environmental conditions at values that are favorable to current forms of life: bacteria are, for instance, at the origin of the significant rise in atmospheric oxygen on Earth some 2.4–2.2 billion years ago (Catling et al. 2001) while also playing a unique role in the fixation of atmospheric nitrogen (Postgate 1998). Owing to their metabolic diversity, microorganisms are capable of transforming numerous organic “waste” compounds into useful compounds for other organisms. They also transform many inorganic and sometimes toxic substrates into nutrients for yet other organisms. And they sometimes live in symbiosis with larger organisms, thereby providing the latter with metabolic pathways they would otherwise lack (e.g. Tannock 1990; Scanlan and Marchesi 2008).

In addition to being interesting for its own sake, microbial diversity is also most rewarding when studied in relationship to general biodiversity and ecology. Because microbes and macrobes are often involved in intimate associations, it is likely that they will display interrelated ecological phenomena. For instance, it is reasonable to expect that they will affect each other’s geographic distributions. In this case, biogeography studies of microorganisms are required to identify models of their geographical distribution patterns and investigate whether these models fit or not with those of the macrobial domain (Martiny et al. 2006). More generally, a broad range of microbial ecological phenomena ought to be investigated, modeled and understood if one is to grasp how microbial diversity evolves both spatially and temporarily, and how this diversity differs or not from that of macroorganisms. This includes studying for instance how microbial diversity might be influenced by the type of habitat or by the heterogeneity of this habitat, as well as assessing the extent to which this diversity follows particular geographical distributions and responds to specific environmental changes (Horner-Devine et al. 2004). Yet, such microbial ecological phenomena also ought to be compared to those that more classically characterize macrobial diversity, thereby showing that classical macrobial ecological models might rightly be extended downward to the microbial realm, or, from the other perspective, might establish limitations to such classical models. This is all the more relevant as microbial ecological phenomena have been found to interact very closely with macrobial ecological phenomena, owing to the tight functional integration that microbes and macrobes have with each other.

In this respect, studies of the geographical distribution of microorganisms have led to profound controversies (e.g. Hedlund and Staley 2003 in the case of bacteria). Whereas some argue that microbial diversity corroborates classical models of macrobial diversity according to which high dispersion rates of organisms should result in the absence of any specific geographic distribution (e.g. Finlay and Clarke 1999 in the case of protists), others strongly argue the opposite, at least for certain species of bacteria such as extremophiles that are associated with very localized environmental conditions (e.g. Papke et al. 2003 in the case of hyperthermophilic bacteria). Still other studies point to correlations of specific microbial geographical distribution patterns with phenotypic traits exhibited by the corresponding microorganisms, and try to compare such phenomena to similar ones inferred from models in classical macrobial biodiversity (e.g. Green et al. 2008; Ragon et al. 2012). Taking

microbes into account alongside macrobes is therefore likely to be very valuable in ecology and biodiversity studies since this will either extend the empirical relevance of current ecological models into the microbial domain, or point to unique features of microorganisms that may necessitate ecological models of a more specific nature.

### Defining microbial diversity: two classical issues revisited

Defining biodiversity at the microbial scale encounters two classical inter-related issues that traditional macrobial biodiversity also encounters. One is the issue of defining the items whose diversity will be measured. This is often called the *units* problem. The other is the issue of choosing the *mathematical measure* of diversity. Clarifying these two issues is required in particular to make sense of the idea of “measure of diversity” or “diversity index”.

As I have mentioned above, biological diversity is often defined as the variety of life at every hierarchical level and spatial scale of biological organization: genes within populations, populations within species, species within communities, communities within landscapes, landscapes within biomes, biomes within the biosphere (Wilson 1992; Harper and Hawksworth 1995; Gaston and Spicer 2004). To make the concept of biodiversity even more comprehensive, some have defined it in such a way as to include also functional groups and functional traits (Díaz and Cabido 2001; Petchey and Gaston 2006), as well as interactions between species (Dyer et al. 2010; Thompson 1996) and types of ecological and evolutionary processes (Noss and Cooperrider 1994). If we follow such construals of biodiversity, the *units* of diversity are extremely varied in type, including not only biological entities such as genes, populations or species, but also biological functions and processes, be they ecological or evolutionary. MacLaurin and Sterelny refer to this problem of choosing proper items of biodiversity as the “units-and-difference” problem (2008). Unless solved, the consequence is a plurality of construals of “biodiversity”, with each one resting on particular units of biodiversity. These units of biodiversity are also so varied that much concern arises when it comes to quantifying them in the field and to formulating relevant conservation policies. Assessing diversity at all levels of organization from genes up to the biosphere, including not just biological entities but also processes (see Baptiste and Dupré 2012) might be a good in-principle-objective, yet simply intractable in practice.

For this reason, a very reasonable fallback solution is to construe biodiversity in a much narrower sense, for instance as “*species* diversity” (e.g. Hubbell 2001; Magurran 2004). The entities “species” certainly appear to be the most commonly used unit in biodiversity studies. Detectability, sampling, measurement, abundance in space and time, and density are all concepts that are commonly defined in relationship to “species” (e.g. Magurran and McGill 2011).

However, even when the units of biodiversity are limited to species, one still faces the issue of properly defining “species”. This concept is well known for its elusiveness (e.g. Mayr 1982; Kitcher 1984; Sober 1984; Wilson 1999), and, despite its very frequent use as the unit of biodiversity, its applicability to biodiversity

studies has been strongly questioned and reassessed (e.g. Rojas 1992; MacLaurin and Sterelny 2008). What is even more important in the case of microbial diversity is the fact that microbes raise novel complications for the concept of “species”. Genealogies of unicellular organisms can be extremely fuzzy, most notably due to a phenomenon known as lateral gene transfer (LGT). LGT occurs, for instance, through absorption of genetic material present in the local environment (“transformation”), through transfer of genetic material from one microorganism to another (“conjugation”), or even through transfer of genetic material mediated by a virus (“transduction”) (Paul 1999). The striking thing is that LGT not only takes place between microorganisms of the same species, but also between microorganisms that belong to different species or even domains (Gogarten et al. 2002; Andersson 2005), thereby causing the relative fuzziness of genealogies in microorganisms. The result is that species boundaries can be very blurred in the microbial realm, and the concept of “species” needs redefining or relaxing if it is to be of any use. Indeed, philosophers and biologists appear divided as to how to define species in light of such phenomena. Some still argue that a realist construal of species is possible and relevant in microbiology, and in biology in general (Cohan 2002); on the opposite bank, others are much less optimistic and prefer to adopt a pragmatic point of view by defining “species” in a nominalist way or even by replacing it by conventionally defined units of evolution (Rosselló-Mora and Amann 2001; Baptiste and Boucher 2009; Doolittle and Zhaxybayeva 2009). In practice, four large families of definitions of “species” seem to coexist in microbiology: species as groups of organisms that reproduce through genetic recombination; species as evolutionary lineages that have been aggregated through ecological selective pressures; species as reconstructed phylogenies on the basis of genetic data; or species defined in a nominalist and operational way, for instance as genomically coherent monophyletic clusters of organisms sharing a high degree of similarity (Ereshefsky 2010). As a consequence, even when the units of biodiversity are narrowed down to species, a researcher would still run the risk of encountering a plurality of construals of “biodiversity”, each based on a particular definition of “species”.

The second major issue underlying the concept of “biodiversity” is that of choosing a *mathematical measure* of diversity. This mathematical measure is akin to a quantificatory tool or a formula to be used to compute a biodiversity index on the basis of a set of quantified attributes of the chosen units of diversity. The mathematical measure is the tool that makes it possible to give a numerical value to biodiversity and to answer the question “*How much* biodiversity is there?” once all the relevant empirical data have been gathered about the units of biodiversity. This issue is different from the issue of defining the units of biodiversity (i.e. of defining what this biodiversity is a diversity of); nevertheless it assumes that the units of biodiversity are agreed upon and that all the relevant variables concerning these units can be quantified in a way or another. On this basis, the issue consists in defining the mathematical formula to be used to calculate the numerical value of biodiversity.

Several mathematical measures exist however, each leading to different ways of quantifying biodiversity. For instance, when units of biodiversity are set to be

*species*, different mathematical measures focus on some attributes of species rather than others (thereby including some variables rather than others), and they compute biodiversity numerical values in some fashion rather than another. Some biodiversity measures focus on the *number* of different species in the system, while others also take into account the relative *abundances* with which individuals are distributed over different species. Still others highlight the characteristic *features* of species (e.g. their functional traits) and how different some species might be from one another in this respect (Purvis and Hector 2000; Magurran 2004). In the first case, the biodiversity measure is based on species richness; in the second, taking into account species abundances leads to measures such as the Shannon-Wiener index, the Simpson index or the Berger-Parker index; for the third case, a focus on species features and how different they are produces measures such as the Weitzman index, the Weikard index or the Nehring-Puppe index.<sup>5</sup>

There is therefore a plurality of possible mathematical measures, and biodiversity research faces a dilemma as to which measure or index to choose in order to quantify biodiversity. However, because of the way they are built, indices emphasize some selected parameters over others. A choice of biodiversity measure thereby determines how much weight a researcher is willing to give to which piece of information. As such, there is no “true” or “correct” biodiversity measure, but merely measures that fit particular objectives, such as assessing the development over time of a nature reserve or comparing two patches of rainforest in terms of possibly useful pharmaceutical substances. By focusing more on relative species abundances, some measures will capture the heterogeneity of a given ecosystem at different points in time; other measures that are, for instance, built upon pairwise species distances, will instead aim at assessing some form of dissimilarity between two ecosystems.

Microbial diversity fares no better than general biodiversity when it comes to addressing this issue of a plurality of diversity measures. There appears to be no single “right” measure for microbial diversity, but rather, there are measures that take into account specific variables over others depending on the researcher’s objectives. For instance, in the context of therapeutic studies (e.g. eradication of pathogens or identification of therapeutic substances) and of technological applications (such as the identification and destruction of contaminants, or the production of energy), microbial diversity tends to be measured with biodiversity indices that focus more on species features and dissimilarities than on species abundance and heterogeneity (Baumgärtner 2006a).<sup>6</sup> The choice of diversity

<sup>5</sup> For instance, if  $n$  is the total number of species of a given ecosystem  $E$ , and if  $p_i$  is the relative abundance of species  $i$  (such that  $\sum p_i = 1$ ), then the species richness of  $E$  is equal to  $n$ . Similarly, the Shannon-Wiener diversity measure of  $E$  is equal to  $\exp(-\sum p_i \log(p_i))$  and the Simpson diversity measure of  $E$  is  $1/\sum p_i^2$ . If one has phylogenetic or taxonomic information about the species of  $E$  such that it is possible to assess, for instance, pairwise distances between species depending on their characteristic features, then one can define measures that include these distances and account for the diversity of  $E$  in terms of features. For more details, see for instance (Magurran 2004).

<sup>6</sup> Indices that focus on species abundance and heterogeneity include the species richness index, the Shannon-Wiener diversity measure or the Simpson diversity measure (see note 5); indices that focus on species features and dissimilarities include the Weitzman index or the Nehring-Puppe index. For more details about these indices, see for instance Baumgärtner (2006b) or Magurran (2004).

measure is therefore very strongly dependent on the goals that motivate this measure in the first place.

### Extending microbial diversity at the frontier of life and non-life

Microbial diversity has been self-evidently named for its focus on “microbes” or “microorganisms”, and these are nothing other, as mentioned above, than “small organisms” that are too tiny to see with the naked eye. As such, microbes cut across the three domains of life, Archaea, Bacteria and Eukarya. However, the science of microbes—microbiology understood in a broad sense—also often includes within its scope of investigation other entities of biological interest such as viruses and specific organized non-cellular entities that include viroids, satellites, virophages, plasmids or even prions.<sup>7</sup> These entities all share the same building blocks as unicellular and multicellular organisms—nucleic acids and/or amino acids, as well as the same genetic code—and are also capable of Darwinian evolution, which explains the interest they raise among biologists. In addition, microbiologists also study unicellular entities whose status as fully living organisms is clearly debated. This is, for instance, the case with such bacterial endosymbionts as *Carsonella ruddii* and *Hodgkinia cicadicola* whose very tiny genomes lack many of the critical life-sustaining genes (Nakabachi et al. 2006; McCutcheon 2010). As a result, microbial diversity can be understood not just as the study of the diversity of small *living* organisms such as bacteria, archaea, fungi, algae, or protists, but also as the study of the diversity of *not-so-clearly-living* entities such as those just mentioned that include viruses, viroids, satellites, virophages, plasmids, prions as well as some tiny unicellular symbionts and, I firmly believe, many still-to-be-identified entities at the frontier of life and non-life. This is debatable however, because these entities are not considered to be alive by some definitions—most notably because they lack a self-sustaining metabolism and a capacity for autonomous reproduction (e.g. Joyce 1994; Ruiz-Mirazo et al. 2004; Moreira and López-García 2009). According to such criteria, these entities should all be excluded from the scope of *biodiversity*

<sup>7</sup> *Viroids* are viral particles that are usually smaller than viruses and that are composed of a short stretch of circular single-stranded RNA. Unlike viruses, viroids do not have any protein coat (e.g. Diener 1971; Dimmock et al. 2007). *Satellites* are viral agents composed of nucleic acid (DNA or RNA) that can only reproduce if their host cells are also co-infected with another specific virus called a helper-virus or master-virus. Satellites may represent evolutionary intermediates of viroids and viruses (e.g. Saunders and Stanley 1999; Dimmock et al. 2007). *Virophages* are viruses that infect other larger viruses. They are sometimes considered a sub-group of satellites, yet some argue for a distinct classification (e.g. La Scola et al. 2008). *Plasmids* are double-stranded, and often circular, DNA molecules that notably occur in bacteria and that can replicate independently of the chromosomal DNA (e.g. Lederberg 1952). *Prions* are often infectious agents that are not composed of nucleic acids but that consist of proteins considered to be in a misfolded form and that have been identified in different mammals and in yeast (e.g. Prusiner 1982). Interestingly, prions have recently been found to be capable of Darwinian evolution (Li et al. 2010). It is the development of sequencing techniques and of biochemical tools in the past decades that has led to a more thorough investigation of these numerous, minute, and organized entities that abound in the vicinity of known living entities at their sub-cellular scale. Because these discoveries have so far been mostly driven by the pathogenicity of the entities in question, it is reasonable to expect that many more such non-pathogenic sub-cellular entities will be identified in the near future.

studies. I argue that there are at least three sets of reasons why one might wish to include such entities within the scope of biodiversity studies, in addition to the fact that they all use the same molecular building blocks as those that clearly-living entities do, and that they are capable of Darwinian evolution as well.

The first set of reasons is about abundance. As a matter of fact, scientists have already started gathering much quantitative information about the diversity of those not-so-clearly-living entities, and most notably of viruses. As of today, nearly 2500 virus species have been identified and a specific taxonomy has been developed.<sup>8</sup> According to some reports, these known species represent but an extremely small fraction of the total virus diversity that has been estimated to be in the range of  $10^5$ – $10^{13}$  species, and is therefore possibly an order of magnitude larger than the diversity of unicellular organisms (Colwell 1997; Rohwer 2003; Rohwer and Barott 2012). Research on virus diversity is hindered by biases similar to those that affect microbial diversity in favor of human and agriculture-related pathogens. Most known viruses to date concern human illnesses or are related to plant and animal diseases that also concern humans because of their economic impacts. However the largest share of virus diversity simply is unknown. Viruses are also extremely ubiquitous. Studies of seawater samples, for instance, have shown an amazing diversity of viruses with some estimates indicating that 200 liters of seawater may possibly contain over 5,000 different virus species (Breitbart et al. 2002). Viruses also appear most significant in terms of abundance: estimates run in the range of  $10^9$  viruses per cubic centimeter of nearshore surface sediments and  $10^8$  viruses per cubic centimeter of ocean water. Correspondingly, the total number of viruses in the oceans would be as high as  $10^{30}$ , making viruses a most significant component of biomass (Suttle 2005). Richness, abundance, and biomass are all are strong quantitative arguments in favor of studying virus diversity.

The second set of reasons is more qualitative and concerns the structural and morphological diversity displayed by such not-so-clearly-living entities. Studies of viral communities have indeed revealed a plethora of genotypes and morphotypes. These include “complex” viruses such as the well-known head-and-tail ones that possess a capsid and a protein tail acting as a syringe. There are also “helical” viruses that result in rod-shaped or filamentous particles, “icosahedral” viruses that possess a closed shell built out of a certain number of identical sub-units, and “envelope” viruses that seclude themselves inside a membrane assembled from lipids and carbohydrates borrowed from the host cells (Dimmock et al. 2007). The diversity of morphotypes inside each of these four large clusters is also huge. For instance, a survey of bacteriophage diversity in a European lake reported 39 morphologically distinct types of head-and-tail viruses (Demuth et al. 1993). Viruses also vary considerably in size, ranging from a few dozen nanometers in the case of typical icosahedral viruses, up to several hundreds of nanometers in the case of giant viruses like the “mimivirus” whose size compares to that of a small bacterium (La Scola et al. 2003).

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<sup>8</sup> The International Committee on the Taxonomy of Viruses recognizes the existence of 2,475 virus species as of late 2011 (see <http://www.ictvonline.org/virusTaxInfo.asp>).

The third set of reasons to include entities such as viruses within the scope of “biodiversity” comes from the intricate ecological roles such entities play. The morphological diversity of viruses is obviously linked to the complex patterns of resistance and susceptibility that characterize the virus-host relationship. Because viruses need to maintain very strong interactions with host organisms in order to reproduce—sometimes in quite symbiotic ways (Roossinck 2005)—it comes as no surprise that viruses play a significant role in a broad range of ecological phenomena. This shows, for instance, in how viruses may affect the geographical distribution patterns of certain plants, and possibly also in how viruses may affect the adaptation of some organisms to their local environment (Wren et al. 2006). Virus diversity is therefore intimately linked to microbial and macrobial diversity. In the exact same way that specific ecological phenomena cannot be understood without taking into account clearly-alive microorganisms—as seen in the “[Why microbial diversity?](#)” section—there are ecological phenomena whose explanation require taking into account such not-so-clearly-living entities like viruses. The minute interactions that take place between all these different types of entities cumulatively have a huge impact on large-scale ecological and biogeochemical cycles (Fuhrman 1999). A most revealing example is that of seawater viruses that cause a significant amount of marine microbial mortality. By doing so, viruses accelerate the transformation of nutrients from particulate (living organisms) to dissolved states (after cell lysis), where they can be incorporated anew by microbial communities. Yet, when viruses lyse microbial cells, they also happen to convert particulate organic carbon into dissolved forms. This, in turn, lowers the levels of cellular carbon, which results in more carbon being respired in the surface waters, and hence decreases atmospheric carbon (Suttle 2005). Another most telling example is that of other marine viruses that infect particular microalgae and induce the synthesis of dimethylsulphide, which in turn has a significant impact on the production of clouds and on global climate (Ayers and Caine 2007; Wilson and Allen 2011).

Including such not-so-clearly-living entities as viruses and the like within the scope of biodiversity studies appears therefore most legitimate: their diversity is important in its own stake for quantitative and qualitative reasons, and they are important in their relationship to the rest of diversity studies, as we have just seen. The concept of biodiversity as such requires that one pays attention to biological entities that are (usually) smaller than those one traditionally assigns to life, such as viruses. And, as a matter of fact, there exist many other types of such not-so-clearly-living entities that can also play very significant ecological roles: as mentioned above, these include viroids, satellites, virophages, not to mention also plasmids, prions, or even other—still to be discovered—entities. Interestingly, the study of viroid diversity, for instance, is already the topic of nascent research studies (e.g. Kofalvi et al. 1997; Wang et al. 2008).

### **The “lower-limit problem”**

A tacit assumption usually underlies the concept of biodiversity, namely the assumption that it is the diversity of what is alive that we are concerned about.

Etymologically speaking, biodiversity is the diversity of the “bios”, that is to say, of *life*. Sticking to this etymology implies that biodiversity ought to take into account anything that is alive and weigh its relative contribution to the diversity of all forms of life; and conversely, that all non-living things ought to be excluded from its scope. Because macrobes are uncontroversially alive, they are dutifully included within the scope of biodiversity. And, when by microbes we mean small multicellular and unicellular organisms, then microbes too are clearly to be included into the scope of biodiversity.

What is not so clear however is what ought to be done with such microbial entities such as viruses, viroids, satellites and so forth. In the previous section, I have just argued that there are at least three sets of reasons to include them within biodiversity studies: their abundance, their morphological diversity and their intricate ecological role. It is, however, debatable whether such entities meet the criteria for being alive. And, in turn, this issue raises the question whether biodiversity should be exclusively concerned with entities that are clearly alive—be they macrobes or microbes—or whether it should also encompass not-so-clearly-living entities at the frontier of life and non-life—contrary to what the etymology of the word would suggest. In other words, these abundant morphologically diverse, ecologically relevant, not-so-clearly-living entities point to the question of *delineating* the entities that biodiversity is concerned with when going deeper and deeper into the microbial domain. This is what I refer to as the “lower-limit problem” of biodiversity. Should biodiversity only include entities that are clearly judged alive? Should it also include entities that are, for instance, studied by microbiology yet whose status as living entities is debatable, such as viruses or some tiny reduced endosymbionts? Should it also include still other entities judged non-living by most, such as plasmids or prions? In sum, one may reformulate the lower-limit problem as a set of two complementary issues: (1) Should one stick to the etymology of the word “biodiversity” and use the concept of “life” as delineation criterion to decide which entities to include or not within the scope of biodiversity? Or (2) should the “bio” of biodiversity be dropped, and in this case which delineation criterion should be used to decide what is to be included into the scope of this new—so to speak—“X-diversity”?

In the first case—using “life” as delineation criterion—the issue is to decide whether viruses and the like are alive or not. If yes, then these entities should be counted as “biodiversity”. If not, then they should simply be excluded from “biodiversity”. They would obviously remain as one of the foci of *diversity* studies—in par, possibly, with geodiversity or landscape diversity studies for instance—yet they would not be included into what counts as *biodiversity*. However—as I will show below—there is also the possibility that the criterion of “life” might not be operationalized in the case of such microbial entities, and that, as a consequence, it might not be possible to decide clearly whether viruses and the like ought to be judged alive or not. In this case then, the “lower-limit problem of biodiversity” would remain unsolved unless another delineation criterion can be used instead of “life”. But which delineation criterion, and why?

The “lower-limit problem” is all the more acute as microbiology continues to enrich the list of these biologically-relevant entities that prosper at the frontier of life and non-life: viruses, viroids, satellites, virophages, plasmids, prions, tiny

endosymbionts and so forth. What were at one point simply “small microbes”—especially infectious ones—have become a rich and diverse group of not-always-so-small entities displaying a wide array of phenotypes, while simultaneously using the most common building blocks of life—nucleic acids and amino acids—and lacking some of the attributes of typical unicellular organisms, such as phospholipid-based membranes, for instance, or critical life-sustaining genes. Because their identification is driven by the use of molecular and biochemical tools—such as genome sequencing and its application to metagenomic studies—and because these tools are continually being improved upon and rendered easier to deploy, it is more than likely that the list of such entities at the frontier of life and non-life will continue expanding in the very near future, together with a deeper understanding of the intricate ecological roles of such entities. And, if this list keeps expanding, then the question of deciding whether it constitutes or not—as a whole or in part—a legitimate aspect of biodiversity will become even more pressing, and all the more so as ecology and microbial studies become increasingly integrated into a single larger field of biodiversity studies.

It is worth underlining also that the “lower-limit problem” is indeed a different problem than the units problem mentioned above, and that it adds to the list of foundational issues that the concept of “biodiversity” faces. The units problem—as we have seen—concerns the choice of the types of items that biodiversity is the diversity of, such as genes, species or traits. And it is attributes of these units that are included as variables in biodiversity measures or indices. As we have also seen, such variables include for instance the number (of genes, species, traits), the number of organisms (of each species or displaying each gene or trait), specific distance metrics (between genes, species, traits) and so forth. However, in all these cases, the units of biodiversity are assumed to *refer to living* entities or organisms. For instance, speaking about measuring functional diversity, Petchey and Gaston say that it “is about measuring functional trait diversity, where functional traits are components of an *organism’s* phenotype that influence ecosystem level processes” (Petchey and Gaston 2006: 742, my italics). In other words, whatever the choice of units may be, these units are supposed to be items possessed by, displayed by, or constituted by *living* organisms. The units problem therefore presupposes the lower-limit problem to be solved or non-existent. Yet, the lower-limit problem specifically concerns the nature of that implicit reference that the units of biodiversity all refer to. In this respect, it is a problem that all units of biodiversity encounter, and that is therefore orthogonal to the units problem. As such, it is an additional conceptual and foundational issue that biodiversity must face.

Not addressing the lower-limit problem puts the concept of biodiversity on quite soft ground. How can we scientifically ever study biodiversity if we do not know what this diversity ultimately refers to? How can we even measure biodiversity if we do not know precisely which units of what to take into account? Of course, it could be argued that “biodiversity” should rather be understood as a “folk concept” with multiple or loose construals that do not require the lower-limit problem to be settled. This position is, I think, untenable as biodiversity is the very focus of a large amount of science, and as the general public as a whole precisely relies on science as a guidance for understanding this very diversity and taking proper relevant

action. It is because biodiversity is a *scientific* concept that it requires such careful examination of its foundational assumptions, and in particular of the lower-limit problem.

### The “lower-limit problem” and “life”

As mentioned above, the first and most tempting way to address the lower-limit problem would be to stick to the etymology of the word “biodiversity” and use the concept of “life” as an empirical delineation criterion. I argue that this avenue is not presently open, and that there are, in addition, good reasons to think that it will remain closed in the future. There is presently no available agreed-upon definition of life that would make possible a clear-cut distinction between living and non-living entities. Rather, there exist many different definitions of life that have given rise to at least as many controversies. Hundreds of definitions have been listed (e.g. Gayon et al. 2010; Palyi et al. 2002; Popa 2004), and the subject matter of defining life is by no means settled. There is, for-lack-of-any-better-solution, a weak consensus among scientists on the so-called “NASA definition of life”—or some of its variants—according to which “life is a self-sustained chemical system capable of undergoing Darwinian evolution” (Joyce 1994: xi). In the context of origins of life studies for instance, this definition suits well proponents of the “RNA-world” scenario and the views that life started with RNA molecules capable of self-replication and evolution (Gilbert 1986). However others have dismissed such a definition as applying only to populations and not to single individuals (Luisi 1998), or as not incorporating any notion of self-containment (Shapiro 1986; Segré et al. 2001). Controversies also arise with chemical cross-catalytic systems like oligo-peptidic autocatalytic networks. Some argue that such chemical systems ought to be considered alive since they are endowed with metabolic activity, and can grow and divide (e.g. Kauffman 1993); yet others argue the opposite on the grounds that such autocatalytic chemical systems are not capable of open-ended evolution (e.g. Luisi 1998; Ruiz-Mirazo et al. 2004). Similarly, advances in synthetic biology raise questions as to whether some of the chemical systems engineered by scientists—associating lipid membranes and catalytic RNAs for instance—ought to be considered alive or not. Some argue that such systems are still far from being alive and need many additional features to be so; others consider these systems as very close to fully living (e.g. Rasmussen et al. 2003; Noireaux et al. 2005; Schrum et al. 2010). Probably one of the most enduring controversies on defining life concerns the status of viruses. Many researchers argue that they are parasitic and definitely not alive by themselves, because they lack in particular the metabolic system that they hijack from their—truly living—hosts (e.g. Luisi 1998; Ruiz-Mirazo et al. 2004; Moreira and López-García 2009). Yet in light of recent findings, others strongly argue the contrary, in particular when it comes to very complex viruses, like the mimivirus, that have genomes over 1 million base pairs—a size comparable to unicellular organisms—and that also turn out to be themselves hosts to other smaller viruses or virophages (e.g. La Scola et al. 2003, 2008; Claverie and Ogata 2009). Conversely, it is also debated whether some of the simplest unicellular organisms that have been found recently ought to be qualified as alive or

not. In particular, bacterial endosymbionts, such as *Carsonella ruddii* and *Hodgkinia cicadicola*, have genomes in the range of some 150,000 base pairs only, in which numerous genes considered essential for life are missing. It has been suggested, therefore, that such organisms may not be true “living organisms” but rather organisms on the brink of becoming organelles of their symbiotic hosts (Nakabachi et al. 2006; McCutcheon 2010). As a result, and even though they may be classified as unicellular organisms, such organisms are no more alive than some of the most complex viruses.

What this shows is that sorting life from non-life is no trivial matter. Competing definitions of life put the cursor at different places. And newly found more-or-less alive microbial entities, such as giant viruses and tiny bacteria, totally blur the borderline between non-living matter and living matter. The implicit assumption that biodiversity makes—that there exists a definition of life that makes the question of sorting the “bios” from the “non-bios” an empirically tractable one—turns out to be most problematic, and questions the very feasibility of defining a lower-limit of biodiversity on empirical grounds. It has been argued that the controversies about defining life stem from the present lack of theory of life and are therefore a consequence of the current limitations of scientific knowledge (Cleland and Chyba 2002). Such a claim suggests that delineating life from non-life, even if currently impossible, will be a tractable problem once a theory of life is found. However, it is not clear at all whether an empirically adequate definition of life ought to be dichotomous or rather continuous. Many have argued in favor of a clear-cut delineation between inanimate matter and life, and this stance has been implicit in the vast majority of definitions of life that have sought to specify the right set of criteria that would be the hallmark of living organisms.<sup>9</sup> Yet, in light of the many newly found entities that populate the space between simple organic molecules and fully living unicellular organisms—prions, plasmids, virophages, satellites, viruses, highly reduced bacterial symbionts, and so on—life appears not so much as a matter of all-or-nothing but rather as coming in degrees and shades of grey. This explains—I firmly believe—why there is just so much controversy and disagreement about defining life in a dichotomous way. The richness of nature at the microbial scale shows that such a clear-cut delineation simply does not exist. Rather, there appear to exist many “more-or-less alive” things that populate the microbial world. Life would therefore be better construed not as a dichotomous property but as coming in degrees (Bedau 2010; Bruylants et al. 2010; Malaterre 2010). In between “clearly-not-alive” entities—such as simple molecules of methane or carbon dioxide—and “clearly-alive” entities—such as an *E. coli* bacterium—a continuous construal of life acknowledges the existence of many “more-or-less alive” entities, each one displaying, with more or less effectiveness, a few or many of the key features of clearly alive entities (e.g. metabolic activity, reproduction, information encoding, individuation and so forth). A continuous construal of life also accounts for what is seen in biodiversity studies, namely the

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<sup>9</sup> This is the case both in the diachronic context of origins of life research that aims at explaining the historic transition from non-living matter to living matter, and in the synchronic context of microbial research that investigates the present frontier of life and non-life. In this paper, the focus is obviously on this second synchronic research context.

fact that these studies have been drawn into the investigation of diversity at smaller and smaller scales, down to microbes, viruses and viroids, because these microbial entities have been found to play extremely significant ecological roles and to be intimately linked to the rest of biodiversity through complex webs of interactions.

On the other hand, if life truly is a matter of degrees, then this casts a serious doubt on the possibility of delineating the “bio” of biodiversity, and of defining an empirically grounded “lower-limit” of biodiversity. This points therefore to the difficulty of addressing the lower-limit problem of biodiversity by sticking to the etymology of the word and using the concept of “life” as an empirical delineation criterion. In turn, this shows the necessity of anchoring the concept of biodiversity on much more stable ground than it has been so far assumed to be. Until this is done, one may think about restricting “biodiversity” to clearly-alive entities—starting with unicellular organisms such as bacteria, archaea and protists—while grouping diversity studies about all not-so-clearly-alive entities such as viruses and so forth into a specifically labeled “sub-microbial diversity” concept. Despite not being a direct answer to the lower-limit problem, and despite splitting microbial diversity studies into two, this move may help by at least signaling the conceptual issue of sorting out the extent to which “biodiversity” indeed is the diversity of “bio”.

## Conclusion

The aim of this paper was to assess the soundness of the concept of “biodiversity” when investigating biological entities at smaller and smaller scales. I have listed several reasons for biodiversity studies to take into account the diversity of the microbial world. However, this raises a foundational problem for the very concept of “biodiversity”, that adds to other well-known foundational problems like those of the units and of the measure of biodiversity: the problem of its clear delineation at the quite fuzzy frontier of life and non-life. I have called this problem the “lower-limit problem” of biodiversity. I have argued that this problem is all the more salient as microbiology continues to enrich the list of entities at the frontier of life and non-life and as ecology and microbial studies are becoming more and more integrated. I have also argued that addressing this lower-limit problem by sticking to the etymology of “biodiversity” and using “life” as an empirically-informed delineation criterion was currently not a viable option, and there were good reasons to think that it would remain so in the future. This argues in favor of downplaying the role of the “bio” within “biodiversity”, yet leaves open the problem of settling on its scope. Some may argue that the solution to the lower-limit problem is bound to involve, to some extent, normative values and judgments, as it has been argued to be the case with the problems of the units and the measures of biodiversity (e.g. Baumgärtner 2006b; Sarkar 2005). Others may argue on the contrary that the solution to the lower-limit problem will rather involve a pluralist stance, making “biodiversity” a concept whose varied construals depend on the objectives of scientists and the specificity of their object of study. How such solutions may work is however open at present.

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