Chapter 5

Transitioning Toward a Universal Species Concept for the Classification of all Organisms

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1. Introduction

One purpose of this paper is to briefly discuss the various stages in the development of microbial taxonomy to illustrate how new technological developments have influenced our understanding of prokaryotic species. This will be followed by a discussion of why a universal species concept for all organisms is a critical need for biology as a scientific discipline. In particular, arguments will be made for the adoption of the phylogenomic species concept as a Universal Species Concept (USC). The final section will provide an outline of how biologists might implement a USC.

It is worth noting here that the idea of a 'species' is a human idea, not a naturally determined or 'god-given' designation. In nature there are many examples of animals such as the dog-wolf group in which multitudinous variations due in part to human influences have produced a very complex array of descendants, all of which comprise a single 'species' according to the Biological Species Concept. Similar examples can be found in plants as well.

2. History of bacteriological treatment of species

Before we proceed, it would be helpful to understand how microbiologists, in particular bacteriologists, have dealt with the species issue from a historical perspective. The classification of prokaryotic organisms, i.e., Bacteria and Archaea, has undergone many changes since microbial life was first discovered. All of these changes have been brought about by technological advances.

The history of microbial taxonomy can be broken down into four periods:

a. Discovery of microorganisms,

b. Advent of pure cultures and phenotypic features,

c. Introduction of molecular analyses and

d. Gene sequencing and genomics.

The brief historical treatment below illustrates how important the introduction of new instruments, techniques and analyses were in the development of the field of microbiology and, in particular, microbial systematics.

Period 1. Discovery of microorganisms 1673 to 1850

One can appreciate that a long time elapsed between the discovery of microorganisms by Antonie van Leeuwenhoek in the late 1600s and the early attempts to describe and name bacteria and other microorganisms. Microorganisms would not have been discovered had it not been for the microscope. Initially they were known only from observations of their morphology and these were hampered because of the poor quality of microscopes that were available at the time, the small sizes of the organisms and the paucity of distinguishing morphological features. A few species were named that were quite distinctive morphologically, however, there were not many. A more thorough treatment of this period and more recent history can be found in [15].

Period 2. Advent of pure cultures and phenotypic features 1850 - today

A critical innovation in the study of microorganisms was the development of procedures to isolate them in pure culture. Because microorganisms are so small, it was initially very difficult to determine their features, aside from their cell size and shape. The cultivation of microorganisms on solid media enabled individual cells to be separated from one another to allow them to grow into colonies that could be studied as 'pure cultures' of a single type or species. This critical breakthrough was first achieved in the mid-1800s in Robert Koch's laboratory.

These pure cultures could be readily grown in abundance in the laboratory thereby enabling the characterization of their cellular chemical composition, physiology, metabolism and life cycles. In time a variety of phenotypic tests were developed that could be used to characterize each individual species. A testament to the success of phenotypic characterization is illustrated by the publication of [2], the first edition of which was published in 1923. It is now in its 9th edition (1994). Phenotypic tests were and still are strongly relied upon for the identification of species.

More recently the 2nd edition of Bergey's Manual of Systematic Bacteriology (BMSB), which is the most encyclopedic treatment of all described prokaryotic species (Archaea and Bacteria) has recently been completed with the publication of Volume V [3]. Thousands of species of bacteria have been named based primarily on phenotypic features. In addition, new species are still being discovered through traditional agar plate isolation approaches as well as novel modifications that enable the growth of previously un-isolated microorganisms. Importantly, the taxonomy of the Bacteria and Archaea in this most recent edition of BMSB relies on the 16S rRNA gene sequences of organisms from the domain level to the genus. So, it

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is a phylogenetic classification for all taxonomic levels except the species. This point will be elaborated on below.

The availability of phenotypic and genotypic features coupled with the abundance of strains that were available of some microbial groups such as the enteric bacteria provided fertile ground to use the phenetic approach for their classification beginning in the 1950s. Computers were also becoming available in the last half of the 20th century so comparisons of genera that had large numbers of species and strains could be analyzed using similarity coefficient analysis and related procedures [16]. However, the phenetic approach has taken a back seat since gene sequencing became available.

Period 3. Introduction of molecular analyses 1960 - 1990

Once DNA was discovered, its features began to be used to distinguish among microorganisms. A classical example of the impact of this approach was the determination of the DNA base composition, i.e., the mol% G + C content of DNA (mol G + C/mol G + C + A + T) x 100. By conducting this molecular analysis it was determined that one group of coccus-shaped bacteria, the *Staphylococcus* genus and related bacteria could be readily distinguished from another group of coccus-shaped bacteria, the *Micrococcus* group. The mol% G C of the former was very low (ca. 30-35 mol%) compared to that of the *Micrococcus* group (ca. 70-75 mol %). Clearly with differences in DNA content that great, the two groups of cocci must be classified in different groups. Currently, based on 16S rRNA gene sequencing, *Staphylococcus* and related cocci are placed in the Firmicutes phylum whereas *Micrococcus* and related organisms are in the Actinobacteria phylum.

The other technique that was developed in the 1960s was that of DNA-DNA hybridization (DDH). In this procedure, if one wishes to compare how similar two organisms are to one another, the DNA is extracted and purified from each of them. This double-stranded DNA is then 'melted' to single strands by heating and the separated solutions are mixed with melted DNA fragments from a test organism. These are allowed to re-anneal by slowly reducing the heat of the solution. The degree to which they re-anneal with one another to form double strands is then analyzed. An organism's own DNA is used as a control which is stated at 100%. Strains that exhibit <70% re-annealing with another strain by this procedure are considered to be members of a separate species, whereas those exhibiting >70% are considered to be members of the same species.

The combination of phenotypic features and DDH gave rise to the 'polyphasic species definition'. In 1987 a committee of prominent microbial taxonomists adopted the polyphasic definition [25]. This dictum which still stands today is that all members of a bacterial species must have a unique phenotype and exhibit greater than 70% DDH by standardized procedures [23]. More recently, it has been found that organisms that are greater than 97-99% similar by 16S rRNA gene sequence must be different species thereby relieving the necessity of carrying out DDH analyses except for the most closely related strains [17, 8]. This finding was helpful because at this time only specialized laboratories are equipped to carry out DDH analysis.

Period 4. Gene sequencing and genomics 1990 - today

In the 1990s gene and protein sequencing became readily available to biologists. This technological advance has fundamentally changed taxonomy because these sequences can be used to trace the evolutionary history of a lineage. Two major early impacts of this on taxonomic were the discovery of the three domains of organisms, the Bacteria, Archaea and Eukarya and the development of the Universal Tree of Life based on 16S and 18S rRNA gene sequencing of representatives from all three domains [27].

Another ad hoc meeting of an international committee of expert bacterial taxonomists which was held in 2002 resulted in a significant modification of the polyphasic species concept [22]. The major change was an allowance to permit the use of multiple locus sequence analysis (MLSA) in which the sequences of typically 5-7 genes are concatenated together and then analyzed phylogeneticaly. This could be used in place of DDH in the polyphasic species definition. This is significant because it is an evolutionary approach that uses sequence based phylogenetic analyses that have been successfully applied already for the identification of bacterial species in some genera such as *Streptococcus* as well as others [e.g., 7]. Since the process of speciation is an evolutionary process a sequence based, phylogenetic approach is well suited for the classification of species.

Since the availability of genome sequences, their analyses have shed considerable light on what comprises a current bacterial species [9, 10]. For example, the determination of ANI (average nucleotide identity) of genomes can be used to replace DDH as a means of determining the boundaries of a bacterial species.

There still remain major drawbacks to the current polyphasic bacterial species definition. First, the current bacterial species definition is not a single concept but a dual concept, a combination of two concepts, one phenotypic and the other molecular. Furthermore, some variations of the concept are not evolutionary. For this reason, the current bacterial species definition is extremely unlikely to become a universal species concept.

Because of these issues, a genomic – phylogenetic (or phylogenomic [1]) species concept was proposed for the Bacteria and Archaea in 2006 [19, 20, 21]. Because genomes contain all the genetic information of an organism, it is provides ideal and sufficient evolutionary information for a species classification based on genome sequences. A partial genome sequence may not necessarily always be sufficient.

One of the rationales for the phylogenomic classification is that it is consistent with the Tree of Life which is based on 16S rRNA and 18S rRNA gene sequences. Although the Tree of Life is phylogenetic, there is insufficient resolution in the 16S rRNA gene to distinguish among many prokaryotic species [Fox et al., 1992; 22]. Therefore, less highly conserved genes must be used at the species level. Hence the phylogenomic species concept relies on sequence analyses of less highly conserved genes [19] as used in the MLSA approach [e.g., 7]. Using the phylo-

1[1]The term, 'phylogenomic' was introduced by [5] and seems appropriate to replace to the term 'genomic – phylogenetic' in the name of the species concept [19] because it is less clumsy. Therefore, phylogenomic was adopted by [20, 21] for the name of the species concept.

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genomic species concept ensures the reliance on phylogenetic analyses from domain to species in the Bacteria and Archaea and could also be applied to the Eukarya.

Phenotypic characteristics will always remain important in taxonomy not only for bacteria but for other organisms. However, they should be confined primarily to the identification of organisms based on known distinctive features of the organism. Phenotype may also be used in nomenclature as many unique phenotypic features can be aptly and often colorfully applied in coining names for novel species. However, phenotypic features should never be used as a basis for classification because, unlike gene and protein sequences, they cannot be analyzed phylogenetically.

3. Why should biologists develop a Universal Species Concept?

Most biologists are not taxonomists as their work is quite separated from that of the taxonomist. Also there is already is a classification of the organisms in their fields so they see little need for a universal species concept. Therefore, many would not regard the development of a universal species concept to be very important. Nonetheless, there are two strong arguments in support of a universal species concept.

First, biologists, as well as all other scientists, use terms about the basic units of their science that are critical to their thinking and comprehension. Perhaps an analogy with chemistry is apropos here. In chemistry the basic units of their science are defined very definitively. Thus, a compound is a chemical with a definite formula that can be written out on a piece of paper. Can you imagine if organic chemists used a different concept for the definition of a compound than the inorganic chemists? This is clearly absurd. However, in biology there is no uniform or definitive idea for how the basic unit of life, the species should be classified. I strongly believe that in order for biology to become a true and rigorous science, a concept for a species that would apply universally to all organisms is a basic requirement. The term 'concept' is used here to indicate that the same *methodological approach* should be used to classify a species across all disciplines. Therefore, the dual species concept for microorganisms in which both phenotype and DDH are used should be abandoned in favor of a single concept, e.g. the phylogenomic species concept. This does not mean that phenotype would not play an important role in taxonomy. It would still play a major role in the identification and naming of species, but not in their classification.

The adoption of a Universal Species Concept would not mean that each species from bacterium to plant is constrained by some human-imposed, artificial boundary but that each species would be determined by the same conceptual approach, such as the phylogenomic approach. The result would be that all species would be classified with the same methodology. For taxonomic purposes experts in each discipline would have to decide on the intraspecific constraints for each species.

Second, now is a propitious time for the adoption of a universal species concept in biology because we have all the information that is needed. For example, like chemists, we can

now actually write out the chemical formula for the genome of many species. Indeed, [26] has recently proposed that a genome sequence should suffice for the naming a bacterial species irrespective of any additional information. Furthermore, he concludes that this is consistent with the International Code of Nomenclature for Bacteria [11] and the phylogenomic species concept. Of course, associated with that formula are genes and proteins as well as all the characteristics that comprise the innate properties of the organism. This includes not only the genes that are expressed only under certain conditions, but those too, that are not always expressed.

Many microbiologists and perhaps other biologists may not immediately flock to adopt a USC although they may agree that this is an important goal for biology. The reason is that it will take some time to make changes. For example, systematists of many individual groups of organisms will need to identify an appropriate set of genes for MLSA and then invest resources and time in order to properly classify the species they are most interested in. For example, the 16S rRNA gene sequence, which has very low resolution, seems to be an inappropriate gene to include in such analyses because of its highly conserved nature. Eventually, I believe that most biologists interested in taxonomy will adopt the phylogenetic approach and the phylogenomic species concept as more appropriate genes and more genomic information become available.

4. The quest for a universal species concept

The Biological Species Concept (BSC) proposed by [12]was a breakthrough in taxonomy. This simple concept states that a species consists of a group of organisms in which a male and a female member may breed to produce progeny which are also fertile.

At that time, bacteriologists were working from a completely phenotypic perspective without any thought that sexuality in bacteria was possible. However, in the late 1940s and 1950s Joshua Lederberg's laboratory demonstrated that enteric bacteria such as *E. coli* could carry out conjugation (Lederberg, 1947; 1957), in which genes and in some cases, the entire genome from an F^+ cell could be transferred to an F^- cell. It was then that microbiologists began to think that perhaps bacteria could also fit into the Biological Species Concept.

Arnold Ravin was a bacterial geneticist who explored the possibility of including bacteria in the BSC [13, 14]. He argued that there was sufficient evidence to conclude that bacteria speciated through evolutionary processes, which even then were regarded as the hallmark of speciation. Moreover, it was clear from the experiments on bacterial conjugation that genes could be transferred from an F^+ cell to an F^- cell and these could be expressed in the recipient cell. Therefore, the major elements needed to fulfill the BSC were available for at least some bacteria. However, the difficulty remained that the process could not be readily demonstrated on a species by species basis among such a highly diverse group of organisms that reproduced primarily by asexual reproduction. The idea seemed impractical even if bacterial sexuality was more widespread. For those reasons bacteriologists have abandoned the idea of using the BSC for bacteria.

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5. What should the universal species concept be?

Following closely on the heels of gene and protein sequencing advances and genomic analyses, a number of microbiologists have argued for a new concept for the bacterial species [1, 4, 6, 15, 19, 24].

Likewise there are several other species concepts that can be applied to organisms. However, if the BSC cannot become a Universal Species Concept, which one should be used? Several of these are dual concepts and therefore have little chance of becoming a universal species concept. The phylogenomic species concept was recommended as a universal species concept [21] because it can be applied not only to microorganisms, but to all other organisms as well. Further, it analyses the evolutionary relatedness among organisms which is a key factor in speciation. Other species concepts seem deficient in comparison.

It should be noted, however, that it is more important for biologists to adopt a Universal Species Concept than for a particular concept be adopted [21]. If there is one that is really better than the Phylogenomic Species Concept what is it?

6. Implementation of the USC by challenge

Now that a universal species concept has been proposed, how should it be implemented? Ideally it would be wonderful to have biologists meet together, discuss the issue and then vote on it. However, this is very unlikely to happen during this current global financial climate and perhaps not anytime soon thereafter.

An alternative approach is to conduct phylogenomic analyses on species that have a questionable classification. If sufficient evidence can be found, based on phylogenomic data that indicate the current taxonomy is flawed, this could be published to challenge the current classification. In contrast, if the analysis confirms the current classification, it would also provide additional validation of the PSC as a USC. In this manner the phylogenomic species concept could be considered the *de facto* species concept for the classification of that and all other species.

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