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Opinion: Response to concerns about the use of DNA sequences as types in the nomenclature of prokaryotes

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Abstract:

In the current Opinion we respond to the major concerns by Bisgaard et al. (2019) [7] and Overmann et al. (2019) [24] and conclude that the adoption of sequences as types for the names of prokaryotes will allow for improvements of the taxonomic framework, increased stability of names derived from robust phylogenomic methods, and enable a full circumscription of the microbial world rather than just the cultivated minority.

Text:

Recent advances in the development of high throughput sequencing platforms and accompanying bioinformatic tools have provided new opportunities for the cataloguing of the global prokaryotic diversity. Low-cost genome sequencing has made routine the use genomic data to circumscribe prokaryotic species and genera [8, 16, 18, 32], and enabled the construction of a global taxonomic framework based on phylogenomic analyses [27]. This molecular revolution is an enormous benefit to prokaryotic systematics and will undoubtedly accelerate the major goal of cataloguing and understanding bacterial and archaeal diversity.

In the last five years, several proposals have been made to further incorporate the improved understanding of microbial diversity obtained from genome sequencing into microbial taxonomy. One proposal that will greatly benefit the cataloguing of cultivated and uncultivated taxa, if accepted, is to allow genomic information to become type for nomenclatural purposes [47, 48]. In this proposal, a genome sequence deposited in one of the International Nucleotide Sequence Database Collaboration (INSDC) repositories can be designated as the type of a new taxon and an alternative to the deposition of a living culture in two different international collections. This proposal does not replace type strains that have already been deposited as the nomenclatural type with sequence data and does not prevent deposition of strains as types in the future.

This proposal would enable the formation of a stable nomenclature for uncultured prokaryotes, whose genomic information is known by means of metagenome assembled genomes (MAGs) or single cell amplified genomes (SAGs) [15]. A robust nomenclatural framework for the uncultured taxa can then be readily incorporated into the taxonomy of the cultured taxa, creating a single, stable nomenclature and taxonomy for all of prokaryotic life. However, the proposal to allow DNA sequences to become types has raised some concerns among taxonomists [7, 23, 24] that deserve comment. Their concerns can be summarized in the following major points, which we paraphrase from [7] and [24] in italics:

- 1- *The scientific data supporting the description of a species may not be reproducible, particularly if the original material (DNA) is damaged or lost or if no scientific distribution of nonproliferating material is anticipated [7].*

Loss of the original DNA would certainly make it impossible to reproduce the description, but this is also true when the type strain is lost. When a description is based on a culture loss of the culture, erroneous or contaminated deposits, and loss of viability causes enormous problems. For instance, 15 out of the 27 Requests for an Opinion currently being addressed by the Judicial Commission of the ICSP deal with the rejection of names or the establishment of neotype strains due to the lack of an authenticated living culture as type material. Cases such as the erroneous deposit by the authors [30], loss of the original culture [29], loss of a deposit [10], distribution of strains that do not match the nomenclatural type [22] or deposits of contaminated strains [45] require designation of neotype strains, rejection of the names or some other solution. In this regard, if genome sequence had instead been considered type, the link to the authenticity of the nomenclatural type would not have been lost.

Moreover, the phenotypic properties of cultured strains change upon adaption to laboratory growth conditions (e.g. [2]). This observation, together with the knowledge that lyophilized and frozen cultures lose viability with time [6, 19], means that the preservation of an identical exemplar at both genotypic and phenotypic levels over decades or longer is not guaranteed, and many type strains have not even survived a few years. If taxonomy deals with the explanation and structure of the natural relations of living beings, then it is desirable that the reference material remains intact. Sequence data has a better chance of meeting this criterion.

A second related concern is the potential disappearance of the type material (i.e. genome sequence data) from the public repositories. However, the interlinked effort of the INSDC (<http://www.insdc.org/>) formed by NCBI, EMBL-EBI and DDBJ, guarantees the safe and indefinite deposits of digitalized data as the information is stored in different formats in the different servers of the organization. Overall, the portability and expected archival permanence of sequence data mean that the ability to reuse and reanalyze such data is a distinct strength. For metagenomes and 16S rRNA gene amplicon surveys, repositories require the submission of the raw reads and so future bioinformatics developments should further improve assembly and binning of the sequences. In the long term, the digital form of a type has more guarantee of an intact preservation than viable frozen or lyophilized cells.

Lastly, MAGs reconstructed correctly from uncultured organisms will be repeatedly retrieved by different laboratories, either from the same metagenome(s) by different tools or from different metagenomes. Thus, there will be independent confirmation of the

existence of these taxa. If not, then the name will seldom be used and have little long-term consequence.

- 2- *To date, functional assessments for genomes are limited and do not necessarily allow recognition or prediction of distinguishing phenotypic traits that may define the taxa. Genomic data do not always correlate with gene expression such that apparent features at the genome level may infer misleading phylogenomic or taxonomic relationships [7].*

The phenotype is a tool and not the goal of systematics. As better methods are developed, they should be implemented. Historically, the best practices [42] encouraged a thorough exploration of the phenotype and establishment of diagnostic traits that distinguish a taxon from its closest relatives. However, current practices in taxonomy seldom follow this recommendation and are mostly based on genotypic data, relegating the phenotype to sparse, often uninformative and in many cases irrelevant traits that have little biological or diagnostic value [43; 44]. The increasing use of MALDI typing [21] is a notable example of useful data that nonetheless yields minimal biological insight beyond their value as a portable 'fingerprint'. Instead, genomic comparisons among members of closely related taxa can detect diagnostic genes, operons or other genetic features. If taxonomy deals with the distinction and diagnosis of taxa, genetic data is much more portable between laboratories than phenotypic data.

- 3- *The problems of defining what is required to present new taxa will increase. It is already current practice to describe novel species on the basis of single strains, while their intraspecific diversity at the genotypic and phenotypic level is a priori unknown [7].*

This is very true, and, unfortunately, a general trend in the last few decades [37, 41]. Research on intraspecific diversity is needed to elucidate the relevant taxonomic traits and real blueprint of a species. However, this problem is not related to the nature of the type, whether DNA sequences or strains. Interestingly, MAGs somewhat circumvent this problem. MAGs are the composite of the co-occurring strains of the same taxon, and the assembled genome may correspond to the core genome of the species and not just to a single and perhaps non-representative member of the taxon [15]. We agree that indeed single strain species descriptions are an important concern, but this is not an argument against the proposal of sequence as type [48].

4- *The proposal of Whitman [47, 48] is likely to lead to the proposal of unknown numbers of novel species based on the description of single DNA sequences only and will lead to taxonomic and nomenclatural chaos [7].*

We do not understand why adoption of sequence as type would lead to chaos. In contrast, controlling the incipient chaos in the informal naming of uncultivated taxa is a major justification for adopting the sequence as type! Moreover, in the developing informatics era, where databases are storing information and machines are learning how to process these data, accelerated integration of data in a taxonomic framework will become informatically-driven, leading to exactly the opposite outcome [37].

In addition, it is unlikely that new versions of the genome sequences will lead to instability. Analyses of mock datasets of known composition reveal that genome refinements will mostly affect only a small number of genes or nucleotide substitution positions [39]. Already there is at least one example where the sequence of an isolate is almost identical to its corresponding MAG (e.g., ANI >99.9%) [14]. Hence, the nomenclature and classification will remain unaffected in the great majority of cases where new versions of the genome become available.

5- *The motivation for researchers to cultivate and preserve strains and to attempt to investigate phenotypes will decrease, resulting in unknown intra- and interspecies diversity, at the phenotypic levels [7].*

Actually, the opposite is the more likely outcome. Many of the current investigations of environmental organisms were preceded by their discovery by 16S rRNA gene sequencing. It was the recognition of their widespread abundance that stimulated their isolation and characterization. We can highlight examples from our own work where the identification of the first extreme halophile from the bacterial domain in a system that never had been previously reported [4] led to its cultivation [3]. Likewise, one of us observed a ubiquitous oil-degrading organism by means of MAGs. Forearmed with knowledge of its importance and growth characteristics derived from bioinformatic functional annotation of the genes present in the MAG, a representative isolate was subsequently obtained based MAG-guided growth media [14]. The recognition of important prokaryotic taxa based upon their ecological, genetic and predicted physiological properties has allowed the scientific community to focus their efforts on isolation and characterization. Moreover, opening a new avenue for microbial systematists does not mean that another is closed, and we both acknowledge and

applaud the considerable ongoing progress being made with novel ‘culturomics’ approaches [20]. Indeed, the Whitman [48] proposals allow for the replacement of a type sequence with a type strain when the taxon is brought into culture.

6- *If the proposal of Whitman [47, 48] and Konstantinidis et al. [15] is implemented, we doubt that new species named only on the basis of genome sequences as type material will be used by the whole scientific community [7].*

The underlying rationale for this statement is not clear, whilst the need for a stable nomenclature for the uncultured is very clear. For example, a Google search on ‘SAR11’, an uncultivated group common in seawater, and a genus name like *Alteromonas* return similar numbers of entries. Because of the lack of rules, synonymy often occurs with informal names, which leads to confusion. Moreover, the accumulating alphanumeric names do not reflect ecological or metabolic distinctiveness and are less optimal for communication than Linnaean names. Thus, we expect binomial naming of taxa named with sequences as type to be both useful and readily adopted.

7- *There are concerns on that the bioinformatics methods are inaccurate in several passages of Overmann et al. [24].*

Currently sequencing technology is very accurate and genome sequences of pure cultures are now widely used in taxonomy. Concerning appropriateness as type, these sequences are already far superior to phenotypic comparisons of strains in terms of precision, stability over time, and ability to be archived. However, MAGs and SAGs have not yet obtained this level of accuracy for a number of reasons, including high intra-population sequence diversity and/or low sequence coverage. It is also clear that MAGs are composites of DNAs from a population of organisms co-occurring in the same sample and are seldom representative of the complete genome of a single organism [15]. Nevertheless, there are many cases where MAGs match with a high ANI (>97%) cultured organisms such as *Escherichia coli* and other human gut microbial species [1, 28]; other examples are *Haloquadratum walsbyi* (with 99.9% ANI identity; [31, 46]) and the recently cultured “*Candidatus* Macondimonas” [14]. Nearly identical MAGs of uncultured taxa can also be recovered from samples from different sites but representing similar environments [14, 38, 46].

While some published MAGs may span much broader lineages than current species definitions [5, 39], the technology continues to improve and new approaches are being discovered. For instance, tools such as MiGA [33] determine whether a MAG is a chimera of distant lineages, if there is contamination due to binning problems or, indeed, large portions of horizontally transferred DNA exist. Altogether it seems that despite the fact that bioinformatics pipelines for quality assessment are not yet perfect, reliable MAGs can be retrieved. These observations, together with a proper scientific evaluation using critical examination of the MAG quality and their inferred ecological and phenotypic properties, will minimize the accumulation of chimeras in databases. Moreover, high standards for allowing a MAG to become type material may be implemented to avoid taxonomic errors [15]. If future resequencing of the same DNA brings better assemblies, they could replace former sequences. It should be emphasized that the International Code of Nomenclature of Prokaryotes only defines Principles, Rules and Recommendations for nomenclature. Taxonomists must then determine which methodological approaches are appropriate e.g. through formulating minimal standards. It seems reasonable to extend the “freedom of taxonomic thought or action” (Principle 1[4]) that applies to traditional methods to sequence based methods.

8- A second point of concern is that a metagenomic sequence obtained from an environmental sample represents the genetic makeup of a certain cell at a particular point in time [24].

While this claim is true for SAGs, this concern also applies to isolates. A strain represents the taxon at the moment of isolation. Strains then have to survive the extremely artificial laboratory habitat, undergoing selection for cultivability. Moreover, as strains are studied under different conditions in different laboratories, the expressed phenotype is only representative of a particular time and culture condition. For example, the well-known difficulty in standardizing conditions for fatty acid and polar lipid profiles reflects these factors. Lastly, regarding this concern, MAGs may actually have an advantage, given that MAGs represent the average genome of the population at the time sampled and not a single cell.

9- A third concern pertains to the applicability of a rather narrow range for numerical threshold values for genome sequence similarity to delineate different bacterial taxa. The emergence of different bacterial groups is due to different evolutionary mechanisms. Rates of homologous recombination (relative to mutation rates) vary widely in different bacterial species [24].

The concern regarding thresholds applies equally to type strains as type sequences. Most of what we know about the evolutionary processes leading to speciation has been obtained from genome sequencing. While this new knowledge certainly informs our taxonomic theories, it is itself irrelevant to the decision as to the nature of type. We agree that distinct lineages show different evolutionary processes and rates. It still seems that, at least at the level of the unit that has been considered a prokaryotic species, there is an evolutionary jump (or genetic discontinuity) between a taxon and its closest relative [13, 25], a result of the speciation process and probably universal molecular mechanisms that drive speciation [9]. Importantly, this evolutionary jump (or gap) is consistent with how species have been named and classified over the last four decades. The exceptions are due to special cases of medical importance and/or imprecision of the traditional taxonomic methods [13]. As has been reiterated before [34, 35], the DDH threshold of 70% or the ANI threshold of 94% [12, 32, 36] should be taken as a relaxed border that can embrace species and not an absolute threshold that forces unnecessary segregation of taxa [35]. When considering the results of pairwise comparisons among 90,000 genomes [13], there is a clear drop (i.e. lack of genome pairs related at this level) in the occurrences of ANI values between 95 and 85%. However, within this gap, there are always outliers that are the exceptions of the rule. We agree that biological diversity is very large and so there will be different evolutionary processes in different taxonomic groups. Therefore, thresholds should not be considered absolute and evidence for an evolutionary gap between close relatives should be part of the criterion for segregating taxa.

10- it is not the primary goal of systematics, and specifically taxonomy, to provide as rapidly as possible species tags just for future reference and without further substantial information. Conventional taxonomy is not mere nomenclatural stamp collecting exercise [24].

Of course, we agree with this statement. However, it is wrong to say that the choice of type reflects the quality of the taxonomic analysis. Certainly, to create a taxonomy that ignores the uncultivated taxa, which are undoubtedly the great majority [40], leads to a misleading perspective and misrepresents the systematics as well as the biology of prokaryotes. In the past, this was unavoidable. Now that we have the technology to obtain a clear view of the fullness of life around us, we should use it. Lastly, we would argue that the study of organisms begins and does not end with their naming. A stable nomenclature is a necessary tool for further progress.

11- A second nomenclatural system independent of the Prokaryote Code is not suited to establish consistent, stable, and non-redundant identifiers for not-yet-cultivated prokaryotic taxa and hence does not solve the problem [24].

Optimally, both cultured and uncultured taxa should be included under the same umbrella, but if the code does not adjust to equally represent the uncultivated taxa, an independent alternative will inevitably be found [15]. Indeed, regrettably, parallel codes of nomenclature already exist for prokaryotes such as *Cyanobacteria* [16], but such coexistence is already a fact that can be handled and integrated in major systems and databases such as the NCBI Taxonomy Database, that serves as the standard nomenclature and classification for the International Sequence Database (INSD) [11]. As long as the ICNP respects a putative code for the uncultivated, as it does with other nomenclature Codes [26], then nomenclatural chaos will not occur.

In conclusion, we rebut the concerns raised by Bisgaard et al. [7] and Overmann et al. [24] and conclude that the adoption of sequence as type will allow for an improved taxonomic framework, with high stability derived from robust phylogenomic methods, and will allow a full circumscription of the microbial world rather than just the cultivated minority.

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