Minutes of the online meeting of the International Committee on Systematics of Prokaryotes [DRAFT of March 2, 2020]

5th January 2020: Iain Sutcliffe, Chair of the ICSP, Northumbria University, Newcastle upon Tyne, U.K.

To the members of the International Committee on Systematics of Prokaryotes

In keeping with Article 4 of the ICSP Statutes, the Editorial Board of the International Code of Nomenclature of Prokaryotes (ICNP) is conducting an open electronic meeting concerning proposals for changes in the ICNP.

The first phase of the meeting will take place from January 5, 2020, until March 1, 2020. It is intended to allow open discussion of the proposals as an email chain among the members of the ICSP and other interested parties. Comments may be made by the 'reply-all' option on your email server. Comments should be less than 500 words in length and should identify the author's name(s) and affiliation(s). Comments should be respectful, and ad hominem comments will be deleted from the record. As comments accumulate, the Editorial Board will transfer them to the ICSP website, and the edited comments will serve as the minutes of the meeting. Please feel free to add interested parties to the email recipient list and solicit comments from interested parties outside the ICSP.

The second phase of the meeting will consist of voting and will take place from March 1 to March 31, 2020. Only members of the ICSP may vote.

The issues for the current discussion are the "Modest proposals to expand the type material for prokaryotes" made by Whitman (2016; IJSEM 66: 2018-2112; naming of https://doi.org/10.1099/ijsem.0.000980) and a related proposal by Whitman et al. (2019; IJSEM 69: 2174-2175; <u>https://doi.org/10.1099/ijsem.0.003419</u>) concerning granting priority to Candidatus names. To simplify the discussion, the ICSP and contributing colleagues are asked to give particular consideration to the following statements, which represent the central concepts. Should they be passed at the voting stage, other rules will be changed as described in Whitman (2016) and Whitman et al. (2019) to make the remainder of the Code consistent with these changes.

Proposal 1 (Whitman 2016). Extend the nature of the type acceptable for valid publication of a species or subspecies name to allow the use of complete or partial genome sequences as type (Whitman 2016). The new rules would be worded [new text is underlined]:

Rule 18a. The type of a species or subspecies must unambiguously identify the taxonomic group and is a designated strain or other material. Whenever possible, the type of a species or subspecies is a designated strain.

(3) [first section] As from 1 April 2020*, sequences of genomic DNA may also serve as the type when it unambiguously identifies the species. When possible, it should be a high quality draft or better genome sequence.

Rule 30.3.c. [new rule] When a sequence is the type, the accession number in a publically available database or the sequence must be given. It is recommended that, when possible, a sample of the DNA be deposited in at least two publically accessible service collections in different countries and the catalog numbers be indicated.

*The original date of January 2016 proposed in Whitman (2016) is changed to reflect the time necessary to bring this matter to a vote. All of the other proposals in Whitman (2016) will be taken as originally worded.

Proposal 2 (Whitman 2016). Articulates a general concept for what can serve as type for a species.

Rule 18a (3). [second section] <u>As new methods are developed, they may serve as the type</u> material so long as they unambiguously identify the species or subspecies and can be readily archived and compared.

Proposal 3 (Whitman 2016). Allows valid publication of the name of a genus in the absence of a type species if the type is too ambiguous to circumscribe a species.

The rule would be:

"Rule 20a. The nomenclatural type (see Rule 15) of a genus or subgenus is the type species <u>or</u> the sequence of one or more genes that unambiguously identifies the genus or subgenus. The type species is the single species or one of the species included when the name was originally validly published. Only species whose names are legitimate may serve as types."

Proposal 4 (Whitman et al. 2019). Upon acceptance of Proposal 1, the priority of the names of *Candidatus* taxa published before 1 April 2020* which are otherwise in accordance with the rules of the Code will have priority based upon their date of publication in the IJSEM unless a synonymous name already exists based upon deposition of type cultures.

Whitman et al. (2019) also provides a simple nomenclature for identifying the nature of the type material:

'When the type is a culture, the superscript "T" will be used immediately following the name or strain identifier. If the type is a sequence, the superscript "Ts" will be used. If the type is a description, preserved specimen or illustration, the superscript "Td" will be used. If a representative of a taxon is brought into culture, the type strain is then designated as described in Rule 18f. The name may be emended by the new authors, and the superscript "Ts" or "Td" is replaced by the superscript "T".

*The original date of 1 January 2020 is changed to reflect the time necessary to bring this matter to a vote.

For further guidance, major publications that discuss these proposals include:

(in favour)

Whitman 2015. Syst. Appl. Microbiol. 38: 217-222 (https://doi.org/10.1016/j.syapm.2015.02.003)

Konstantinidis et al. 2017. ISME J 11: 2399-2406 (https://www.nature.com/articles/ismej2017113)

Rossello-Mora and Whitman 2019. Syst. Appl. Microbiol. 42: 5-14 (https://doi.org/10.1016/j.syapm.2018.07.002)

(against)

Overmann et al. 2019. Syst. Appl. Microbiol. 42: 22-29. (https://doi.org/10.1016/j.syapm.2018.08.009)

Bisgaard et al. 2019. Diagn. Microbiol. Infect. Dis. 95: 102-103. (https://doi.org/10.1016/j.diagmicrobio.2019.03.007)

COMMENTS:

Comments are present in the order they were received and may have been lightly edited. Please email Barny Whitman [whitman@uga.edu] or Lenie Dijkshoorn [L.Dijkshoorn@lumc.nl] for questions, suggestions, errors and omissions.

January 13 Henrik Christensen, Member of Judicial Commission, University of Copenhagen, Copenhagen, Denmark

We have recently published a note that presents a warning (Bisgaard et al. 2019) about the proposal of using DNA sequences as type material to name new species (Whitman). If implemented the proposal to use DNA sequences as type material may have far-reaching consequences for all microbiologists, ID specialists, vets and other specialists dealing with bacterial names, not to speak about the companies that develop species identification tools and strains for biotech production of probiotics, vaccines and enzymes. The risk is an unstable nomenclature violating Principle 1 of the "code" (".. 1) Aim at stability of names, 2) Avoid or reject the use of names which may cause error or confusion 3) Avoid the useless creation of names .. "). I have become involved in this problem as an active scientist working with bacterial taxonomy at the university. I will contact other taxonomic colleagues as well to revive the discussion.

You are of course welcome to contact me for further explanations and discussions of the problem.

January 13

Lenie Dijkshoorn, Executive Secretary ICSP, Leiden University Medical Center, Leiden, The Netherlands

I fully support the letter from Henrik. There is an urgent need for contemplation for workers in the field who use names in daily work.

January 13 William B Whitman, ICSP Delegate, University of Georgia, Athens USA Iain Sutcliffe, Chair of ICSP Northumbria University, Newcastle upon Tyne, U.K. Ramon Rossello-Mora, Vice-Chair of Judicial Commission of the ICSP, Grup de Microbiologia Marina IMEDEA, Illes Balears, Spain

Genome sequencing has revolutionized prokaryotic systematics by greatly improving the identification of species, elucidating the functional properties of taxonomic groups, and resolving many of the ambiguities in the phylogeny of the higher taxa. Following from the principles described in the International Code of Nomenclature of Prokaryotes, gene sequences are also suitable type material for the description of prokaryotic species. As put forth in principle 4 of the Code, the primary purpose of naming is to supply a means of referring to specific prokaryotes. The Code possesses two mechanisms to insure uniqueness and stability of names. First, it gives priority to the earliest name of the entity. Second, each name is associated irrevocably with some type material. The only name that can be used that includes this type material is the name with priority. The relationship of the name to the type material is further determined by the formal description (also called the protologue), which defines how a taxon is delineated in reference to the type material. Gene sequences clearly possess sufficient specificity and information to serve as type material and delineate taxa. In fact, it has been the common practice to differentiate species based upon sequence similarity since the mid-sixties and formally recommended by Wayne et al. (1987).

A stable nomenclature is essential for all scientific disciplines. While this need was met with the adoptions of the Approved Lists in 1980 and the Code of 1990, subsequent changes in 2001 restricted the Code to organisms that can be deposited as pure strains in culture collections. These changes removed the protection of the Code from the names of prokaryotes that cannot be easily cultured. Proposal 1 would restore the original intent of the Code. By allowing gene sequences to serve as type material for prokaryotic species, this simple change will create stability in naming of *Candidatus* taxa, endosymbionts, and many uncultivated prokaryotes. It is already well established that the use of sequence data, increasingly in the form of whole genome sequences, produces reliable and stable classifications. Thus, proposal 1 will meet an important need within microbiology and allow the creation of a unified nomenclature for all prokaryotes, in contrast to the current "International Code of Nomenclature of *Cultivated* Prokaryotes". Proposal 2 states the rationale for Proposal 1. Proposal 4 implements proposal 1 for *Candidatus* taxa and provides a simple system for identifying the nature of the type material.

Proposal 3 recognizes that on some occasions, the sequence data may be of sufficient quality to delineate a genus but not a species. An example might be 16S rRNA sequences, but it is

inevitable that larger amounts of genome sequence data will also be used. In these cases, a genus name may be validly published without designated type species. Because the genus name provides the root for higher taxa, genus names are required creation of stable higher taxonomies.

Wayne et al. 1987. Int J Syst Bacteriol 37:463-464.

January 14 Kostas Konstantinidis, Member of Judicial Commission, Georgia Institute of Technology, Atlanta Georgia, U.S.A.

In response to Christensen and Dijkshoorn:

I do NOT share the same view with you on this issue but before i offer my arguments for this, i would like to ask Henrik (and/or Lenie):

Why you believe the genome/DNA sequence as Type would make for an unstable system OR will make the identification of taxa of medical importance more challenging (since almost all these taxa are known by cultures and, hence, there will be no change to them really if genome sequences are accepted as Type)? Could you offer a couple specific examples to back up these claims?

I would argue that the Bisgaard et al. 2019 paper was vague about these key points, so the underlying rationale is not clear to me yet.

January 15 Edward Moore, ICSP delegate, University of Gothenburg, Gothenburg, Sweden

The proposals formulated by Iain (Jan 05) to modify the International Code of Nomenclature of Prokaryotes (The Code) have been percolating for a number of years now. They have been presented in publications and discussed as 'considerations' or 'modest proposals', etc. However, prior to January 5, no formal proposals were presented to ICSP for consideration to vote for adopting. Now, the ICSP Executive Board has formulated formal proposals for consideration – below. This is an essential step forward, which is good to try to resolve the issues, as well as the concerns of the proponents and opponents of the proposals.

My comment here is to the proposed or designated schedule (it is not clear to me) for the 'second phase' of the open electronic meeting, i.e., for voting (March 01-31) on the proposals. I point out that the members of the ICSP are representatives of the various Microbiological Societies of the different countries. As such, our decisions and votes on issues should reflect the considerations or, the consensus – in the best cases, of our respective national Societies. Particularly, this issue of revising The Code warrants informing member microbiologists of the national Societies, and their consideration, as well, rather than only of the individual ICSP members.

In any case, now we have formal proposals to be considered for voting. Unfortunately, the proposed/designated schedule for the 'first phase of the meeting, i.e., open discussion, etc.,' does not allow for consideration by the overall members of the Societies. I know of presentations of some of these issues, as concepts, only in sessions at the last 2 FEMS

meetings. I do not know if the issues have been presented and discussed within the different Society meetings – they have not been discussed before within the Swedish Microbiological Society (SFM).

Now, the problem is that the dates of Societies' meetings, where these proposals could be brought up and debated, are after the proposed 'deadline' for voting on these proposals, in most cases. The annual meeting of the VAAM in Germany is in the second week of March; they do have a Fachgruppe für Systematik und Identifizierung – I do not know if they are considering the proposals in their session. However, the SFM in Sweden is meeting in May; the MS in the UK is meeting in April; the ASM in the USA is meeting in June; the Spanish Society is meeting in July 2021, although a Systematics and Taxonomy meeting is scheduled for April, 2020! These meetings, and the annual meetings of other Societies, as well, are after the 'deadline' for ICSP voting on the proposals.

I propose that the dates of the 'first phase' of the open electronic meeting for open discussion be prolonged, to allow communication of the formal proposals for revising The Code to be circulated to the members of the national Societies. In any case, I commend the ICSP Executive Board for presenting the formal proposals and initiating the open electronic meeting.

January 15 Iain Sutcliffe, Chair of the ICSP, Northumbria University, Newcastle upon Tyne, U.K.

I should like to reply promptly to these comments since they relate to the timeline & decision making process rather than the scientific issues under discussion.

Firstly, my apologies for any ambiguity in my email of Jan 5th: This is now a 'designated' schedule i.e. voting by ICSP members will begin on March 1st and close on 31st.

Secondly, regarding the timeline and current 'window' for discussion. It is important to stress that the Whitman (2016) proposals were published online on 1st May 2016 i.e. 3 years 8 months ago, which I would have thought is more than sufficient time for interested parties to have encountered these proposals (it is also unambiguous in the original text that these are formal proposals to emend the Code that require an ICSP vote).

Notably, the paper has attracted 26 citations according the IJSEM website (34 by googlescholar), including at least one dedicated commentary outside of the specialist systematics literature (e.g. Bisgaard et al. 2019 in a clinical journal). Moreover, as you note, these issues were highlighted in the last two FEMS meetings and they have also been addressed in specialist meetings (e.g. BISMIS, Bergeys International Society for Microbial Systematics). Thus it is demonstrable that the proposals have had 'reach'.

My personal view is that this is a more than sufficient time for these proposals to have come to the attention and gather responses of the scientific community. Moreover, ICSP members and other interested parties have had the past 44 months to engage in discussions with colleagues and 'gauge the mood'. There are still 12 weeks for further activities of this sort and I am pleased that you have widened the debate by adding recipients to this email trail.

Thus, I believe that the majority vote decision of the ICSP Executive Board to now bring this matter to the vote is the correct one.

January 16 Mei-Chin Lai, ICSP delegate, National Chung Hsing University, Taichung, Taiwan

I agree with "Proposal 1" that genome sequences should be included and suggest that the "completeness" of genome sequences need to be over or around 97%.

January 16 Henrik Christensen, Member of Judicial Commission, University of Copenhagen, Copenhagen, Denmark

In response to Konstantinidis's comments of January 14

Unfortunately there was a space limitation with the paper of Bisgaard et al., and we also wanted to keep the text short. I agree that it would have been relevant to give some examples.

Question:

Why you believe that the genome/DNA sequence as Type would make for an unstable system OR will make the identification of taxa of medical importance more challenging (since almost all these taxa are known by cultures and hence, there will be no change to them really if genome sequence is accepted as Type)? Could you offer a couple specific examples to back up these claims?

I would argue that the Bisgaard et al. 2019 paper was vague about these key points, so the underlying rationale is not clear to me yet.

Answer:

On behalf of my co-authors I will try to give a more extended answer here.

<u>1. Risk for an increase in the number of heterotypic synonyms.</u> A new species B is proposed (validly published) and only one DNA sequence serves as type material and only one sequence is known from the species. B is closely related to an existing well known species A of high clinical importance. This can happen since species of type A have a high diversity at the population level and, in such cases, ANI can be lower that 0.95 for some populations of the species. If only comparisons between A and B are based on type strains (or type DNA material), less than 0.95 ANI can be obtained, and a claim made for a new species. If a medical clinical microbiologist identifies an isolate by whole genomic sequencing as species B, this species is not known to be of clinical importance to him, and he might get confused about the disease associated, how the infection can be treated with antibiotics, and how it can be prevented. The consequence can be a wrong treatment of the patient. The problem already exists with cultured type strains, and it is expected to increase if the proposal of using DNA sequences as type material is adopted.

<u>2. Identification of clinically important streptococci</u>. An example provided by a co-author of the Bisgaard paper is related to the problems in the clinic to differentiate *Streptococcus pneumoniae* (important pathogen) from *S. pseudopneumoniae* and *S. mitis* (commensals). These species are closely genetically related, and their virulence can only be establisbed based on cultivation. This co-author even extents the case to ANY bacteria for which vaccines are being developed. Strain material is essential to test for the specificity of vaccines, for strains of existing species as well as for strains of new species in the future.

A more general statement was made by another co-author: It is only possible clinically to link a name of a culture-positive organism to additional data available through publications, to subjects such as diagnosis, prognosis, and treatment. Allowing for species to be name based upon DNA alone will not be helpful from a clinical standpoint.

An even more general statement made by the same co-author reaches beyond the clinical field relates to the scientific demand for reproducibility of experiments. The deposit of genomic DNA or, worse, simple submission of wgs data to a public database does not allow reproduction and confirmation of the conclusions of the authors about the taxonomic status of new isolates and strains simply because there will be no proof that the wgs data are coming from the proposed species.

January 17 Edward Moore, ICSP delegate, University of Gothenburg, Gothenburg, Sweden

In response to Lai's comments of January 16

Thank you for your mail and your comment.

Please note: The proposals would establish new rules that would **<u>substitute</u>** whole genome sequence data for the strain as the type material required for the valid publication of names of new bacterial species. IJSEM already requires including whole genome sequence data for the valid publication of new species names, since 2018.

The proposed rule changes do **<u>not</u>** make a proposal for the coverage or the quality of the whole genome sequence data that would serve as the type material.

Question: Why do you propose 97% 'completeness' or coverage of genomes; why not 20%, i.e., the amount necessary for ANI analyses, or complete genomes, including plasmids?

Thank you in advance for your consideration.

If proponents of the new rules proposals disagree with my assessment, please correct my response to Mei-Chin.

January 17

Frans Reubsaet, Diagnostic Laboratory for Bacteriology and Parasitology (BPD), Center for Infectious Disease Research, Diagnostics and laboratory Surveillance, National Institute of Public Health and the Environment (RIVM), The Netherlands

In response to Moore's comments of January 17

At this moment most genomes are analyzed by Illumina platforms. We experienced that pollution with other DNA is no hypothetical. Second, even the de novo created sequences are artificial. So if the decision is made in favour of whole genome sequences, sooner or later it will become clear that poor data will not prevail.

January 17 William B Whitman, ICSP Delegate, University of Georgia, Athens USA

Regarding the discussion between Ed and Mei-Chin, I'd like to clarify a few points regarding proposal 1, which would allow gene sequences to serve as type. If passed, strains would still remain the preferred type [see highlighted text below]. Thus, sequence data would only substitute for strains when strains are unavailable.

Rule 18a. <u>The type material of a species or subspecies must unambiguously identify the</u> <u>taxonomic group and is a designated strain or other material.</u> Whenever possible, the type of a species or subspecies is a designated strain.

This proposal also does not require a whole genome sequence but only enough sequence to unambiguously identify the species. This wording was chosen to allow naming of endosymbionts where the whole genome sequence is not available. There are many examples of this in IJSEM, but a recent one describes a *Borrelia* species, Loh et al. 2017 [doi.10.1099/ijsem.0.001929] where the diagnosis was made on the basis of the sequences of five genes: 16S rRNA, *flaB*, *groEL*, *gyrB* and *glpQ*.

(3) <u>As from April 2020, sequences of genomic DNA may also serve as the type material when it</u> <u>unambiguously identifies the species.</u> When possible, it should be a high quality draft or <u>better genome sequence.</u>

The second sentence [highlighted] constitutes a recommendation stating a preference for genome sequences. There is substantial precedence for the Code to make recommendations as well as rules. For instance, the 1990 Code recommended deposition of strains as type material. The current Code recommends the descriptions should conform to the minimum standards for the group (Recommendation 30). Because minimum standards for whole genome sequences were proposed in IJSEM in 2018 [Chun et al. 2018. Proposed minimal standards for the use of genome data for the taxonomy of prokaryotes. Int J Syst Evol Microbiol 68: 461-466. doi.org/10.1099/ijsem.0.002516], clear directions regarding implementation of this recommendation already exist.

January 18 Kostas Konstantinidis, Member of Judicial Commission, Georgia Institute of Technology, Atlanta Georgia, U.S.A.

In response to Christensen's comments of January 16

In my view, there are two distinct issues, one is the concept of using the DNA as an alternative type material and the other is the technical aspect on what the minimum standards will be for this. My read of Whitman's comment of January 17 is: no change in how we do business for cultured organisms (that is, depositing an isolate to 2 culture collections is the recommended way to name/describe new taxa) and genome sequence becomes an equally appropriate alternative type material for uncultured taxa and fastidious organisms that are difficult to be maintained in culture collections or get lost (if i have misread this, please somebody to correct me!). This way, we will be able to start describing the "uncultivated majority" using similar standards to those used for cultures, and i would argue that this would even promote the culturing of the important uncultivated taxa because of more interest/attention to them once they are described taxonomically. Karthikeyan et al. 2019 is a great recent example of this from my

group (we are now in the process of depositing the isolate to culture collections); i am aware of several similar examples if you want to see more. I short, i see this as a win-win situation for all of us and no threat whatsoever for the culture collections. To the contrary, I think allowing genome sequence to serve as type material may further promote culturing efforts! So, I am in favor of Whitman's proposal personally as I understand the proposal.

As far as the technical standards, be sure that all (or most, at least) of us that would like to have genome sequence as type material, do NOT want to do this with lower standards. We want to have as high standards as the isolate genomes, if not higher. I do believe it is doable. I explain a bit more below for those that want to read more on the technical issues and then address your specific concerns further below. I will also try to publish officially the points below in peered review press so you can refer to them and offer your arguments in favor or against toward helping to establish, hopefully, the standards that we can all adopt and use in practice soon! But the essence of what i am writing below can also be found in the paper cited by Sutcliffe above, Konstantinidis et al. 2017 (https://www.nature.com/articles/ismej2017113).

On the technical standard issue:

Several scientists have argued that the MAG and SAG information is not of similar quality to the information derived based on isolate-based experiments in the lab and thus, does not represent well the organisms under investigation (Bisgaard 2019, Overmann 2019). While this is, at least partly, true, it is not critical enough to prevent progress towards cataloguing the taxonomic diversity of uncultivated organisms, for several reasons. First, prokaryotic taxonomy has always relied on imperfect methods; MAGs/SAGs are not an exception to this. Take, for instance, the DNA-DNA hybridization (DDH) method, the "golden standard" for species demarcation. The genome-aggregated average nucleotide (ANI) value of shared genes among two related genomes (Konstantinidis and Tiedje 2005) has been shown to correlate well with their DDH values, and deviations in the values were common and largely attributable to the experimental noise of the former as opposed to the latter method (Goris 2007). Second, there are approaches to assess guality beyond reasonable doubt such as visual examination of read-recruitment pots (Rodriguez-R 2016) in combination with the quality checking pipelines (Parks 2015, Rodriguez 2018), and in our view only genomes of high enough guality based on these tests should be taxonomically described (Konstantinidis 2017). Third, the standards to use have been outlined already previously by us (Konstantinidis 2017) and others (Bowers 2017), and are of similar stringency to those used for isolate genomes. Further, long-read sequencing for routine taxonomic descriptions, even on environmental samples, is coming up soon [e.g., (Andersen 2019)], and is strongly expected to circumvent several of the low quality issues reported for MAGs and SAGs in the literature, e.g., identify and fix genome sequences that may be chimeric. It has been argued that when DNA sequence type material is replaced by new versions due to new sequencing technologies and/or tools for genome assembly, the species descriptions would have to be consequently revised, resulting in an unstable classification (Bisgaard 2019). However, this is unlikely to be true for most -if not all- taxa because such new versions will mostly affect only a small number of genes or nucleotide substitution positions in the genome as analysis of mock datasets of known composition has revealed (Sczyrba 2017) or the sequencing of the isolated Candidatus Macondimonas diazotrophica that was almost identical to its corresponding MAG (e.g., ANI >99.9%) (Karthikeyan 2019). It is even less likely that the affected genes by new genome versions would represent the species-diagnostic traits because these genes are often the hypothetical, mobile or prophage-associated genes found in multiple copies (and short contigs) in the genome (Pena-Gonzalez 2019). Hence, the genealogy of the genome and thus, its nomenclature and classification, will remain unaffected in the great

majority of cases where new versions of the genome become available. In a few cases that the new genome version will include major changes in gene content, the old version could be replaced by the new version in a process analogous to replacing the (usually lost) type strain of a (named) species by a neotype strain for isolated organisms.

References cited: Andersen et al. 2019. <u>Syst Appl Microbiol</u> **42**: 77-84. Bisgaard et al. 2019. <u>Diagn Microbiol Infect Dis</u> **95**: 102-103. Bowers et al. 2017. <u>Nat Biotechnol</u> **35**: 725-731. Goris et al. 2007. Int. <u>Syst Evol Microbiol</u> **57**: 81-91. Karthikeyan et al. 2019. <u>ISME J</u> **13**: 2129-2134. Lawrence and Ochman. 1998. <u>Proc Natl Acad Sci U S A</u> **95**: 9413-9417. Overmann et al. 2019, <u>Syst Appl Microbiol</u> **42**: 22-29. Parks et al. 2015. <u>Genome Res</u> **25**: 1043-1055. Pena-Gonzalez et al. 2019. <u>Appl Environ Microbiol</u> **85**(24). Rodriguez et al. 2018. <u>Nucleic Acids Res</u> **46**(W1): W282-W288. Rodriguez-R and and Konstantinidis. 2016. <u>PeerJ Preprints(</u>e1900v1). Sczyrbaf et al. 2017, <u>Nat Methods</u> **14**: 1063-1071.

January 20

Ramon Rossello-Mora, Vice-Chair of Judicial Commission of the ICSP, Grup de Microbiologia Marina IMEDEA, Illes Balears, Spain

In response to Christensen's comments of January 16

I recall Cowan 1965 [3]: The adequacy of characterization of a bacterium is a reflexion of time; it should be as full as modern techniques make possible. Unfortunately, one now regarded as a adequate is likely, in ten years time, to be hopelessly inadequate. I think taxonomy must adapt to the modern times. To Christensen concerns:

1. Risk for an increase in the number of heterotypic synonyms.

This is independent of genomes as type material. Close to 90% of the species descriptions in IJSEM are single strains [18], mostly without genome provided, nor DDH, using 98.7% 16S rRNA threshold. The use of strict or narrow values (e.g. 70% DDH) has been incorrectly used to force unnecessary classifications [13, 14]. I anticipate that with the genome sequencing, the recognition of heterotypic synonyms will increase. However, genome sequence as a reference will provide a much more stable framework than the simple use of 16S and API strips. The evidences of an evolutionary gap between species ([7, 17], will facilitate circumscriptions as the database grows.

Diseases are not always linked to species identity. Just looking to e.g. Bacillus cereus group [8], some traits are linked to a strain and could even be horizontally transferable (e.g. cry genes diagnostic of B. thuringiensis). Other clinically relevant traits as e.g. hemolysin or enterotoxin genes could be genus widely distributed [9]. For instance, sequencing B toyonensis genome allowed the (i) detection of clinically relevant genes and (ii) understanding of their non-functional nature. This is a good example of the contrary of what is mentioned.

There are many other cases in where it is clear a strain-specific and not species-specific virulence factors e.g. Legionella pneumophila [2], Vibrio toranzoniae [11], Pseudomonas aeruginosa [4], Streptococcus uberis [20], Ralstonia solanacearum [21], and so on...

Treatments against clinical infections are mostly done using antibiotic treatment, and sensitivity may be (i) strain specific, (ii) susceptible of horizontal gene transfer and/or (iii) susceptible of spontaneous mutation. Unstable characters, as linked to plasmids (e.g. degradation of naphthalene; [15]) have always been considered not suitable for taxonomic purposes. It is known that characters like phage sensitivity, immunoreactivity [16] and antibiotic susceptibility are often strain-specific and, may be of a lot of relevance for clinical issues but not for taxonomy.

2. Identification of clinically important streptococci bacteria for which vaccines are being developed.

I agree that for vaccine development living material is needed, but immunoreactivity may be strain specific, and virulence factors that can be horizontally transferred. I doubt that clinical microbiologists will abandon cultivation just because the reasons to isolate an organism are very distant from those of the classification purposes.

It would be good to check how many new descriptions in IJSEM are related to clinical cases and with medical relevance. And how many of them have their virulence factors elucidated. I anticipate that if any, very few.

We never underestimated the value of cultivation and evaluation of clinical relevant traits, but the investigation in infection and disease's research is significantly different from classification. I trust that if a study reveals a clinically relevant yet uncultivated organism, this will lead to focus efforts in obtaining pure cultures as occurred with Salinibacter [1], Macondimonas [10]; and many more examples of ecologically relevant organisms [6, 7, 12, 19].

References:

- 1- Anton et al. 2002. IJSEM. 52:485-491
- 2- D'Auria et al., 2010. (<u>https://bmcgenomics.biomedcentral.com/articles/10.1186/1471-2164-11-181</u>)
- 3- Cowan. 1965. J Gen Microbiol 39: 143-153
- 4- Choi et al, 2002 (https://jb.asm.org/content/184/4/952)
- 5- Harbison et al., 2016 (https://academic.oup.com/femsle/article/363/15/fnw151/2197705)
- 6- Henson et al., 2018 (https://www.nature.com/articles/s41396-018-0092-2.pdf)
- 7- Jain et al., 2019 (https://www.nature.com/articles/s41467-018-07641-9)
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January 20 Anne Willems, Ghent University, Ghent, Belgium

[In response to Christensen's comments of January 16]

I have a question regarding what would be the consequences of the newly proposed rules in the following situation: In case a species would be described with a genome sequence as type material, for example in the absence of a culture or in case of a MAG, and later on cultures belonging to that species do become available: can and should a type strain be designated then for that species even though it's genome sequence may not perfectly match with the one first proposed? Would the type strain replace the type genome that was first proposed?

January 20 Iain Sutcliffe, Chair of the ICSP, Northumbria University, Newcastle upon Tyne, U.K.

In response to Willem's comments of January 20

This circumstance is directly addressed in the Whitman (2016) proposals, by minor amendment to Rule 18f, which would allow for the replacement of a type sequence of genomic DNA with a type strain (see text in blue below).

This is one of the ancillary changes referred to in my original email. Apologies for not being clearer.

Rule 18f. If a <u>sequence of genomic DNA</u>, description or illustration constitutes, or a dead preserved specimen has been designated, the type of a species [Rules 18a(1) <u>and 18a(3)</u>] and a later strain of this species is cultivated, then the type strain may be designated by the person who isolated the strain or by a subsequent author. This type strain shall then replace the <u>sequence of genomic DNA</u>, description, illustration or preserved specimen as the nomenclatural type. The designation of a type strain in this manner must be published in the IJSEM, the authorship and date of priority of publication being determined by the effective and valid publication of the name by the original authors (Rule 24b). (underlined text are new additions to the current rule)

January 21 Pierre-Edouard Fournier, ICSP Delegate, UMR VITROME, Marseille, France

As a clinical microbiologist, I have been using partial, and then complete genomic sequences for bacterial identification on a routine basis for diagnostic purposes for many years. As a consequence, I support the proposal to use genomic sequences as type material for new taxa when a culture cannot be obtained.

However, and as discussed recently with lain, I have a few concerns that include:

- Defining quality criteria that will be applied to DNA sequences prior to being used as type material is crucial and may be very difficult for metagenomic data. There are few sequencing systems commercially available currently, but so many sequence analysis softwares and strategies...

- There is a risk of discouraging culture efforts, and notably the deposit in two type culture collections, of strains of previously described *Candidatus* species whose type material is a DNA sequence. There is a risk that microbiologists who cultivate strains belonging to previously described *Candidatus* species only deposit them in a single culture collection, as requested for publishing in most journals, and do not make the effort to publish them as type strains in IJSEM as described in Iain's message below. To avoid this, maybe the cultivators' names should be added to validation lists, not as "discoverers" of the new species but as the first "cultivators".

- New *Candidatus* species will be proposed mainly on the basis of DNA sequence data, as no strain will be available at the time of description. Currently, many new species descriptions use overall genome relatedness indexes and "universal" thresolds such as 70% for dDDH and 95-96% for OrthoANI. However, these thresholds do not apply to all taxonomic groups and may, therefore over- or underestimate the biodiversity of some groups of prokaryotes. When cultivable strains are available, phenotypic data may help with a more precise classification. With a reduced number of phenotypic characteristics evaluable, which will be the case with uncultivated species, this may not be possible.

January 21 William B Whitman, ICSP Delegate, University of Georgia, Athens USA

[In response to Fournier's comments of January 21]

With regard to Pierre-Edouard's comment about recognition being given to the cultivators, a mechanism already exists to do just that. Changing the type from a sequence to a strain should be recognized as a change in the species circumscription, which would be recognized by an emendation of the species description [see Rule 35]. Emendations are indicated in the defining publication that accompanies the species name. This has already been done for at least one species whose type was a description.

February 6 Suresh Korpole, Head, Microbial Type Culture Collection (MTCC) CSIR-Institute of Microbial Technology, Chandigarh

I am Suresh Korpole, working at Microbial Type Culture Collection, CSIR-Institute of Microbial Technology would like to make a submission pertaining to microbial systematic studies. We have been experiencing problems in submission of strains at foreign culture collections with the implementation of Biodiversity Act and Nagoya Protocol. India is a participating country of Budapest Treaty. Though our National Biodiversity Authority (NBA) allows us to deposit the proposed type strains and type strains at abroad culture collections, there are certain issues that are preventing the free supply of microbial strains (as NBA request to provide intimation on further supply of strains for any commercial exploitation). We can submit the strains with terms such as any commercial exploitation involving the deposited microbe must be shared equal benefits. In fact, it must be informed to the depositor, which I think is correct as per IPR related regulations. However, editors at IJSEM insist not to add any conditions during the deposition of strains at culture collections, which is in contradiction to the rules of Government at Indian territory. Therefore, it is becoming very difficult to practice microbial taxonomy related research in India that habitat various biodiversity hotspots. As proposed by Prof. William B. Whitman (Whitman 2016; IJSEM, 66; 2018-2112), we sincerely request to amend the rule for the description of novel species and allow use of complete or draft genome sequence as type description. Since, the genome sequence provides all information (including the in silico DNA identity) on phenotypic features, the requirement of essential deposition of strains in two different countries culture collections may be discontinued and request to allow the publication with strain deposited in a single culture collection in the country of researcher residing with genome sequence available for global researchers. This will certainly boost the research ability of enthusiastic researchers residing in countries like India.

Thank you all for going through my views and looking forward to hear a positive news on amending the strain deposition requirements and accepting genome sequence as type description.

February 6

A. Nemec, Professor of Medical Microbiology,Laboratory of Bacterial Genetics, National Institute of Public Health, Prague, Czech Republic

I do not support Whitman's proposal. If accepted, this change will further broaden room for proposals for novel names with little or no biological meaning. Labelling single isolates with nomenclatural tags has already become a common practice, which is supported by the majority of bacterial taxonomists but considered meaningless or even ridiculous by many non-taxonomists. It is foreseeable that if the proposal is approved, any novel (partial) genome sequence showing ANI values of <95% against those of type strains associated with validly published names will have a chance to become easily a type for a novel species name. And it will be even possible to automate it as there will be no need for analysis of live cultures, e.g. just using publicly available sequences. As even a single cell can be sequenced, it will change taxonomy to a digital form. Although this progress must be expected, I do not understand why sequences should be labelled by formal binomial names, which definitely will occur given this practice for single isolates. I believe that formal binomial names should be reserved for biologically well-defined discrete and internally coherent population entities. I dislike how statistical thresholds (ANI etc.) are universally/ technocratically applied to natural bacterial communities in the absence of a universal concept of bacterial species. People are just labelling

taxonomically unique (in terms of the quantitative thresholds) singletons without any idea about the taxonomic/population nature of what they are labelling. I can repeat here a comment used in my nomenclatural reviews: The nomenclatural code does not explicitly define how many strains are needed for such a purpose, but it states (Rule 27) that the valid publication of a name must be accompanied by a description of a taxon. However, every description of a general category (species) based on a single individual (strain) is in principle meaningless, providing no information about species-specific or diagnostic traits. Furthermore, in the absence of a generally accepted biological concept of species for bacteria, a bacterial species is defined only stochastically, i.e. as a cluster of highly similar/related individuals in the multidimensional phylophenetic space, which are separated (in terms of quantifiable similarity/ relationship) from other such clusters. The analysis of a taxonomically new single organism then cannot give any information about the nature of a new hypothetical cluster or position of the strain within that cluster.

February 7 Edward Moore, ICSP delegate, University of Gothenburg, Gothenburg, Sweden

[In response to Korpole's comments of February 6]

The suggestion by Suresh Korpole raises an interesting potential solution for countries like India, Brazil, Bolivia, Colombia, etc., which have installed stringent restrictions on transfer of their genetic resources. At first consideration, adopting the proposed changes to the International Code of Nomenclature of Prokaryotes (The Code) may seem to solve some problems of national restrictions on transport of resources out of the countries of origin.

Two points:

1) If the Indian regulations governing transport of national resources out of India are based on the Nagoya Protocol (NP) for Access and Benefit Sharing (ABS), the issue described may NOT be solved by the proposed rule changes. That is because the regulations on ABS govern "genetic resources". This includes, of course, DNA sequence data.

Does India not restrict WGS data as they restrict biological materials? If not, why not? That is, if the WGS data provides all relevant phenotypic, metabolic, etc. information (I suggest that it does NOT), then not restricting WGS data defeats the purpose of restricting transport of strains. But, then, it is not necessarily expected that the national regulations of any country will be completely logical!

2) We have worked for many years with Indian microbiologists. We receive many strains without restrictions for deposit in an international collection. It is my understanding that transport of strains out of India for taxonomic studies is NOT restricted. I have copied this mail to colleagues in India with whom we have worked for many years. I ask any of them to provide clarification on national restrictions on transport of bacterial strains out of India, i.e., for taxonomic purposes.

If the only problem for Suresh Korpole is a clause in the IJSEM agreement that does not allow a stipulation on commercial development, the IJSEM agreement should be considered, rather than immediately changing The Code. If such a stipulation are not allowed by IJSEM, I suggest that the IJSEM may be in violation of European law. The NP for ABS states that the individual countries regulate the sampling, handling, transport and, particularly, commercial development of their national genetic resources. Any IJSEM restriction on national regulations of commercial

development of nomenclatural type material is most likely illegal – such restrictions certainly make no sense, from point of view of taxonomy.

In order to consider the argument of Suresh Korpole in favour of adopting the proposed changes of The Code, I suggest that clarification is needed on the Indian laws regulating microbial strains that are used for taxonomic purposes and also on the IJSEM restrictions on accepting nomenclatural type material.

In any case, changing The Code to try to accommodate the national laws of all countries is illogical.

February 7 Ulrich Nübel, Leibniz Institute DSMZ-German Collection of Microorganisms and Cell Cultures, Germany

I share many of the concerns raised previously about the proposal to allow sequences as types for bacterial nomenclature and I sincerely hope for a wise decision of the ICSP to reject that proposal in its present form. From my perspective, changing the International Code of Nomenclature for Prokaryotes in accordance to the proposal would primarily result in a relaxation of scientific standards, rather than any 'modernization'. Such a change is likely to discredit bacterial taxonomy in the long run, and would probably damage the science of microbiology in general.

The reproducibility of experimental results is a fundamental requirement of any scientific approach. Therefore, throughout the life sciences, making available investigated materials to peers that raise a valid interest is a mandatory requirement as soon as a manuscript gets published in a scientific journal. This ensures that results can be double-checked and reproduced by colleagues, and complemented by additional analyses in the future. The field of molecular microbial ecology may be unique in that this requirement is rarely enforced and it is uncommon to share or exchange environmental samples across laboratories. Transferring this unique negligence to bacterial taxonomy by abolishing the formal requirement to share the underlying investigated material upon describing a novel species will reduce reproducibility severely. These concerns are not addressed by the deposition of DNA (which is not even mandatory in the proposal) and certainly not by depositing sequences in public databases.

While the genome is a part of an organism, a genome sequence is not. Rather, a genome sequence is an experimental result derived from a sample of that organism. In this respect, a genome sequence is even comparable to a microscopical drawing. For good reasons, drawings are not permitted as nomenclatural types any more. While a genome sequence may well contain more information than a drawing, it is still not even guaranteed that all information present in a microscopical drawing can be derived from a genome sequence. Much like a drawing, a genome sequence can be derived from an organism, but not vice versa, and this non-reversibility is due to an inherent loss of information during the sequencing process. I do not question the value of genomic information in general, but for the study of an organism's biology or phenotype, the physical material is indispensable.

The discussion on the replication crisis in science is still ongoing. It thus can only be detrimental to microbiology if a system is deliberately generated that is prone to artifacts and that decreases reproducibility.

Note: I am not an expert in taxonomy. My current research interests are the genomic epidemiology of pathogenic bacteria and the genetic determinants of bacterial secondary metabolite synthesis.

February 7 Kostas Konstantinidis, Member of Judicial Commission, Georgia Institute of Technology, Atlanta Georgia, U.S.A.

Allow me a few short comments on the issues raised in today's emails against DNA/genome sequence serving as Type material:

 A genome sequence is indeed required to be publicly deposited as part of the new proposal for validation/checking purposes (see Whitman 2015). I would also argue that checking/validating a genome sequence can be more accurate/precise and more highthroughput than validating a culture; e.g., the latter is typically done by checking
i) the 16S sequence, which has low resolution at species level, and ii) the diagnostic phenotype, which is often lab-specific, and <u>not necessarily representative of a major in-situ activity</u>.

2. The single-strain species description issue is NOT specific to DNA/genome sequence but applies the same to cultures. In fact, I would argue that a MAG that represents an abundant population is NOT a single-strain description but the average genome of the population/many cells and thus, carries much more weight than a single strain for identifying diagnostic traits etc. A SAG (single-cell amplified genome) is similar to a single strain and descriptions based on single SAGs should be discouraged, in my view.

We recently published an opinion article that gives more details for the responses above if you have the time to read [Konstantinidis et al. 2020. Environ Microbiol: <u>https://sfamjournals.onlinelibrary.wiley.com/doi/full/10.1111/1462-2920.14934</u>]

In short, I personally remain convinced that the arguments against using genome sequence as type are rather weak overall.

February 8

Fanus Venter, ICSP delegate, University of Pretoria, South Africa

I want to address the restrictions on the export of cultures and respond to the request by Ed Moore to provide clarity.

Although I do not know the details of the Indian regulations my understanding from discussions with colleagues during the BISMIS meeting in Pune in 2016 was that their regulations are similar to what we have in countries such as South Africa and Brazil. But let me provide clarity by explaining the South African situation.

It is possible to export biological material (in my case bacteria that need to be deposited in a culture collection) for "*research purposes other than bioprospecting*" once you have obtained a permit from the provincial authorities from where the culture was obtained. The export permit requires that every time this culture is supplied to a third party (e.g culture collection to client) permission should again be obtained from the same provincial authority (9 different departments in the case of SA). This restriction is not acceptable under the current regulation of the Code.

This restriction is still required even though the MOUs of many culture collections exclude the commercial use of their cultures.

So why have we been able to still describe new species? Although the Nagoya Protocol is only effective after 12 October 2014, our first national regulations were already published in 2008 and European culture collections will not accept cultures isolated after 2007 without the necessary permits. We are "lucky" that cultures isolated before 2008 can still be used as type material as they are not subjected to the conditions of the South African regulations. This is often a fact we take into account when selecting the type strain, but as it is now more than 12 years ago, it becomes more difficult and our work is slowly coming to a standstill unless we can get the regulation amended.

For this scenario having DNA sequences as type material (obtained from an existing strain) will be of great benefit for countries known for their biodiversity.

I will address the use of Digital Sequence Information under the Nagoya Protocol in another email.

February 8 Fanus Venter, ICSP delegate, University of Pretoria, South Africa

To provide some context to an issue raised by Ed Moore:

"That is because the regulations on ABS govern "genetic resources". This includes, of course, DNA sequence data."

The issue of how "Digital Sequence Information" should be treated under the Nagoya Protocol is not clear and is currently one of the major issues which will be discussed at COP 15 (Conference of the Parties to the Convention on Biological Diversity) in Kunming, China in October 2020.

Details on the history and current process leading up to COP 15 as well as the views of a number of countries and organizations can be found at <u>https://www.cbd.int/dsi-gr/</u>

In short:

The issue of how "Digital Sequence Information (DSI) on Genetic Resources" should be regulated under the Nagoya Protocol was first raised at COP 13 in 2016. The matter was not resolved at COP 15 in 2018 and is now one of the main issues that will have to be negotiated at COP 15 later this year. Although there is a common understanding among the country representatives that it would not be ideal to restrict the use of sequence data for research purposes, there are concerns related to how sequence data used for commercial applications could be traced back to the country of origin to ensure benefit sharing.

The negotiation on DSI will certainly be linked to the renegotiation of the Convention of Biological Diversity, (the so called post 2020 framework) and it is important that biologists interact with their respective government delegations long before October 2020 to ensure an agreement that would not restrict our research efforts.

February 8 Prabhu Patil, Institute of Microbial Technology, Chandigarh, India

I am Prabhu Patil, Scientist working in the Institute of Microbial Technology, Chandigarh, India that hosts MTCC. My training is in bacterial genetics and never knew what is type strain, type species and what is bacterial taxonomy. But because of my association with MTCC, my group is doing core genome-based taxonomic and phylogenetic studies of bacteria, particularly members of *Xanthomonas* genus and the order *Xanthomonadales*. In earlies studies we reported that even clones have been reported into different species! And in the latest study, which is in the biorxiv preprint server, our analysis revealed that *Xyella*, even though a highly reduced genome, is a variant lineage of genus *Xanthomonas* using deep genome-based phylogenetic and taxonomic analysis.

The advent of the web or the internet and genomics era has transformed the field of bacteriology. There is an urgent need and scope to come with terms before things go out of control. Also, considering the way bacterial evolve and regulate the genes, I have two suggestions

1) To allow the use of genome sequence-based approaches to delineate and proposal a strain into new species, genus, and higher taxonomic levels. Hence use genome sequence, as type or reference material (submitting raw reads and assembly in NCBI or EMBL or DDBJ)

2) Allow the proposal of a novel species just based on genome sequence analysis (like ANI, dDDH, AAI), if a researcher has the genome sequence of two or more non-clonal or diverse isolates belonging to the proposed species!

This will democratize plus avoid bureaucracy and also make the field of taxonomy crossdisciplinary and attractive to a new generation of researchers from both basic and applied areas.

February 8

Markus Göker, Leibniz Institute DSMZ -- German Collection of Microorganisms and Cell Cultures, Braunschweig, Germany

First, I would like to draw the attention of the ICSP to the discussion in mycology about exactly the same kind of proposal (*IMA Fungus* volume 9, pages167–175(2018). The publication available at:

https://eur02.safelinks.protection.outlook.com/?url=https%3A%2F%2Fdx.doi.org%2F10.5598%2 Fimafungus.2018.09.01.10&data=02%7C01%7Ciain.sutcliffe%40northumbria.ac.uk%7C4a 79512564f948ede37808d7ac291316%7Ce757cfdd1f354457af8f7c9c6b1437e3%7C0%7C0%7 C637167164730146992&sdata=BzijQSzIrICCBqK7br2%2BEV1FjUWRf8jdaFbHDE3qYys %3D&reserved=0 is critical, negative response to the proposal that sequences can serve as nomenclatural types in mycology that was signed by more than 400 mycologists.

Second, I would like to emphasize that the ballot is not just about the use of genome sequences as types. The forthcoming decision may indeed be regarded as a decision about whether or not genome sequences are permitted as nomenclatural types. This is inaccurate, however, because the decision is about a specific implementation of this idea by specifically modifying the ICNP. Even researchers sympathetic towards genome sequences as nomenclatural types must consider the consequences of these specific modifications.

The suggested phrasings are presented as modest changes which only increase the options of microbiologists. But the proposed changes will not only cause genome sequences to be used as types of microorganisms that cannot be cultivated but also as types of microorganisms that could well be cultivated. Because journals like IJSEM now require a genome sequence for proposals of new taxa there is no extra effort needed to use this sequence as a type. But deposits may be a burden sometimes. The procedures are time-consuming and have become even more bureaucratic lately due to the need for compliance with the Nagoya protocol. If sequences are accepted as types, these efforts shall no longer be necessary. Rather, you only need to sequence an isolate's genome before you can leave it to moulder in a private collection (or autoclave it right away), and then go ahead and validly publish a species description anyway. Predictably, authors will then in most cases take the line of the least resistance and not deposit. Journals cannot effectively control whether or not it would have been possibly to obtain a pure culture and deposit it in two collections. Thus the net effect is the large-scale replacement of strains as types by genome sequences as types. This holds although it does not seem to be the intention of the authors of the proposal.

The proposed modifications include ambiguous clauses ("whenever possible", "when possible", "when it unambiguously identifies") in relatively huge numbers and at crucial positions. Similarly, the term to "unambiguously identify" is also used but remains undefined. It appears to be dependent on empirical results and on taxonomic opinion, which is subject to change and must not be governed by the Rules of nomenclature and must not govern them.

The Proposal for Rule 18a (3) appears to imply that methods are material. I am not sure whether this makes any sense. All in all it seems to me that these modifications would introduce ambiguity into the ICNP that would make it increasingly difficult to determine whether or not certain taxonomic proposals are in accordance with the Rules. Again, this may not be the intention of the authors of the proposed changes of the ICNP.

February 8 Joachim Wink, Working Group Microbial Strain Collection, Helmholtz Centre for Infection Research, Germany

Higher benefit from a separate naming system for uncultivated microorganisms

The use of genome sequences as types of validly published names under the ICNP is sometimes regarded as a necessity for microbial ecology. However, it is unclear whether and if so to which extent ecology could actually benefit. Ecologists were always able to name isolates or sequences quite independently of the ICNP and such names acquired a certain stability simply be their reuse in the literature and in databases. The status of being validly published according to the ICNP does not necessarily increase the stability of naming because taxa with validly published names can be reclassified, yielding other validly published names. Names such as SAR11 for a group of uncultivated bacteria where used stably, were easily recognizable and supported the communication of scientific results.. Such names are not even formed in Latin, let alone validly be published.

While SAR11 was discovered in 1990, the first cultivated representative was not available before 2002 and could prominently be published in Nature. Once a (pure) culture is not a prerequisite for assigning a validly published name any more obtaining a (pure) culture will not be interesting any more and thus hardly ever pursued.

In 2012 Brinkhoff and coworkers (DOI: 10.1038/ismej.2011.190) identified and described the Marine Myxobacterial Cluster (MMC) which includes non cultivated Myxobacteria from sediments. The cluster was found on many different places and was described by partial genome sequences. The many efforts in trying to cultivate these organisms failed. For everyone working with Myxobacteria it's clear what the MMC and it is important to be able to directly separate them from the cultivable ones.

The proposal by Whitman included special annotation for distinct kinds of nomenclatural types. But these are not a part of the taxon name. Since taxonomic literature is hardly read, most people only deal with names. Thus the proposed approach would create a lot of confusion by mixing distinct kinds of types. Previous revisions of the Code have intended to reduce this kind of confusion by restricting the kinds of nomenclatural type that can be used.

A separate formal registry system for names of uncultivated microorganisms is clearly preferable. Such a dual nomenclature is often criticized for creating confusion. Yet an informal way of naming clades in ecology always existed in parallel to the valid publication under the ICNP. Significant confusion cannot arise if the kind of name can easily be inferred from the name itself. Names for uncultivated organisms should simply avoid using Latin Linnaean binomials. This may even be advantageous because Latin is nowadays hardly known and non-Latin names such as SAR11 and MMC are already in use. Confusion that arose in cyanobacterial taxonomy under two codes or in mycology when distinct names for anamorphs and teleomorphs coexisted could not occur in such a system.

February 9 Jörg Overmann, Leibniz-Institut DSMZ-Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, Braunschweig Germany

[In response to Korpole's comments of February 6 and Moore's comments of February 7]

I would like to contribute to this particular point of the discussion by pointing out some legal facts:

1. According to Indian Legislation (BD Act, 2002), Indian Researchers can apply to deposit bacterial strains in public collections outside of India using Form C. HOWEVER, all non-Indian persons or entities that would like to subsequently access this strain MUST OBTAIN prior approval of NBA according to Section 3 of the BD Act (see attached note, point 3.). This means that Indian strains are NOT publicly accessible even if deposited in international public collections and any access not authorized individually by the Indian NBA is illegal.

2. Given the current state of discussion regarding the inclusion of Digital Sequence Information into the ABS regime of the Nagoya Protocol, it can be expected that the access to genome sequence information will be regulated soon as well. Even at present, certain countries have legislation and/or policies in place that do not permit the free exchange of sequence information. Next October, the COP will probably decide on new regulations that likely will impose severe restrictions on the exchange of DSI on a multilateral international level. That is, it is well possible that from next year on, a deposit of DSI in public databases that potentially could serve as type for the description of a new species will not be legally possibly.

It is obvious, that the amendment of the Code to include genome sequences as type material will not solve any of the above problems.

February 10

Brian J. Tindall, Member of Judicial Commission, Leibniz-Institut DSMZ-Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, Braunschweig Germany

[In response to Korpole's comments of February 6]

The deposit of strains that serve as nomenclatural types in at least two collections in two different countries (Rule 30 3b) was introduced because there was at least one scientist depositing strains in two collections in the same country and explicitly required that the depositor be consulted before strains could be released. In essence a "safe deposit".

Rule 30 4 also states:

Organisms deposited in such a fashion that access is restricted, such as safe deposits or strains deposited solely for current patent purposes, may not serve as type strains.

Here emphasis is on "such as" in the knowledge that there may be reasons why a person or institute may not be supplied with a particular organism (could include plant, animal or human pathogens where the appropriate laboratory facilities are not available). A discussion document was made available to the ICSP executive board, and there was extensive correspondence with Editors-in-Chief and those responsible for publishing the IJSEM over a period of 18 years.

The deposit of a strain in more than one collection is also a form of "backup". Imagine the scenario should GenBank be on one server without any backups.

While strains deposited solely for current patent purposes were clearly not permitted, there are numerous examples where strains deposited solely for current patent purposes have been accepted as nomenclatural types (including very recent instances): https://doi.org/10.1099/ijsem.0.003527

In essence any strain originating in India that is deposited in conformity with the requirements laid down by the National Biodiversity Authority and is deposited either in India or in a foreign country will be subject to the same restrictions [http://nbaindia.org]. Placing a blanket ban on not accepting collection accession numbers from collections located in India but then allowing the deposit of the same strains in collections outside of India to serve as nomenclatural types does not solve the problem of "restrictions".

There would appear to be a number of misunderstanding that have arisen over the years that need to be clarified.

February 10 Ramon Rossello-Mora, Vice-Chair of Judicial Commission of the ICSP, Grup de Microbiologia Marina IMEDEA, Illes Balears, Spain

I agree with the many definitions of taxonomy, that indicate this discipline to be:

- The biological discipline that identifies, describes, classifies and names extant (and extinct) species and other taxa (e.g. Padial et al., 2010, zoologists).

- The theory and practice of classifying organisms (Mayr; 1969).

- The identification and interpretation of natural groups of organisms (i.e., taxa) based on characters (such as morphology, genetics, behavior, ecology) (International Commission on Zoological Nomenclature; <u>https://www.iczn.org/outreach/faqs/</u>).

- Taxonomy is the scientific study of biological species and is thus a fundamental sub-discipline of biology. Taxonomists catalogue, describe and classify species, compare their traits in order to name species and categorise these species according to their natural phylogenetic relationships (Amann et al., 2014). This document is signed by several of the relevant taxonomists of plants and animals in Germany.

And so on...

While of utmost relevance, taxonomy is not dealing with the preservation of living beings, but on the what my admired Cowan described the *Trinity of Classification, Nomenclature and Identification* (Cowan, 1965). It seems stupid to have to say that Botanists construct herbaria and Zoologists collections of dead exemplars of animals, and their duties are not ultimately to preserve plant seeds or animal eggs. The storage of preserved exemplars of their subject of study is to have an image of what is considered the morphological prototype hosting the name. Almost all taxonomies rely on the Taxonomic Species Concept that deals with the morphological traits as the basis of classification (although they would like to be able to apply the Biological SC, that is nearly only applicable to vertebrates). Conspicuously, DNA can serve as type material for animals in accordance with the zoological code

(<u>https://www.iczn.org/outreach/faqs/</u> check for the question Can DNA be a type specimen? A question updated in 2012!!!).

Contrarily, whether like it or not, the taxonomy of prokaryotes (especially the species category) has been constructed on the basis of genetic traits, since the mid 60's on the in vitro whole genome comparisons and since the 90's on the 16S rRNA gene sequence. Like it or not, phenotype has been abandoned, and in most of the publications (especially look at IJSEM) relies on API strips, some Biolog tests, fatty acids and some other (not always) chemotaxonomic parameters. The diagnostic tables explaining what is positive and what is negative do not inform at all on what these organisms are.

Like it or not, the future of taxonomy will rely on *in silico* genome analyses, to circumscribe taxa, to reconstruct phylogenies, to infer metabolisms and phenotypes that could be tested in the laboratory, and discover functions hidden in "hypothetical proteins". The information of a genome surpasses in taxonomic relevance any of the currently used tests to reveal phenotype.

I think taxonomy deals with the construction of a classification system that is of **universal** use and explains the natural relations between organisms. It is not the explanation of what can grow isolated in the laboratory under artificial conditions and can be preserved in freezers or lyophilized ampules.

Amann, et al.,

(https://www.leopoldina.org/uploads/tx_leopublication/2014_Stellungnahme_Taxonomie_EN_fin al_01.pdf)

Cowan. 1965. J Gen Microbiol 39: 143-153

Mayr, (1969) Principles of Systematic Zoology, Graham Hill, New York

Padial et al., 2010 (<u>https://frontiersinzoology.biomedcentral.com/articles/10.1186/1742-9994-7-16</u>)

February 10

Brian J. Tindall, Member of Judicial Commission, Leibniz-Institut DSMZ-Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, Braunschweig Germany

[In response to Patil's comments of February 8]

The first part of point 1) is best answered by Principle 1 (4) of the International Code of Nomenclature of Prokaryotes [<u>https://doi.org/10.1099/ijsem.0.000778</u>]. Nothing in this Code may be construed to restrict the freedom of taxonomic thought or action.

There is nothing to stop you from employing your genome sequence based classification approach based on a scientific justification of the way this is done. However, you must also accept that others may take a different approach and treat your data/taxa in a different fashion and come to different conclusions. They may also use exclusively other data, or combine additional data with yours. In your case you have used genome sequences, whereas previous work has centered on other data.

Point 2) if you have isolates then one of them would be designated the nomenclatural type. At the same time Rule 27 2c and 2d states:

c) The properties of the taxon being described must be given directly after (a) and (b). This may include reference to tables or figures in the same publication, or reference to previously effectively published work.

d) All information contained in (c) should be accessible.

In other words the digital genome sequence information should be "accessible" when included. This was introduced because one particular lab had not been making digital sequence information available. If you study the growth properties of the nomenclatural type one would select the appropriate (culture collection) strain, whereas if you are comparing genomes you would access the digital genome sequence information that is documented as being derived from the strain that is the designated nomenclatural type. This guarantees a link between the nomenclatural type (as a strain) and the data derived from studying it.

Thank you for also pointing to your paper: <u>https://doi.org/10.1101/2020.02.04.933507</u>

This highlights two issues. Firstly your evaluation is based on a POCP value of 50% as the lower threshold for delineating the genus, while you seem to be using 60% AAI as the lower threshold for genus delineation. There are, however discussions that indicate 50% POCP may be too low, raising it to 60, 65 or 70% would provide a different interpretation of the same data. Similarly, if an AAI value between 60-80% delineates a genus then this could be taken to mean "anywhere" between 60-80%. In the absence of the similarity values it is not easy to interpret the coloured heat map, but again raising the AAI value to 70, 75 or even 80% would allow a different interpretation of the same data. However, the data indicates that one should go back and look at the classification of the group, which is often the case as new data or new taxa become available.

February 10 S Shivaji, L V Prasad Eye Institute, Hyderabad, India

My passion for the field of microbial diversity and taxonomy of cold habitats dates back to the early 1980s, and over the years my lab has published several papers including the description of about a hundred new bacterial and fungal species. It has always been the endeavour of the international community to bring in stringency while describing a new species like data on DNA-DNA hybridization, lipid profiles, fatty acid profiles, 16S rRNA gene sequences apart from all the other classical data and conventional growth, physiological and biochemical data. With the advent of genome sequencing there is a need to relook at the criteria for describing a new species.

I would like to make the following suggestions for a new species, genus, and higher taxonomic level identification. :

1. Retain all the above especially phenotypic and chemotaxonomic characteristics.

2. Adopt whole genome sequence as mandatory, including bioinformatic analysis with respect to whole genome similarity, resistome, unique pathways etc.

3. Candidate species where convincing phenotypic data is available along with genomic data.

4. Deposition of the type strain in a recognized culture collection centre anywhere in the world including the country of origin.

5. Valid certificate of deposition, viability and availability.

I am confident that this would facilitate the work in the exciting area of microbial diversity and taxonomy without any hurdles.

February 14

Brian J. Tindall, Member of Judicial Commission, Leibniz-Institut DSMZ-Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, Braunschweig Germany

It would appear that publications are appearing that make reference to current Requests for an Opinion and the "loss" of nomenclatural types. Unfortunately, a closer examination highlights other issues that need to be clarified. I have taken 4 examples at random.

In the case of:

Enterobacter siamensis

1) the sequence available from the NBRC (from NBRC 107138) is not identical with HQ888848 (documented as being obtained from the type strain).

2) there are two deposits of HQ888848, HQ888848.1 and HQ888848.2. These two sequences are clearly not identical.

3) neither HQ888848.1 nor HQ888848.2 are identical with the sequence available from the NBRC website, making it difficult to assess whether either HQ888848.1 or HQ888848.2 were ever obtained from the designated type strain or the strain that was deposited. Consequently one cannot rely on the 16S rRNA sequence data and one should check all other data published to see whether it was derived from the strain currently available.

In the case of:

Seliberia and Seliberia stellata.

This organism was first described in 1963 and Mortimer P. Starr obtained a strain that was mentioned in a publication in 1974 from one of the authors of the original description (via G. A. Zavarzin) that was held in the International Collection of Plant Pathogenic Bacteria (a collection that appears no longer to exist). It is unclear whether the current strain in circulation is the original strain, since it appears to come from D. Nikitin rather than the original authors. When originally described 5S/16S rRNA cataloguing/gene sequencing technology was not available

and the Request for an Opinion relies solely on these results, without making any reference to other properties of the strain from the original publication. Just as *Pseudomonas radiora* has been shown to be a member of the genus *Methylobacterium*, or that *Brevibacterium halotolerans* is a member of the genus *Bacillus*, no other evidence has been presented that *Seliberia stellata* is not in the same genus as species, currently in the genus *Bradyrhizobium*. Consider also *Hydrogenomonas eutropha* moving via *Alcaligenes*, *Ralstonia*, *Wautersia* and *Cupriavidus*.

Schmidt and Starr make reference to polar growth and the formation of rosettes (not uncommon in members of the Alphaproteobacteria) as well as similarities to members of the genera *Nitrobacter* and *Rhodopseudomonas*.

In the case of:

Moorella thermoautotrophica

An extensive publication deals with this issue and other issues that also arise that also relate to the accuracy of deposited digital sequence information: https://doi.org/10.3389/fmicb.2019.03070

In the case of

Spirillum volutans

Originally described by Ehrenberg in 1832, no strains were isolated at the time. The designated type strain ATCC 19554 appears to be longer viable, but a 16S rRNA sequence has been deposited as GU585672. A second strain, from Pringsheim, ATCC 19553 might be a suitable candidate as a neotype. This also illustrates the wisdom of "back ups"in more than one collection.

Applying good scientific practice it would seem appropriate to assume that those who deposit digital sequence information or prokaryote strains would take appropriate measures to ensure that what is being deposited is authentic.

February 14

Brian J. Tindall, Member of Judicial Commission, Leibniz-Institut DSMZ-Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, Braunschweig Germany

To put things into perspective when evaluating comments made in current publications on the number of Requests for an Opinion dealing with "problematic" nomenclatural types I would like to refer to two publications:

1) Sequencing orphan species initiative (SOS): Filling the gaps in the 16S rRNA gene sequence database for all species with validly published names. https://doi.org/10.1016/j.syapm.2012.12.006

Among other aspects the project identified some 230 16S rRNA gene sequences that "had to be discarded due to bad sequence quality". These were "replaced" (ie "neo-type" sequences) by better versions. There are additional 16S rRNA gene sequences there were not picked up in that project that have needed to be replaced and a conservative estimate is that this would total 250. If we had to write a Request for an Opinion or propose neotypes to correct each of these sequences then this would mean 250 such publications. "Updating" digital sequence information would require similar mechanisms.

I note also that *Alterococcus agarolyticus* AF075271 started out its life as a member of the family *Enterobacteriaceae* (AF075271.1) as indicated in the original publication, but now enjoys a re-incarnation in the family *Opitutaceae* (AF075271.2) where it seems to rightfully belong. This is an "update" that few people are aware of.

2) Meeting report: GenBank microbial genomic taxonomy workshop (12–13 May, 2015) <u>https://dx.doi.org/10.1186%2Fs40793-016-0134-1</u>

This touches on the issue of the authenticity of strains for which deposited digital sequence information is available and includes data from type strains that are held in culture collections. Sifting through the different databases indicates that there are instances where the genome comes from a strain of a species that is not the species (sometimes also genus, family, order or even class) that the Latin binomial attached to it appears to claim.

See also: Phylogenomics and systematics in *Pseudomonas* https://www.frontiersin.org/articles/10.3389/fmicb.2015.00214/full

Re-evaluation of the taxonomy of the Mitis group of the genus *Streptococcus* based on whole genome phylogenetic analyses, and proposed reclassification of *Streptococcus dentisani* as *Streptococcus oralis* subsp. *dentisani* comb. nov., *Streptococcus tigurinus* as *Streptococcus oralis* subsp. *tigurinus* comb. nov., and *Streptococcus oligofermentans* as a later synonym of *Streptococcus cristatus*.

https://doi.org/10.1099/ijsem.0.001433

Expression of Concern: *Micromonospora craniellae* sp. nov., isolated from a marine sponge, and reclassification of *Jishengella endophytica* as *Micromonospora endophytica* comb. nov. <u>https://doi.org/10.1099/ijsem.0.003487</u>

February 14

Brian J. Tindall, Member of Judicial Commission, Leibniz-Institut DSMZ-Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, Braunschweig Germany

In reply to Prof. Wink's comments on an "alternative" system. Those familiar with the NCBI taxonomy section, Prokaryote nomenclature up-to-date or NamesforLife will be aware that behind all Latin names or in the NCBI for names such as "SAR11 cluster bacterium JGI ETNP_125m_186_B03" there are numerical Codes and as such the system referred to by Prof. Wink is already available, perhaps with the one small issue that appropriate reference points (ie nomenclatural types) are not currently defined.

While there may appear to be advantages of using Latin names that refer to "meaningful" ecological or metabolic properties the Code states:

Principle 4

The primary purpose of giving a name to a taxon is to supply a means of referring to it rather than to indicate the characters or the history of the taxon.

General Consideration 8

The International Code of Nomenclature of Prokaryotes is an instrument of scientific communication. Names have meaning only in the context in which they were formed and used.

However, *Rhodococcus equi* makes no exclusive claim that it is the only red coccus or that there may not be non-pigmented strains, nor does it preclude the fact that it can be isolated from sources other than horses. Removing it to another genus where the name makes no reference to red or coccus would then destroy the information contained in the name, but not the fact that among its properties it may be a red coccus. Latin names may be easier for us to remember, but do not appear to be suitable for bioinformatics work.

Current numerical nomenclatural systems already exist (but without nomenclatural types designated for names not covered by the ICNP), can be easily implemented, dovetail immediately with names validly published under the ICNP and would not interfere with Latin names as currently used. Perhaps one of the major issues is to educate those working outside of taxonomy at present to implement a nomenclatural type based system and to be consistent in the use of nomenclatures (whether Latin based or numerical), including the principle of propriety that is also not always applied consistently in the Latin based system.

February 20

Lily Eurwilaichitr, Thailand Bioresource Research Center (TBRC), National Science and Development Agency, Thailand

We are aware of a proposal to the ICNP to allow the use of partial or complete genome sequences as type. After much consideration, we strongly believe that genome sequence alone should not be accepted as type for the following reasons.

1. If sequence can be adopted as type, it will pose an immense risk of losing the type strain collection, as type strains are no longer needed at culture collections.

1.1. This lack of the physical presence of type strains will impair public access to the culture of type strains. This, in turn, will hinder future study and distribution of those strains for further usage and applications. It also will be difficult to obtain cultures of strains for reference purposes.

1.2. Without the need to deposit type strains in culture collections, there is a higher chance that the culture of type strains will be lost or inaccessible from an individual's collection.

2. Apart from sequence data, other important information related to type strains will be lost or insufficient for further utilization. Sequence data alone most likely does not paint a complete knowledge for the genome and would be inadequate for the effective utilization of those strains.

3. The sequencing technology is not yet stable and is still evolving.

3.1. Different sequencing platforms often result in different outcomes with regards to numbers of OTUs and lengths of sequencing reads. It poses a challenge for the standardization of the quality of the sequences to be accepted as type.

3.2. As sequencing is still relatively expensive and relies on high technological expertise, it can be considered a disadvantage for many researchers. The difference in availability of the sequencing instruments and financial capability in different countries will most likely further produce a larger gap between researchers in the already developed countries and those in the developing countries. This will also discourage researchers without much instrument and financial support to discover and propose new species.

4. Acceptance of sequence as type will dissuade the culture-as-type study. This will pose a challenge in the proposal of new species. Researchers have to search and compare the culture-as-type and genome-as type information in the report of new species. In addition, there will be a complication in prioritizing the culture-as-type versus genome-as type for proposal of new species name.

5. There is not yet a proper and simple way for the public to validate or verify if the sequence data is accurately from a living organism. This is especially difficult without the strain being deposited in a reliable culture collection.

6. If the unculturable genome assembled from metagenomic study is accepted as type, there is no clear method to verify that the assembled sequence is from a single organism and there is no clear benefit from the sequence as the organisms are still unculturable.

For these reasons, we currently oppose the proposal of the sequence as type and feel that it should be reconsidered.

February 20 Joachim Wink, Working Group Microbial Strain Collection, Helmholtz Centre for Infection Research, Germany

I'm still following the discussion related to the use of DNA as type material for bacterial species descriptions and I have some additional remarks on the role of the deposition of type strains.

I now have worked over 30 years in the field of taxonomy of *Actinomycetes* especially members of the genus Streptomyces. If you go back to the golden area of antibiotics, many novel species were described. As it was not necessary to deposit them in an open collection, in many cases every new antibiotics producer was described as a novel species. Basing on the huge number of species within this genus until today, not all the taxonomic positions of these different species has been clarified. The members of the genus Streptomyces have also very large genomes with a size of 8 to 10.000 kb. There are many reports about the horizontal gene transfer within this genus, so also within one species the different isolates show differences in their genomes. If the species will only be defined by their genome than we will came back to a similar situation as we had during the use of antibiotic production as the only taxonomic marker.

February 20 Markus Göker, Leibniz Institute DSMZ -- German Collection of Microorganisms and Cell Cultures, Braunschweig, Germany

Here I am returning to my first comment, specifically to the claim that the proposed changes of the ICNP would not only cause genome sequences to be used as types of microorganisms that cannot be cultivated but also as the nomenclatural types of microorganisms that could easily be cultivated.

I had argued that even if a pure culture is already available, the possibility to use a genome sequence as type instead would cause this culture to not be deposited any more in a collection, at least in the majority of the cases. Because journals like IJSEM now require a genome sequence for proposals of new taxa, there is no extra effort needed to use this sequence as a nomenclatural type. The efforts to deposit a strain shall no longer be necessary. Predictably, authors will then in most cases take the line of the least resistance and not deposit strains. Journals cannot effectively control whether a culture is available and could be deposited, especially if this is not declared. Even the IJSEM has difficulties to control whether deposits are patent deposits or are subject to restrictions, such as strains from Brazil or India, and thus cannot serve as deposits of type strains, causing the need to deny the status of being validly published afterwards.

It has been argued by supporters of the proposal to modify the ICNP that type strains can be provided later on for a name that was validly published based on a sequence as nomenclatural type and then exchange the nomenclatural type. The proposed changes to Rule 18f are supposed to cater for that, and during the online debate it was mentioned that providing better types could easily be done within an emendation of a taxon description. However, no evidence was provided for the likelihood of such an event.

Obtaining better type material later on is indeed unlikely according to 400 mycologists (doi:10.5598/imafungus.2018.09.01.10): "An undesired side-effect that should also be considered is that, in practice, few researchers will be devoted to re- describing (or actually describing) species that have been previously named based on just a DNA sequence. This has several causes, but among them, there is an important bias in research journals disfavoring the publication of re- descriptions of already known taxa, versus the description of new taxa. Another reason is time constraints, since it is not uncommon that specialists do not have the time to properly describe all of the numerous undescribed species they are aware of. This makes them focus on those that are more likely to be published as new species and not on those that have been already described, even if previous descriptions are faulty or defective. Anyhow, having numerous names only based on DNA sequences and few descriptions of the actual organisms would create an enormous number of (validly published) names applied to taxa for which virtually no information exists."

In fact, the number of published emendations is already now much smaller than the number of names validly published under the ICNP.

Recieved February 21 Comments from members of the Subcommittee on Taxonomy of Mollicutes, submitted by Dan Brown, ICSP delegate, University of Florida, USA

January 7

J. Dennis Pollack, Ohio State University, ret. (USA)

I don't think genome sequence is sufficient to constitute the type material of a new species.

January 7

Glenn Browning, University of Melbourne

I would accept a closed genome sequence with good depth, but not partial or draft sequences.

January 8 Alain Blanchard, University of Bordeaux (France)

I would accept a closed genome sequence with good depth, but not partial or draft sequences. In addition, in the pdf "It is recommended that, when possible, a sample of the DNA be deposited in at least two publically accessible service collections in different countries and the catalog numbers be indicated" is ambiguous. Indeed, the DNA of most of the uncultured bacteria is usually obtained with a high level of contamination from the host (e.g. plant DNA for phytoplasmas). At least, the DNA sample that should be provided to the collections should be of the same quality as the one that was used to obtain the full genome sequence.

January 8 Chih-Horng Kuo, Academia Sinica (Taiwan)

I support the use of genome sequence as the type material. For more detailed considerations:

1. The complete & closed chromosome sequence should be required; plasmid(s) may be missing in the assembly but those probably are not critical for taxonomy.

2. Sometimes completing the chromosome sequence is just not practical, and draft genomes could provide some very useful information. The major concern is what would be the quantitative standards for "high quality" draft. If the community can come to a consensus, then accepting draft genomes would be fine.

3. In addition to the assembled genome, the raw sequencing data sets must be made available. In case the genomes are mis-assembled, other people can identify & verify the problem.

4. Making the DNA samples available is important but may not be always possible. Even when possible, the quantity may be quite limited. So perhaps this should be recommended and not an absolute requirement.

January 9 Mitchell Balish, Ohio University (USA)

A genome sequence could potentially stand as type material for a new species if at least the following criteria are met:

1) There must be evidence that the sequence is either complete (excluding episomal elements) or nearly so, accounting for difficulties in sequencing repetitive regions, etc. Evidence for completeness of a sequence that isn't closed could derive from the completeness of sets of genes encoding the proteins involved in well-established metabolic (or other) pathways, like glycolysis and protein translation (as appropriate).

2) To establish that a genome represents a new species, some stringent threshold of difference from other species – excluding elements like transposons, prophages, and pathogenicity islands – must be reached. The quantification of this difference should be established not by looking at one gene or a small number of genes; it should be derived from information integrating the entire genome (minus the aforementioned variable elements), like total percent nucleotide identity or protein similarity, or even shared gene content. Candidate criteria along these lines are proposed by the authors who are in support of the use of genome sequence as type material. It is important that the criteria are applied very strictly and regularly. I suspect many things we call different species would actually fail to meet these criteria; but I think it's better to err on the side of not calling something a new species, at least until phenotypic characterization establishes otherwise.

January 10 Joachim Frey, University of Bern (Switzerland)

I fully agree with the comments of Mitch Balish.

1) The genome must be complete. Currently combining sequencing from a long read run (e.g. PacBio) with short reads run (Illumina) are standard to get a best possible full genome sequence. Both the final full genome sequence and the short reads must be made accessible by depositing at GenBank/EMBL and SRA (short reads archive).

2) The entire genome sequence except transposons IS, CRISPR etc must be used.

3) If the type strain is deposited, (if the [organism] can be grown) the study should be reproducible. I do not know if depositing DNA will become a standard but it would certainly be useful.

January 13 Assunta Bertaccini, University of Bologna (Italy)

The DNA sample provided to the collections should be of the same quality as the one that was used to obtain the full genome sequence.

The complete & closed chromosome sequence should be required; making the DNA samples available may not be always possible so perhaps this should be only a recommendation but realistically based (I mean the scientific community should be sure of the existence of the strain).

Evidence for completeness of a sequence that isn't closed could derive from the completeness of sets of genes encoding the proteins involved in well-established metabolic (or other) pathways, like glycolysis and protein translation (as appropriate).

To establish that a genome represents a new species, some stringent threshold of difference from other species – excluding elements like transposons, prophages, and pathogenicity islands – must be reached. The quantification of this difference should be established not by looking at one gene or a small number of genes; it should be derived from information integrating the entire genome (minus the aforementioned variable elements), like total percent nucleotide identity or protein similarity, or even shared gene content. Candidate criteria along these lines are proposed by the authors who are in support of the use of genome sequence as type material. It is important that the criteria are applied very strictly and regularly. The genome must be complete. Both the final full genome sequence and the short reads must be made accessible and depositing DNA would certainly be useful.

I don't agree with Chih-Horng Kuo about draft genomes and raw sequencing data sets these data could/should be handled only by expert colleagues who can verify them in the most appropriate manner.

January 15 Ana Sofia Ramirez Corbera, Universidad de Las Palmas de Gran Canaria (Spain)

The genome sequence is sufficient to constitute the type material of a new species, but I would also add the necessity of detecting it several times (in different places or the same place at different times) as an equivalent of the need to have some isolations of the same species.

January 18 Christine Knox, Queensland University of Technology (Australia)

It is time to have an alternative to serotyping and DNA-DNA hybridization assays in order to define a new type species. 16S rRNA sequencing and then a closed and complete genome sequence of the strain to be designated the type strain is the way forward. It would be good to have deposits of both the culture (when possible) and the DNA.

There will be difficulties if more than one strain is described. It may not be possible to provide multiple WGSs. Sequencing and alignment of selected genes then may define phylogenetic relationships but this cannot be used to describe type strains.

January 30 Dmitriy V. Volokhov, US Food and Drug Administration (USA) [edited for length]

A high-quality draft (genome scaffolds) or better complete genome sequences should be provided for Candidatus species.

I disagree that ONLY complete genome sequences should be acceptable; researchers could have a lot of situations when assembly of complete genome sequences for Candidatus species may not be possible.

At least two different genome assembly algorithms should be used for Candidatus species.

The DNA sample for Candidatus species provided to the collections should be of the same quality as used to obtain the full genome sequence. But what will be acceptance criteria of this "same quality"?

I disagree that ONLY DNA and/or DNA sequence deposition for cultivable [organisms] will be sufficient instead of deposition of live culture of type strain.

A single strain per each species could be sufficient in a case when the novel species found to be genetically unique in comparison to other well-known species.

There will be difficulties if more than one strain is described for the same species if multiple WGSs are not provided. In this case, MLST can be used as define phylogenetic relationships among strains. MLST should not be used to describe type strains for Candidatus species.

[end of comments from members of the Subcommittee on Taxonomy of Mollicutes]

February 21 Comments of Marco Riojas, American Type Culture Collection, Manassas VA USA

The proposed changes to the ICNP recommend that sequences of DNA may serve as type material if it "unambiguously identifies" the taxon.

Reliance upon the phrase "unambiguously identify" is shortsighted and willfully disregards the progressive revisionism that is key to science. Decades ago, *Bacillus anthracis* could be "unambiguously" differentiated from near neighbors by its pathogenicity, i.e. its ability to cause the disease anthrax. This definition ultimately proved incorrect, as we now know that the virulence genes are plasmid-borne, and that *B. cereus* strains with these genes can cause essentially the same disease. Similarly, a gene or set of genes can be thought to be specific to a certain taxon and incorrectly used as definitive identification of that taxon. One such example is the botulinum neurotoxin (BoNT), originally thought to be specific to and therefore indicative of *Clostridium botulinum*. Since then, BoNT genes or homologs have been found in *C. argentinense*, *C. baratii*, *C. butyricum*, *Weisella oryzae*, *Chryseobacterium piperi*, and *Enterococcus faecium*.¹ Genes or sets of genes can "unambiguously identify" a taxon… until they no longer do.

If these genes are mere identification criteria published in a standard (non-*IJSEM*) journal, they can simply be a) nullified by a subsequent publication proving the genes do not unambiguously identify the taxon, or b) supplanted by a newer set of genes with better specificity. However, if these genes are officially designated as type sequences, it is unclear how a retraction of type

status would occur. In case a), it would seem destined for referral to the Judicial Commission in order to allow a type sequence to be "undesignated". In case b), the proposed changes to Rule 18f allow for replacement of a sequence of genomic DNA with later cultivated type strain; however, they do not allow for replacement of a type sequence with a different sequence (or more generally, "material"). If sequence is to be allowed to serve as type, a protocol must exist for the inevitable situation where sequence must be replaced with sequence. An additional proposal to modify the ICNP should be made to this effect. At the very least, the current proposals should be tabled until such time as a coherent implementation can be evaluated *in toto*.

1. Poulain B and Popoff MR. Why Are Botulinum Neurotoxin-Producing Bacteria So Diverse and Botulinum Neurotoxins So Toxic? *Toxins (Basel)* **11**, doi:10.3390/toxins11010034 (2019).

February 21 Marco Riojas, American Type Culture Collection, Manassas VA USA

It is universally acknowledged that science faces a reproducibility crisis. The proposed changes to the ICNP threaten to exacerbate this crisis.

An essential foundation of prokaryotic taxonomy is the availability of type strains to the entire scientific community. Currently, type strains of novel taxa must be deposited in two culture collections in different countries [Rule 30(3)(b)].¹ This ensures that scientists around the world can order the same strain, reproduce experiments, verify results, and build upon the science relating to this organism.

By allowing valid publication of taxa without making a viable culture available, researchers will be unable to reproduce research related to this organism. If the type sequences proposal is accepted, yes, researchers will be able to download the type sequence and analyze it. However, because the input sequence will be identical, the results will almost certainly be identical as well. The ANI results I generate on my computer will not differ from those anyone else generates. This is not proper scientific reproducibility; this is simply running the same thing multiple times.

Under the current system, criteria subsequently found to be insufficient or ambiguous (as addressed in my previous response) can be ameliorated by returning to the preserved type strain and determining new criteria. However, this will not be possible if non-biological criteria (e.g., sequences) are accepted as type. This is the primary advantage of the current culture-based system. The type strain is the definition of the species; it is a <u>specific organism</u> that is the taxonomic reference point. As new technologies are developed, scientists can return to the type culture to reexamine it using the latest techniques. Thus, the current system allows the taxonomy to evolve and adapt to the future. On the other hand, the type sequence (or other material) will exist as a fixed snapshot in time. As new criteria or novel technologies develop (e.g., a metabolome, or some as yet undiscovered [futuretech]-ome), one cannot return to the type DNA sequence to identify new criteria under the new system (with the possible exception of extracting a hypothetical proteome via translation of the gene sequences). The DNA sequence will always be the DNA sequence. Thus, despite the comments expressing the proposals as bringing the nomenclatural system into the future, dissociating nomenclatural types from viable cultures would in fact have the opposite effect.

In order to preserve the adaptability of our systematic scheme, nomenclatural types should continue to be viable cultures, as currently required. The proposals under consideration should be rejected.

1. Parker CT, Tindall BJ, and Garrity GM. International Code of Nomenclature of Prokaryotes. <u>*Int J Syst Evol Microbiol*</u> **69**, S1-S111, doi:10.1099/ijsem.0.000778 (2019).

February 23 Comments of Fanus Venter, ICSP delegate, University of Pretoria, South Africa

After spending some time to have a relook at all the comments raised so far I would like to make a few comments. It is clear from the discussion that people feel strongly about the issues and that their viewpoints are clearly shaped by their current field of research or work environment.

I think the concerns towards these proposals have been well articulated. For me the main issues are quality of the sequences (completeness and contamination), incorrect assignment of taxa and the accompanying instability of the system, the ability to replicate findings, descriptions with limited phenotypes as well as concerns that cultures will no longer be shared (only for organisms that have been cultured). Various participants have responded to these concerns and I don't want to address these again. I would rather focus on the implications if we do not accept these proposals and continue with "business as usual"

For me these proposals are primarily to create a reliable phylogenetically based taxonomy/classification system for all Bacteria and Archaea. The desire to be able to place the uncultured bacteria within our existing classification and the ability to refer to them by a binomial name will remain. I foresee that if these proposals are not accepted, we will see the establishment of a parallel nomenclature code to deal with the uncultivated prokaryotes. This idea has support especially among the researchers working in the field of environmental microbiology and ecology. As this "Code" will potentially deal with the majority of bacteria, it will have a major impact on all fields of microbiology including traditional bacterial taxonomy.

The second benefit that accepting these proposals would have, is that it will allow taxonomists in many of the developing countries to continue to catalogue their unique prokaryotic diversity. The resolve of many of the developing countries to exercise their sovereign rights over their biological resources to ensure benefit sharing when used for commercial gain, will remain. To ensure that benefit sharing is done these countries will still enforce measures to keep track of who outside their country has access to these resources. If genome sequences will not be accepted as alternative type material, the ICSP will have to address this issue by re-evaluating their requirement for deposits of cultures with no restrictions on access. I am of the opinion that the need to keep track of access to cultures differ from "safe deposits" and should be allowed. I have been in discussions with our national government for a number of years now and can assure everybody that changing the Code will be far easier than addressing national regulations that deal with all biodiversity to make exceptions for microbiologists to deposit type material.

I would therefore urge the members of the ICSP to carefully consider the concerns and consequences of accepting / rejecting the proposals when casting their votes.

February 23 Brian J. Tindall, Member of Judicial Commission, Leibniz-Institut DSMZ-Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, Braunschweig Germany

In response to the comments of S Shivaji on February 10.

I noted the split between "chemotaxonomy" and "phenotype". There is no reason why chemical data should not be included as part of the phenotype, just as the ribosome or ATP synthase has a phenotype. While the phenotype is often referred to as "unreliable" or "uninformative" this often depends on how work is done or which parameters are studied. Genetic information can be "unreliable" if different labs submit different digital sequence information for what they claim to be the same strain or "uninformative" if it is a gene that appears to be easily lost or gained in a population.

One of the key issues is that one has forgotten is that as defined by Colwell the "polyphasic" method has moved on. Originally defined on data available at the time and clearly a phenetic approach (ie. overall similarity and not limited to phenotype as often mistakenly assumed), the polyphasic method can include relevant phenotypic information as well as relevant gene based information. Co-relating the two is the next major task in the biological sciences. Annotation of genes usually requires knowledge of the phenotype. Debates with Peter Sneath missed the point that the early rRNA ctalaogue Sab values were phenetic and not (phylo)genetic = cladistic. The strength of the system that developed was that work on the lipids of what was to become the Archaea went back to 1962 and supported a completely different data set, just as early 16S rRNA catalogue and cytochrome sequences (Nature papers in the late 1970s) showed the same picture or that the respiratory lipoquinone data collected from the late 1950s onwards and published in a review by Collins and Jones quickly allowed one to make sense of re-arrangements in the genus Pseudomonas and the concept of the alpha-, beta- and gammasubclasses. The latter being also supported by lipopolysaccharide work. Both the gene based and phenotype based systems point to an evolutionary basis for their distribution and development over geological time. A broad based "polyphasic" approach is a multi-disciplinary approach that takes us to the limits of our current methods and understanding of biology.

Unfortunately, the "phylogenetic" system (priority being given to sequence based interpretation) has also had its down side. Work by Imhoff in the 1990s on the chemical composition of the genus *Rhodobacter* has only recently resulted in a realization that the "phylogenetic interpretation" can be refined by relevant phenotypic (chemical) data. Major theories on the nature of "genera" in the planctomycetes, or *Methanogenium* were quietly silenced with the help of the chemical data. The genus *Peptoclostridium* Yutin and Galperin 2013 was put into perspective by Gerritsen et al. 2014. Placing *Deinobacter* in the genus *Deinococcus* was also a major disservice to the existing chemical data on this "genus", and we continue to founder on a clear definition of the genus *Clostridium*, where chemical data (with its underlying genetic information) points to a radical split.

February 23 Markus Göker, Leibniz Institute DSMZ -- German Collection of Microorganisms and Cell Cultures, Braunschweig, Germany

The "discussions related to the use of DNA as type material for bacterial species descriptions (<u>https://www.the-icsp.org/</u>, accessed on 2020-02-23) are not necessarily easy to follow because the order of the contributions may obscure the logical interrelations of the specific arguments. It

thus makes sense to sort the contributions according to arguments. This in turn may best be achieved by grouping the arguments into pros and cons of the distinct nomenclatural codes under discussion: The current INCP (Parker et al., 2019) vs. the ICNP modified as suggested by Whitman (2016). See (1) below.

I have added a third approach, a separate naming system for uncultivated taxa that takes into account the concerns raised by Oren & Garrity (2018) for comparative purposes. This does not mean that this approach is the one I favour. I think it should be discussed more broadly. Indeed, the ballot is not just about the use of genome sequences as nomenclatural types. Rather, the decision is about a specific implementation of this idea by specifically modifying the ICNP. Even researchers sympathetic towards genome sequences as nomenclatural types must consider the consequences of these specific modifications. Alternatives for modifying the ICNP also exist. For instance, impure cultures or dead specimens could be allowed under certain circumstances. Such alternatives should also be taken into account and more broadly discussed.

The contributions taken into account in the attached file are those I am aware of as of today (2020-02-23). They are referred to using their author(s) and date. Not all of the e-mails from the debate may have been sent to me.

The juxtaposition in the attached file is opinionated but even those who disagree with me may find the separation into distinct arguments to be of use. My own conclusion would favour the ICNP, combined with a distinct system for uncultivated organisms, and the current ICNP over the proposal by Whitman (2016).

It has also been argued that the decision should be postponed because most of the affected microbiologists are unaware of it (Christensen-01-13; Dijkshoorn-01-13; Moore-01-15). For an opposing view see Sutcliffe-01-15. I would prefer to put the debate on a broader basis even if this implied a (potentially considerable) delay. Most microbiologists I talked to were unaware of the fact that the decision is scheduled for March 2020.

(1) http://goeker.org/downloads/Pros and cons of sequences as types MG 2020-02-23.pdf

February 23

Brian J. Tindall, Member of Judicial Commission, Leibniz-Institut DSMZ-Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, Braunschweig Germany

Given the fact that these discussions involve the International Committee on Systematics of Prokaryotes and the International Journal of Systematics and Evolutionary Microbiology, it would be appropriate to highlight the science of systematics. Systematics is a fundamental part of the biological sciences and can be succinctly described as the cradle of comparative biology. Sadly, one often sees this science reduced to the naming of biological entities. The latter element is nomenclature and is part of the elements: nomenclature (the naming of classified biological entities), classification (the science of grouping biological entities based on their properties and theoretical and philosophical considerations), and characterization (the collecting of data on the biological entities that is potentially limited only by the methods available to us). Together these are regarded as comprising taxonomy, where a taxonomic system is a prerequisite for the identification of a biological entity either as a member of an existing taxon (irrespective of rank) or novel at one or more ranks. Identifications typically rely on a limited data set that may none-the-less allow predictions to be made about features not included in the identification system, but included as part of the original taxonomy. As such taxonomies are open ended and nomenclatures serve as pointers to the classification and properties of the biological entity in question. Limiting those properties to only digital sequence information or reducing the classification to ANI, AAI or POCP values could be considered to be a reductionist, minimalistic approach that also precludes alternative methods or interpretation, as well as excluding relevant biological information.

Systematics certainly uses the underlying taxonomic system, but it should neither be reduced to taxonomy nor nomenclature. It is a fallacy to assume that either systematics (in the wider sense) or taxonomy has either a limited goal or inherently limits the data sets I consider myself to be a systematist with some 44 years of standing and reading relevant papers in Journal of Biological Chemistry, Molecular Microbiology, PNAS, Journal of Molecular Evolution. Journal of Lipid Research, Genome Biology or Systematic Biology contributes to the scope of systematics and the need to appreciate the current limitations that seem to have been self-imposed that many seem to have identified as the root cause of problems, but where the alternatives do not address the needs of systematics, nor does it break with what could be considered to be a limited view of the purpose of either taxonomy or its component parts (nomenclature, classification, characterization).

Systematics is indeed a multi-disciplinary science and genomics is also one element in appreciating biological diversity. Given the magnitude of the task it would be far more beneficial to get the diverse range of experts together and to illuminate biology from its very different angles that would enrich both systematics and the appreciation of taxonomy with its underlying infrastructure. I recall a paper I wrote 27 years ago where I cited Dobzhansky and the fragmentation of the biological sciences. Little has changed in the intervening years.

February 23 Andrey Yurkov, Leibniz Institute DSMZ-German Collection of Microorganisms and Cell Cultures GmbH, Braunschweig, Germany

On behalf of the World Federation for Culture Collections (WFCC) I would like to share our opinion on the proposal.

The proposal heavily relies on the use of and access to sequence information (referred to as Digital Sequence Information, or DSI) and this topic is a matter of ongoing discussions among CBD parties. Considering the fact that access to the sequence data (DSI) can be equated to the access to genetic resources under the CBD, we have prepared the following opinion letter. We would like to emphasise that it is important to ensure that the reference material, whether a culture or sequence, or both, is properly safeguarded and is accessible. Participants of this discussion should be informed about the discussion on the DSI and already existing national regulations and policies regarding whole-genome sequencing. Permitting sequence data to replace cultures as a nomenclature type should not be seen as a method for overcoming problems of restricted access to countries genetic resources. This is very important consideration.

[The following letter was attached to this comment]

February 23 İpek Kurtböke, University of Sunshine Coast, Australia

On behalf of the WFCC's Executive Board, I would like to forward our views on the proposed changes in the *International Code of Nomenclature of Prokaryotes*.

WFCC is not a decision-making authority in prokaryotic, eukaryotic and viral taxonomy; however, as an end user it is impacted by the decisions made by relevant international committees. In this instance replacing type strain deposition by only nucleotide sequence data we believe can weaken the robustness of bacterial taxonomy for the following reasons:

1] Reliable identification of the type strains for a given genus and species deposited after that for the genus in question:

It should employ polyphasic methods including genome sequences. Accordingly, only physically available reference material can ensure error free identification via reanalysis of the material. Moreover, due to the current availability of powerful identification methods replacing the previously popular ones, the older data can be reassessed at any time. The use of such powerful techniques to resequence a species is not possible without the preserved biological material.

2] Compliance with international rules and regulations:

Collections must follow national and international laws such as biosafety, biosecurity (including dual-use organisms), and quarantine regulations as well as complying with the import and export restrictions. All these regulations contain appendices with lists of names of organisms. Any access, utilization and sending of an organism requires a risk assessment with the confirmed identity of the organism. Restricting deposition and identification to whole-genome sequences alone would limit vital information on the growth characteristics, physiology, morphology and many more features of the organism. Technology-based approach can also discriminate collections and institutions from developing countries who may not have access to this technology.

3] Deposition of the genome and other molecular data:

Genome information has weighty importance however, quality control has to be ensured via high standards for whole-genome sequences at the global level. Culture collections so far have made significant progress in developing standards for authentication, preservation and tracking of the origins of the deposited material. They regularly exchange information and share knowledge and experience with collections through international, regional meetings and networks. WFCC thus would welcome open discussions on commonly used standards for genome data, its acquisition, analysis, storage and access to ensure reliability.

We also would like to draw attention to the currently encountered difficulties in gaining access to the sequence data (Digital Sequencing Information or DSI). While access to the (reference) material is regulated by national and international agreements and laws, present views on the access to DSI vary substantially. Restricted access to the DSI thus can be in conflict with the principle of "unrestricted access" of the ICNP. As a result, if deposited biological material is available, the DSI can be recreated in another location for the same species.

4] WFCC values molecular investigations related to unculturable diversity including detection of new phylum/class/family/genera/species through such information. Since in these instances live

material generation is not possible molecular information can be utilized and the "*candidatus*" status can be created for these species until they can be isolated from environmental sources.

In conclusion, the WFCC's Executive Board members recognize the importance of molecular data and DSI, however, the integrity of such information can only be ensured and validity can be confirmed with the presence of deposited biological material.

February 23

Brian J. Tindall, Member of Judicial Commission, Leibniz-Institut DSMZ-Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, Braunschweig Germany

[Comments In response to comments of Venter of February 23, edited for length. Full text posted at www.goeker.org/icsp/Tindall comments on the comments posted by Venter 23_02_2020]

There is a general issue of the quality of data associated with publications proposing names of new taxa or new combinations for existing taxa.

The use of the term "phylogenetic" is often misleading because it is now taken to include only gene or protein sequences and effectively tries to exclude the "phenotype". Unless I have misunderstood something, genes primarily encode for RNA, that in turn (with the exception of tRNA and rRNA) may be translated into protein sequences that themselves are either structural entities or enzymes. Enzymes may be single entities (i.e. an amylase) or part of a biochemical pathway (TCA cycle). As such most of the genome encodes for phenotypic features, providing one gets away from the definition "phenotype " = biochemical/physiological tests". I noted some years ago the work on the ribosome that culminated the Nobel Prize work (two publications in Science) highlighted the importance of the structural aspects i.e. the phenotype of the expressed genes. The Hennigian definition of "phylogenetic systematics" is about character analysis and became known as cladistics. This is in contrast to phenetics that is based on overall similarity and may include phenotypic and genetic data (see Cain and Harrison's original definition. The third alternative is to combine the two.

If one substitutes "evolutionary framework" for "phylogenetic" this might be more realistic. Evidence is that different genes have different "phylogenies" as a result of their different structural and functional roles (that is also reflected in codon usage and amino acid usage). Whole organism "phylogenies" in the prokaryotes have a network like structure (ie vertical and horizontal inheritance (gene loss and gain, gene duplication with change of function) that we are trying to press into a tree like structure.

We already have parallel systems - see my earlier e-mail. Given the fact that it can now take up to 4 months to get a name published on the Validation Lists there is also a two tier system, with names published in original articles in the IJSEM being given favour to those names being published in other journals.

There is nothing in the text that I sent around that was written 12 years ago that infringes the rights of the sovereign states to determine what happens to the biological diversity over which they exercise sovereign rights. However, by restricting access to the biological entities themselves (including of course parts of it such as DNA specimens) or the digital sequence information already creates a two tier system whereby one set of nomenclatural types are readily available for verification/further work and the others not. Spain makes exceptions to comply with the Code.

As in the case of one former member of the EU, there are now consequences for future funding (perhaps even for the EBI-EMBL in Hinxton) and decisions have been made to withdraw from a common science forum perhaps to the detriment of scientists involved. One has to accept that.

February 24 Edward Moore, ICSP delegate, University of Gothenburg, Gothenburg, Sweden

I try to address here the specific proposal for one of the proposed modifications (underlined), i.e., addition of a third clause (3) to Rule 18a:

<u>"(3) As from 1 April 2020*, sequences of genomic DNA may also serve as the type when it</u> <u>unambiguously identifies the species. When possible, it should be a high quality draft or better</u> <u>genome sequence</u>".

While most discussion has concerned the usefulness of WGS data for characterising and identifying bacteria, the purpose of rule 18a of The Code has been to define what should be the reference and Rule 30(3-b) insures (since 2001) that the references serving taxonomy and systematics are available to the scientific community. I emphasise that WGS data are not the reference of a taxon – they are the result of an analysis of the reference – as already pointed out by Ulrich Nübel (Feb. 07). As such, any particular WGS data are dependent upon varying factors – none of which have been defined by proponents of the new rules.

So, it is important to try to address this issue of the proposed Rule 18a(3), i.e., what should serve as nomenclatural type material as the 'ultimate' reference for prokaryote taxa. More specifically, the implications of implementing the new rule 18a(3), as Markus Göker (Feb 08) stressed. As Nübel (Feb 07) pointed out, the reproducibility of analyses for proposing and validly publishing new taxonomic names cannot be insured with WGS electronic data. Yet, reproducibility of analyses is essential (required?) for reliable science, including reliable taxonomy. Being able to reproduce the analyses of research is generally accepted as essential for publication. This goes to the crux of the argument in considering Rule 18a(3), regardless of any issues of what should be done for characterising taxa. And, this has not been addressed by proponents of the proposed new rules. Furthermore, it was not addressed in two ICSP meetings (2017 and 2019), although I and others raised this question.

Do proponents of the new rules not believe that it is necessary to be able to reproduce the characterisations of bacterial taxa? Do proponents of the new rules not believe that it is necessary to safe-guard the reference material for bacterial taxa?

The so-called, 'chain-of-custody' of the WGS data cannot be confirmed, beyond the expertise and the word of the depositor of the WGS data into a public database. Given the overall levels of 'crap' genomic data in the public databases, I submit that such trust would not be sensible.

It would be good to receive a discussion from any of the proponents for changing the rules about how you see these issues. I think these points have been somewhat lost in the discussions about how bacteria should be analysed.

February 25 John Hays, Medical Microbiology & Infectious Diseases, Erasmus University Medical Center Rotterdam

With respect to the proposed change of the Code of Nomenclature of Prokaryotes, I would like to add the following suggestion (whatever the result of the proposed changes):

When defining 'the sequence of one or more genes that unambiguously identifies the genus or subgenus' a minimal set of NOMENCLATURE-SPECIFIC METADATA' must be included with the sequence according to the following principles:

'FAIRDATA-2'

Findability (where has the sequence been deposited?)

Authentication (when, where and by which department and institution the sample was taken, isolated and sequenced?)

Interoperable (is the sequence derived from an OTU or from whole genome/gene sequencing?) Reusable (is their clinical material or cultured bacterial isolate available for further studies?) Depth (what is the minimum sequencing depth for the published sequence?)

Association (what is the current most closely related genus, species or subspecies related to the new sequence? – Include information on how this was determined)

Technology (which manufacturer and sequencing technology was used and which version?) Algorithm (which software package and version was used to obtain the sequence?) Number of sources- (the sequence has been confirmed from a minimum of 2 different independent sample sources and/or scientific institutions).

A letter could be added to the name or strain identifier to indicate that FAIRDATA-2 information is available and would act as a potential marker of quality of the sequence.

February 24 Brian J. Tindall, Member of Judicial Commission, Leibniz-Institut DSMZ-Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, Braunschweig Germany

[Edited for length. Comments posted 24th February (full text posted at www.goeker.org/icsp/Tindall comments on the proposed changes posted 24_02_2020]

<u>Rule 18a.</u>

The Code is neutral on a number of points, including whether a nomenclatural type "must unambiguously identify the taxonomic group". The "nomenclatural type if that element of a taxon with which a name is permanently attached", but at the same time does not preclude that it may be considered later on that a name is a heterotypic synonym of another name. This wording should be deleted. It would be appropriate to substitute "nomenclatural type" in all instances in the Code where the term "type" is used alone.

The use of the term "material" implies a physical object. In the case of genome sequences there is a difference between the sequence chemically encoded on a piece of DNA and the digital sequence information that is obtained by experimental procedures and deposited in an electronic database as an electromagnetic signal in binary code.

Rule 18a (3) [first section]

For "sequences of genomic DNA" read "digital sequence information that is obtained by

experimental procedures and deposited in an electronic database". Remove "unambiguously identifies the species" since this is outside of the remit of the Code. In essence "digital sequence information" is the same as a description.

Rule 30.3.c.

We are not talking about a physical sequence, but digital sequence information obtained by experimental procedures and deposited in an electronic database as an electromagnetic signal in binary code. This is essentially a description at the level of the genome. The term "catalog" is incorrect and should be replaced by "accession". DNA deposited in at least two publically accessible service collections constitutes as preserved specimen. It is also questionable what purpose this would serve, since, in contrast to a written description, illustration or preserved specimen on a microscope slide of the organism the only way of examining the preserved DNA with regards its physical nature (i.e. by determining the nucleotide sequence by current methods) would be to destroy it. See also Sneath and Neimark: https://doi.org/10.1099/00207713-45-1-188 https://doi.org/10.1099/ijs.0.63718-0

<u>Rule 18a (3</u>

This is already covered by Principle 1 (4), but also makes the mistake that a method cannot serve as a nomenclatural type. This can be deleted.

<u>Rule 20a</u>.

This links back to the issue of "unambiguously identifying" a taxon, which is not part of the remit of the Code. It also makes a claim that one or more genes may unambiguously identify the genus or subgenus. Since different authors may evaluate the same information differently this would not preclude the establishment of heterotypic synonyms, ie the two taxa were not unambiguously identified. "Or the sequence of one or more genes that unambiguously identifies the genus or subgenus" should be deleted.

Proposal 4

As indicated previously, at the rank of species and subspecies this would apply to the nomenclatural type and not to the corresponding name. In the past all changes to the Code were documented in articles in the IJSEM, in the minutes of the appropriate committees/commissions and applied from their date of publication of the version of record.

February 25

Brian J. Tindall, Member of Judicial Commission, Leibniz-Institut DSMZ-Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, Braunschweig Germany

We should not get off track. The Code is about names that are attached to taxa and not how taxa are defined or differentiated:

General Consideration 4:

Rules of nomenclature do not govern the delimitation of taxa nor determine their relations. The Rules are primarily for assessing the correctness of the names applied to defined taxa; they also prescribe the procedures for creating and proposing new names.

Principle 1

The essential points in nomenclature are as follows.

4. Nothing in this Code may be construed to restrict the freedom of taxonomic thought or action.

Principle 8

Each order or taxon of a lower rank with a given circumscription, position, and rank can bear only one correct name, i.e., the earliest that is in accordance with the Rules of this Code.

Rule 23a

Each taxon above species, up to and including order, with a given circumscription, position, and rank can bear only one correct name, that is, the earliest that is in accordance with the Rules of this Code

Rule 24a

Note 3. Synonyms may be homotypic synonyms (i.e., more than one name has been associated with the same type) or heterotypic synonyms (i.e., different names have been associated with different types that in the opinion of the bacteriologist concerned belong to the same taxon).

I have already indicated that "*unambiguously identify*" (see extracts from the Code above) is outside the remit of the Code and Principle 8, Rule 23a and Rule 24a Note 3 lay down what happens to names when it becomes apparent that nomenclatural types need to be associated with other names.

"Changing digital sequence information"

Rule 18g

Change in characters of type and neotype strains. If a type or neotype strain has become unsuitable owing to changes in its characters or for other reasons, then the matter should be referred to the Judicial Commission, which may decide to take action leading to replacement of the strain.

In essence this could be re-worded to cater for other instances, but it is unclear to me how deposited digital sequence information could "change in characters", unless one has changes taking place on the electronic storage media. However, digital sequence information is also not physical "material". I really think we must get back to the term "nomenclatural type" before we all get really confused.

However, if digital sequence information is not the nomenclatural type, but part of the description then one can emend the description and specify either a new accession number or use xxnnnnn.1 and xxnnnn.2. There is definitely a case for always using the ".1", ".2" or ".3" designation since without the version number, sequence accession numbers are not unique identifiers and it takes the guess work out of knowing which version was used..

Dr. John Hayes' point on FAIRDATA-2 would be more appropriate as a recommendation under Rule 27 2 d). As a Rule it would mean that if not implemented this would hinder the valid publication of the name - undesirable.

February 25 William B Whitman, ICSP Delegate, University of Georgia, Athens USA

Tindall's point about Principle 1 is key. Not only does the requirement for type strains restrict the freedom of taxonomic thought, it actually prevents naming of most of the prokaryotes on our planet.

For a more comprehensive discussion of these topics, please see the recent paper by Rossello-Mora et al. [1]. In brief, this paper makes the following points:

- 1) With the current methodology, DNA sequencing is reproducible. Certainly, it is at least as reproducible as comparisons to type strains, which are frequently lost, misidentified, difficult to obtain, or have other problems.
- 2) Phenotype is a tool and not the goal of systematics.
- 3) The choice of type material is irrelevant to whether or not intraspecies diversity is known. Definitions of species based upon a single strain are common, and the intraspecies diversity is not known. If a sequence was the type, one could also identify a cluster of related sequences illustrating the intraspecies diversity.
- 4) Claims of taxonomic 'chaos' are greatly overstated. What some people call chaos, others call growth in knowledge and understanding.
- 5) Naming the uncultured will stimulate attempts cultivate these prokaryotes.
- 6) Names with sequences as nomenclatural types will be widely used by the microbiological community. It will meet an important demand.
- 7) Concerns about the bioinformatics tools available for creating MAGs are overstated.
- 8) The choice in type material is irrelevant to whether or not narrow thresholds are used to delineate taxa. It is possible to use flexible threshold for sequences as well as strains.
- 9) Using DNA sequences as the type for species meets a real need in microbiology and is not mere nomenclatural stamp collecting.
- 10) A single naming system that includes both the cultured and uncultured taxa will have enormous synergies for all fields of microbiology. It will break down barriers between disciplines and lead to new understanding about the microbial world.
- Rossello-Mora R, Konstantinidis KT, Sutcliffe I et al. (2020) Opinion: Response to concerns about the use of DNA sequences as types in the nomenclature of prokaryotes. Syst. Appl. Microbiol., <u>https://doi.org/10.1016/j.syapm.2020.126070</u>

February 25 Iain Sutcliffe, Chair of the ICSP, Northumbria University, Newcastle upon Tyne, U.K.

It is my personal opinion that **the members of the ICSP should vote to accept the Whitman** (2016) proposal to include sequence as type, and the related proposal of Whitman et al. (2019), of which I am a co-author. This is a matter of simple principle if those who are voting believe that our nomenclatural Code should be relevant to and inclusive of the entire prokaryotic world. The Code must be emended so that it becomes a Code genuinely for the "Nomenclature of Prokaryotes" rather than only for the "Nomenclature of <u>Some (but not all) Cultivated</u> Prokaryotes".

A vote to reject the proposal of sequence as type will be self-defeating for those who advocate that type strains ensure nomenclatural stability: such a vote will propagate a chaotic and fragmented proliferation of informal names, such that invalid names will eventually again outnumber valid names, undoing the pioneering achievement of the production of the 1980 Approved Lists.

Nevertheless, the concerns of the clinical microbiology community are not to be dismissed lightly. However it seems unlikely that allowing sequence as type for uncultivated taxa will diminish enthusiasm for cultivation of pathogens and host-associated taxa. Indeed, projects such as the 'culturomics' studies of the IHU Méditerranée Infection Marseille illustrate well how culture-independent understanding of the microbiome has renewed and not stifled enthusiasm for culture itself. The valuable work of culture collections will continue to underpin these endeavours.

Uncertainties about quality control are secondary to matters of principle here. The Code governs nomenclature and not methodology. As now, the community will have responsibility for methodological QC and will impose its standards on what is acceptable through the peer review process. Minimal standards have already been well articulated (e.g. Bowers et al. 2017; Chun et al. 2018).

Large-scale problems with the semantics of the Code are also not to be expected. I believe that the Whitman (2016) emendations on which we will vote are not fundamentally *revolutionary* so much as *restorative*, given that earlier versions of the Code served well in the two decades between the 1980 Approved Lists and the 2001 revisions (that introduced the restricted definition of the type of a species as being a living culture).

It is not overly dramatic to assert that this is an existential crisis for the ICSP: not accepting sequence as type will undoubtedly lead to a divorce between traditional taxonomists and others researching microbial diversity, notably (but not just) ecologists, that could do long lasting harm, with the Code marginalised and considered as being only relevant to a minority of the prokaryotic world. Such a schism will ossify the conservative tendency of traditional prokaryotic taxonomy, condemning the field to mundane 'handle turning', as manifest in its most common output form: the formulaic single strain species description published in IJSEM. It will then be no surprise when promising and able young microbiologists abandon ship in favour of other more stimulating fields.

February 25 Stephen L. W. On, ICSP Delegate, Lincoln University, New Zealand

I do not find these proposals "modest". I find them radical. They reform the current rules of prokaryotic classification and nomenclature and are fundamentally and dangerously flawed.

- 1. No sequencing technology is error free. To propose a new taxonomic unit based upon data that is potentially flawed from the start is not a good beginning to defining reference material for comparison.
- 2. Even where multiple core genes derived from whole genome sequences are used to classify cultured strains, the position in trees can differ markedly. This is well established for single gene trees (a notable example includes *Helicobacter cinaedi* where different strains were initially considered different species on the basis of their position in a phylogenetic tree!) but occurs with WGS/multi-gene analyses too. We have a paper in review where we find clinically relevant species have the potential to be misclassified into newly configured genera because of the relative positions of neighbouring taxa and inclusion of additional core genes.
- 3. Tools (POCP, AAI) have been described that use WGS data to help delineate genus boundaries. I can direct you to a proposal that has completely misunderstood those guidelines. There is ample potential for this to occur repeatedly.
- 4. Similar tools (isDDH, ANI) analyse WGS data to mimic traditional DNA-DNA hybridization experiments. The formulas described to predict homology values differ in their efficacy according to taxonomic group and in some cases yield conflicting results. Some of these discrepancies are listed in our 2017 minimal standards paper for *Campylobacter, Arcobacter, Helicobacter* and *Wolinella* species.

I have contributed to several studies where misidentified or poorly classified strains of clinical relevance have been re-examined with a polyphasic approach and their taxonomic position correctly assigned. Nonetheless, the potential for misleading other investigators remains as a result of their publication; it has only been possible to re-examine the taxonomic position of the isolates in question since they represent culturable entities. It is a matter of genuine concern to me that material as described here could be used to designate an organismal type with no true recourse to independently re-examine it.

In short, neither the technical nor analytical tools we currently use for sequence analysis can be said to provide wholly unambiguous or unequivocal assessments of the taxonomic position of a given entity. Detailed examination of a cultured strain (or one that can be reasonably determined as distinct, such as related in the original Candidatus proposal) allows for such determinations, and provides a necessary "sanity check" on whether or not a proposal is sound *– and of benefit to the wider scientific community whom we serve*. Indeed, Society members in the food sector have said "the applied practical implications and ramifications have not been thought through. "Inadequately defined reference material will only yield an increasingly muddled resource that has the potential to confuse, mislead and, at worst, put at risk the health of humans, animals, plants and /or the environment.

Detailed references / unpublished data supplied on request. I am vociferously opposed to these proposals.

February 25 Edward Moore, ICSP delegate, University of Gothenburg, Gothenburg, Sweden

I try to address further, specific points of **<u>Proposal 1</u>** of the proposed modifications (underlined):

Rule 18a. <u>The type of a species or subspecies must unambiguously identify the taxonomic</u> <u>group and is a designated strain or other material</u>. Whenever possible, the type of a species or subspecies is a designated strain.

(3) [first section] <u>As from 1 April 2020*, sequences of genomic DNA may also serve as the type</u> when it unambiguously identifies the species.

These proposals offer the option of submitting WGS Digital Sequence Information or, a "... designated strain.", i.e., "... whenever possible ...". This 'option' would lead to instability in classification and nomenclature, rather than more stability, as the proponents of the new rules propose, and would have consequences on the overall state of bacterial taxonomy.

These consequences would be due to the 'option' of least resistance being taken by researchers submitting proposals for new taxa. Given the option to submit DSI or ponying up for the costs of shipping "Dangerous Goods" to 2 different public Collections and waiting on the Collections to complete their 'authentication' protocols, the Researcher will, of course, opt for submitting to DDBJ or EMBL or GenBank. However, that option does not allow for 'authentication' of the characterization of bacteria, besides checking A, C, G and Ts.

We do not even need to speculate on whether researchers will 'opt' to submit their new strains, in addition to the WGS DSI. We have a 'control' study: In 2010, within the activities of an EU-project, Erko Stackebrandt (2010) conducted a survey of prominent microbiological journals,

835 articles, for the number of bacterial strains investigated. How many of the strains were deposited into public Collections?

"Of the about 20,200 strains listed, only 190 strains (0.94%) were deposited."

According to publication policies of most journals, authors are expected to make honest efforts to provide biological materials to researchers upon request.

"In an anonymous request to obtain strains from 100 randomly selected authors of the journals screened, only 19% indicated their willingness to provide strains, 5% confirmed deposition of these strains in public collections after publication; of the others contacted, 61% did not respond and 15% responded that the strains had died or were included in patented processes, and hence were unavailable."

In my own experience, requests to corresponding authors for strains receive responses less than 50% of cases and success in receiving strains in less than 10% of cases.

The microbiological journals that 'expect' authors to provide biological materials to researchers are the journals that offer possibilities for 'effective publications' of new bacterial taxa. However, as far as I know, only IJSEM requires deposit of the type strain of new taxa to be deposited into public collections, i.e., for safe-keeping and for access by the scientific community. Removing the obligation of deposit of type strains into public Collections will decimate the resources of reference materials available to the scientific community.

Stackebrandt. 2010. Trends in Microbiology; doi:10.1016/j.tim.2010.05.001

February 26 Stefan Emler, SmartGene Services, Lausanne Switzerland

We, the undersigned, see conceptual and practical flaws with the proposed changes to ICNP, to allow species definitions on the basis of gene/genome sequences alone.

We, professional microbiologists and physicians, rely on taxonomy and nomenclature for unambiguous communication in our work, delivering care to patients and animals, and security to food and to diagnostic and pharmaceutical products.

Conceptual Flaws: New taxons based solely on genome or gene sequences increase uncertainty about species nomenclature overall

A) Disconnected data: Currently, ICNP requires several independent parameters to describe a species (DNA, proteins, membrane, metabolism, plasmids...) plus submitting a strain to a culture collection; this multi-factorial discipline assures stability and traceability of the nomenclature upon which we rely. Genomes alone provide insufficient information to delineate key attributes of a species.

B) Natural diversity: Different genera display different evolutionary speeds; and different genes different evolutionary clocks. Kingdom-wide ANI cut-offs, as proposed for novel "species", do not accommodate such natural diversity. A CLSI expert committee (Guideline MM-18 Second

Edition (2018)) concluded that generalized nucleotide similarity cut-offs, though easy to apply, are unreliable for species discrimination, even for 16S rDNA. Evolution and natural intra-species diversity do not follow standard rules, but require specific assessment.

Practical Flaws: Uncertainty in nomenclature is compounded by inconsistent data and erroneous annotations

If biological material is unavailable for a putative "species", confirmation of findings by other institutions and orthogonal methods becomes impossible and strain collections can no longer reproduce the features described.

NGS techniques and IT tools often generate variable output, absent a standardized approach and dependent on expertise. Thus, many genomes in the public domain today reveal inconsistencies in sequence content, assembly and annotation. In the absence of submitted strains, sequencing artifacts can be indistinguishable from real genetic variation.

Genomes submitted with inaccurate species and genus names further compound the uncertainty of species boundaries. We expect such noise in the system to proliferate, as new sequences are assigned identifications when matching unverified records; such erroneous annotations will contaminate future results by association.

Alternative Approach:

We believe that the current, multi-factorial discipline for designating a new species is valuable and should be maintained.

In addition, to address uncultured organisms in meta-genomics, we propose creating a "classification of genome-based OTU of uncultured bacteria" <u>outside</u> the ICNP, for which genomic data alone <u>would be sufficient</u>. Transparent quality and plausibility controls at the point of data submission should be established, both for the sequence and for the annotation. Classification criteria should be defined and followed as suggested by Konstantinidis et al; we propose adding the requirement that such referenced OTUs be derived independently from at least two different sources, as suggested by Patil. Over time, natural variation of each classified OTU should be codified, to differentiate it reliably from other close OTU's .

Submitted OTU's should be made available publicly, without a species name but associated to a genus or family in the annotation. Users could search for these entities specifically or exclude them, as is the case already with "uncultured" organisms in GenBank/NCBI.

Name	FIrst Name	Amiliation	City/country
Bloemberg	Guido	FAMH Microbiology, Head National Centre for Enteropathogenic Bacteria and Listeria (NENT) Institute for Food Safety and Hygiene Vetsuisse Faculty, University of Zurich	Zürich/Switzerland
Brown- Elliott	Barbara A.	Associate Professor of Microbiology, Associate Director of Microbiology, Mycobacteria/Nocardia Laboratory, The University of Texas, Health Science Center at Tyler	Tyler, TX 75708/USA
Egli	Adrian	Professor, Head Clinical Bacteriology and Mycology, Research group leader, Department of Biomedicine, University Hospital Basel	Basel/Switzerland

Undersigned and co-signatories:

Ellis	David	President SmartGene, Inc., Head of Business Development and operations North-America	Raleigh NC 27624 – 9543/USA
Fingerle	Volker	National Reference Center for Borrelia, Bavarian Health and Food Safety Authority (LGL)	Oberschleissheim/Ger many
Gessner	Andre	Protessor, Institute Director, Institute of Clinical Microbiology and Hygiene, University Hospital Regensburg	Regensburg/Germany
Hiergeist	Andreas	Head of NGS facility, Institute of Clinical Microbiology and Hygiene, University Hospital Regensburg	Regensburg/Germany
Imkamp	Frank	Molecular Diagnostics, Institute of Medical Microbiology, University of Zurich	Zürich/Switzerland
Junier	Pilar	Professeure assistante, Laboratoire de microbiologie, Institut de biologie	Neuchâtel/Switzerland
Margos	Gabriele	National Reference Center for Borrelia, Bavarian Health and Food Safety Authority	Oberschleissheim/Ger many
Patel	Robin	Chair, Division of Clinical Microbiology, Consultant, Divisions of Clinical Microbiology and Infectious Diseases, Director, Infectious Diseases Research Laboratory, Elizabeth P. and Robert E. Allen Professor of Individualized Medicine, College of Medicine and Science, Mayo Clinic	Rochester, MN 55905/USA
Reischl	Udo	Professor, Head of Moleulcar Diagnostics, Institute of Clinical Microbiology and Hygiene, University Hospital Regensburg	Regensburg/Germany
Risch	Martin	FAMH, CEO Labormedizinisches Zentrum Dr Risch	Vaduz/Fürstentum Liechtenstein
Schrenzel	Jacques	Associate professor, Head of the Bacteriology Laboratory, Division of Infectious Diseases, Geneva University Hospitals (HUG)	Genève/Switzerland
Vaneechout te	Mario	Professor, Laboratory Bacteriology Research, Department Diagnostic Sciences GE32, Faculty of Medicine & Health Sciences,Ghent University, Flanders	Gent/Belgium
Wallace Jr.	Richard J.	Chief Infectious Disease, Professor of Medicine, Departments of Medicine and Microbiology and Center for Pulmonary Infectious Disease Control, The University of Texas Health Center	Tyler, TX 75708/USA
Walther- Antonio	Marina	Department of Surgery & Department of Obstetrics & Gynecology, Assistant Professor, Microbiome Program, Center for Individualized Medicine, Mayo Clinic	Rochester, MN 55905/USA
Wengenack	Nancy L.	Director, Mycology and Mycobacteriology Laboratories, Professor of Microbiology and Laboratory Medicine and Pathology, Mayo College of Medicine	Rochester, MN 55905/USA
Zbinden	Reinhard	Professor, Director Institute of Medical Microbiology, University of Zurich	Zürich/Switzerland

February 27 Reto Lienhard, Laboratoire Borrelia (CNRT/ NRZK), La Chaux-de-Fonds

Being too late for the joint comment you received from Stefan Emler, I would like to add my consent to the opinion submitted by Dr Emler.

February 27

Florian Maurer, Head, National and WHO Supranational Reference Center for Mycobacteria Research Center Borstel, Leibniz Lung Center, Borstel, Germany

In support of Emler:

I fully support this initiative, please consider me as co-signatory no. 20.

February 27 Dan Brown, ICSP delegate, University of Florida, USA

[In response to comments of Moore, February 25]

The mycoplasmologists showed how to overcome this 10 years ago: "Creation of a Bacterial Cell Controlled by a Chemically Synthesized Genome" DG Gibson et al., Science 2010.

February 27 Reinhard Rachel and Harald Huber, Universitaet Regensburg, Germany

[edited for length]

Over the last decades, a taxonomic framework emerged that is based on genetic information and on the description of the corresponding organisms including details on their morphology, physiology, behaviour, and structure. The proposed changes of the International Committee on Systematics of Prokaryotes to "describe" a microorganism by simply depositing genetic material is a dramatic change in the field of taxonomy and systematics. Having described many bacterial and especially archaeal species, we wish to voice our opinion on this matter.

For us, the most problematic consequences will arise if it will be possible to "describe" a new genus or even higher taxa based on the deposition of only a single gene (e.g. 16S rRNA sequence). In public databases, hundred thousands of such sequences are meanwhile available. What would happen, if somebody makes use of these data to "describe" thousands of "new" genera? Surely, this leads to an inflation of new genera or higher taxa without any scientific value! Moreover, as the description of a new genus would become possible on sequence-information alone, the current lack of an understanding of the physiology and morphology of these organisms will worsen. The new era of metagenomics brought about insights into the genomic diversity of the microbiological world, but at the same time, the scientific community realises that the lack of additional information on these organisms severely hampers our understanding of ecological habitats and microbiological diversity. This issue will dramatically intensify with the new rules on classification: why should anybody go the hard way and enrich, isolate and characterize a new organism, which takes at least months, if it is possible to "describe" new genera without any greater efforts within a few days?

In our opinion, these changes will be the beginning of the end of a reputable, knowledgebased taxonomy and systematic. A description of an organism based on sequence data alone will not increase our knowledge about microbial diversity but leads to an implosion of systematics. The tremendous amount of metagenomic data makes it attractive to use them for taxonomic purposes. If the corresponding organism is in culture, these data are indeed highly valuable. But without an organism, what should be the benefit? The successful amplification of a gene does not necessarily prove that this gene is active in a cell, nor that it is the only active variant in this cellular context. Open questions remain: where does this gene come from? Is this gene actively transcribed and translated, is it regulated, and how is it regulated? The concept "one gene–one protein" has been shown to be unprecise and insufficient in too many cases, thus the presence of one gene alone is not sufficient to define a cell, a species, or a genus. Hence, we strongly believe that the deposition of genetic material and by extension the description of a single gene does not justify the description of a new genus. Consequently, we strongly object the proposed changes in the taxonomic code and encourage the International Committee to reconsider their envisaged changes.

February 27 US Culture Collection Network

On Monday February 24, the US Culture Collection Network held a virtual meeting to compose a response to the proposed ICNP rule change. Participants in the meeting included 21 individuals from US and European collections, and experts in taxonomy and nomenclature. Additional comments were received by email and incorporated into the response. The full document has been posted on the USCCN blog site, <u>http://usccn.blogspot.com</u>. A 500-word summary is below.

Co-authors of the USCCN response document:

Kyria Boundy-Mills, Executive Board, World Federation of Culture Collections; Steering Committee, US Culture Collection Network; Curator, Phaff Yeast Culture Collection, University of California Davis

Marco A. Riojas, Scientist, ATCC/BEI Resources

Manzour H. Hazbón, Senior Scientist, ATCC/BEI Resources

C.M. Lucy Joseph, Curator, UC Davis, Department of Viticulture and Enology Culture Collection

George M. Garrity, Michigan State University and NamesforLife, LLC **Ulrich Nübel**, Leibniz Institute DSMZ, Germany

Summary:

The USCCN has identified numerous serious concerns regarding the proposed changes to the ICNP currently under consideration, particularly with respect to their purpose and strategy. (Full text: usccn.blogspot.com)

- 1. General Consideration 4 and Principle 1 specifically restrict the ICNP to the formation and application of names to prokaryotes, guaranteeing freedom of taxonomic thought. The current proposals by Whitman intermingle nomenclature with taxonomy in such a way as to clearly violate both General Consideration 4 and Principle 1.
- 2. Recent similar proposals in the nomenclatural codes governing animals, algae, fungi, and plants have not been supported for numerous reasons, many equally applicable to prokaryotic nomenclature as well.
- 3. Serious issues exist with the proposed use of gene sequence data.
 - Many sequences have been deposited that are attributed to the wrong organism.
 - Deposited DNA sequences are not permanent: many have been modified or removed.
 - Genome sequence can differ dramatically based upon which assembly method is used.
 - Accepting gene sequences that cannot be replicated from a living type strain presents temptation for unscrupulous researchers to invent fraudulent sequences.

The current proposals would have many detrimental effects on US culture collections, their users, and science as a whole, including:

1. Eliminating access to the living materials required to reproduce or extend characterization of organisms. Culture collections currently fill this critical need by providing such material.

- 2. The proposed changes impact users of collections and nomenclature in the medical, food safety, biosecurity, and many other fields.
- 3. Funding agencies such as NSF and DoE require attribution and provenance of data generated by users. Removing the requirement to preserve living type organisms can lead to problems with use and public release of data, e.g. data policies implemented by DoE, one of the major generators of genome sequence data in the world.

The USCCN recommends several actions regarding the current proposed changes and the overall process:

- 1. The proposed changes should not be adopted at this time. At the very least, the currently recommended changes should be tabled until additional proposed changes are made that address the serious flaws in the current proposed implementation, and all these changes should be evaluated as a whole.
- 2. The ICSP should be more proactive about reaching out to potentially affected stakeholders to inform them when proposals are being brought to an official vote. Parties (e.g., culture collections and clinical and microbiological societies) impacted by future proposed changes should be contacted in a timely manner so they can be more involved in discussions.
- 3. Rather than creating unnecessary disruption of the nomenclatural system, greater emphasis should be placed on developing methods for culturing, characterization, and preservation of currently unculturable and fastidious organisms.
- 4. Specimens containing uncultured organisms should be preserved and made available to the scientific community. Professionally managed collections could preserve fastidious organisms, such as obligate symbionts within their host. It may be useful to consider a future proposal for amendment of the ICNP to allowing nomenclatural recognition of organisms in currently unrecognized formats (e.g., *in vivo* in animal models).

February 27 Gerard Verkley, on behalf of the board of the European Culture Collections' Organisation (ECCO)

On behalf of the Executive Board of ECCO, I would like to transfer our position on the proposed changes in the ICNP.

The proposed changes in the ICNP could have serious consequences in the activities of our member collections and their role in biodiversity preservation and distribution for life science researchers.

ICNP rule 30 3b requires deposit of a designated type-strain in culture collections in two different countries. Unfortunately, the proposal to allow the use of complete or partial genome sequences as type, will in our view take away the firm basis of the taxonomic system, i.e. the type-strains. Strains allow for a polyphasic approach in characterizing species including by DNA-based and phenotypic analyses (virulence, antimicrobial resistance, metabolic catalytic activities, etc), and critical review later on as new techniques develop to improve taxonomy and classification. Without type-strains, further research, reproducibility testing and characterization of the taxa, including re-sequencing, will not be possible, and the system will slowly but certainly be undermined. Furthermore, the proposal could open the door for short track (even automated) publication of massive numbers of new names, that will be very difficult to assess.

Motivation to deposit any biological material in public collections (type-strains, DNAextracts or environmental samples) will decrease. The proposal allows for later replacement of a type sequence by a type strain, but we expect that in practice this will be forgotten or ignored. This should concern all stakeholders, especially in the light of climate change and the accelerated loss of biodiversity.

Sequencing methods cannot discriminate between live bacteria and transient DNA. Depending on the method used for extracting DNA and sequence (bioinformatics) analyses, discrepant results can be obtained. Determining the activity and physiological state of a microorganism using DNA sequencing is challenging.

Increasing regulatory pressure from Access and Benefit Sharing (ABS, Nagoya protocol) and other legislations make it more difficult for scientists to deposit strains in collections abroad. ECCO and other organisations aim to support depositors by developing best practices and model transfer agreements (MTA). The situation is already serious but could deteriorate even faster if the proposal were accepted. Sustainability of public culture collections is of great importance for all stakeholders. Recent promising developments in culturomics will depend on public collections to preserve key-or type-strains of the vast numbers of (new) species isolated.

It has been suggested that the proposal could be a way to overcome the problems with regard to restricted access to biological material under the ABS regimes implemented in various countries/regions, as (at least for a long time) sequence information has been regarded to be out of scope of the Convention on Biological Diversity (CBD) and the Nagoya protocol. Ongoing discussions to include digital sequence information (DSI) under the Nagoya protocol might result in a positive decision at the 15th Conference of the Parties in October 2020, which could then also have major consequences for the accessibility of sequence-based types.

February 27 Erika Tóth, ICSP delegate, Hungarian Society for Microbiology

I am Erika Tóth (Department of Microbiology, ELTE, Hungary) and I have followed the discussion of collegues connected to the topic for long. We have discussed the pro and contra arguments with collegues also in Hungary about the use of genome as type material, here I summarize our opinion.

Basically we share the opinion of those collegues who consider doubts about using only the genome as type material, and at many points I agree with the letter of US Culture Collection Network that it should not be allowed at this time and so non-cultivable bacteria cannot be described solely based on the genome (MAG) sequence.

The genomic sequence does not answer all of the questions, we may have often only assumptions for certain metabolic abilities/processes, but until we investigate the property in question, in silico results remain only an assumption (measured or thought?).

Moreover, we also have doubts about the quality of the sequences deposited, how much they will be reliable, how can it be controlled, etc.

In my opinion to use the genome data as type material would lead to a little "chaos". On the other hand, some research groups would jump at the opportunity, and we are afraid that this way taxonomy as a discipline would be degraded into a branch of bioinformatics...

Therefore, in our opinion is that to keep the bacterial strain as the type material is still important.

February 28

Brian J. Tindall, Member of Judicial Commission, Leibniz-Institut DSMZ-Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, Braunschweig Germany In response to the comments of Dan Brown, February 27

There was a lot of media hype about this, but I had the good fortune to hear an excellent, balanced and realistic presentation by Dr. Gibson, who drew attention to the complexity of the task that included the need to synthesise parts of the complete genome and to then "assemble" them in another living cell. The vacated cell and the genome introduced are from entities that are considered to be different species, but show almost identical 16S rRNA sequences and the genomes also appear to be highly similar. One interesting point raised by Dr. Gibson is that a single point mutation in what turned out to be a "critical gene" was sufficient to prevent the "vacated cell - foreign genome hybrid" from replicating. It is unclear what would happen if one used the genome from another mycoplasma such as *Eperythrozoon suis*. Technologies do of course advance with time.

February 28

Brian J. Tindall, Member of Judicial Commission, Leibniz-Institut DSMZ-Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, Braunschweig Germany

In the interest of clarity could I please request, as a matter of utmost urgency and before voting commences that the ICSP secretary, Dr. Lenie Dijkshoorn circulate to this e-mail list, a list of all persons entitled to vote on the current issues being discussed. The current ICSP website does not seem to be up-to-date. I have been informed as a member of one of the IUMS member societies that Dr. Richard Hahnke now represents the German DGHM, and reading the e-mail from Dr. Stephen On (to a smaller circle) he appears to represent the New Zealand Society for Microbiology and does not appear to be on the last e-mail list (I have added him). Neither are listed on the ICSP website. Dr. F. A. Rainey appears under the ICSP-EB, but I cannot find any reference to him under the ICSP membership, nor his IUMS member society affiliation. Prof. Milton da Costa is sadly no longer with us, but is listed as representing the Portuguese Society for Microbiology. There may have also been other recent changes to the ICSP of which we are not aware.

The following information should be included.

1) the name of the person

- 2) the name of the IUMS member society that they represent
- 3) the country where that IUMS member society is registered

February 28 Tomohiko Tamura, Biological Resource Center (NBRC), National Institute of Technology and Evaluation (NITE), Japan

We disagree the proposal to allow the use of complete or partial genome sequences as type of bacteria in the following points:

We greatly agree with the idea of Dr. Markus Göker on February 8. As noted in the comments, the proposed changes will not only cause genome sequences to be used as types of microorganisms that cannot be cultivated but also as types of microorganisms that could well be cultivated.

As Dr. Ulrich Nübel commented on February 7, the reproducibility of experimental results is a fundamental requirement of any scientific approach. More than 10% of strains deposited in the

culture collection are contaminated, which is found by tests performed after receipt. If there is contamination, we ask the depositor to resend the correct strain. The quality control of the culture collection supports the foundation of scientific research using microorganisms. Since contamination has occurred in the cultured strains, genome sequences will also contain contamination. According to the Rule 30 (3) (c) [New rule] by Whitman (2016), the following has been proposed: "It is recommended that, when possible, a sample of the DNA be deposited in at least two publically accessible service collections in different countries and the catalog numbers be indicated." However, researcher cannot get enough DNA from uncultivated microorganisms for deposit. If DNA has been deposited, how the DNA is used for. It is very difficult to distinguish the contamination and target from only genomic sequences with current technology. Thus, we think it is difficult to ensure the reproducibility of experimental results by third party by the DNA deposition. In addition, since the accuracy of the genome sequence cannot be guaranteed, it is difficult to recognize/verify the existence of "species", which is the minimum unit of an organism based on it. So, there are problems with using sequences of genomic DNA as the type material.

On the other hand, there are many uncultured organisms on the earth whose existence can be confirmed by the genome sequencing. We believe that the information about uncultivated organisms should be disseminated and shared for the development of science. In the same way as so far, we should promote the registration of these genome sequences with International Nucleotide Sequence Database Collaboration: INSDC and disseminate them in academic papers if there is a high possibility of a neoplasm on the genome sequence. However, the studying uncultured microorganisms by genome sequence and the giving a scientific name for cultured organism by polyphasic approach should be considered separately. After the adoptions of the Approved Lists in 1980 in the International Code of Nomenclature of Prokaryotes, this system is very easy to understand for many researchers. The management of scientific names which include two different type materials by polyphasic approach and by genomic DNA can often result in pre-1980 confusion.

February 28 Johannes Sikorski, Leibniz-Institut DSMZ-Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, Braunschweig Germany

[In response to the comments of Tindall, February 28]

I support this request. The list of members (<u>http://www.the-icsp.org/icsp-members</u>) has been updated the last time apparently more than 2 years ago (Members of the ISCP_30/10/2017).

February 28 Comments of Fanus Venter, ICSP delegate, University of Pretoria, South Africa

[In response to the comments of Tindall and Sikorski, February 28]

We had an EB meeting yesterday where changes in the membership of the ICSP was discussed. The current members (as reflected on the website) will be in office until the end of March based on the new statutes. Iain has requested that I add some of the additional names of people that recently joined the ICSP (linked to societies that had no representation as part of the current committee) and that should be done by Monday. This list will however change again when the new committee start with their work in April.

February 28 George M Garrity, Michigan State University, East Lansing, Michigan USA

The current online discussion on Whitman's modest proposals raise an interesting issue: what remedies exists if and when the views that are expressed by a representative to the ICSP differ from those of the organization that they represent? Can the representative be recalled by the member society or their vote rescinded? If not, can any vote by the ICSP be considered binding?

February 28 Aharon Oren, ICSP delegate, The Hebrew University of Jerusalem, Jerusalem, Israel

In the 'standard' appointment letter, there is a sentence stating that the member "will serve until unless our society notifies [the ICSP] differently". A society can therefore discontinue the membership of its delegate in the ICSP if it is not happy with his/her performance. I doubt whether this can be done retroactively and that a vote can be rescinded by a society.

February 28 Iain Sutcliffe

Thanks Aharon, this is a useful comment.

My interpretation is that each ICSP member has been delegated by their Society in view of their expertise and ability to vote on behalf of the Society (otherwise every ICSP vote – contentious or otherwise - would be paralysed by the time it would take for delegates to liaise with their Society seeking guidance on how to vote).

As you point out, were a Society to then deem that it's delegate had not acted appropriately in representing them, then they have the ability to rescind the delegate status.

February 28 Edward Moore, ICSP delegate, University of Gothenburg, Gothenburg, Sweden

[In response to the comment of Dan Brown of February 27]

Thanks for the reminder about this seminal publication! [DG Gibson et al., Science 2010] Of course, that publication was a milestone in microbiology, biochemistry, genetics, genomics, etc.! I did not realise that this process was actually being used in routine applications. That is exciting, of course. But, it seems a complicated effort to avoid depositing bacterial strains. Perhaps, such a process should be considered for the 'not-as-yet-cultivated' bacteria. How should 'not-as-yet-uncultivated' bacteria be determined – peer-review of manuscripts submitted for publication in IJSEM?

February 28 Edward Moore, ICSP delegate, University of Gothenburg, Gothenburg, Sweden

I further support the request of Brian Tindall for an up-date on the ICSP Delegates list. Additionally, considering the importance of the proposed rules changes and how controversial they are, evidently, I propose that the Minutes of the online discussion for January and February be compiled as pdf files at the closed of the Discussion phase of the proposals (as Barny Whitman has done for the comments from Jan. 05 to Feb. 25) and send to the contacts for all listed national societies. That gives the chance for the national societies to consider the arguments (pro and con) and to arrive to a consensus opinion. If those representatives or the societies do not want to address the issues, that will be fine. But, at least, it will give them a chance to consider the proposals and the implications and to designate a Delegate to the ICSP to make their vote.

I will send the compiled comments to the microbiological societies in Sweden and compile responses in preparation for my vote as the ICSP Delegate for the Swedish SFM.

February 28 George M Garrity, Michigan State University, East Lansing, Michigan USA

Interesting, but what if the delegate fails to notify the member society of issues on which a vote of some significance is pending? Also, what happens if the member society ceases to exist?

February 28 Marco Riojas,

In the interest of fairness, I think it may also be valuable to review the status of the full member societies granted a voting delegate to the ICSP.

IUMS indicates that "Membership... is open to societies or institutions [formatted as list for clarity]

- 1. having major interests and activities in microbiology,
- 2. having a membership of at least 20 microbiologists, and
- 3. holding at least one open scientific meeting a year."

February 28

Brian J. Tindall, Member of Judicial Commission, Leibniz-Institut DSMZ-Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, Braunschweig Germany

Article 2 of the ICSP statutes states:

Full Members. Each Member Society of the BAM Division of the IUMS is entitled to appoint one Full Member to the ICSP by means of a letter to the Executive Secretary of the ICSP. Full

Members may be appointed at any time. A Member Society is not obliged to appoint a member, and such positions will be considered to be vacant until a member is appointed.

1. The tenure of a Full Member is for one three-year term. A term begins upon appointment by a Member Society and ends either upon appointment of another Full Member by the Member Society or on 1 April 2020, or every three years thereafter. Full Members shall be eligible for reappointment by his or her Member Society for an indefinite period.

Certainly one problem is that the IUMS website lists societies that are no longer members, organizations that either have not existed for 5 years or more and organizations that have since been re-organized and re-named. Perhaps some are even missing. A very difficult area to establish a fundamental aspect of Article 2 of the ICSP statutes. However, my experience is that the IUMS secretary-general is always very helpful, providing accurate information. We did have a case of someone sitting in on meetings as a representative of an IUMS member society when the UK Company House records listed the society as having been previously dissolved months before. IUMS also had no record of that society ever having paid membership in the years previous. Strange things have happened, although one would not expect that to be the case.

Article 3

Functions of the ICSP

a) To represent the diversity of interests of different microbiological disciplines on matters concerning the nomenclature of prokaryotes.

Different microbiological disciplines seems to sum up quite well the present e-mail list, although only a small minority can vote. However, I am sure that one can one rely on the ICSP membership to represent their interests.

Article 18

Officers of the ICSP, including members of the Executive Board, the Judicial Commission, and the Subcommittees of Taxonomy may be removed from office by a vote of the members of the ICSP. Reasons for removal from office include failure to perform the responsibilities of the office, using the office for purposes other than those specified in the Statutes, and violation of the conflict of interest statement.

I routinely publish a "conflict of interest statement" on all my publications to avoid any misunderstandings, but with the introduction of the "conflict of interest statement" in the last version of the statutes no one has ever asked me for such a statement. I did ask the Judicial Commission for such a statement some years back and if I recall got only two replies. Mine you can extract from the end of my publications.

Prof. Oren's document is very explicit, but is not on the member society letterhead as I would expect from the wording of Article 2. That would clearly authenticate the document - nothing is to be implied or inferred from this observation. What would happen if someone claimed to represent the U K Federation for Culture Collections (hasn't existed for say 10 years), state that they have a new secretary, since we all know that Dr. Fergus Priest sadly passed away and send a delegate to the ICSP?

My original request seemed reasonable.

February 28

Iain Sutcliffe, Chair of the ICSP, Northumbria University, Newcastle upon Tyne, U.K.

At the present time, the vote will necessarily proceed on the basis of the current membership of the ICSP.

As we have only recently revised and approved our statutes (see <u>https://www.microbiologyresearch.org/content/journal/ijsem/10.1099/ijsem.0.003117</u>), we have a clear process by which delegates are appointed. If a member is delegated, we have to accept them, though I will check that all delegates represent organisations listed at <u>https://www.iums.org/index.php/regular-iums-members</u>.

Clearly there are some problems in terms of representation (for example there is only one delegate from China despite the huge volume of taxonomic activity there). However, reviewing how members are appointed and representation of different 'user groups' would (if they are so minded) be a task for the incoming Executive Board, which is due to be elected by September 1 2020, once the ICSP membership is itself reconstituted on 1 April 2020.

February 28 Edward Moore, ICSP delegate, University of Gothenburg, Gothenburg, Sweden

I address further, a specific point of **Proposal 1** of the proposed modifications (underlined):

Rule 30.3.c. [new rule] When a sequence is the type, the accession number in a publically available database or the sequence must be given. It is recommended that, when possible, a sample of the DNA be deposited in at least two publically accessible service collections in different countries and the catalog numbers be indicated.

The proposal for "... a sample of the DNA be deposited in at least two publically accessible service collections in ..." has been made.

Please note: When strain Collections start to run low on the number of ampoules or vials of preserved biomass of particular bacterial strains, they will typically sacrifice a sample of the strain to rejuvenate the culture. From the cultures, they will carry out QC and prepare new stocks of the preserved strain to make available to the scientific community.

If we adopt the proposed rules changes on allowing WGS Digital Sequence Information or "... when possible, ..." samples of genomic DNA to serve as the type material for new taxa and no biological strain material is deposited, how will we obtain new genomic DNA after the deposited sample of DNA runs out?

Again, this goes to the issue of maintaining the reference material for access by the scientific community. I see no solution to this potential problem if the proposed rules changes are adopted.

February 28 Marco Riojas and Manzour H. Hazbón, American Type Culture Collection, Manassas VA USA

[The following statement has been edited to 500 words. The full statement is available at: http://www.goeker.org/icsp/]

The proposed changes to the ICNP would greatly harm culture collections and the scientific community. They contain numerous flaws, and their incorporation will have many unintended consequences.

<u>Proposal 1</u>

Allowing DNA sequences as types minimizes the importance of viable biological specimens and reduces biology to sets of very limited data. The full scope of an organism's complex, unpredictable biology cannot be extrapolated from its genome. Post-translational modifications, epigenetics, metabolome, the phenotypic/functional consequences of the interaction between complex biochemical pathways – these characteristics would all be lost under the current proposals. Logistically, DNA sequences in databases are frequently updated/deaccessioned. Assignment of a genetic accession or set of accessions would inevitably result in conflicts where a type sequence was no longer available, leading to systematic chaos. The ICNP nomenclatural system and the taxonomy that derives from its governance of validly published taxa require stability greater than current DNA databases can provide. Acceptance of the proposal would do irreparable harm to prokaryotic systematics. The nomenclatural type of species and subspecies must remain the type strain.

Proposal 2

The scientific arguments against Proposal 2 mirror those against Proposal 1. The full scope of an organism's biology cannot be captured by any specific methodology. However, Proposal 2 suffers from an additional mistake that makes it unworkable and unimplementable. The proposed text states that new <u>methods</u> may serve as types. A method is a procedure that is performed, resulting in collection of scientific data. Microscopy and WGS are methods; microscopic observations and genomic sequence are the data collected through these methods. While the proposal's presumed intention is that such <u>data</u> may serve as type in the future, the text that has been submitted to the ICSP for a vote is logically flawed and cannot be implemented into the ICNP. At a minimum, this proposal should be rejected in the current vote, and properly constructed text should be submitted as a future proposed change.

Proposal 3

This proposal contradicts the taxonomic system universally used today. A genus is composed of a species or a group of defined and related species. Creating genera without species is illogical and serves no practical purpose. This would create more confusion among scientists, as the definition of a genus is already at best loosely defined.

Proposal 4

The current system of nomenclature already allows the description of publication of organisms that are not culturable as *Candidatus* taxa. The only element missing from the *Candidatus* system is nomenclatural priority. However, granting *Candidatus* organisms such priority would inevitably degrade the quality of science performed around these taxa. Rather than requiring that these organisms be properly isolated and cultured, this proposal would encourage researchers to shortcut the proper characterization of these taxa.

The value of genomics cannot be overstated. However, this is not the issue currently under consideration. These methods and the data generated should not be conflated with the necessity or appropriateness for them to serve as nomenclatural types. The proposed changes to the ICNP are scientifically misguided and logistically unworkable and should be rejected.

Dr. Johannes Elias, Institute of Microbiology, DRK Kliniken Berlin

While the proposed acceptance of genomic sequences as type material is commendable, further clarifications seem necessary to address valid concerns. Particularly point 2 in the replique of Bisgaard et al (2020) regarding the quality of DNA sequences deserves further attention.

Specifically, the following questions need to be elaborated on:

What minimal proportion of the sequenced genome is to be accepted as type material? Clearly, multi-locus exceed the ability of single-locus approaches in separating taxa (Sheppard et al, 2008; Bennett et al, 2007).

How is the term "unambiguously" in proposed Rule 18a (3) defined? What minimal quality criteria have to be met to "unambiguously" identify a species? In addition to minimal genomic proportion above, are genomes assembled from short reads to be complemented by long-read (i.e. 3rd generation) methods?

References:

Bennett JS, Jolley KA, Sparling PF, Saunders NJ, Hart CA, Feavers IM, Maiden MC. Species status of *Neisseria gonorrhoeae*: evolutionary and epidemiological inferences from multilocus sequence typing. BMC Biol. 2007 Sep 7;5:35.

Bisgaard M, Christensen H, Clermont D, Dijkshoorn L, Janda JM, Moore ERB, Nemec A, Nørskov-Lauritsen N, Overmann J, Reubsaet FAG. The use of genomic DNA sequences as type material for valid publication of bacterial species names will have severe implications for clinical microbiology and related disciplines. Diagn Microbiol Infect Dis. 2019 Sep;95(1):102-103.

Sheppard SK, McCarthy ND, Falush D, Maiden MC. Convergence of *Campylobacter* species: implications for bacterial evolution. Science. 2008 Apr 11;320(5873):237-9.

February 28 Iain Sutcliffe, Chair of the ICSP, Northumbria University, Newcastle upon Tyne, U.K.

It has been what could comfortably be described as a "bit of day" regarding the extensive correspondence within this email thread. I regret that matters of 'process' rather than scientific discussion have become muddled together and I wish to clearly disaggregate the two by addressing the former.

Yesterday the Executive Board of the ICSP had an online meeting in which there was good agreement that the level and volume of scientific discussion had been very encouraging and that the matters at hand had reached a varied audience well beyond the usual reach of ICSP business. No doubt debate will continue is the next month.

As clear opinions have been expressed, at some considerable length, from a range of sources, I believe that all the voting members of the ICSP must be fully aware of the significance of the matters at hand and the arguments for/against.

In view of this I see no reason to suspend the vote, which I repeat is being conducted fully in line with our procedures as outlined in our statutes. Any ICSP member who believes that the arguments in favour of the proposals under consideration are not yet convincing (or that the wording of any of the proposals is problematic) is able to express this concern by simply voting against the proposals to which they object.

Please also note that the four proposals as articulated on 5th Jan will be voted on separately so it is not 'all or nothing' – for example someone may be in favour of proposal 1 but still able to vote against proposal 2.

February 28 Edward Moore, ICSP delegate, University of Gothenburg, Gothenburg, Sweden

Dear Colleagues of the ICSP, the ICSP EB and the JC,

I hope that you all take my next suggestion in the manner in which it is offered, i.e., as an honest assessment and not a 'political' move. That is, my assessment is that we (ICSP) are not ready to consider the proposed rules changes to The Code at this time.

It was my argument to lain Suttcliffe at the beginning of this Discussion that 2 months was not enough time to get the information out to the microbiological community and to consider the implications of the proposals, if adopted. Iain disagreed with me and I disagreed with him. Impasse.

It occurred to me also that during the Discussion, there were at least, 2 different debates ongoing. Proponents of the rules changes argue issues of identification and classification. Opponents of the rules changes argue issues of nomenclature and classification. And, opponents of the rules changes argue for comprehensive (remember 'polyphasic') characterisations. The proponents of the rules changes seem to accept that comprehensive characterisation is not necessary if WGS DSI is available.

Proponents of the rules changes cannot believe that WGS data provides all relevant information for characterisation of a taxon. For that, you must disregard phenotypic features of a taxon; you must discount epigenetics effects; DNA methylation effects; post-transcriptional and post-translational modification effects, etc.

You must also be satisfied with not having the reference strains for *in vivo* analyses of virulence factors, vaccine development, production of toxins, secondary metabolites, fatty acids and other components of cell membranes, etc. Do proponents of the new rules propose that these, and more, essential features are insignificant, even though we know that natural selection acts on the phenotype, not the genotype or the genome? Thus, it boils down to a debate about identification (proponents) vs. characterisation (opponents).

OK. Nowhere do I find where the proponents of the new rules address concerns about maintenance of biological reference material as nomenclatural types, although that concern has been raised numerous times!

As Brian Tindall (Feb. 02), George Garrity (Feb. 27) and others pointed out, the proposals do not consider correctly General Consideration 4 and Principle 1 of the ICNP.

Finally, the wording of the proposals should be considered before actual voting – for example:

Proposal 2 (Whitman 2016).

Rule 18a (3). [second section] <u>As new methods are developed, they may serve as the type</u> material so long as they unambiguously identify the species or subspecies and can be readily <u>archived and compared.</u>

This makes no sense; it is clear that "... new methods ..." cannot "... serve as ... type material ...". We can assume what is meant but, it certainly is not ready to vote on.

Therefore, I – as a Delegate on the ICSP – move that these proposals for changing The Code be tabled at this time. Delaying the vote would allow proponents of the new rules to re-consider the rules and the wording of the proposals. Delaying the vote would allow for these proposals to be presented to Societies' members for consideration and debate – e.g., at annual meetings held this year. Furthermore, delaying the vote would allow the proposals to be presented and debated at the IUMS Congress in Korea in October, with a Discussion phase after the Congress.

Regardless of the ICSP rules of procedure, I think my proposed option to table the vote of the rules changes to The Code needs to be considered.

February 29

Brian J. Tindall, Member of Judicial Commission, Leibniz-Institut DSMZ-Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, Braunschweig Germany

Given the importance of genome sequencing in modern biology there is clearly a need to address the issue of how to best deal with the accumulating data. In many cases I am confident that scientists actively working in this field and also in dialogue with biologists in other areas will form themselves into multi-institutional, multi-disciplinary teams. Since much of this will take place in the laboratories/in silico one should not be concerned about the future of the biological sciences. See Mayr "The growth of biological thought" or Hull "Science as a process" for a past evaluation.

The present proposals to change the Code seek to rectify a number of problems, many of which lie in the way data are collected, their reliability and the resulting interpretation. Both these areas lie outside of the jurisdiction of the Code, although there is a clear need to address these issues and it is here that journals and the scientific community have a role to play. The impression has been given that by not making changes to the Code that attempt to address these issues will reflect badly on the ICSP. This is not the case, since by recognising the role of the Code in governing only nomenclature the ICSP would also be recognising its neutrality with regards the data collected and how that data is used in a classification. At the same time many of the proposals to improve the quality and scope of the data collected should be addressed and implemented where possible.

The current proposals to change the Code are problematic for a number of fairly simple reasons.

1) genome sequences as alluded to in the proposals are "digital sequence information", are certainly not a physical object and are not "material".

2) "digital sequence information" is essentially a string of letters that reflect the IUPAC coding for bases GCGGTTAGCCCTTTGA. As such this describes what has been observed experimentally on the genetic information encoded on the genome ie is effectively a description.

3) the use of the term "unambiguously identifies" takes the Code into the realms of evaluating the significance or value of a particular data set that also lies outside of a Code that deals solely with nomenclature.

4) proposals to make methods the nomenclatural type are clearly a misunderstanding and have no place in the Code.

5) the deposition of DNA in culture collections, even if it can be replicated in a foreign source constitutes " a dead preserved specimen".

Nomenclature is a part of taxonomy and taxonomy part of systematics. Systematics concerns itself with biology and is neither limited to either phenotypic data or genomic data. The genome encodes for everything that is (potentially) expressed (the phenotype in the wisest sense) and ensures the transfer of vital information from generation to generation, but it is the phenotype on which the living, cellular entity ultimately relies.

February 29 Vikas Gautam, WHO Surveillance Centre for VPD (Bacterial), PGIMER, Chandigarh, India

As a researcher, I've also named a new genomospecies and encountered few issues during publication.

I am contacting you to notify you about possible changes to the rules of microbial nomenclature that might affect the operations of culture collections worldwide. In case some of your colleagues may better be suited to deal with this issue please accept my apologies for bothering you and feel free to forward this message to them.

Valid publication of names of species and subspecies of Archaea and Bacteria currently requires the deposit of the type strain in at least two culture collections in two distinct countries. The proposal to modify the International Code of Nomenclature of Prokaryotes (ICNP) to permit genome sequences as types instead may dramatically reduce the incentive for researchers to deposit material in collections. It may be required to deposit in one country only (may be own Nation of discovery)

Evidence indicates that most microbiologists, even those dealing with taxonomy and nomenclature, are unaware of the forthcoming ballot by the International Committee on Systematics of Prokaryotes (ICSP) about this proposal. I think whatever your opinion regarding this issue, we all should agree that it must be avoided to let a decision of far-reaching consequences be made by a handful of people without a proper representation of the affected scientific communities.

March 1 Barry Bochner, Biolog USA

I tend to agree with comments by Nemec and the culture collections (DSMZ, ATCC). After skimming through the posted comments, I think a reasonable compromise is that bacterial strains inferred by sequencing should have their own valid taxonomy and taxonomic tree. Instead of being granted species status and a Latin name, the "inferred by sequencing" entities could be given a unique number. That should allow both approaches to move forward unhindered. Culture, deposit, sequencing, and phenotyping of type strains Is a preferred and substantially better option, when that option is available. At some point in the future it will probably become possible to easily synthesize whole genomes and to accurately predict the physiology and metabolism of a bacterium from its genome sequence, but we are still years or decades away from having those capabilities.

March 1 William B Whitman, ICSP Delegate, University of Georgia, Athens USA

I would like to reiterate a point I made on January 17 that seems to have been lost during the debate. While proposal 1 allows DNA sequences to serve as type, it also states that "Whenever possible, the type of a species or subspecies is a designated strain." Thus, it is reserved for special circumstances. To address the issue of reproducibility, it also states that "It is recommended that, when possible, a sample of the DNA be deposited in at least two publically accessible service collections."

Likewise, there has been some concern that phrases "whenever possible" and "it is recommend" are too ambiguous. However, the Code has to cover a large number of different circumstances, and it has never specified the technical details of the description. The Code only specifies nomenclature. The description is the responsibility of the authors and reviewers.

Claims that these proposals will lead to chaos assume that many of our colleagues will use them to avoid the verification system for cultures that is currently in place. In my experience, most of our colleagues are doing their best, and I believe that this claim is overstated. Occasionally, we all make mistakes, but names based upon erroneous types do not have to used. In this regard, it does not matter if the type is a strain or a sequence.

Perhaps most importantly, many of the comments against the current proposals fail to address the important issue of the large number of well-characterized and cultured organisms whose names cannot be validly published because the culture collections are either unable or unwilling to take them into their collections. The example of SAR11 raised by Joachim Wink [February 8] illustrates this point well. It is arguably the most abundant bacterial species on earth. It was first cultivated in 2002. Its genome has been sequenced, and many of its physiological properties are well described. Nevertheless, it has remained *candidatus* because of the failure of culture collections to accession it. Because *candidatus* names are outside the Code, its proposed name lacks priority, and its nomenclature is unstable. There is really no justification for this sorry state of affairs.

In conclusion, the requirement of deposited type strains works well for some prokaryotes but not all. The current proposals do not prevent the deposition of type strains. However, it makes the Code accessible to all microbiologists.

March 1 Edward Moore, ICSP delegate, University of Gothenburg, Gothenburg, Sweden

A last comment for the February Minutes.

During the last two months, in private e-mails about the proposed rules changes, I have been accused at least twice, directly, of having bias against the rules changes because I am affiliated with a Strain/Culture Collection – the CCUG is one of the largest Collections with focus on strains of clinical relevance (pathogens, etc.) in Europe. Further, it was pointed out to me that people working in Collections need to find other ways of working, rather than relying on 'collecting strains', like so many butterfly collectors.

Evidently, Collections are seen as forcing our services on poor microbiologists to deposit their strains and carry out unnecessary analyses! Fyi: The CCUG has sold less than 50% of the Type strains that we have accepted from researchers depositing their strains for publication purposes. For every Type strain that a Collection receives and never distributes, the costs associated with accession are not recovered. However, the CCUG and, I think, other Collections of ECCO (Feb. 27) and USCCN (Feb. 27) consider their work to be a service.

The CCUG has almost 75,000 strain entries, approx. 75% were isolated from clinical samples. Among the nearly 75,000 strains, 3,791 are Type strains (5%); 812 of the Type strains do not yet have determined WGS (that is being addressed through on-going WGS projects). During 2019, CCUG sold, donated or otherwise distributed 2,032 strains to the scientific and industrial and educational communities. Of 2,032 strains distributed, 846 (42%) were Type strains; of which, 219 had WGS data in public databases. The Type strain of *E. coli* (CCUG 24T) was ordered more than 10 times – the WGS data for that strain has been in the databases for more than 20 years. And, yet, researchers still order the strain for their research.

If we had been operating under the proposed rules changes, no strain material would be available for the researchers who ordered the Type strains – even those with publicly-available WGS. Presumably, the researchers who ordered strains with available WGS did so because the information in the WGS DSI, alone, was not adequate for their research. The CCUG is providing strains to researchers for various types of basic research, to industry for commercial development including new vaccines, to educational institutes for teaching purposes; a varied range of applications – all of which require reference strain biomaterial.

I point out finally that the IJSEM has required WGS data on Type strains for valid publications of names to be deposited in public databases only for approx. one year. ALL of the WGS data available in the databases to date are there ONLY because the Type strain biomaterial was available for the sequencers.

So, think carefully about the implications of removing the requirement for deposit of Type strains to be made available for future researchers and applications. Researchers working with MAGS need a different – probably, bar-coding or other numerical system. They should not be trying to force their 21st century data into an 18th century binomial classification system – do you really want to assign 4,000 new binomial names for every gut or soil microflora study? The arguments

of proponents of the rules changes are all about identification and classification – not the impact on nomenclature.

Thanks for your consideration – Happy Leap-Day!

March 1 Markus Göker, Leibniz Institute DSMZ -- German Collection of Microorganisms and Cell Cultures, Braunschweig, Germany

[In response to comments of Whitman on March 1]

Awaiting editing for length

March 1 İpek Kurtböke, University of Sunshine Coast, Australia

In the case of a contaminated sample resulting in wrong sequence consequences can be serious. Molecular data only should only apply to "unculturables". All validly described species should be available physically.

March 2 William B Whitman, ICSP Delegate, University of Georgia, Athens USA

[In response to the comments of Ipek Kurtböke of March 1]

We largely agree, but I would stipulate that the 'uncollectable' is a better term than 'unculturable'. This would include prokaryotes that have been cultured by not accessioned by culture collections in addition to the truly uncultured. In fact, this is what proposal 1 says.

The problem with excluding the uncollectables from the Code is that their names have no standing and no priority. The ability of culture collections to accession an organism should not be the defining criterion for it to be validly named.

March 2 Aharon Oren, ICSP delegate, The Hebrew University of Jerusalem, Jerusalem, Israel

[In response to comments of Whitman on March 1]

The name *Pelagibacter ubique* was not validly published and cannot be validly published as '*ubique*' is an adverb and not an adjective or a substantive (noun), and therefore it does not quality as a specific epithet according to Rule 12c of the ICNP.

March 2

John Hays, Medical Microbiology & Infectious Diseases, Erasmus University Medical Center Rotterdam

There appears to be a lot of international interest in this subject. Is it worthwhile thinking about publishing a Position Paper on this subject and the replies received?

[On March 2, positive responses for this idea were received from Jörg Overmann, Brian Tindall, Ulrich Nübel, Marco Riojas, and Jean Swings]

March 2 Jean Swings, Laboratory for Microbiology, University of Gent, Belgium

Although I have no vote in the ongoing discussion, I have read the comments with great interest. The summary provided by Markus Göker is very useful. It is healthy to have such a discussion because as in every scientific discipline, taxonomy and its nomenclature needs principles, standards, rules and regulations.

Although the Code is about nomenclature , we all know that it has a strong implicit impact on taxonomy/systematics . It is not "neutral". This is certainly the case for non- taxonomists among microbiologists, but the elements brought into the debate prove that this is also the case for taxonomists, as summarized yesterday by Ed Moore.

For our eminent predecessors, the genome sequence was the unreachable holy grail. Their physicochemical DNA and RNA hybridizations introduced completely new approaches in taxonomy and phylogeny and attracted us as young scientists. Nowadays the holy grail lies in our hands. I agree with Brian Tindall that the best future scenario for taxonomy/systematics (including nomenclature) is as an attractive scientific field with strong relevant links to genomics, bio-computing, microbial ecology, population genetics and evolution instead of being reduced to the strict application of a number of calculations with pre-set values, principles and rules . Above all should it provide a practical guide to all its users.

In our discipline we need talented young scientists, but they will not be necessarily be stimulated by Byzantine and semi-legalistic discussions on nomenclature and procedures. It is clear to me that a type culture plus its high quality complete genome sequence is the gold standard in taxonomy and nomenclature. Maybe taxonomy (including nomenclature) needs to be better adapted into a tiered Mendeleev-like system where all cultures plus all complete genome sequences get their place and recognition in order to serve the community? This discussion and this vote is maybe not ripe as stated by Ed Moore.

March 2 İpek Kurtböke, University of Sunshine Coast, Australia

I fully agree with Prof. Swings. "Type culture plus its high quality complete genome sequence is the gold standard in taxonomy and nomenclature" is the way to go.

March 2 George M Garrity, Michigan State University, East Lansing, Michigan USA

There have been a number of papers arguing on behalf of the proposal published in the literature already, largely by Whitman and colleagues. However, publication of rebuttals are fewer in number and length. In one instance, the discussion was curtailed by the publisher while allowing for a second publication in favor to appear in response to the rebuttal. Selecting a venue for such any publication will be important to ensure editorial neutrality.

This is a far reaching issue with significant ramifications. There are legal and social issues that warrant further careful consideration. Biological nomenclature plays an important role in decision making in a wide range of activities outside of systematics (eg. medicine, public health and safety, law, commerce). The views of those communities deserve to be heard and documented without rebuttal from the advocates of Whitman's proposal(s).

In my opinion, the prudent approach would be a collection of position papers that would fully explore all of the issues that will affect not only taxonomists, but all of the affected parties. As suggested by Ed Moore, the issue be discussed/debated at the annual meetings of microbiological societies this year (and perhaps next given the potential effect of coronavirus pandemic on scientific meetings).

Whatever the outcome, it should be a decision that comes from informed stakeholders that are fully informed of all of the issues.