Minutes of the online meeting of the International Committee on Systematics of Prokaryotes [DRAFT of February 25, 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 5<sup>th</sup>: This is now a 'designated' schedule i.e. voting by ICSP members will begin on March 1<sup>st</sup> and close on 31<sup>st</sup>.

Secondly, regarding the timeline and current 'window' for discussion. It is important to stress that the Whitman (2016) proposals were published online on 1<sup>st</sup> 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> taxonomic group and is a designated strain or other material. 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> <u>When possible, it should be a high quality draft or better</u> <u>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(e1900v1)</u>. 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)
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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

In this email 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 anm 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*.<sup>1</sup> 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 Comments of 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)].<sup>1</sup> 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 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 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 xxnnnnn.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>