Microbial taxonomy run amok

Robert A. Sanford[1], Karen G. Lloyd[2], Konstantinos T. Konstantinidis[3], and Frank E. Löffler*[4,5]

[1] Department of Geology, University of Illinois at Urbana-Champaign, Urbana, IL 61801, USA

[2] Department of Microbiology, University of Tennessee, Knoxville, TN 37996, USA

[3] School of Civil and Environmental Engineering & School of Biological Sciences, Georgia Institute of Technology, Atlanta, GA 30332, USA

[4] Center for Environmental Biotechnology, Department of Microbiology, Department of Civil and Environmental Engineering, Department of Biosystems Engineering and Soil Science, University of Tennessee, Knoxville, TN 37996, USA

[5] Biosciences Division, Oak Ridge National Laboratory, Oak Ridge, TN 37831, USA

*Correspondence:

frank.loeffler@utk.edu (F.E. Löffler) & rsanford@illinois.edu (R.A. Sanford)

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Abstract

DNA sequencing has led to an explosion in discovery of microbial phylogenetic novelty, to which the traditional system of prokaryotic taxonomy has not yet adapted. A lack of expansion of the International Code of Nomenclature of Prokaryotes (ICNP) to effectively capture this information has created a "wild west" situation where non-validated names are published or appear in taxonomic databases. The rapid propagation of variant and questionable naming methods has led to widespread confusion and undermines prior accomplishments. We exemplify inconsistencies that have arisen from this practice that endanger the interoperability of scientific findings. The immediate solution for this problem is to adopt universal best practices that are accepted by expert researchers, major publishers, international microbiological societies, and the ICNP.

Main text

Scientific inquiry in the microbial sciences relies frequently on interpreting an enormous amount of DNA and RNA sequence information [1, 2]. One of the most significant research areas that has blossomed due to next generation sequencing technologies is the study of diverse natural microbial communities. The large sequence datasets can be assembled and then binned using bioinformatic techniques into **metagenome-assembled genomes** (MAGs) [3], or **single cell amplified genomes** (SAGs) generated from microbial cells physically separated from their environment [4]. A meaningful analysis of sequence datasets requires determining the taxonomic composition of the genomes of the sample under study. To make taxonomic assignments, the analyses of these datasets either focus on a single **phylogenetic marker** (i.e., the 16S rRNA gene) or a suite of universally conserved genes with taxonomic information [5]. Curated nucleic

acid **sequence databases** have been developed for this purpose such as the Ribosomal Database Project (RDP) [6], Greengenes [7], and SILVA for 16S rRNA genes [8], and the Genome Taxonomy Database (GTDB) [9] and the National Center for Biotechnology Information (NCBI) taxonomy [10, 11] for sequenced genomes, MAGs and SAGs. The RDP assignments are primarily based on the available taxonomy of 16S rRNA gene sequences representing cultured organisms following taxonomic roadmaps outlined in Bergey's Manual of Systematics of Archaea and Bacteria [12], and secondarily, a few well-studied uncultured groups. The SILVA database and the GTDB include taxonomic names proposed by individual researchers for DNA sequences of uncultured taxa to stay current with the literature.

Traditional prokaryotic **taxonomy** relies on cultivation and in-depth characterization of **axenic cultures**, and the International Code of Nomenclature of Prokaryotes (ICNP) established rigorous recommendations for validly publishing new or revising existing **nomenclature** [13, 14]. Over the past decades, this system of rules has built a robust taxonomic framework facilitating research, communication, and policy around the globe. This traditional system is challenged to accommodate an increasing number of sequences that lack cultured representatives, and names that are coined outside the bounds of the code established by the ICNP [15, 16]. Naming **uncultured (uncultivated) taxa** is justified, since the sequences belong to real organisms that must be categorized and named in order to be studied efficiently.

MAGs and 16S rRNA gene amplicons representing uncultured taxa often dominate both the diversity and abundance of microbial cells in environmental samples [1, 2, 17]. Furthermore, a number of organisms can be grown in the laboratory but are either fastidious such as the highly abundant ocean microbe SAR11 [18] or depend on intricate syntrophic interactions such as the

anaerobic methanotrophs ANME-1 [19]. Even though these organisms are technically cultured, they do not meet the strict requirements of facile manipulation in axenic culture required for official naming status by the ICNP [13]. Recently, the ICNP voted not to include genetic material from uncultured or non-axenic organisms as type material, which is a prerequisite for validly naming such taxa based on the well-established and rigid ICNP nomenclature rules that anchor microbial taxonomy. Hence, ***Candidatus*** names, which are often given to such uncultured or non-axenic taxa, remain provisional, are not regulated (and thus, often do not comply with naming rules), and could be overwritten when somebody validly publishes a name for a representative of the taxon based on an isolate [20]. This lack of inclusion could result in less centralized approaches for developing a robust nomenclature for uncultured organisms. Proposals for centralization have been put forth [15, 16, 21, 22], but none of them has yet been adopted, leaving current research in limbo between competing nomenclatures.

The GTDB has developed a new taxonomy based on relative evolutionary distance (RED) scores of high-quality genomes, with the main goal of establishing the same ranks across taxa to encompass similar intra-rank evolutionary diversity [9]. The SILVA database has partially incorporated the GTDB nomenclature, especially at the higher ranks (e.g., phylum and class levels), and has included additional new taxon names not found in the published literature. Unfortunately, these efforts to remain up to date with independently generated nomenclatures for uncultured or non-axenic cultures do not follow the rigorous ICNP rules and increasingly impact data analysis and data interpretation of cultured organisms, for which a nomenclature was already established. Consequently, confusion among researchers at a level that will damage research progress around the globe is emerging. As a representative example, GTDB proposes

that *Shigella flexneri* is renamed as *Escherichia flexneri* because the divergence of *S. flexneri* from typical *E. coli* genomes is not high enough to justify a separate genus description. This change of established nomenclature will impact clinical practice and effective communication among researchers worldwide. RED scores are one aspect of taxonomic classification but should not be used as the sole criterion for (re)classification. Perhaps more important, name changes with far-reaching implications should only be made in consultation with experts working with these organisms. We provide selected examples below, further illustrating how sequence data analysis using the current database resources generates results, even for cultured organisms, that are not supported by the published taxonomy or the scientific understanding of the biology of the organisms under study.

For taxonomic classification of datasets that comprise sequences of uncultured, or, better, **not-yet-cultured organisms**, or of organisms that can be maintained in the laboratory but no isolates (i.e., axenic cultures) are available, the SILVA SSU rRNA gene database 138 (released December 16, 2019) is the preferred resource for many researchers analyzing sequence data. SILVA stays at the cutting edge of the (un)official nomenclature and states that it is a "comprehensive on-line resource for quality checked and aligned ribosomal RNA sequence data" (www.arb-silva.de). Greengenes utilizes nomenclature proposed from phylogenetic methods applied prior to 2013 and the RDP has not been updated since 2016 (although a new release is forthcoming). Therefore, both databases do not include more recently proposed and/or validated names at the time of this writing and are therefore of limited utility for taxonomic analysis of environmental samples.

SILVA 138 aligns with the GTDB in reporting name changes at higher taxonomic ranks, including the complete reclassification of taxa previously associated with the class *Deltaproteobacteria* (Table 1). This nomenclature deviates from established names, phenotypes, and genotypes in the delineation of names for genera, despite being a lineage with many cultured isolates with validly published names. Following the nomenclature established and approved by the ICNP, the order *Desulfuromonadales* within the *Deltaproteobacteria* comprises the families *Desulfuromonadaceae*, *Pelobacteraceae*, and *Geobacteraceae*, each comprising several well-established genera such as *Geobacter* within the *Geobacteraceae*. Until the most recent release, GTDB conserved existing nomenclature (i.e., family and genus names); however, it distinguished phylogenetically distinct genera of sufficient intra-genus evolutionary diversity into lettered subsets (e.g., *Geobacter-A, Geobacter-B,* etc.). This approach is also applied to higher taxonomic ranks, and such names remained useful as they provided a means to identify taxa as evolutionarily distinct even though they shared the same genus epithet. In contrast, the latest releases of the SILVA 138 database and the GTDB do not follow this logic and both have changed their previously established nomenclature, creating new genus names that have never been published in the peer-reviewed literature. The family *Desulfuromonadaceae* now comprises the genera *Desulfuromonas*, '*Candidatus* Deferrimonas', and "Trichloromonas" ("Thrichloromonas" in GTDB) (Table 1). There is no reference in the peer-reviewed literature to the genera '*Candidatus* Deferrimonas' or "Trichloromonas" at the time of this writing; the only place where these genus names appear is in the SILVA 138 database and in the GTDB. In addition, both of these "new genera" contain previously characterized bacterial isolates, *Desulfuromonas soudanensis* and *Desulfuromonas michiganensis* for '*Candidatus* Deferrimonas' and "Trichloromonas", respectively. The ICNP classifies the family *Desulfuromonadaceae*

within the order *Desulfuromonadales*; however, this rank was eliminated in SILVA 138, for no obvious reason. The GTDB maintained the order *Desulfuromonadales* but included the new family "Trichloromonadaceae", which lacks documentation in the peer-reviewed literature and does not appear in SILVA 138.

Within the new order *Geobacterales*, SILVA and GTDB list two families: the *Geobacteraceae* and the new family "Pseudopelobacteraceae" (Table 1). Associated with the family "Pseudopelobacteraceae" are several validly published *Pelobacter* species, which are now grouped under the new genus "Pseudopelobacter". Another new genus is "Syntrophotalea" in the family *Syntrophotaleaceae*, which also comprises published *Pelobacter* species (Figure 1). Neither of these families or genera have any referenced use in the peer-reviewed literature at the time of this writing. Within the SILVA 138 database, the family *Geobacteraceae* now includes the genera *Geobacter*, "Citrifermentans", "Geotalea", and "Trichlorobacter" (Table 1, Figure 2). The GTDB also adopts these genus names, but places "Trichlorobacter" in the "Pseudopelobacteriaceae" family instead. "Citrifermentans" contains the well characterized species *Geobacter bemidjiensis,* which recently was renamed *Geomonas bemidjiensis* [23]. Of note, the only genera documented in the peer-reviewed literature are *Geomonas, Geobacter* and "Trichlorobacter", but the latter had been reclassified as *Geobacter* [24]. The genus designations "Citrifermentans" and "Geotalea" do not appear in the peer-reviewed literature or in any database other than in SILVA 138 and the latest version of the GTDB, even though two known cultured representatives are assigned to these genera, *Geobacter bemidjiensis* and *Geobacter uraniireducens*. The name "Citrifermentans" may indicate that members of this proposed genus ferment citrate but this cannot be verified because no information is available in the literature, let alone a validly published taxonomic description. Table 1 compares the SILVA, NCBI (also in

RDP), and GTDB nomenclatures of the *Geobacterales* and *Desulfuromonadales*, illustrating that the SILVA 138 database and the GTDB incorporate taxa that do not follow a centralized nomenclature. This issue is not limited to these orders and inconsistent naming occurs in other orders and families listed in both databases.

The ICNP protects the taxonomic framework that has been painstakingly developed by researchers around the globe over the past decades. Deep sequencing will continue to reveal new diversity requiring a rational approach to include sequences without cultured representatives in an overarching taxonomic framework. For the foreseeable future, the discovery of new sequences will continue to outpace the traditional practice of **polyphasic taxonomy**, which generates the problem: researchers need up-to-date databases for sequence analysis, putting database curators under pressure to constantly refine and update the taxonomic framework for the classification. The current ICNP rules for validly publishing names for new taxa cannot accommodate this need as lengthy and labor-intensive experimental efforts are required. Roadmaps have been proposed for naming uncultivated archaea and bacteria, an effort that highlights the need for a sensible path forward to include not-yet-cultured organisms in a taxonomic framework [15, 16, 21, 22]. Clearly, such an effort requires consensus and buy-in from all stakeholders, and solo efforts cannot deliver productive solutions. Changing the established taxonomic framework and nomenclature must follow scientific discourse and rules, including the peer-review process. Curators of online databases are in a position of power because they control a product many researchers around the world demand for data analysis. It would be a disservice to the scientific community at large if the curators do not follow best professional practice, or otherwise risk the constructed taxonomic framework to crumble without

providing the services and standardization the global scientific community is looking for. The taxonomic framework of axenic cultures has been robust and provided invaluable services to the international research community and policy makers, but it is apparent that the system has to adjust in order to continue to fulfill its purpose and stay relevant. Opportunities exist to reconcile fast-paced sequence discovery with a responsive taxonomic framework, and specific plans have been proposed [16]. These efforts must adopt universal best practices that are accepted by the global research community with buy-in from database curators, major publishers, professional and international microbiological societies, and the ICNP.

Although it is generally accepted that a new taxonomic framework is needed to avoid aberrations, reaching consensus among the various stakeholders has shown to be challenging with slow progress. Considering the fast pace at which researchers discover new genetic diversity of microorganisms, slowness is not an option if the goal is to maintain and build a meaningful taxonomic framework. Even without consensus and an agreed upon framework with guidelines and rules, adherence to professional best practices can bridge this current phase of uncertainty and help coalesce the community. A number of points can help to ensure that taxonomy stays relevant and can fulfill its purpose for the global research community, practitioners and policy makers.

- Transparency: A rationale for each name change must be communicated in the peer-reviewed literature. Name changes should have links to the previous nomenclature to allow continuity of research, and ideally, take place in consultation with, or review by, the respective experts.
- The expansion of the existing classification should, to the extent possible, use identifiers while maintaining the original nomenclature.

- New names without track record in the peer-reviewed should not be introduced.
- Naming based on polyphasic taxonomy versus sequence-only based description should be apparent.
- A conservative approach should be applied and the existing polyphasic taxonomy upheld to the extent possible.
- *Candidatus* names should be established for uncultured taxa based on the existing naming guidelines and then be given equal priority to names of validly published isolates. This practice will support a robust taxonomic framework during the current period, in which the sequence-based discovery of microbial phylogenetic novelty outpaces cultivation and polyphasic characterization. Of note, reclassification and name changes are possible should subsequent polyphasic characterization and additional phylogenetic information justify revisions [13].

Although incomplete, this list can guide database curators to implement a best practice approach that should avoid "wild west" conditions before comprehensive reform of the existing ICNP catalog of recommendations (rules) has been accomplished to accommodate the needs of modern research. The expectation is that database curators engage in open dialog with all stakeholders to build a best practice approach that can assist the ICNP in setting up a revised and workable framework.

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Glossary

Axenic culture: A single, defined population growing in the absence of other organisms. Also referred to as isolate or pure culture.

***Candidatus*:** Provisional category to classify not-yet-cultured taxa of any rank.

Classification: The hierarchical grouping of organisms based on phylogenetic and phenotypic data (i.e., the arrangement of organisms into groups [taxa] on the basis of their relationships).

Metagenome-assembled genome (MAG): A single-taxon genome assembly based on DNA-sequence contigs binned from a metagenome.

Nomenclature: The application of a set of rules and conventions that govern the naming of organisms. For bacteria and archaea, the International Code of Nomenclature of Prokaryotes (ICNP) establishes these rules.

Not-yet-cultured organisms: A term recognizing that uncultured taxa (taxon, singular) can potentially be cultivated with sufficient understanding of organismal biology.

Phylogenetic marker: A single gene (e.g., the 16S rRNA gene) or a set of conserved genes used for taxonomic and phylogenetic profiling of a microbiome or an isolate.

Polyphasic taxonomy: A consensus approach that utilizes multiple phenotypic, morphologic and genetic data in order to taxonomically describe taxa.

Sequence databases: Repositories that store and make available digital nucleic acid and/or protein sequences. Databases differ in scope (comprehensive versus specialized), are generally interlinked, and form the basis for big data biology.

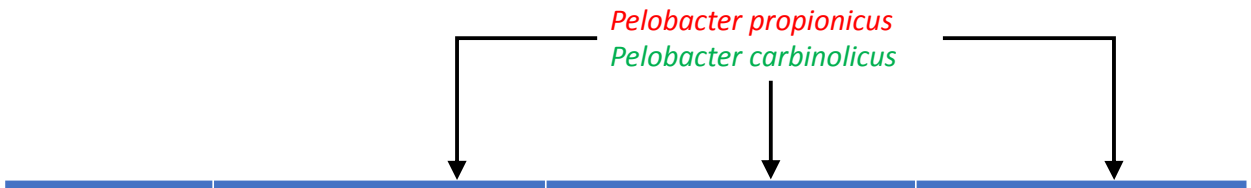
Single cell amplified genome (SAG): Genome information generated from a single cell that has been lysed and its genome amplified and sequenced.

Taxonomy: From Greek, taxis, arrangement, and nomia, method; taxonomy has three pillars, identification, classification and nomenclature, and is the science and practice of identifying and circumscribing, naming, and classifying microorganisms based on genomic and/or phenotypic characteristics.

Uncultured (uncultivated) taxa: Real organisms existing in nature that have so far resisted targeted cultivation *in situ* or in the laboratory (see not-yet-cultured organisms above). Note that "unculturable taxa" is a misnomer because if the organisms exist in nature, conditions allowing their growth exist and they can potentially be cultured.

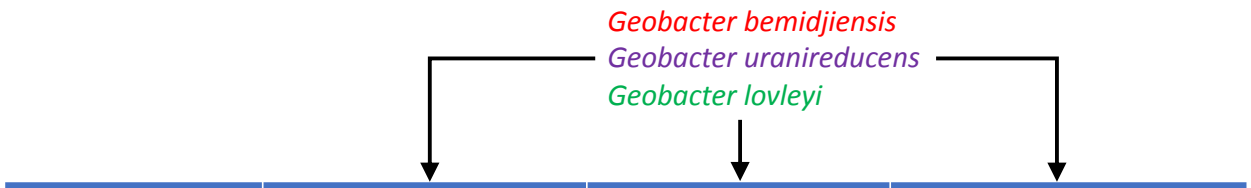
Table 1. Comparison of the taxonomies of the family *Geobacteraceae* between the SILVA SSU rRNA gene 138 database, the RDP 11.5 database and the GTDB (Release 04-RS89). Yellow highlighted cells represent new higher rank names listed in the GTDB. Green highlights indicate new family and genus designations only found in the GTDB and the SILVA 138 database lacking any reference in the peer-reviewed literature. Text in red font indicates new names only found in the GTDB or names that do not match between SILVA and the GTDB. Text in blue font indicates a new published genus name *Geomonas* with no match in the GTDB or the SILVA database, and the asterisk indicates a validly published reclassification of *Geobacter bemidjiensis* [23].

Reference Genome	Database	Phylum	Class	Order	Family	Genus
Geobacter	SILVA	Desulfobacterota	Desulfuromonadia	Geobacterales	Geobacteraceae	Citrifermentans
bemedjiensis	NCBI	Proteobacteria	Deltaproteobacteria	Desulfuromonadales	Geobacteraceae	Geomonas*
	GTDB	Desulfobacterota	Desulfuromonadia	Geobacterales	Geobacteraceae	Citrifermentans
Geobacter	SILVA	Desulfobacterota	Desulfuromonadia	Geobacterales	Geobacteraceae	Geotalea
uraniireducens	NCBI	Proteobacteria	Deltaproteobacteria	Desulfuromonadales	Geobacteraceae	Geobacter
	GTDB	Desulfobacterota	Desulfuromonadia	Geobacterales	Geobacteraceae	Geotalea
Geobacter	SILVA	Desulfobacterota	Desulfuromonadia	Geobacterales	Geobacteraceae	Trichlorobacter
lovleyi	NCBI	Proteobacteria	Deltaproteobacteria	Desulfuromonadales	Geobacteraceae	Geobacter
	GTDB	Desulfobacterota	Desulfuromonadia	Geobacterales	Pseudopelobacteriaceae	Trichlorobacter
Pelobacter	SILVA	Desulfobacterota	Desulfuromonadia	Geobacterales	Pseudopelobacteriaceae	Pseudopelobacter
propionicus	NCBI	Proteobacteria	Deltaproteobacteria	Desulfuromonadales	Desulfuromonadaceae	Pelobacter
	GTDB	Desulfobacterota	Desulfuromonadia	Geobacterales	Pseudopelobacteriaceae	Pseudopelobacter
Pelobacter	SILVA	Desulfobacterota	Desulfuromonadia		Syntrophotaleaceae	Syntrophotalea
carbinolicus	NCBI	Proteobacteria	Deltaproteobacteria	Desulfuromonadales	Desulfuromonadaceae	Pelobacter
	GTDB	Desulfobacterota	Desulfuromonadia	Desulfuromonadales	Syntrophotaleaceae	Syntrophotalea
Desulfuromonas	SILVA	Desulfobacterota	Desulfuromonadia		Desulfuromonadaceae	Trichloromonas
acetexigens	NCBI	Proteobacteria	Deltaproteobacteria	Desulfuromonadales	Desulfuromonadaceae	Desulfuromonas
	GTDB	Desulfobacterota	Desulfuromonadia	Desulfuromonadales	Trichloromonadaceae	Thrichloromonas
Desulfuromonas	SILVA	Desulfobacterota	Desulfuromonadia		Desulfuromonadaceae	*Candidatus* Deferrimonas
soudanensis	NCBI	Proteobacteria	Deltaproteobacteria	Desulfuromonadales	Desulfuromonadaceae	Desulfuromonas
	GTDB	Desulfobacterota	Desulfuromonadia	Desulfuromonadales	Trichloromonadaceae	Deferrimonas



	NCBI	SILVA	GTDB
Phylum	Proteobacteria	Desulfuromonadota	Desulfuromonadota
Class	*Deltaproteobacteria*	*Desulfuromonadia*	*Desulfuromonadia*
Order	Desulfuromonadales	* Desulfuromonadales	Geobacterales Desulfuromonadales
Family	Desulfuromonadaceae	Pseudopelobacteriaceae Syntrophotaleaceae	Pseudopelobacteriaceae Syntrophotaleaceae
Genus	*Pelobacter*	Pseudopelobacter Syntrophotalea	Pseudopelobacter Syntrophotalea

Figure 1. Illustration of different taxonomic assignments of the two well characterized *Pelobacter* species *Pelobacter propionicus* (red font) and *Pelobacter carbinolicus* (green font) in the SILVA database and the GTDB compared to the NCBI taxonomy. The font colors track the name changes in the SILVA database and the GTDB. Highlighted in yellow are the name changes of lower taxonomic ranks (i.e., phylum, class and order) associated with the GTDB and the SILVA database. Note that SILVA has not designated an order for *Pelobacter carbinolicus* (indicated by the asterisk) but has done so for *Pelobacter propionicus.* Both SILVA and the current version of the GTDB use two unpublished family and genus names to accommodate *Pelobacter propionicus* and *Pelobacter carbinolicus*.



	NCBI	SILVA	GTDB
Phylum	Proteobacteria	Desulfuromonadota	Desulfuromonadota
Class	*Deltaproteobacteria*	*Desulfuromonadia*	*Desulfuromonadia*
Order	Desulfuromonadales	Geobacterales	Geobacterales
Family	Geobacteraceae	Geobacteraceae	Geobacteraceae Pseudopelobacteriaceae
Genus	*Geobacter* *	*Citrifermentans* *Geotalea* *Trichlorobacter*	*Citrifermentans* *Geotalea* *Trichlorobacter*

Figure 2. Illustration of different taxonomic assignments of the three well characterized *Geobacter* species *Geobacter bemidjiensis* (red font), *Geobacter uranireducens* (purple font), and *Geobacter lovleyi* (green font) in the SILVA database and the GTDB compared to the NCBI taxonomy. The font color tracks the name changes in the SILVA database and the GTDB. Highlighted in yellow are the name changes of lower taxonomic ranks (i.e., phylum, class and order) associated with the GTDB and the SILVA database. The GTDB places *Geobacter lovleyi* into the Pseudopelobacteriaceae, a family that has not been validly published. The asterisk indicates a validly published reclassification of *Geobacter bemidjiensis* to the new genus *Geomonas* [23]. As a result, four additional genus names are now associated with these three well known *Geobacter* species, but only one of these new genus designations has been validly published.

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Outstanding Questions Box

Is a unified taxonomic framework based on the current practice realistic considering that sequence-based discovery of new phylogenetic diversity will continue to outpace the cultivation of novel taxa?

What immediate steps can be taken to maintain the integrity of prokaryotic taxonomy?

Do opportunities exist to bring together a comprehensive group of international experts, and if not, what mechanisms exist to facilitate such meetings?

Can a committee of experts represent the interests of all stakeholders?