Peter Kampfer and Stefanie Glaeser review prokaryotic taxonomy in the sequencing era with an emphasis on the MLSA in classification. John Bowman reviews proteomic applications in microbial taxonomy while Joachim Wink reveals how taxonomy and natural product research can work together. Artem Men, Kirby Siemering and Susan Forrest visit new toolboxes for microbial systematics taking us to metagenomics and beyond. Johannes Groenewald, Marizeth Groenewald and Pedro Crous tell us novel advancements in fungal and yeast systematics. The expertise then moves on to viruses and looks at the classification and systematics of bacteriophages and viruses with Stephen Abedon, Hans Ackermann and Adrian Gibbs.

An article on the World Federation for Culture Collections (WFCC) is also included in this issue co-authored by Philippe Desmeth and myself, stressing once more the importance of depositing newly described species, and culture collections in microbial systematics. Information on the mission and activities of the WFCC and the World Data Centre of Microorganisms (WDCM; http://new.wfcc.info) dating back to Professor Skerman's days at the University of Queensland in the 1960s is also included. I am hoping that collections and individuals will become members of the Federation from Australia, which also offers the Skerman Award every three years to a microbiologist for a significant contribution to the field of systematics.

I would also like to draw attention to the newly established *Bergey's International Society for Microbial Systematics* (BISMiS) and encourage microbiologists to become a member of the Society (http://www.bergeys.org/index.html).

Finally, I would like to thank all the contributing experts and hope that this issue will further strengthen the importance of microbial systematics in Australia and perhaps encourage young members to define a career path in the field.

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Biography

Dr Kurtböke is currently the Vice-President of the World Federation of Culture Collections (WFCC). She has been working in the field of biodiscovery and has been an active member of the international actinomycete research community since 1982. She is engaged with studies related to actinomycete diversity and taxonomy, particularly uses of actinophages to define suborder boundaries of the order *Actinomycetales.* She currently conducts research and teaches in the field of applied microbiology and biotechnology and is senior lecturer at the University of the Sunshine Coast, Queensland.

Molecular taxonomic parameters

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The parameters in place for the circumscription of taxonomic ranks increase with the description of lower ranks; only one or a few, mostly genomic properties, for phyla, classes and orders, while those for families, genera and, above all, for species, are described with increasing complexity, including molecular, chemotaxonomic, morphological and biochemical properties. Even the attempt to list a few examples for species-rich genera or for a phylogenetically diverse range of taxa would go beyond the scope of this communication. Rather, the presently applied molecular approaches for delineation should be revisited here. For a broad overview on the use of the wide spectrum of phenotypic methods recommended today the reader is referred to a recent publication by Tindall *et al.*[1].

Since the early 1980s 16S rRNA gene sequence identities have been included in the description of mainly higher taxonomic ranks. The phylogenetic superiority of this molecule over other genes to place an organism next to its nearest neighbour has been well covered in the literature[2]. The primary structure of this gene, however, is too conservative to differentiate among strains of species as well as among closely related species (for

example, see certain members of the genus *Aeromonas*[3]). Specific proteins[4,5] as well as multilocus sequence analysis (MLSA) of housekeeping genes are increasingly used to obtain a refined picture of relatedness where 16S rRNA gene analysis fails[6]. Either concatenated or individually, phylogenetic analyses between three and ten of such orthologous genes contribute significantly to the clarification of membership of strains and species to species and genus, respectively. The increasing availability of draft and complete genome sequences will facilitate the search for such genes, and especially in the absence of a wide range of universal markers, for sets of taxon-specific genes. By and large, independent approaches for tree construction from completely sequenced genomes[7,8] support the main phylogenetic lineages of prokaryotes, though the order of branches may change.

The description of higher ranks did not follow a coherent strategy as they were defined along the evolution of the 16S rRNA gene tree. While some families and orders were validly named or already had a standing in nomenclature before the molecular taxonomic revolution, others were created whenever a new lineage emerged (for example, the family *Planctomycetaceae* and the order *Planctomycetales* were named in 1987 by Schlesner and Stackebrandt[9], while the class Planctomycea[10] and the phylum Planctomycetes[11] (http://www.taxonomicoutline.org/) were created when the phylogenetic uniqueness of this lineage became manifest within the radiation of the 16S rRNA gene tree. In order to evaluate the comparability of ranks between different organisms, defined mainly on the basis of the 16S rRNA genes as outlined in *Bergey's Manual of Systematic Bacteriology,* second edition[12], an independent assessment of the higher taxa was published by Konstantinidis and Tiedje[13]. The genetic relatedness between 175 genomes was measured using the average amino acid identity (AAI) of all genes shared between any two strains (orthologues and paralogues). Plotting 16S rRNA gene identity against AAI, the distribution showed, on average, a 30.7% overlap between the ranks, meaning that a high proportion of strains actually belong to different ranks than to the one devised by 16S rRNA gene identity. Especially neighbouring ranks (for example, the order and the class) overlapped to a higher extent than non-adjacent ranks. Major taxonomic changes will be necessary in the future in order to adjust the single-gene 16S rRNA sequence-based hierarchy of ranks to the emerging genome-based taxonomy.

The strain level is the only one for which the DNA-DNA hybridisation (DDH) approach is taxonomically meaningful[14,15]. The level of actual genome sequence identity among two strains must be higher than 96%[16] to reach a DDH similarity value of higher than 70%. This latter value is one of the agreed standards in bacterial systematics, requested to demonstrate high genomic relatedness among strains of a species. It also means that strains affiliated to a species may show differences of about 4% in their genome sequences; thus they are allowed to differ significantly in terms of genomic, hence phenotypic diversity. This finding is regularly encountered when strains are screened for physiological test, for example, by commercial kits such as API (bioMérieux) or BIOLOG Inc. panels. Comparison of genome size and genome architecture in strains of the same species also supported this notion impressively (for example, *E. coli* O157:H7 with a genome size of 5.44 Mb possesses 1,346 genes not found in *E. coli* K-12 with a genome size of 4.64 Mb).

In place since the mid-1960s, the DDH approach, despite its superiority over other methods to unravel close relationships, has always been an orphaned issue. Stackebrandt and Ebers[17] have compiled some of the drawbacks which make DDH seem like a method salvaged from the past: hybridisation results are, among other factors, influenced by a significant number of physicochemical parameters, genome size, large plasmids, and DNA purity; reciprocal values may differ by up to 15%; unlike sequences, which must be deposited in public databases for inspection of quality, no reviewer of a new species description is in a position to assess the background information leading to given DNA reassociation values; last, but not least, the data are not cumulative. These authors concluded that most microbial taxonomists are not in a position to perform these studies by themselves but need collaboration with the few specialised laboratories worldwide.

The relationship between rRNA gene sequence identities and DDH values is not linear but curvilinear. The plot of both parameters, however, clearly indicated that at rRNA gene identities of 97% and below the corresponding DDH values were never higher than 70%. This finding led to the recommendation and aid for systematists to abolish the need to perform DNA-DNA reassociation in those cases where novel strains showed only moderate rRNA similarities (≤97%) with its nearest neighbour[15]. The correlation plot was updated in 2005[17], suggesting to revise the previous recommendation: rather than 97.0%, a 16S rRNA gene sequence similarity threshold value around 98.5% should be defined at which DDH experiments are obligatory for testing the genomic uniqueness; hence the species status of a novel isolate.

The advent of genome sequence comparison and suppression subtractive hybridisation[18] to identify strain-specific sequences seems to offer an alternative for delineating closely related taxa. However, even in times of generating draft genome sequences becoming faster and cheaper, the costs will nevertheless be prohibitive for the majority of taxonomists in the foreseeable time. The advantage, however, lies in the availability of genome sequences, deposited in public databases, as well as the use of open access algorithms, which should make future intrageneric and intraspecies comparison more transparent. A new method, the pairwise comparison of complete or draft-incomplete genomes, has recently been explored, that may develop into an alternative to the classical DDH approach[19]. Expanding MLSA to a higher level, the average nucleotide identity (ANI) of shared orthologous genes or of large genome fragments between two strains was found to be a robust means of comparing the genetic relatedness among strains[20]. Surprisingly, ANI values of approximately 95% to 96% correspond to the traditional 70% DNA-DNA threshold value for delineating species according to the current definition. How then do DDH and ANI values compare to 16S rRNA gene sequence identities in the most crucial

taxonomic area which is the phylogenetic distance between two closely related species? Pairs of organisms with higher than 95% ANI also show higher than 98.5% 16S rRNA gene identity[20]. None of the restrictions mentioned above for DDH apply for the determination of ANI (and AAI) values. The general applicability of the method and the availability of rapid BLAST tools to align entire draft and full genomes to calculate ANI values[21] are the main obvious advantages for scientists and reviewers of publications. The speed at which draft genome sequences will be generated in the very new future leads to the prognosis that the present emphasis on DDH for species delineation will shift to genome- and gene sequence-based approaches.

It must be noted, however, that progress in bacterial taxonomy should not be rushed. The replacement of DDH by ANI or any other genomic method does by no means imply the abolishment of phenotypic properties in the classification process. Polyphasic taxonomy as performed since 1980 has proven its superiority over all classification attempts applied in the 100 years before. Any changes of the recommended data set and approved methods to be used in the classification process need to be evaluated and sanctioned by the guardians of taxonomy (members of Subcommittees of the International Committee on Systematics of Prokaryotes). The users of taxonomy are encouraged to make use of the emerging wealth of genomic information; they should not refrain from convincing editors and reviewers on the usefulness of the new approaches by demonstrating their scientific potential and applicability in practice in publications on the description of novel taxa.

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Biography

Erko Stackebrandt, PhD is the former director (1993–2009) of the German Collection of Microorganisms and Cell Cultures GmbH, Braunschweig, retired full professor of the Technical University, Braunschweig and is now living in Paris, France. Dr Stackebrandt is a trained microbiologist and is involved in systematics, evolution and molecular phylogeny of Archaea and Bacteria. His PhD thesis (1972-1974) was supervised by Otto Kandler and he was a curator of the German Culture Collection DSM, Munich (1972–1977). Dr Stackebrandt did his postdoc with Carl Woese at the University of Illinois, Urbana Champaign 1978 and with Karl Schleifer at the Technical University, Munich 1979–1984. He was head of the Departments of Microbiology at the University of Kiel (1994–1990) and at the University of Queensland, Brisbane, Australia (1990–1993).

Dr Stackebrandt's main interests are in the development and application of novel molecular tools for the characterisation and identification of prokaryotes, phylogeny and systematics. He has been involved in research projects funded by the German Science Foundation, Ministry for Science and Technology, European Space agencies, the European Commission, and various biotech companies, working on pure cultures and microbial communities. He has participated in the description and taxonomic revision of more than 450 bacteria taxa (species, genera, families, orders and classes) and has written >600 papers in refereed journals and >80 book chapters.

Awards

Heisenberg stipendiat (1982–1983)
Award for the habilitation thesis from the Technical University Munich 1982
Corresponding member of the Academy of Science at Göttingen since 1988
International Travelling Lecturer Award, Australia 1990
Bergey's Award, 1991; Bergey Medal 2009
USFCC/J Roger Porter Award 2002
Honorary member of the Hungarian Society for Microbiology
Fellow of the American Academy of Microbiology
Associate Editor (previous) of *International Journal of Systematic Bacteriology* (IJSB), *Anaerobes*, *Actinomycetologica*, and *Encyclopaedia of Life*. Chief Editor/Editor of *IJSB/IJSEM* (previous), *Archives of Microbiology*, *Current Microbiology*, *The Prokaryotes 3rd and 4th edn*.