Letter to the Editor

Headaches for Taxonomists: the *M. avium*-*M. intracellulare* Complex

In a recent study, Wayne and colleagues from the International Working Group on Mycobacterial Taxonomy describe a phenotypic cluster that is phenotypically very similar to *M. avium* and *M. intracellulare* but may be a separate genospecies (6). This cluster consisted of 19 strains with a mean internal matching score of 88% and a matching score of 84 and 82% with clusters representing *M. intracellulare* and *M. avium*, respectively. The members of this cluster did not agglutinate with one of the *M. avium* complex typing sera or agglutinated as one of the higher-numbered serovars. Of 12 strains tested, all but one failed to react with a nucleic acid probe specific for *M. intracellulare* or *M. avium* (Gen-Probe, San Diego, Calif.), which uses a hyper-variable and species-specific region within the 16S rRNA molecule as the target. Wayne and colleagues point out correctly that the separation of *M. avium* and *M. intracellulare* must have been relatively recent, as reflected in the shallow branching of the 16S rRNA tree (5) and given both phenotypic convergence and phenotypic divergence. They suggest that the described similarity cluster represents a novel species within the *M. avium*-*M. intracellulare* complex.

A recent comparative 16S rRNA analysis to characterize the species affiliation of different strains of the phenotypic *M. avium*-*M. intracellulare* complex resulted in a detailed phylogenetic resolution of what is currently assigned to this complex (1). Despite the phenotypic similarity, this study has demonstrated the existence of several phylogenetically distinct taxa. In contrast to genetically homogeneous species, e.g., *M. tuberculosis* (4), *M. intracellulare* was characterized by three different 16S rRNA sequences originating from a common ancestor. Unique 16S rRNA sequences with respect to sequence identity were defined for the following groups of *M. intracellulare* strains: (i) ATCC 13950 (*M. intracellulare* type strain), ATCC 35762, ATCC 35769, ATCC 35761, ATCC 35848, ATCC 35763, ATCC 35772, and ATCC 35764; (ii) ATCC 35847; and (iii) ATCC 35770, suggesting that what is currently described as *M. intracellulare* is composed of at least three genetically different taxa. Owing to the heterogeneity within the 16S rRNA molecule, which includes a region targeted by a nucleic acid probe specific for *M. intracellulare* (Gen-Probe), strains ATCC 35847 and ATCC 35770 cannot be expected to hybridize with this probe. Although strains ATCC 35847 and ATCC 35770 have been ascribed to serovars 7 and 18, this affiliation is hampered by the limitations of serotyping (6). Interestingly, a recently developed nucleic acid probe termed SNAP MAX (Syngene, San Diego, Calif.), which for proprietary reasons has unfortunately not been disclosed by the manufacturer, has been shown to recognize strains which are phenotypically similar to *M. avium* and *M. intracellulare* but do not react with the nucleic acid probe by Gen-Probe specific for *M. intracellulare* or *M. avium* (2, 3), such as strains ATCC 35847 and ATCC 35770.

Given this molecular evidence for genotypic diversity within the *M. avium* phenotypic complex, it would be of interest to know what relationship at the 16S rRNA level the cluster defined by Wayne and colleagues shows to strains ATCC 35847 and ATCC 35770 and to determine the relation-

ship of this putative novel species to the already defined genotypes.

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Author's Reply

I thank Drs. Kirschner and Böttger for their perceptive comments on our recent paper on the taxonomy of mycobacteria (5). I have followed the work from their laboratory with great interest and pleasure over the past several years, as they have been putting the molecular frosting on the phenotypic cake that was started over 30 years ago with Ruth Gordon's recognition of the necessity for a polythetic approach to the taxonomy of mycobacteria (2). My response to their letter is not a rebuttal, since I see no significant disagreement among us. It is rather a more general response to the question of the conventions we use in deciding whether to apply the species or the subspecies form (or an infrasubspecific form) of nomenclature to a newly recognized taxon.

First, I will comment briefly on the specific issue of the status of our cluster 4, which we suggested might represent a third species in the *M. avium* complex. We did not formally

propose species status for this cluster, noting that we did not have the semantide-based data to justify this, and that we were starting a semantide cooperative study to clarify the phylogeny of this and other phenotypic clusters. In fact, as I noted in the J. R. Porter lecture last year (3), evidence from the RNA probe studies by Ferguson et al. (1) and from our serologic study of catalase now suggest that this cluster might best be regarded as a subspecies of *M. intracellulare*. This brings me to my more general comments.

The terms species and subspecies in bacteriology are conventions that define certain forms of nomenclatural usage. Their practical value depends upon general agreement on those conventions. A recommended convention for species nomenclature is that it circumscribes bacteria that fall within a 70% DNA homology group, as determined under stringent hybridization conditions (4); judging from the frequency with which it is cited, that convention seems to be acceptable to most investigators. In recent years, the technical facility with which selected regions of rRNA can be sequenced and compared has led to great enthusiasm in the application of this elegant technique to bacterial taxonomy and has provided some beautiful insights into bacterial phylogeny. Nevertheless, questions remain about hierarchical interpretation of differences that are observed in highly selected regions of one class of RNA and their correlation with the structural relationships between the entire genomes as inferred from DNA hybridization. This is not to discount the validity of RNA selected-region-based taxonomy but only to point out the need for some agreement on a convention that is consistent in its impact on nomenclature. This, then, is a plea that investigators who are actively pursuing rRNA sequence-based taxonomy also develop the capability for determining DNA homologies or establish collaborative ties with other laboratories in which that technology is

established. This would help provide the basis for some convention for interpreting the differences in rRNA sequences that are seen both within and outside of the boundaries of species as defined by the 70% homology convention.

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