

Mycobacterium abscessus, a taxonomic puzzle

Enrico Tortoli,^{1,*} Tomas A. Kohl,² Barbara A. Brown-Elliott,³ Alberto Trovato,¹ Sylvia Cardoso-Leão,⁴ Maria Jesus Garcia,⁵ Sruthi Vasireddy,³ Christine Y. Turenne,⁶ David E. Griffith,⁷ Julie V. Philley,⁷ Stefan Niemann,² Richard J. Wallace Jr³ and Daniela M. Cirillo¹

Dear Editor

We read with interest the paper of Adekambi and coworkers [1] suggesting the reinstatement of *Mycobacterium abscessus* subsp. *abscessus* (A), *M. abscessus* subsp. *massiliense* (M) and *M. abscessus* subsp. *bolletii* (B) as independent species.

This paper is in clear contradiction with our own recent work [2] published in 2016.

The claim of the authors (1st page) that postgenomic analysis 'unambiguously supports the reinstatement of species' seems to us at least highly questionable; in none of the studies they cited in their paper [3–8], with the exception of one study [9] done in collaboration with the same authors, is the status of subspecies questioned.

A major argument supporting the suggestion of Adekambi *et al.* was published by him in 2008 [10]. The major conclusions of this work were that 1) an *rpoB* gene similarity $\leq 97.7\%$ correlates with a DNA–DNA Hybridization (DDH) $< 70\%$; and 2) the DDH may be inferred by the formula: $5.98(\textit{rpoB} \text{ similarity}) - 2516.1$.

In the 3840 bp sequence of the *rpoB* gene obtained when the sequences of the type strains of A, B and M (deposited by Adekambi *et al.* in GenBank; AY147164, AY859692 and AY593981), have been cut to start and end at the same nucleotide positions, we obtained the following similarity values: A versus M=98.07%; A v B=97.86%; M v B=98.51%; each of them corresponding to DDH>70%.

Adekambi *et al.* questioned our data produced, performing the wet lab DDH test with five replicates [11]. Instead they proposed results (Table 2 of [10]) inferred from *rpoB* similarity using the above mentioned formula. In our view, it is not valid to replace a complex assay that takes into account the whole genome (which is still considered the gold standard), with a formula based on just a single gene.

Bioinformatic algorithms are available and validated in multiple studies, which can be used to infer the DDH from genomic data. The best known, in addition to Average Nucleotide Identity (ANI) [12], are the Genome to Genome Distance (GGD) [13, 14] and the Genomic Signature-Delta Difference (GS-DD) [15].

In our work we calculated the GGD, which is the equivalent *in silico* of DDH, using the software available at <http://ggdc.dsmz.de/ggdc.php> and obtained the following DDH-equivalent results: A v M=84.7%; A v B=86.10%; M v B=87.70%; all clearly >70% and not supportive of the status of independent species [12].

We also calculated the GS-DD using the software available at www.cmlb.uga.edu/software/delta-differences.html, which again did not support the hypothesis of independent species (Table 1).

We disagree, furthermore, in the assertion of Adekambi *et al.* (third page) in which we are said to have stated that ANI values >98.3% are needed to support the status of subspecies. Fig. 1 of our paper [2] reports the results of our analysis of 46 genomes (43 clinical isolates+3 type strains) showing the ANI variability which, among strains belonging to the three taxa (A, B and M), ranged from 96.6 to 97.6%. Within each single taxon (A or B or M), this variability ranged from 98.5 to 99.9%. Therefore the subspecies threshold was $\leq 97.6\%$ with values >98.3% reflecting the intra-subspecies variability.

Adekambi *et al.* report several examples of species characterized by ANI pairwise values >96%. In a recent investigation at the level of whole genomes of 148 species of the genus *Mycobacterium*, we too faced this anomaly [16]. We confirmed the close ANI pairwise similarity between *Mycobacterium intracellulare* and *Mycobacterium yongonense* as well as *Mycobacterium chimaera* and *Mycobacterium paraintracellulare*. Far from considering this finding a proof of the legitimacy of species characterized by pairwise ANI

Author affiliations: ¹Emerging Bacterial Pathogens Unit, IRCCS San Raffaele Scientific Institute, Milan, Italy; ²Molecular and Experimental Mycobacteriology, Forschungszentrum Borstel, Leibniz-Zentrum für Medizin und Biowissenschaften, Borstel, Germany; ³Department of Microbiology, Mycobacteria/Nocardia Research Laboratory, The University of Texas Health Science Center at Tyler, Tyler, TX, USA; ⁴Departamento de Microbiologia, Imunologia e Parasitologia, Escola Paulista de Medicina, Universidade Federal de São Paulo, São Paulo, SP, Brazil; ⁵Department of Preventive Medicine, Public Health and Microbiology, Autonoma University of Madrid, Madrid, Spain; ⁶Diagnostic Services Manitoba, Winnipeg, Manitoba, Canada; ⁷Department of Pulmonary Medicine, The University of Texas Health Science Center at Tyler, Tyler, TX, USA.

*Correspondence: Enrico Tortoli, e.tortoli@libero.it

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Table 1. δ^* values calculated comparing the genomes of A, B, M and *M. chelonae*

	<i>M. chelonae</i>	ABSCCESSUS	BOLLETHI	MASSILIENSE
<i>M. chelonae</i>	22	26	24	25
ABSCCESSUS		24	23	23
BOLLETHI			22	22
MASSILIENSE				23

δ^* value obtained by comparison of a genome with itself represents the threshold value that approximates the species separation for the considered genome. δ^* values equal to, or lower than, the value of a genome with itself, identify genomes of the same species [20].

values >96 %, we concluded (supported also by GGD and GS-DD data), for *M. yongonense*, *M. chimaera* and *M. paraintracellulare* (in adjunct to the not validly published *M. indicus pranii*) that the status of species was inappropriate and that would be instead reclassified as subspecies of *M. intracellulare* ([17], Castejon M, Menendez MC, Comas I, Tortoli E, Garcia MJ. Whole-genome sequence analysis in that *Mycobacterium yongonense* and '*Mycobacterium indicus pranii*' are members of the species *Mycobacterium intracellulare*. Designation of *Mycobacterium intracellulare* subsp. *intracellulare* and *Mycobacterium intracellulare* subsp. *yongonense*, unpublished).

The example of the *Mycobacterium tuberculosis* complex seems to us unfortunate; it is well known that the members of this complex actually represent clones [18], and that their classification as species is simply a convention emphasizing differences in the host specificity but lacking taxonomic validity. The case of *Mycobacterium marinum* and *Mycobacterium ulcerans* is different. The close ANI similarity does not take into account the large (1000 kb) difference in genome size, and the presence, in the latter, of the plasmid encoding a toxin (mycolactone) responsible for the unique pathological picture of disease due *M. ulcerans* compared with *M. marinum*. Again, this latter difference is a major clinical difference that overrides the degree of taxonomic similarity.

Another argument to support, according Adekambi *et al*, the hypothesis of independent species, is the number of unique genes. This ranges, in A, B and M, from 15 to 36. In contrast, in a recent study [19] from our laboratory evaluating genomes of 41 species belonging to the genus *Mycobacterium* we detected an average of 1027.35 unique genes per species (median 832). This is two magnitude orders higher in comparison with the quoted differences in A, B and M from Adekambi *et al*.

The concluding remark stating that the reinstatement of M and B as species 'will enable the clinicians to manage the patients appropriately' is also unclear; we do not see, in fact, how the status (species as compared to subspecies) of M and AB can affect the management of patients.

We will accept any decision made by *Int. J. Syst. Evol. Microbiol.* An editorial decision is needed to settle this controversy that threatens the credibility of taxonomic science.

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Conflicts of interest

The authors declare that there are no conflict of interest.

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