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Genome sequence-based criteria for species demarcation and definition: Insights from the genus *Rickettsia*

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Statement of the Problem: *Rickettsia* species are strictly Gram-negative intracellular α-proteobacteria, responsible for many diseases like epidemic typhus, Rocky Mountain spotted fever and can be transmitted to mammalian hosts by hematophagous arthropods vectors. Considering its strictly intracellular lifestyle and the few expressed phenotypic characters, traditional identification methods used in bacteriology are inapplicable. For this aim, we used the genomic comparison to analyze the *Rickettsia* genome sequences in terms of gene content as well as similarity among strains and species, for better species and genus delineation. We also attempted determining genome-based boundaries between species and genera.

Methodology & Theoretical Orientation: A genome-based taxonomy analysis including digital DNA-DNA Hybridization (dDDH) relatedness, Average Nucleotide Identity (ANI) and Average Genomic Identity of Orthologous genes sequences (AGIOS) was performed. We compared 78 *Rickettsia*l genomes and 61 genomes used as outgroup (*Orientia* (11 strains), *Ehrlichia* (22 strains) and *Anaplasma phagocytophilum* (28 strains)) and determined the degree of homology between pair genomes.

Findings: We proposed genomic guidelines for the classification of rickettsial isolates at the genus and species levels. To be classified as a member of the genus *Rickettsia*, an isolate should exhibit degrees of genome sequence homology with any of the 28 validated species studied of >80.6% for OrthoANI and AGIOS values. To be classified as a new *Rickettsia* species, an isolate should not exhibit more than one of the following degrees of genomic sequences similarities with the most homologous validated species: >92.3, >99.2 and >98.6% for dDDH, OrthoANI and AGIOS respectively.

Conclusion & significance: These genomosystematic guidelines can be proposed as an alternative to the multi-locus sequence typing-based taxonomic scheme for the classification of rickettsiae.

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