There is inadequate evidence to support the division of the genus *Borrelia*

There are surely scientific, genetic or ecological arguments which show that differences exist between the relapsing fever (RF) spirochaetes and the Lyme borreliosis (LB) group of spirochaetes, both of which belong to the genus *Borrelia*. In a recent publication, Adeolu and Gupta (Adeolu & Gupta, 2014) proposed dividing the genus *Borrelia* into two genera on the basis of genetic differences revealed by comparative genomics. The new genus name for the LB group of spirochaetes, *Borreliella,* has subsequently been entered in GenBank for some species of the group and the List of named prokaryotic bacterial species (Oren & Garrity, 2015). However, rapidly expanding scientific knowledge and considerable conflicting evidence combined with the adverse consequences of splitting the genus *Borrelia* make such a drastic step somewhat premature. In our opinion, the basis of this division rests on preliminary evidence and should be rescinded for the following reasons:

1) The proposed split of the genus rests on differences in conserved signature indels (CSI) and conserved signature proteins (CSP) between LB and RF spirochaetes. A major flaw in the study published by Adeolu and Gupta (Adeolu & Gupta, 2014) is the exclusion of a *Borrelia* clade containing RF-like species that utilize hard ticks as vectors and reptiles as reservoir hosts (Loh *et al.*, 2016; Takano *et al.*, 2011).

To identify proteins that are uniquely present in various groups of *Borrelia*, BLAST searches (Altschul *et al.*, 1990) were performed by Adeolu and Gupta (Adeolu & Gupta, 2014) using each protein in the genomes of *Borrelia burgdorferi* B31 and *B. recurrentis* A1 (GenBank) as queries. Out of 1,041 and 1,390 protein coding genes, (i.e. the number of proteins reported in GenBank accession numbers NC 011244 and NC001318) present in *B. recurrentis* A1 and *B. burgdorferi* B31, respectively, 15 CSI (7 for LB, 8 for RF) and 25 CSP (21 for LB, 4 for RF) were found to be unique for the respective groups. However, two of the four CSPs that are apparently unique for the RF group species are not found in

all members of this group and therefore do not represent true signature proteins. Hence, just **two** CSPs and 8 CSIs are unique to the RF group.

The same holds true for the LB group of spirochaetes. Five of the 21 CSPs present only in the LB group of spirochaetes are not found in all species of the *B. burgdorferi* s.l. complex. Furthermore, 12 of these CSPs are hypothetical proteins with unknown functions, and so this challenges the utility of these CSPs as unique signature proteins. These facts coupled with the omission of the entire clade of reptile-associated species (Fig 1) underscore our criticism and highlights the uncertainty around the proposed genus split. Presumably this is only the tip of the iceberg, as more RF-like and LB species continue to be detected and described every few years (Loh *et al.*, 2016; Pritt *et al.*, 2016). In this context, it is our opinion that it would be prudent to retain the generic name *Borrelia* for both LB and RF spirochaetes.

2) Their shared spirochaetal morphology (with some variations within both groups such as the number of flagella, number and regularity of spirals), comparable genome structure, similar GC content (nearly 30 %), and common vector-borne lifestyle (using ticks as vectors in natural transmission cycles, with one exception, *B. recurrentis* which is transmitted by *Pediculus humanus*) make the genus *Borrelia* cohesive. Initial work suggested that relapsing fever species are transmitted by soft ticks whilst species belonging to the *B. burgdorferi* sensu lato species complex were transmitted by hard ticks (Barbour & Hayes, 1986). This view had to be modified because several *Borrelia* species that cluster phylogenetically with RF spirochaetes were revealed to be transmitted by hard ticks. Importantly, *B. miyamotoi* (Fukunaga *et al.*, 1995) which has been shown to cause a relapsing fever-like illness (Platonov *et al.*, 2011) referred to as hard tick relapsing-fever (HTRF (Krause *et al.*, 2016)) is transmitted by hard ticks in the genus *Ixodes. B. miyamotoi* occurs sympatrically with LB group spirochaetes and, indeed, the four primary *Ixodes* spp. ticks that transmit *B. burgdorferi* s.l. spirochaetes to humans likewise are the principal vectors of *B. miyamotoi*. Further RF-like and LB spirochaetes are being discovered and described (Loh *et al.*, 2016; Pritt *et al.*, 2016),

the ecological and genetic differences between these groups will most certainly become even more blurred in the future.

Underpinning this point, we have performed a comparative genomic analysis that demonstrated the close genetic relationship between LB and RF group spirochaetes. MUMmer v. 3 (Kurtz *et al.*, 2004) was implemented to align DNA sequences of the main chromosomes of the LB spirochaetes *B. burgdorferi* sensu stricto (s.s.) B31 (GenBank accession no. NC_001318.1), *B. bavariensis* NMJW1 (GenBank accession no. NC_018747.1) and the RF spirochaete *B. duttonii* Ly (GenBank accession no. NC_011229.1, a genetically more complete spirochaete than *B. recurrentis* used above). MUMmer is an ultrafast alignment tool and is designed to find exact matches for a minimum specified length (here, 20 bp being chosen) between two or more input sequences. Sequences were uploaded in fasta format and MUMmer was run using standard parameters.

Comparison of *B. bavariensis* NMJW1 (green triangles) or *B. duttonii* Ly (blue diamonds) with *B. burgdorferi* s.s. B31 resulted in nearly a straight line (from the bottom left to the top right) indicating a high degree of similarity between them (Fig. 2) and that no major re-arrangement had occurred in either of the two strains compared to B31. For sake of clarity, only forward-sequence comparisons are shown. The dots scattered across the plot are matches of the mimimum 20 bp sequence to other regions in the genome. Such “mismatches” were found in both comparisons, i.e. *B. bavariensis* versus *B. burgdorferi* s.s. and *B. duttonii* versus *B. burgdorferi* s.s. (Fig. 2). We conclude that the genospecies compared here display a high degree of synteny.

3) As for the clinical symptoms caused by *Borrelia* species, the symptomology that differentiates RF spirochaetes from the LB group of spirochaetes has been blurred by recent case descriptions. For example, a patient with clinical symptoms resembling those of Lyme neuroborreliosis was diagnosed as being infected with the RF group species *B. miyamotoi* (Boden *et al.*, 2016). Interestingly, infection with the recently described genospecies of the *B. burgdorferi* s.l. complex, *B. mayonii*, produced high

spirochaetal blood densities, akin to that seen following infection with species of the RF group (Pritt *et al.*, 2016).

Thus, splitting the genus does not provide any assistance as far as clinical evaluation is concerned. It does not help end user communities including those in clinical medical practice, public health or those studying the ecology of the bacteria.

Collectively, in view of the inadequate genetic evidence supporting the genus split and the biological features shared between RF and LB group spirochaetes, at present we strongly oppose the proposed division of the genus *Borrelia*. This division complicates an already complicated situation which will serve only to lead to further confusion among scientists, clinicians, public health authorities and the general public. Taken together, we believe that such a change is inadvisable based on currently available biological and clinical evidence, and therefor respectfully request that it be repealed.

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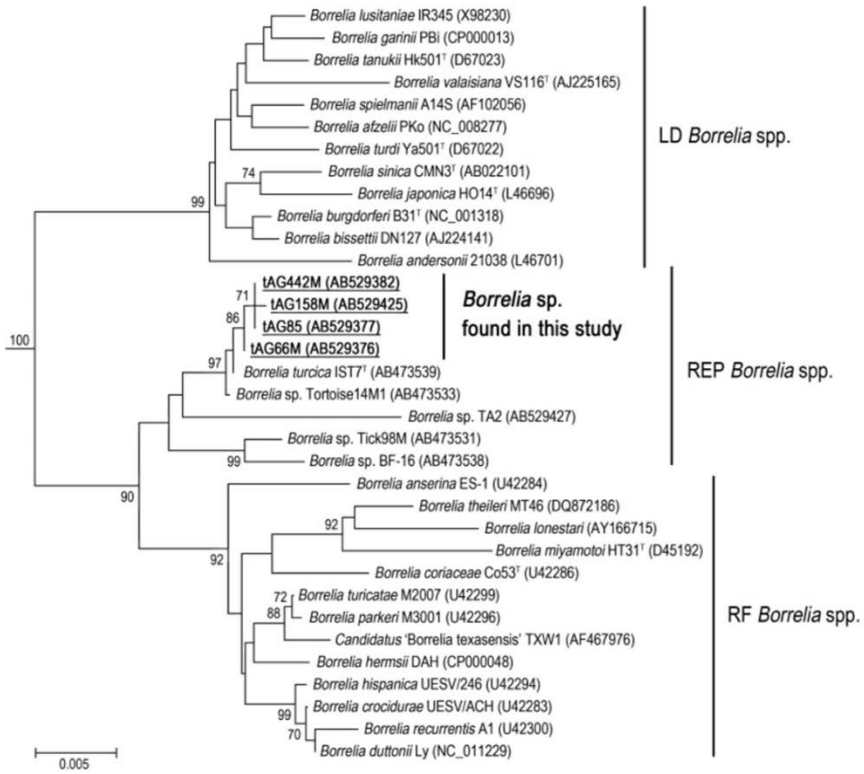

Fig. 1. Phylogenetic analysis based on *16S rDNA* of the genus *Borrelia*. The phylogenetic trees were constructed based on NJ methods, and bootstrap tests were carried out according to the Kimura 2-parameter distances method. All positions containing alignment gaps and missing data were eliminated only in pairwise sequence comparisons (pairwise deletion option). There were a total of 1565 positions in the final data set. The percentage of replicate trees in which the associated taxa were clustered together in the bootstrap test (1000 replicates) was calculated. The phylogenetic branches were supported in > 70% by the bootstrap analysis. The bar indicates the percentage of sequence divergence. *Spirochaeta americana* (AF373921), *Treponema pallidum* (NC_000919) and *Cristispira* sp. (U42638) were used as outgroups.

Figure 1 Phylogeny of *Borrelia*. Figure modified from Takano et al. 2011. Environmental Microbiology Reports 3(5), 632-637 with permission of John Wiley and Sons.

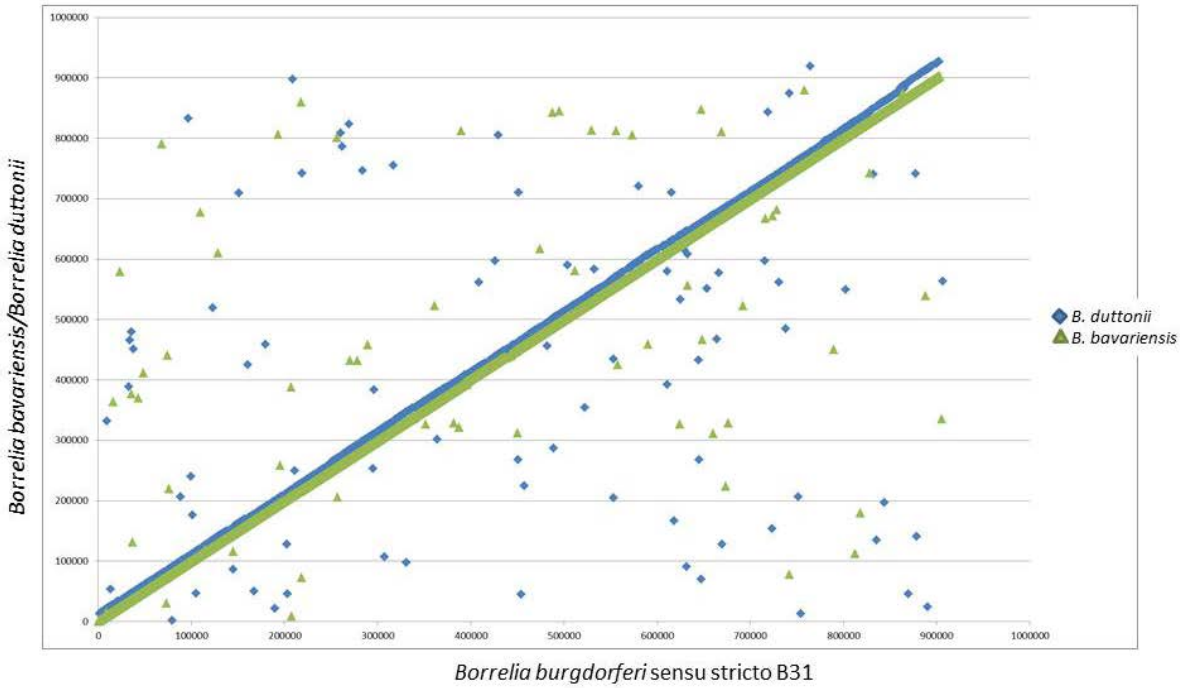

Figure 2 Similarity dot plot of the main chromosome of *B. duttonii* (blue) and *B. bavariensis* (green) compared to *B. burgdorferi* B31 (compiled in MUMmer v. 3). The figure underlines the high similarity at the main chromosome of RF group spirochetes and LB group spirochetes.