

RESEARCH ARTICLE

The genus *Borrelia* reloaded

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Abstract

The genus *Borrelia*, originally described by Swellengrebel in 1907, contains tick- or louse-transmitted spirochetes belonging to the relapsing fever (RF) group of spirochetes, the Lyme borreliosis (LB) group of spirochetes and spirochetes that form intermittent clades. In 2014 it was proposed that the genus *Borrelia* should be separated into two genera; *Borrelia* Swellengrebel 1907 emend. Adeolu and Gupta 2014 containing RF spirochetes and *Borrelia* *liella* Adeolu and Gupta 2014 containing LB group of spirochetes. In this study we conducted an analysis based on a method that is suitable for bacterial genus demarcation, the percentage of conserved proteins (POCP). We included RF group species, LB group species and two species belonging to intermittent clades, *Borrelia turcica* Güner et al. 2004 and *Candidatus Borrelia tachyglossi* Loh et al. 2017. These analyses convincingly showed that all groups of spirochetes belong into one genus and we propose to emend, and re-unite all groups in, the genus *Borrelia*.

OPEN ACCESS

Citation: Margos G, Gofton A, Wibberg D, Dangel A, Marosevic D, Loh S-M, et al. (2018) The genus *Borrelia* reloaded. PLoS ONE 13(12): e0208432. <https://doi.org/10.1371/journal.pone.0208432>

Editor: Sven Bergström, Umeå University, SWEDEN

Received: May 4, 2018

Accepted: November 5, 2018

Published: December 26, 2018

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Data Availability Statement: Relevant data are within the paper and its Supporting Information files. All genome files are available from the NCBI GenBank database (accession number(s) are given in the manuscript).

Funding: This study was part-funded by the Australian Postgraduate Award (AG), the International Postgraduate Research Scholarship (SML), the Australian Research Council (LP160100200 to CO), Bayer HealthCare (Germany) and Bayer Australia, the Murdoch University Small Grant Scheme (CO), the Robert-Koch-Institute via the German National Reference

Introduction

The spirochete genus *Borrelia*, named after the French biologist Amédée Borrel, was originally described in 1907 by Swellengrebel [1], with *B. anserina* (Sakharoff 1891) Bergey *et al.* 1925 designated as the type species. Since then numerous species and strains have been described, and members of this genus are well recognized as the aetiological agents of Lyme borreliosis (LB) and relapsing fever (RF) in humans. Lyme borreliosis and RF genospecies have long been recognized to have different clinical, biological, and epidemiological characteristics, and phylogenetic data is concordant with this, demonstrating that these two groups are genetically similar yet distinct, and form independent monophyletic sister clades that share a common ancestor [2].

Nevertheless, LB and RF *Borrelia* share a common set of genetic and biological characteristics that unify these organisms as a group compared to other related spirochetes. Namely, all LB and RF *Borrelia* species are spirochetes with an obligate parasitic lifestyle, are transmitted between vertebrate hosts by arthropod vectors (ticks and louse), and can be transstadially transmitted within their arthropod vectors. Various vector associations of *Borrelia* have been found in nature, with the genus *Ixodes* mainly vectoring LB species while argasid ticks often vector the RF group. However, some members of the RF group are associated with hard ticks

Centre for Borrelia (VF), and ESCMID via a ESGBOR Study Group grant 2015 (GM). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing interests: The authors have declared that no competing interests exist.

of the family Ixodidae (e.g. *B. lonestari*) [3], with the human body louse *Pediculus humanus* (*B. recurrentis*) [4] or the genus *Ixodes* (e.g. *B. miyamotoi*) [5]. The genus *Ixodes* represents an ancient genus of the family Ixodidae sharing numerous original features with argasid ticks. Both, LB and RF spirochetes are dependent on their vertebrate and arthropod hosts for the majority of their nutritional requirements, and share a unique genomic structure comprised of a single highly conserved linear chromosome and numerous extrachromosomal linear and circular plasmids that can be highly variable between strains [6–8].

Recently, a third group of *Borrelia* organisms has been described that are associated with reptile and echidna (*Tachyglossus aculeatus*) hosts, and do not phylogenetically cluster within either the RF or LB clades. Instead these novel borreliae form their own independent lineages which sit as an outgroup to, and shares a most recent common ancestor with, the RF clade [9]. This novel clade currently has two designated species, *B. turcica* [10] and ‘*Candidatus Borrelia tachyglossi*’ [9, 11, 12], and several other genetic variants that are yet to be formally taxonomically classified [13]. Known vectors for this group include hard ticks of the genera *Amblyomma*, *Bothriocroton*, and *Hyalomma* [10, 11, 13].

Recently Adeolu and Gupta [14] proposed to divide *Borrelia* into two genera to reflect the genetic and phenotypic divergence between LB and RF species, however, this proposal remains under debate [15, 16], and has not been widely utilized in the literature. The justification for this proposal was largely based on the identification of conserved signature insertions/deletions (indels) (CSIs) and conserved signature proteins (CSPs) that are differentially present in the LB or RF *Borrelia* genogroup, as well as average nucleotide identity (ANI) values calculated between whole genomes of 18 *Borrelia* species including eight LB species and ten RF species. Although it is uncontested that these differences exist between LB and RF *Borrelia*, we propose that the methodology used to identify these group-specific differences is subjective and has a highly limited power to delineate LB and RF *Borrelia* into separate genera.

The methodology employed by Adeolu and Gupta [14] reported only “CSIs that are specific for different groups within the *Borrelia*”, and CSPs only “if either all significant [BLAST] hits were from well-defined group of *Borrelia* or which involved a large increase in E-values from the last hit belonging to a particular group of *Borrelia* to the first hit from any other group”. This methodology specifically identifies only CSIs and CSPs that are exclusive only to one *Borrelia* genogroup, and precludes the detection of CSIs or CSPs that may be shared non-exclusively between both genogroups (i.e. contests the hypothesis that LB and RF belong in different genera). This data presented in isolation misrepresents the extent of genomic divergence between LB and RF *Borrelia* and fails to consider widespread genomic similarities between these two groups. Additionally, although ANI has previously been used to investigate prokaryote taxonomy [17], a comprehensive review of this method revealed that although ANI can accurately quantify the genetic relationships between strains belonging to the same species, it was not suitable to differentiate prokaryotic genera. This is due to significant overlapping of intergenera ANI and interspecies ANI values [18], which leads to unreliability in the method.

Alternatively, Qin et al. [18] presented a more heuristic method for delineating prokaryotic genera that measures the percentage of conserved proteins (POCP) between whole genome pairs, reasoning that the degree of protein conservation reflect both genetic and phenotypic relatedness more substantially. Qin et al. [18] demonstrated that among 235 prokaryotic species from 97 genera that POCP values had a higher predictive power than ANI to delineate genera. They showed that with few exceptions POCP values of $\geq 50\%$ could be considered a threshold for prokaryotic genus delimitation, pending other genomic factors that influence POCP, such as large differences in genome size.

Here we investigate the validity of the proposed delineation of LB and RF *Borrelia* into separate genera by performing pairwise analysis of POCP values between 30 *Borrelia* type strain

genomes (where possible), including two new *Borrelia* genomes from the novel reptile and echidna-associated clade, *B. turcica*, and ‘*Candidatus Borrelia tachyglossi*’, which have yet to be analyzed in this context. We also re-examine the CSIs previously used to support the delineation of LB and RF *Borrelia* in the genomes of *B. turcica* and ‘*Candidatus Borrelia tachyglossi*’ to establish whether these molecular markers are useful to establishing the relationship of *B. turcica*, and ‘*Candidatus Borrelia tachyglossi*’. Our analyses indicate that insufficient genomic divergence exist between LB and RF *Borrelia* to consider them separate genera, and that *Borrelia* CSIs are limited in their ability to unambiguously distinguish the taxonomic identity of *B. turcica* and ‘*Candidatus Borrelia tachyglossi*’.

Materials and methods

Strain included in this study

In order to accurately assess *Borrelia* intra-genus POCP, the proteomes of 30 *Borrelia* species strains, including $n = 17$ strains from the LB group, $n = 11$ from the RF group, and $n = 2$ from the reptile and echidna-associated group, were retrieved from GenBank (National Center for Biotechnology Information (NCBI), Bethesda (MD), <https://www.ncbi.nlm.nih.gov/>) or sequenced and assembled from low passage type cultures except ‘*Candidatus B. tachyglossi*’ which was sequenced from a single tick [12, 19]. A tree summarizing the phylogenetic relationship based on 791 homologous proteins is shown in Fig 1. A full summary of strains used is presented in Table 1. To determine the levels of inter-genera POCP within the order Spirochaetales, an additional 54 proteomes, including $n = 8$ *Brachyspira*, $n = 21$ *Leptospira*, $n = 5$ *Spirochaeta*, and $n = 20$ *Treponema* species, were retrieved from GenBank (S1 Table) and included in the POCP analysis.

Sequence analyses

POCP analysis was performed according to Qin et al. [18] and as described in [20]. Briefly, for each genome pair reciprocal BLASTP [21] was used to identify homologous proteins between genome pairs. Proteins were considered to be conserved if the BLAST matches had an E-value of $< 1e-5$, $>40\%$ sequence identity and $>50\%$ of the query sequence in each of the reciprocal searches. The POCP value for a genome pair was then determined as $[(C_1+C_2)/(T_1+T_2)] \times 100$, where C_1 and C_2 are the number of conserved proteins between the genome pair, and T_1 and T_2 are the total number of proteins in each genome being compared [18]. Scripts used for these analyses are available upon request.

The CSIs presented in Adeolu and Gupta [14] that are differentially present in LB and RF genomes were reinvestigated in the genomes of *B. turcica* and ‘*Candidatus Borrelia tachyglossi*’ to establish whether these molecular markers are useful for classifying their taxonomic relationships. To identify CSIs, the conserved amino acid sequences flanking the CSIs were searched against the proteomes of all 30 *Borrelia* genomes used here using BLASTP [21]. Hits from the matching protein in all 30 *Borrelia* proteomes were aligned with MUSCLE [22], and visually inspected for the presence of CSIs. The presence of previously defined CSPs in the genomes of *B. turcica*, and ‘*Candidatus Borrelia tachyglossi*’ was determined using BLASTP searched as described in Adeolu and Gupta [14].

Results and discussion

In order to determine whether the 50% POCP threshold for genus delineation was appropriate for spirochete taxa, we used pairwise POCP analysis to determine the inter-genera POCP values for 84 spirochete genomes from the genera *Borrelia*, *Brachyspira*, *Leptospira*, *Spirochaeta*,

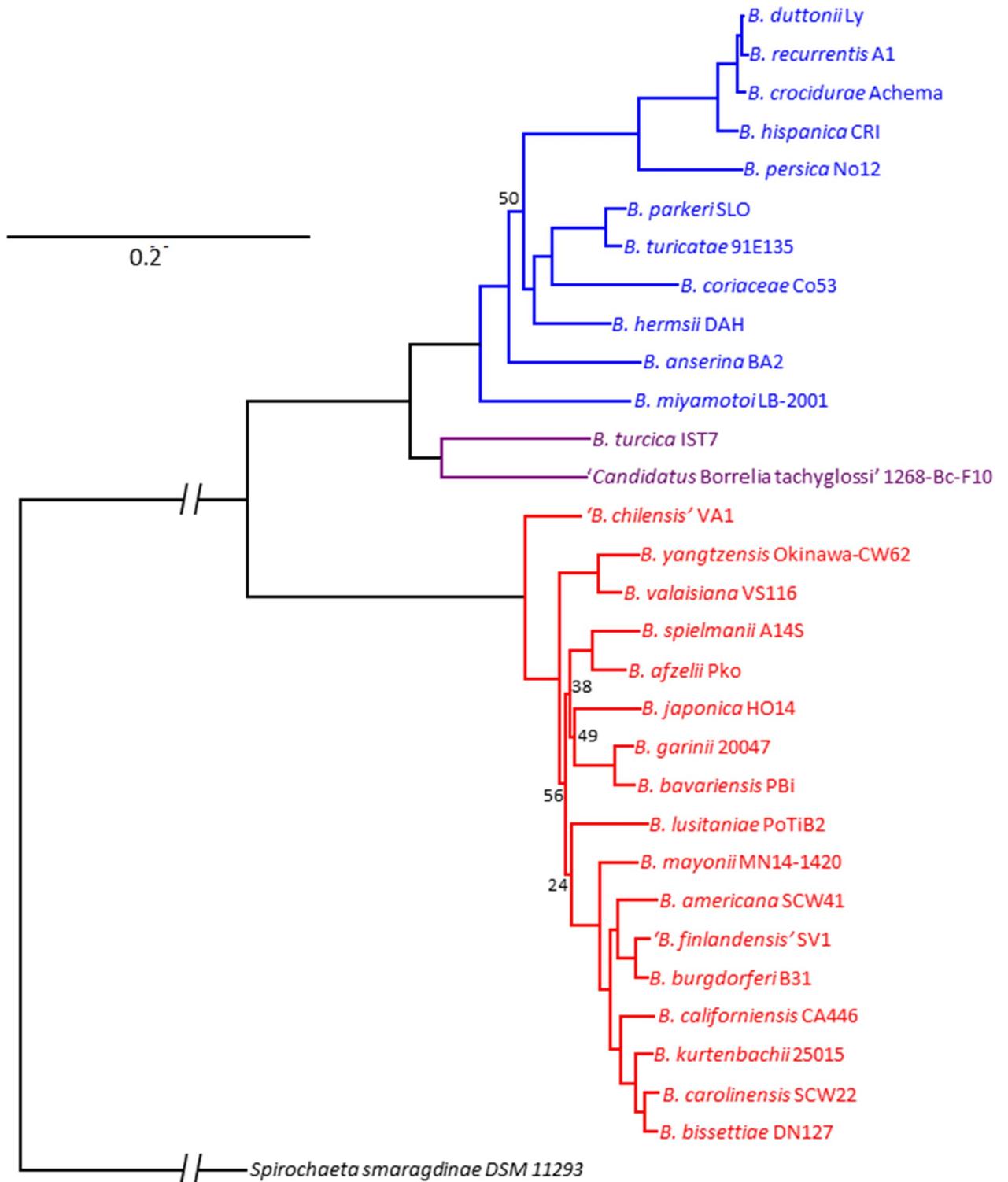


Fig 1. Phylogenetic reconstruction of *Borrelia* species based on 791 aligned protein homologs built with the PEPR pipeline and FastTree2 with 100 jackknifed resampling replicates. All node support values are 100 except where indicated.

<https://doi.org/10.1371/journal.pone.0208432.g001>

Table 1. Borrelia species and strains included in study.

	type strain	strain included	sequence source/GB accession number	available at culture collection			
				ATCC	DSMZ	CIP	JCM
Lyme borreliosis group							
<i>Borrelia afzelii</i> (Canica et al. 1994, sp. nov.)	VS461 ^T	PKo	NC_008277		DSM-10508	CIP 103469	
<i>Borrelia americana</i> (Rudenko et al. 2010, sp. nov.)	SCW41 ^T	SCW41 ^T	SAMN05328445	BAA-1877	DSM-22541		
<i>Borrelia bavariensis</i> (Margos et al. 2013, sp. nov.)	PBi ^T	PBi ^T	CP028872	BAA-2496	DSM-23469		
<i>Borrelia bissettae</i> (Margos et al. 2016, sp. nov.)	DN127 ^T	DN127 ^T	NC_015921		DSM-17990	CIP 109136	
<i>Borrelia burgdorferi</i> (Johnson et al. 1984, sp. nov.)	B31 ^T	B31 ^T	NC_001318	35210	DSM-4680		
<i>Borrelia californiensis</i> (Margos et al. 2016, sp. nov.)	CA446 ^T	CA446 ^T	SAMN05328472	BAA-2689	DSM-17989	CIP 109133	
<i>Borrelia carolinensis</i> (Rudenko et al. 2011, sp. nov.)	SCW22 ^T	SCW22 ^T	SAMN05328473	BAA-1773	DSM-22119		
" <i>Borrelia chilensis</i> "	VA1	VA1	CP009910				
" <i>Borrelia finlandensis</i> "	SV1	SV1	NZ_ABJZ00000000				
<i>Borrelia garinii</i> (Baranton et al. 1992, sp. nov.)	20047 ^T	20047 ^T	CP028861	51383	DSM-10534		
<i>Borrelia japonica</i> (Kawabata et al. 1994, sp. nov.)	HO14 ^T	HO14 ^T	SAMN05328497	51557			JCM 8951
<i>Borrelia kurtenbachii</i> (Margos et al. 2014, sp. nov.)	25015 ^T	25015	SAMN05328498	BAA-2495	DSM-26572		
<i>Borrelia lusitaniae</i> (Le Fleche et al. 1997, sp. nov.)	PotiB2 ^T	PotiB2 ^T	SAMN05328499		DSM-107168	CIP 105366	
<i>Borrelia mayonii</i> (Pritt et al. 2016, sp. nov.)	MN14-1420 ^T	MN14-1420 ^T	NZ_CP015780	BAA-2743	DSM-102811		
<i>Borrelia spielmanii</i> (Richter et al. 2006, sp. nov.)	PC-Eq17 ^T	A14S	NZ_ABKB00000000		DSM-16813	CIP 108855	
<i>Borrelia valaisiana</i> (Wang et al. 1997, sp. nov.)	VS116 ^T	VS116 ^T	SAMN02436326		DSM-21467	CIP 105367	
<i>Borrelia yangtzensis</i> (Margos et al. 2015, sp. nov.)	Okinawa-CW62 ^T	Okinawa-CW62 ^T	SAMN08904503	DSM-24625			JCM 17189
Reptile associated group							
<i>Borrelia turcica</i> (Güner et al. 2004, sp. nov.)	IST7 ^T	IST7 ^T	CP028884-91		DSM-16138		
Australian Borrelia							
<i>Candidatus Borrelia tachyglossi</i> (Loh et al. 2017)		1268-Bc-F10*	CP025785-90				
Relapsing fever group							
<i>Borrelia anserina</i> (Sakharoff 1891) Bergey et al. 1925, species.	nd	BA2	NZ_CP005829				
<i>Borrelia coriaceae</i>	nd	Co53	NZ_CP005745	ATCC 43381			
<i>Borrelia crocidurae</i> (Leger 1917) Davis 1957, species.	nd	Achema	NC_017808				
<i>Borrelia duttonii</i> (Novy and Knapp 1906) Bergey et al. 1925, species.	nd	Ly	NC_011229				
<i>Borrelia hermsii</i> (Davis 1942) Steinhaus 1946, species.	nd	HS1	NZ_CP014349	BAA-2821	DSM 4682	CIP 104209	
<i>Borrelia hispanica</i> (de Buen 1926) Steinhaus 1946, species.	nd	CRI	NZ_AYOU00000000				

(Continued)

Table 1. (Continued)

	type strain	strain included	sequence source/GB accession number	available at culture collection			
				ATCC	DSMZ	CIP	JCM
<i>Borrelia miyamotoi</i> Fukunaga et al. 1995	HT31 ^T	LB-2001	NC_022079				
<i>Borrelia parkeri</i> (Davis 1942) Steinhaus 1946, species.	nd	SLO	NZ_CP005851				
<i>Borrelia persica</i> (Dschunkowsky 1913) Steinhaus 1946, species.	nd	No12	NZ_AYOT00000000				
<i>Borrelia recurrentis</i> (Lebert 1874) Bergey et al. 1925, species.	nd	A1	NC_011244				
<i>Borrelia turicatae</i> (Brumpt 1933) Steinhaus 1946, species.	nd	91E135	NC_008710				

GB = GenBank; ATCC = American Type Culture Collection; DSMZ = Deutsche Stammsammlung für Mikroorganismen und Zellkulturen; JCM = Japan Collection of Microorganisms; nd = no data

*sample ID

<https://doi.org/10.1371/journal.pone.0208432.t001>

and *Treponema*. Among all spirochete genomes investigated inter-genera POCP values ranged between 4.8% and 36.8% (mean 10.1%), indicating a low degree of protein conservation occurs between spirochete genera (S1 Table). These spirochete inter-genera POCP values are at a minimum of 13.2% lower than the 50% value determined by [18], suggesting this value is an appropriate and highly conservative threshold for spirochete genera delineation.

Compared to the low level of protein conservation measured between spirochete genera, POCP values were significantly higher among *Borrelia* species. *Borrelia* POCP values were highest among the LB genospecies, which ranged between 81.1–94.4% (mean 90.2%), while POCP values between RF species were generally lower and more variable, ranging between 65.3–93.1% (mean 81.1%) (Figs 2 and 3). Despite sharing a most recent common ancestor with RF *Borrelia*, *B. turcica* and ‘*Candidatus Borrelia taylori*’ genomes shared higher POCP values with many LB *Borrelia* species compared to RF species, such as *B. chilensis* (78.9% and 87.7%, respectively), *B. americana* (77.4% and 82.2%, respectively), *B. japonica* (77.0% and 82.0%, respectively), and *B. mayonii* (76.8% and 81.42%, respectively). However, *B. turcica* and ‘*Candidatus Borrelia taylori*’ genomes did have higher levels of protein conservation with *B. anserina* (79.1% and 88.5%, respectively) and *B. miyamotoi* (‘*Candidatus Borrelia taylori*’ only: 87.7% POCP) (Figs 2 and 3). Most significantly, all *Borrelia* pairwise POCP values consistently remained well above the 50% POCP threshold for genus delineations proposed by Qin et al. [18], with a minimum value of 64.8% (*B. crocidurae* Achema vs. *B. chilensis* VA1), and a maximum value of 88.8% (*B. miyamotoi* LB-2001 vs. *B. chilensis* VA1) (mean value: 73.6%) (Figs 2 and 3).

The original proposal to delineate LB and RF *Borrelia* was largely based on the occurrence of 53 CSIs that have different forms in LB and RF genogroups [14]. It was subsequently defended by suggesting that novel *Borrelia* species that group with, or as an outgroup to RF *Borrelia* would be expected to contain RF-specific CSIs and generally none specific to the LB group [23]. An examination of these 53 CSIs in *B. turcica* and ‘*Candidatus Borrelia taylori*’ genomes shows although the majority of CSIs present in these genomes correspond to the RF-specific form of the indels, 9/53 (17.0%) and 11/53 (20.8%) of the CSIs in *B. turcica* and ‘*Candidatus Borrelia taylori*’, respectively, correspond to LB-specific forms (Fig 4; Table 2).

Thus, the results of our analysis using genospecies that were originally defined as belonging to the genus *Borrelia* showed a very clear pattern. The results demonstrate that LB and RF

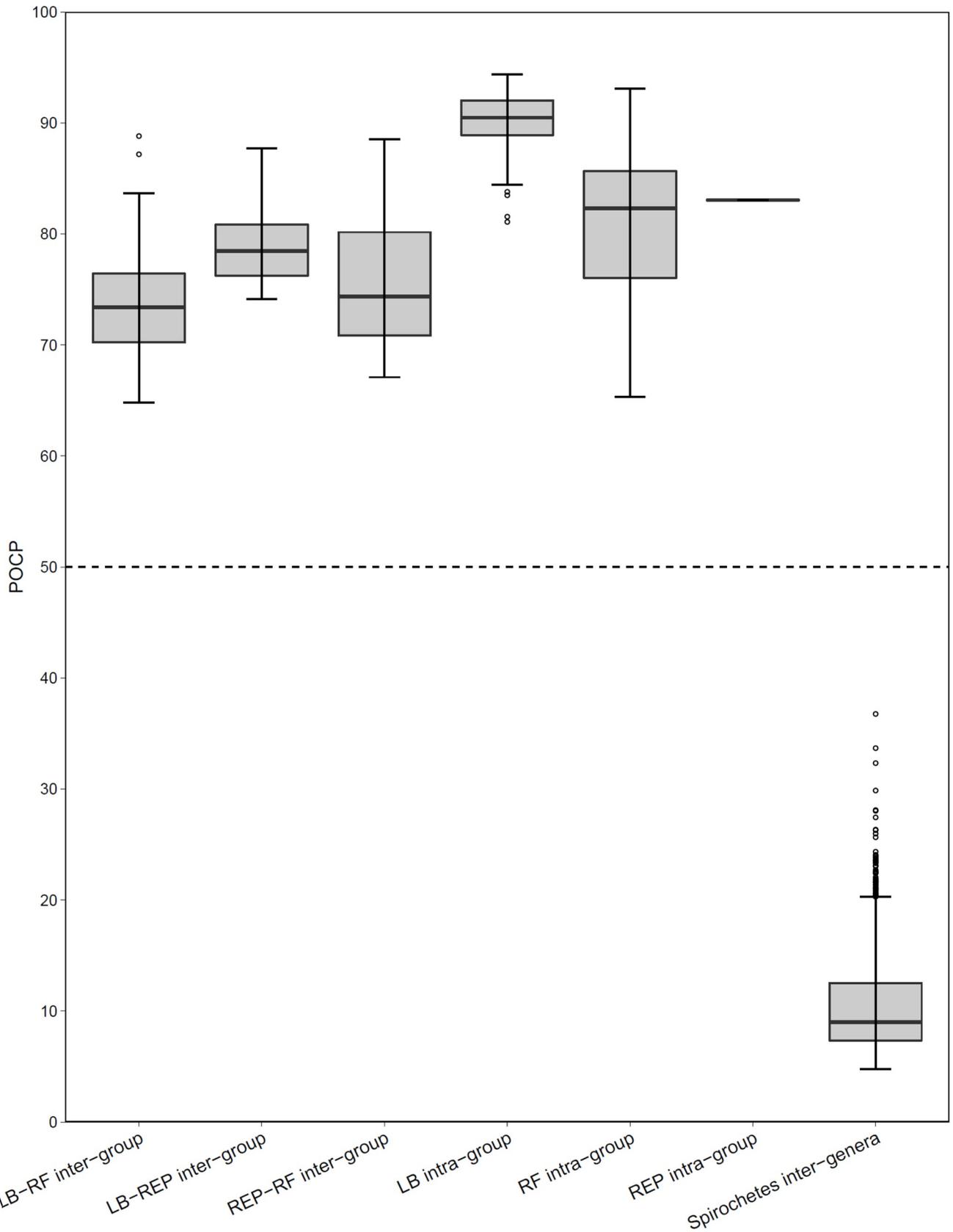


Fig 2. Boxplot of inter- and intra-specific POCP values. Inter-specific and intra-specific comparisons included Lyme borreliosis (LB) and relapsing-fever species (RF), reptile-associated species (REP) including the echnida-associated species ‘*Candidatus B. taylorii*’. The inter-genera comparison included the members of the genera *Borrelia*, *Brachyspira*, *Leptospira*, *Spirochaeta*, and *Treponema*.

<https://doi.org/10.1371/journal.pone.0208432.g002>

Borrelia genogroups lack sufficient proteomic differentiation to be classified as different genera according to the POCP threshold determined by Qin et al. [18]; the analysis of inter-genus POCP supported the classification of the five closely related Spirochaetales genera. Therefore, we propose to formally reestablish the genus *Borrelia* in its original form including species of the LB, RF, and reptile- and echnida-associated genogroups. Additionally, up to 20% of the CSIs identified as having genogroup-specific forms were not concordant with phylogenetic position of *B. turcica* and ‘*Candidatus Borrelia taylorii*’ as predicted previously [23]. Although categorical molecular markers such as these have been previously used in clarifying prokaryotic taxonomy, here these markers appear to have limited utility in resolving the taxonomic classification of novel *Borrelia* species.

The reptile- and echnida-associated *Borrelia* clade to which *B. turcica* and ‘*Candidatus B. taylorii*’ belong is a very recently described group of *Borrelia* for which several novel variants have been described based on single- or multi-gene phylogenetic analyses. Although this group clearly shared a more common ancestor with RF *Borrelia*, the presence of LB-specific CSIs and high protein conservation with LB species suggests this *Borrelia* may share common genetic and biological characteristics with LB species. Both, PCOP and CSI supported the continuum of *Borrelia* species between LB and RF which now includes *B. turcica* and ‘*Candidatus B. taylorii*’. These data suggest that the genus *Borrelia* in the form it was originally described and is proposed here represents a continuum with RF and LB group species at the extreme ends of the genus, and reptile and echnida-associated, and other *Borrelia* species (perhaps still to be discovered) sharing a unique mixture of features from both RF and LB groups.

In our study we included as many type strains as possible, as type strains are the representatives of the species and can be obtained from microbial culture collections. However, for two of the species belonging to the LB group of spirochetes, genomic data of the type strains were

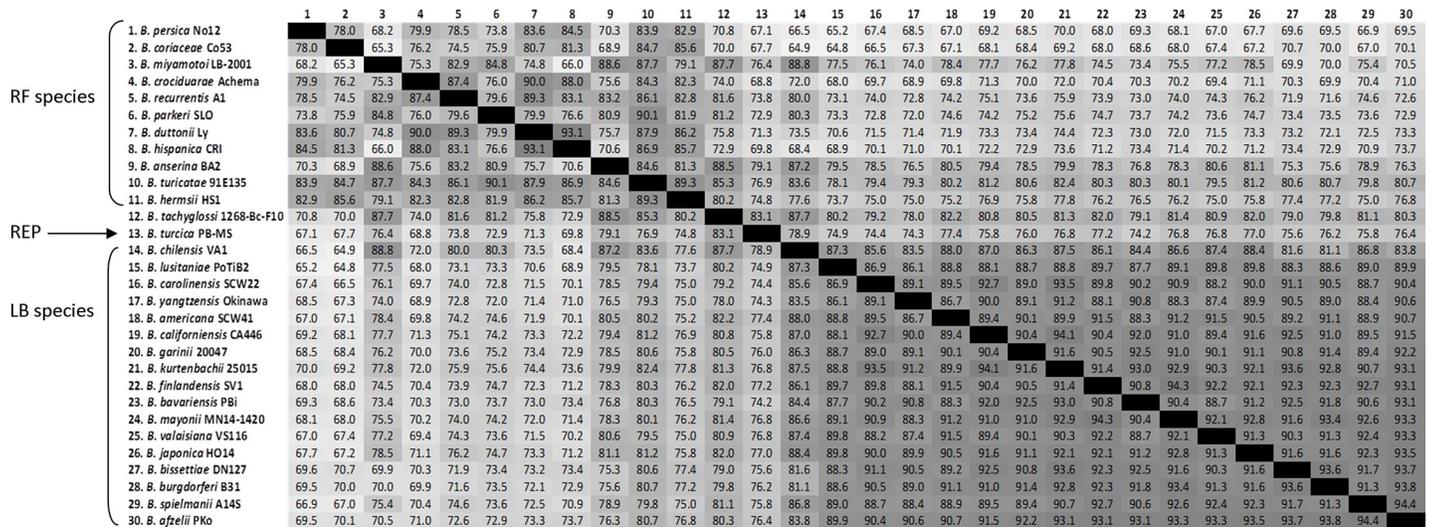


Fig 3. Percentage of conserved proteins (POCP) matrix generated by the method described in [18]. POCP values of species belonging to the LB group, RF group of spirochetes, the reptile-associated species *B. turcica* and echnida-associated species *B. taylorii* are above the genus threshold of 50%, indicating that all belong into one bacterial genus, *Borrelia*.

<https://doi.org/10.1371/journal.pone.0208432.g003>

Table 2. Designation of LB and RF-differentiating CSIs in ‘*Candidatus Borrelia taylorii*’ and *B. turcica* genomes.

Gene	Size of CSI (aa)	‘ <i>Candidatus Borrelia taylorii</i> ’	<i>Borrelia turcica</i>
<i>RecA</i>	1	RF	RF
Nicotinamide-nucleotide adenylyltransferase	1	LB	LB
Hypothetical protein (BB0838)	3	LB	LB
Trigger factor <i>Tig</i>	2	RF	RF
Chemotaxis protein <i>CheY</i>	1	RF	RF
DNA polymerase III subunit beta	1	RF	RF
Translation factor Sua5	2	N/A	RF
Ferrous iron transporter	1	RF	RF
Glucose-6-phosphate isomerase	1	RF	RF
Hypothetical protein (BRE16)	3	RF	RF
Hypothetical protein (BDU327)	6	RF	RF
Hypothetical protein (BT0471)	1	LB	LB
L-lactate permease	1	RF	RF
1-phosphofructokinase	1	RF	RF
GTP-binding protein	2	RF	RF
Sodium/pantothenate symporter	1	LB	LB
Hypothetical protein (BRE32)	2	RF	RF
Hypothetical protein (Q7M33)	1	RF	RF
Hypothetical protein (BRE47)	5	RF	RF
L-proline transport system ATP-binding protein	1	RF	RF
Penicillin-binding protein	1	RF	RF
Hypothetical protein (Q7M131)	1	RF	RF
Hypothetical protein (BT0110)	2	RF	RF
Hypothetical protein (BB0110)	2	RF	RF
Glutamate racemase	6	RF	RF
16S ribosomal RNA methyltransferase <i>RsmE</i>	1	RF	RF
DNA mismatch repair protein <i>mutL</i>	3	RF	RF
Putative lipoprotein	3	LB	LB
Membrane protein	1	LB	RF
Hypothetical protein (BRE314)	1	RF	RF
Methylgalactoside ABC transporter ATP-binding protein	1	RF	RF
Hypothetical protein (BRE355)	1	LB	LB
Sensor transduction histidine kinase	1	RF	RF
DNA polymerase III subunit delta	2	LB	RF
Hypothetical protein (Q7M860)	2	RF	RF
Hypothetical protein (KK90081)	1	RF	RF
Hypothetical protein (Q7M140)	2	LB	LB
Hypothetical protein (BG0159)	1	LB	LB
Outer membrane protein	1	RF	RF
Transglycosylase SLT domain-containing protein	1	RF	RF
Cell division protein <i>FtsZ</i>	1	RF	RF
Excinuclease ABC subunit C	1	RF	RF
Hypothetical protein (BG0519)	1	RF	RF
Hypothetical protein (BBIDN1270545)	5	RF	RF
Hypothetical protein (BBUN400354)	3	RF	RF
Hypothetical protein (BBUZS70553)	1	RF	RF
Hypothetical protein (BB0554)	1	RF	RF

(Continued)

Table 2. (Continued)

Gene	Size of CSI (aa)	' <i>Candidatus Borrelia tachyglossi</i> '	<i>Borrelia turcica</i>
Hypothetical protein (BB0554)	2	RF	RF
Hypothetical protein (BBUCA803285)	1	RF	RF
Methyl-accepting chemotaxis protein	2	LB	LB
Chemotaxis protein	1	RF	RF
Chemotaxis protein	1	RF	RF
Hypothetical protein (L14403475)	1	RF	RF

<https://doi.org/10.1371/journal.pone.0208432.t002>

Borrelia garinii Baranton et al. 1992, *Borrelia japonica* Kawabata et al. 1994, *Borrelia kurtenbachii* Margos et al. 2014, *Borrelia sinica* Masuzawa et al. 2001, *Borrelia spielmanii* Richter et al. 2006 which we consider to be the correct name of the taxon. *Borrelia bavariensis* (Margos et al. 2013) Adeolu and Gupta 2015, *Borrelia carolinensis* (Rudenko et al. 2011) Adeolu and Gupta 2015, *Borrelia garinii* (Baranton et al. 1992) Adeolu and Gupta 2015, *Borrelia japonica* (Kawabata et al. 1994) Adeolu and Gupta 2015, *Borrelia kurtenbachii* (Margos et al. 2014) Adeolu and Gupta 2015, *Borrelia sinica* (Masuzawa et al. 2001) Adeolu and Gupta 2015, *Borrelia spielmanii* (Richter et al. 2006) Adeolu and Gupta 2015 should be considered to be synonyms.

Conclusion

The data presented in this study very clearly demonstrate that all groups investigated, i.e. RF group spirochetes, LB group spirochetes, reptile- and echnida-associated *Borrelia* species belong to the same genus as values for POCP were consistently above the proposed threshold for genus delimitation. We propose to re-establish the genus *Borrelia* in its original form.

Emended description of the genus *Borrelia* (Swellengrebel 1907) (approved lists 1980)

Organisms are helical (0.2–0.3 μm by 10–35 μm). Periplasmic flagella overlap in the central region of the cell. Cells are flexible and motile with rotational and forward/backwards movement. Organisms are host-associated and microaerophilic. They are vectored by argasid ticks, prostrate ixodid ticks, metastrate ixodid ticks and the human body louse. The genome is fragmented into a linear main chromosome, and linear or circular plasmids. The G/C content of the genomic DNA is 27–32 (mol%).

Members of this genus are the causative agents of relapsing fever, Lyme borreliosis or of unknown pathogenicity.

The type species is *Borrelia anserina* (Sakharoff 1891) Bergey et al. 1925(Approved Lists 1980).

Description of *Borrelia afzelii* Canica et al 1994

The description is the same as in Canica et al 1994. DNA-DNA hybridization, RFLP of the *rrs* gene as well as reactivity of monoclonal antibodies differentiates *B. afzelii* from other *Borrelia* species [26, 27]. *B. afzelii* strains are also distinguishable from all other LB species by using Multilocus sequence analysis [24].

Type strain VS461^T (= DSM 10508^T = CIP 103469^T)

Description of *Borrelia lusitaniae* (Le Fleche et al. 1997, sp. nov.)

The description is the same as given in Le Fleche et al. 1997.

Type strain PotiB2^T (= CIP-105366^T, = DSM-107168^T)

Supporting information

S1 Table. Additional proteomes from the order Spirochaetales included in this study. A distance matrix of POCP values is given for genera included and shows that the within genus

percentages are generally higher than 50% except for *Treponema*. (XLSX)

Acknowledgments

The authors acknowledge Cecilia Hizo-Teufel, Christine Hartberger und Sylvia Stockmeier for excellent technical support. The sequencing service was provided by the Norwegian Sequencing Centre (<http://www.sequencing.uio.no>), a national technology platform hosted by the University of Oslo and supported by the “Functional Genomics” and “Infrastructure” programs of the Research Council of Norway and the Southeastern Regional Health Authorities. The authors gratefully acknowledge the services of DSMZ.

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References

1. Skerman VBD, McGowan V, Sneath PHA. Approved List of Bacterial Names. Washington (DC): ASM Press; 1980.
2. Takano A, Goka K, Une Y, Shimada Y, Fujita H, Shiino T, et al. Isolation and characterization of a novel *Borrelia* group of tick-borne borreliae from imported reptiles and their associated ticks. *Environ Microbiol.* 2010; 12(1):134–46. <https://doi.org/10.1111/j.1462-2920.2009.02054.x> PMID: 19758349.
3. Burkot TR, Mullen GR, Anderson R, Schneider BS, Happ CM, Zeidner NS. *Borrelia lonestari* DNA in adult *Amblyomma americanum* ticks, Alabama. *Emerg Infect Dis.* 2001; 7(3):471–3. Epub 2001/06/01. <https://doi.org/10.3201/eid0703.010323> PMID: 11384533; PubMed Central PMCID: PMC2631781.
4. Cutler SJ, Moss J, Fukunaga M, Wright DJ, Fekade D, Warrell D. *Borrelia recurrentis* characterization and comparison with relapsing-fever, Lyme-associated, and other *Borrelia* spp. *Int J Syst Bacteriol.* 1997; 47(4):958–68. <https://doi.org/10.1099/00207713-47-4-958> PMID: 9336893.
5. Fukunaga M, Koreki Y. The flagellin gene of *Borrelia miyamotoi* sp. nov. and its phylogenetic relationship among *Borrelia* species. *FEMS Microbiol Lett.* 1995; 134(2–3):255–8. PMID: 8586277.
6. Casjens SR, Mongodin EF, Qiu WG, Luft BJ, Schutzer SE, Gilcrease EB, et al. Genome stability of Lyme disease spirochetes: comparative genomics of *Borrelia burgdorferi* plasmids. *PLoS ONE.* 2012; 7(3):e33280. Epub 2012/03/21. <https://doi.org/10.1371/journal.pone.0033280> PMID: 22432010; PubMed Central PMCID: PMC3303823.
7. Marosevic D, Margos G, Wallich R, Wieser A, Sing A, Fingerle V. First insights in the variability of *Borrelia recurrentis* genomes. *PLoS neglected tropical diseases.* 2017; 11(9):e0005865. Epub 2017/09/14. <https://doi.org/10.1371/journal.pntd.0005865> PMID: 28902847; PubMed Central PMCID: PMC5612729.

8. Miller SC, Porcella SF, Raffel SJ, Schwan TG, Barbour AG. Large linear plasmids of *Borrelia* species that cause relapsing fever. *J Bacteriol.* 2013; 195(16):3629–39. Epub 2013/06/12. <https://doi.org/10.1128/JB.00347-13> PMID: 23749977; PubMed Central PMCID: PMC3754566.
9. Loh SM, Gillett A, Ryan U, Irwin P, Oskam C. Molecular characterization of 'Candidatus *Borrelia tachyglossi*' (family Spirochaetaceae) in echidna ticks, *Bothriocroton concolor*. *Int J Syst Evol Microbiol.* 2017; 67(4):1075–80. Epub 2017/05/06. <https://doi.org/10.1099/ijsem.0.001929> PMID: 28475032; PubMed Central PMCID: PMC5817193.
10. Guner ES, Watanabe M, Hashimoto N, Kadosaka T, Kawamura Y, Ezaki T, et al. *Borrelia turcica* sp. nov., isolated from the hard tick *Hyalomma aegyptium* in Turkey. *Int J Syst Evol Microbiol.* 2004; 54(Pt 5):1649–52. <https://doi.org/10.1099/ijms.0.03050-0> PMID: 15388724.
11. Loh SM, Gofton AW, Lo N, Gillett A, Ryan UM, Irwin PJ, et al. Novel *Borrelia* species detected in echidna ticks, *Bothriocroton concolor*, in Australia. *Parasit Vectors.* 2016; 9(1):339. Epub 2016/06/16. <https://doi.org/10.1186/s13071-016-1627-x> PMID: 27301754; PubMed Central PMCID: PMC4908759.
12. Gofton AW, Margos G, Fingerle V, Hepner S, Loh SM, Ryan U, et al. Genome-wide analysis of *Borrelia turcica* and 'Candidatus *Borrelia tachyglossi*' shows relapsing fever-like genomes with unique genomic links to Lyme disease *Borrelia*. *Infect Genet Evol.* 2018; 66:72–81. Epub 2018/09/22. <https://doi.org/10.1016/j.meegid.2018.09.013> PMID: 30240834.
13. Takano A, Fujita H, Kadosaka T, Konnai S, Tajima T, Watanabe H, et al. Characterization of reptile-associated *Borrelia* sp. in the vector tick, *Amblyomma geoemydae*, and its association with Lyme disease and relapsing fever *Borrelia* spp. *Environmental microbiology reports.* 2011; 3(5):632–7. Epub 2011/10/01. <https://doi.org/10.1111/j.1758-2229.2011.00280.x> PMID: 23761345.
14. Adeolu M, Gupta RS. A phylogenomic and molecular marker based proposal for the division of the genus *Borrelia* into two genera: the emended genus *Borrelia* containing only the members of the relapsing fever *Borrelia*, and the genus *Borreliella* gen. nov. containing the members of the Lyme disease *Borrelia* (*Borrelia burgdorferi* sensu lato complex). *Antonie van Leeuwenhoek.* 2014; 105(6):1049–72. Epub 2014/04/20. <https://doi.org/10.1007/s10482-014-0164-x> PMID: 24744012.
15. Margos G, Marosevic D, Cutler S, Derdakova M, Diuk-Wasser M, Emler S, et al. There is inadequate evidence to support the division of the genus *Borrelia*. *Int J Syst Evol Microbiol.* 2017; 67(4):1081–4. Epub 2016/12/09. <https://doi.org/10.1099/ijsem.0.001717> PMID: 27930271.
16. Stevenson B, Fingerle V, Wormser GP, Margos G. Public health and patient safety concerns merit retention of Lyme borreliosis-associated spirochetes within the genus *Borrelia*, and rejection of the genus novum *Borreliella*. *Ticks Tick Borne Dis.* 2018. Epub 2018/08/30. <https://doi.org/10.1016/j.ttbdis.2018.08.010> 30154058. PMID: 30154058
17. Konstantinidis KT, Tiedje JM. Genomic insights that advance the species definition for prokaryotes. *Proc Natl Acad Sci U S A.* 2005; 102(7):2567–72. <https://doi.org/10.1073/pnas.0409727102> PMID: 15701695.
18. Qin Q-L, Xie B-B, Zhang X-Y, Chen X-L, Zhou B-C, Zhou J, et al. A Proposed Genus Boundary for the Prokaryotes Based on Genomic Insights. *Journal of Bacteriology.* 2014; 196(12):2210–5. <https://doi.org/10.1128/JB.01688-14> PMID: 24706738
19. Becker NS, Margos G, Blum H, Krebs S, Graf A, Lane RS, et al. Recurrent evolution of host and vector association in bacteria of the *Borrelia burgdorferi* sensu lato species complex. *BMC Genomics.* 2016; 17(1):734. Epub 2016/09/17. <https://doi.org/10.1186/s12864-016-3016-4> PMID: 27632983; PubMed Central PMCID: PMC5025617.
20. Adamek M, Alanjary M, Sales-Ortells H, Goodfellow M, Bull AT, Winkler A, et al. Comparative genomics reveals phylogenetic distribution patterns of secondary metabolites in *Amycolatopsis* species. *BMC Genomics.* 2018; 19(1):426. Epub 2018/06/03. <https://doi.org/10.1186/s12864-018-4809-4> PMID: 29859036; PubMed Central PMCID: PMC5984834.
21. Gish W, States DJ. Identification of protein coding regions by database similarity search. *Nat Genet.* 1993; 3(3):266–72. Epub 1993/03/01. <https://doi.org/10.1038/ng0393-266> PMID: 8485583.
22. Edgar RC. MUSCLE: a multiple sequence alignment method with reduced time and space complexity. *BMC Bioinformatics.* 2004; 5:113. Epub 2004/08/21. <https://doi.org/10.1186/1471-2105-5-113> PMID: 15318951; PubMed Central PMCID: PMC517706.
23. Barbour AG, Adeolu M, Gupta RS. Division of the genus *Borrelia* into two genera (corresponding to Lyme disease and relapsing fever groups) reflects their genetic and phenotypic distinctiveness and will lead to a better understanding of these two groups of microbes (Margos et al. (2016) There is inadequate evidence to support the division of the genus *Borrelia*. *Int. J. Syst. Evol. Microbiol.* <https://doi.org/10.1099/ijsem.0.001717>.Barbour). *Int J Syst Evol Microbiol.* 2017; 67(6):2058–67. Epub 2017/02/01. 10.1099/ijsem.0.001815. PMID: 28141502
24. Margos G, Vollmer SA, Cornet M, Garnier M, Fingerle V, Wilske B, et al. A new *Borrelia* species defined by Multilocus Sequence Analysis of Housekeeping Genes. *Appl Environ Microbiol.* 2009; 75(16):5410–6. <https://doi.org/10.1128/AEM.00116-09> PMID: 19542332

25. Richter D, Postic D, Sertour N, Livey I, Matuschka FR, Baranton G. Delineation of *Borrelia burgdorferi* sensu lato species by multilocus sequence analysis and confirmation of the delineation of *Borrelia spielmanii* sp. nov. *Int J Syst Evol Microbiol*. 2006; 56(Pt 4):873–81. <https://doi.org/10.1099/ijs.0.64050-0> PMID: 16585709.
26. Baranton G, Postic D, Saint Girons I, Boerlin P, Piffaretti JC, Assous M, et al. Delineation of *Borrelia burgdorferi* sensu stricto, *Borrelia garinii* sp. nov., and group VS461 associated with Lyme borreliosis. *Int J Syst Bacteriol*. 1992; 42(3):378–83. <https://doi.org/10.1099/00207713-42-3-378> PMID: 1380285.
27. Canica MM, Nato F, du Merle L, Mazie JC, Baranton G, Postic D. Monoclonal antibodies for identification of *Borrelia afzelii* sp. nov. associated with late cutaneous manifestations of Lyme borreliosis. *Scand J Infect Dis*. 1993; 25(4):441–8. PMID: 8248743.