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TAXONOMIC DESCRIPTION

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Borrelia lanei sp. nov. extends the diversity of *Borrelia* species in California

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Abstract

The diversity of *Borrelia* species discovered in California appears to be particularly high. A divergent group of *Borrelia* strains collected from *Ixodes* ticks in California was described by Postic and co-workers and designated 'genomospecies 2' (Postic D, Garnier M, Baranton G. *Int J Med Microbiol* 2007;297:263–271; Postic D, Ras NM, Lane RS, Hendson M, Baranton G. *J Clin Microbiol* 1998;36:3497–3504). We performed multilocus sequence analysis (MLSA) using eight housekeeping loci (*clpA*, *clpX*, *nifS*, *pepX*, *pyrG*, *recG*, *rplB* and *uvrA*) on 12 strains of this *Borrelia* genospecies to confirm that these strains form a distinct group within the *Borrelia burgdorferi* s. l. complex (Margos G, Hojgaard A, Lane RS, Cornet M, Fingerle V *et al. Ticks Tick Borne Dis* 2010;1:151–158). Phylogenetic and genetic distance analyses based on sequences of the MLSA housekeeping genes corroborated the distinctness of this group; genetic distances to all other members of the *B. burgdorferi* s.l. complex were 96 % or lower. We propose the name *Borrelia lanei* sp. nov. for this genospecies in honor of Professor Robert S. Lane, University of California Berkeley, for his contributions to *Borrelia* and tick research. The type strain for *Borrelia lanei* sp. nov., strain CA28-91^T, has been deposited to two culture collections (=DSM 17992^T=CIP 109135^T).

Borrelia burgdorferi sensu lato (s. l.) is a heterogeneous bacterial species complex that contains the *Borrelia* species causing human Lyme borreliosis (LB). The species complex currently consists of about 20 validated genospecies. In nature, these parasitic bacteria are maintained in transmission cycles among vertebrate reservoir hosts and ticks of the *Ixodes persulcatus* species complex or other *Ixodes* species, such as *Ixodes spinipalpis* [1–3].

The strain designated as the *Borrelia lanei* sp. nov. type strain, CA28-91, was isolated from a pool of 10 questing *Ixodes pacificus* ticks collected during a field survey in Southern California by the California Department of Health Services and the Rocky Mountain Laboratories, National Institute of Allergy and Infectious Diseases [4]. Additional strains of this genospecies have been isolated from questing *I. spinipalpis* and from ticks collected from rabbit vertebrate hosts in Northern California [5, 6]. In 1998, Postic and co-workers undertook molecular analysis of strains from California based on the 16S rRNA gene locus and the 5S–23S intergenic spacer (IGS) region. These investigations suggested that strains CA2 and CA28-91 constituted a new genomic group within the *B. burgdorferi* s. l. complex. Further molecular investigations using the 16S rRNA gene locus, the 5S–23S IGS, housekeeping genes and an outer surface protein gene confirmed that, indeed, strains CA2 and CA28-91 clustered together in phylogenies based on concatenated sequences of several loci as well as the 16S rRNA gene sequences [5]. This prompted the authors to consider strains CA2 and CA28-91 as representative of a novel *Borrelia* genospecies, which they designated 'genomospecies 2'. Strain CA28-91 was proposed as the type strain and deposited in two microbial culture collections.

Interestingly, phylogenetic analysis based on sequences of only the 5S–23S IGS showed that the two strains, CA2 and CA28-91 [6], and also in combination with closely related strains (i.e. CA2, CA426, CA388 and CA28, CA400, CA393,

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Abbreviations: BSK, Barbour-Stoenner-Kelly; IGS, intergenic spacer; LB, Lyme borreliosis; MLSA, multilocus sequence analysis; MLST, multilocus sequence typing; nov., novel; s.l., *sensu lato*; sp., species; s.s., *sensu stricto*; ST, sequence type.

The GenBank/EMBL/DDBJ accession numbers for type strain CA28-91 for sequences are available via GenBank accession nos. *rrf-rrl* AJ006375; *flaB* DQ393351; *hbb* DQ393371; *groEL* DQ393392; *recA* DQ393410; sequences for the MLST housekeeping genes are available for MLST ST 719-730 at the *Borrelia* MLST website at www.pubMLST.org (ID numbers 1949–1960).

One supplementary table is available with the online Supplementary Material.

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CA399) branched in different parts of the tree [5]. These data suggest that this locus alone may not always be reliable as a species marker.

In this study, we used multilocus sequence analysis (MLSA) based on eight chromosomally located housekeeping genes (i.e. *clpA, clpX, nifS, pepX, pyrG, recG, rplB* and *uvrA*) to evaluate the status of strains supposed to belong to 'genomospecies 2'. PCR and sequencing of PCR products was performed as described previously ([7], see also https://pubmlst.org/borrelia/). Sequences were compared to sequences present in the database at https://pubmlst.org/borrelia/ and amongst each other. Sequences that differed by one or more nucleotide from other sequences were considered novel alleles and received a new allelic number. Novel allelic profiles were assigned consecutive new sequence type (ST) numbers. The software MEGA 5.0 [8] was used for concatenation of sequences, alignment, determination of genetic distances and generation of phylogenetic trees. The Kimura two-parameter model was used to conduct genetic distance analyses [9]. In phylogenetic analysis, the percentage of trees in which the associated taxa clustered together is shown next to the branches. Initial tree(s) for the heuristic search were obtained automatically by applying neighbour-joining (NJ) and BioNJ algorithms to a matrix of pairwise distances estimated using the maximum composite likelihood (MCL) approach, and then selecting the topology with superior log likelihood value. Codon positions included were 1st+2nd+3rd+Noncoding. All positions containing gaps and missing data were eliminated. There were a total of 4768 positions in the final dataset.

Phylogenies were reconstructed using the maximum-likelihood model. The general time-reversible model with uniform rates across sites was chosen as the evolutionary model [10], nearest-neighbour interchange for tree optimization, and 500 bootstrap repetitions to estimate branch support. Sequences of the following LB spirochaete species and strains were included in the genetic distance and phylogenetic analysis: *Borrelia afzelii* VS461^T, *Borrelia americana* CA-8B-89, '*Borellia andersonii*' 21123, *Borrelia bavariensis* PBi^T, *Borrelia bissettiae* DN127^T and CA28, *Borrelia burgdorferi sensu stricto* (s. s.) B31^T and Z41293, *Borrelia californiensis* CA443, *Borrelia carolinensis* SCW-22^T, '*Borrelia chilensis*' VA1, *Borrelia garinii* 20047^T, *Borrelia japonica* HO14^T, *Borrelia kurtenbachii* 25015^T, *Borrelia lusitaniae* PoHL1, *Borrelia mayonii* MN14-1420^T, *Borrelia sinica* CMN3^T, *Borrelia spielmanii* A14S, *Borrelia tanukii* Hk501^T, *Borrelia turdi* Ya501^T, *Borrelia valaisiana* VS116^T, *Borrelia yangtzensis* QLM4P1, *Borrelia* sp. CA690 and, as outgroups the relapsing-fever species, *Borrelia duttonii* Ly, *Borrelia hermsii* DAH, *Borrelia turicatae* 91E135, *Borrelia miyamotoi* USA LB2100 and *Borrelia miyamotoi* Japan HT31^T.

We included some of the strains previously used by Postic and co-workers [5] and added additional strains so that the number of strains included in our study totalled 12 (see Table 1). Phylogenetic analysis of the concatenated sequences of the housekeeping genes confirmed the relationship of strains incorporated in the new *Borrelia* species and their distinctness from all other previously described *Borrelia* genospecies. In the phylogeny all strains of the novel genospecies clustered together and apart from all other *Borrelia* genospecies (Fig. 1). The closest-related genospecies of the *B. burgdorferi* s.l. species complex were *Borrelia* species from North America with *B. mayonii*, a human pathogenic genospecies recently discovered in the Midwest of the USA [11, 12], showing the lowest genetic distance to type strain CA28-91^T (0.040, which equals 96 % similarity) followed by *B. burgdorferi* s. s. strains Z41293 and B31 (0.042 and 0.045, respectively) and *B. americana* (0.049) (Fig. 1 and Table S1, available in the online Supplementary Material). All other *Borrelia* genospecies had higher genetic distance values (Table S1).

Within *B. lanei* sp. nov. some strains (LGP59, MMU64, CA2, WILK42), all collected in Mendocino County, showed borderline genetic distances (0.017) compared to the type strain CA28-91^T and genetic distances just above the species threshold to several other strains of the group (yellow label in Table S1). The same strains formed a distinct branch in the phylogeny (Fig. 1) which, nevertheless, formed a sister clade to *B. lanei* sp. nov. strains and not to any other *Borrelia* species. Thus, in spite of slightly high genetic distances for some strains, genetic distances to other strains of the group were below the species threshold. This led us to consider all 12 strains included into our analysis as being members of the new *Borrelia* species, *B. lanei* sp. nov.

The strains included here had been isolated from *I. pacificus* and *I. spinipalpis* ticks (Table 1) [5, 6, 13, 14]. One tick was collected from a jack rabbit and one strain was isolated from a brush rabbit suggesting a potential transmission cycle between lagomorphs and *Ixodes* ticks. Interestingly, *B. lanei* sp. nov. was recently detected in *I. spinipalpis* ticks also collected from a rabbit, but in Canada [15]. However, solid evidence does not exist for either vector or host adaptations, thus, the transmission cycle(s) maintaining *B. lanei* sp. nov. need to be explored in future studies.

DESCRIPTION OF *BORRELIA LANEI* SP. NOV.

Borrelia lanei sp. nov. (la.ne'i. N.L. gen. n. *lanei* in honour of Professor Robert S. Lane for his outstanding contributions to *Borrelia* and *Ixodes* research).

The cells are helical, approximately 0.2 µm by 20 µm, and stain well with Giemsa stain. Unstained cells can be visualized by dark-field microscopy. The bacteria are flexible and motile with rotational and forward/backwards movement. Cells can be cultured *in vitro* under microaerophilic conditions [16] using liquid media such as Barbour–Stoenner–Kelly (BSK) medium. Optimal growth occurs at 33–34 °C. The mean DNA G+C content of the type strain is 27 mol%.

The type strain CA28-91^T was isolated from a pool of 10 questing *I. pacificus* ticks in 1991. It has been deposited at the German Microbial Strain Collection (=DSM 17992^T) and at the Institut Pasteur, Paris, France (=CIP 109135^T). *B.*

Table 1. *Borrelia lanei* sp. nov. strains included into analysis

MLST ST	Strain name	Country of origin	Region	Biologic source of isolate	Tick stage	Vertebrate host	Year of collection	Collected by	Isolated by	Reference	GenBank/ MLST ID
719	CA28-91^T	USA	Kern Co., California	*Ixodes pacificus*	Adults	Unknown	1991	California Department of Health	California Department of Health	[4, 5]	DQ393351.1; DQ393371.1; DQ393392.1; DQ393410.1; AJ006375; ID 1949
720	LGP59	USA	Mendocino Co., CA	*I. pacificus*	Nymph	Unknown	2004	R. J. Eisen, L. Eisen	J. Mun	[14]	ID 1950
721	MMU64	USA	Mendocino Co., CA	*I. pacificus*	Nymph	Unknown	2004	R. J. Eisen, L. Eisen	N.Fedorova	[14]	ID 1951
722	CA2	USA	Mendocino Co., CA	*Ixodes spinipalpis*	Adult female	Jack rabbit	1986	R. S. Lane	R. S. Lane	[5]	DQ393352.1; DQ393391.1; L301231; ID 1952
723	WILK42	USA	Mendocino Co., CA	*I. pacificus*	Nymph	Unknown	2004	R. J. Eisen, L. Eisen	N. Fedorova	[14]	ID 1953
724	CA400	USA	Contra Costa Co., CA	*I. spinipalpis*	Nymph	Unknown	1993	K. A. Padgett	K. A. Padgett	[5]	AY177638 ID 1954
725	CA388	USA	Contra Costa Co., CA	*I. spinipalpis*	Nymph	Unknown	1993	K. A. Padgett	K. A. Padgett	[5]	AY176361 ID 1955
726	CA426	USA	Contra Costa Co., CA	*Sylvilagus bachmani*	Unknown	Brush rabbit	1995	C. A. Peavey	C. A. Peavey	[5]	AY177640 ID 1956
727	UCAsia508	USA	Alameda Co., CA	*I. spinipalpis*	Nymph	Unknown	2009	J. E. Kleinjan	N. Fedorova	Unpublished	ID 1957
728	CA399	USA	Contra Costa Co., CA	*I. spinipalpis*	Nymph	Unknown	1993	K. A. Padgett	K. A. Padgett	[5]	AY182046 ID 1958
729	CA910	USA	Alameda Co., CA	*I. spinipalpis*	Nymph	Unknown	2014	J. E. Kleinjan	J. E. Kleinjan	Det. N. Fedorova	ID 1959
730	CA393	USA	Contra Costa Co., CA	*I. spinipalpis*	Nymph	Unknown	1993	K. A. Padgett	K. A. Padgett	[5]	AY177637; ID 1960

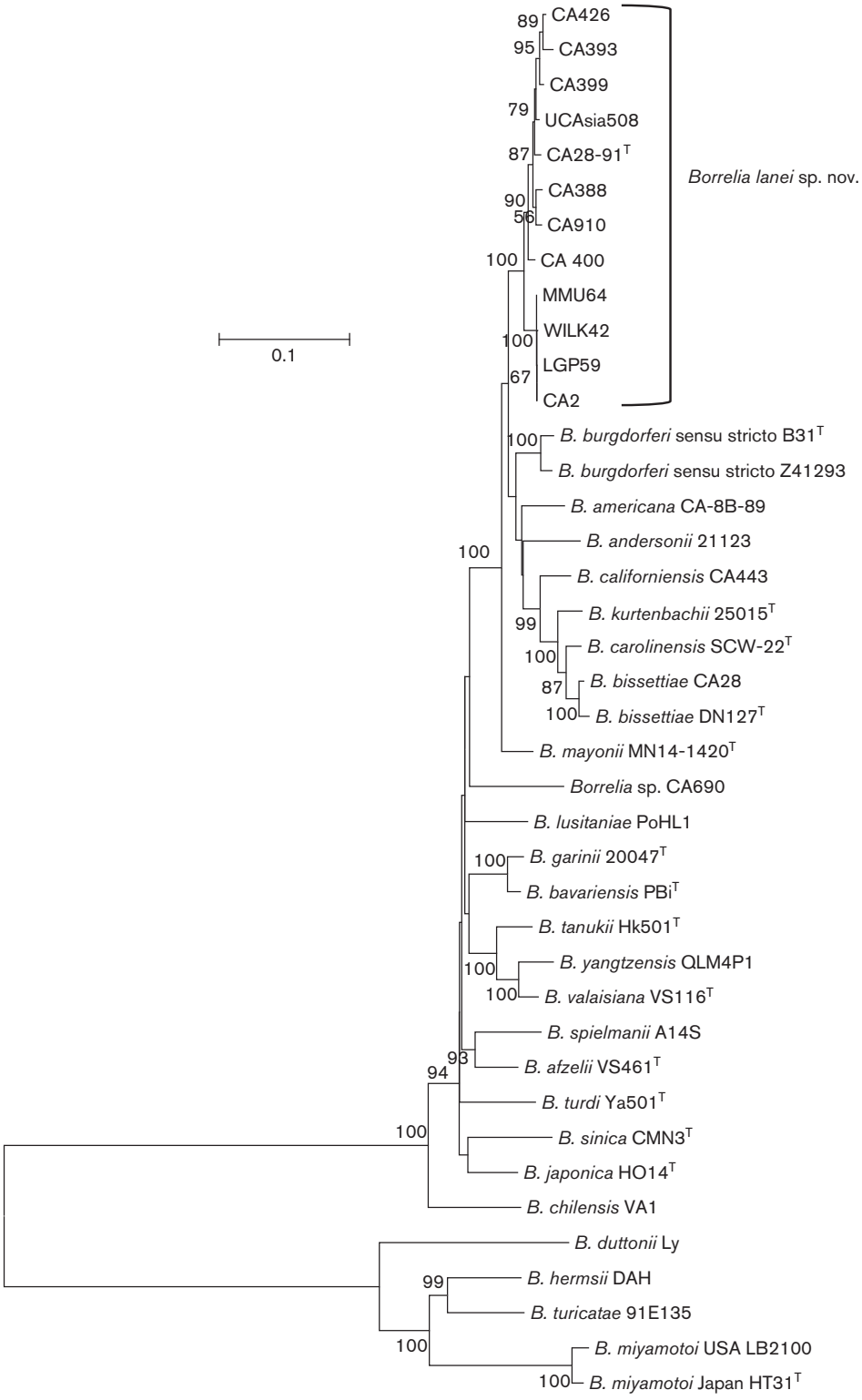

Fig. 1. Molecular phylogenetic analysis of *B. lanei* sp. nov. The evolutionary history was inferred by using the maximum-likelihood method based on the general time reversible model [10]. The tree with the highest log likelihood (−33644, 4319) is shown. A discrete Gamma distribution was used to model evolutionary rate differences among sites [4 categories (+*G*, parameter=0, 6690)]. The rate variation model allowed for some sites to be evolutionarily invariable [(+*I*), 32, 8618 % sites]. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site (scale bar). *B. lanei* sp. nov. strains form a cluster containing two sister clades. Both sister clades form a separate cluster distinct from all other *Borrelia* genospecies known to date underlining this species is divergent.

lanei can be distinguished from other *Borrelia* genospecies via sequences of the *rrs* locus and by MLSA [5, 6, 17]. This bacterium is maintained in nature in diverse transmission cycles likely involving lagomorph reservoir hosts and certain *Ixodes* species ticks such as *I. spinipalpis* and *I. pacificus*.

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Conflicts of interest
The authors declare that there are no conflicts of interest.

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