Unique Strain of *Borrelia miyamotoi* in *Ixodes pacificus* Ticks, California, USA

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DOI: http://dx.doi.org/10.3201/eid2212.152046

To the Editor: *Borrelia miyamotoi* causes a recently recognized tickborne zoonosis in Eurasia and North America (*1*). The species has been detected in *Ixodes persulcatus* ticks in Asia and Russia, *I. ricinus* ticks in Europe, and *I. scapularis* and *I. pacificus* ticks in North America. In most of these regions, *B. miyamotoi* is sympatric with Lyme disease agents, such as *B. burgdorferi*, and both pathogens are transmitted locally by the same species of *Ixodes* ticks. *B. miyamotoi* generally is less prevalent than *B. burgdorferi* in nymphs and adults in North America (*2*), except in California, where the prevalences of the 2 species in populations of nymphal and adult *I. pacificus* ticks are similar (*3*–*6*).

Genomes of isolates of *B. miyamotoi* from *I. persulcatus* and *I. scapularis* ticks have been sequenced (*7*). Comparatively less was known about *B. miyamotoi* in *I. pacificus* ticks. Limited sequence data of 16S ribosomal RNA and flagellin genes and the 16S–23S intergenic spacer (IGS) were sufficient to identify the *I. pacificus*–borne spirochete as a sister taxon to *B. miyamotoi* from elsewhere (*3*,*4*). Until *B. miyamotoi* is isolated from *I. pacificus* ticks, determination of additional sequences from *I. pacficus* ticks from California addresses 2 issues of phylogeographic and potential epidemiologic importance: Is the California population of *B. miyamotoi* more akin to the strain across the Pacific Rim or to the strain thousands of kilometers to the east in North America? Will the noted pattern of exclusive association between the genotype of *B. miyamotoi* and the species of *Ixodes* vector continue to hold (*1*)?

We evaluated DNA extracts of *B. miyamotoi*–infected *I. pacificus* ticks collected by and stored at 2 laboratories in the San Francisco Bay area of California. Ticks had been collected while questing either on low vegetation or in leaf litter. To confirm *B. miyamotoi* in candidate extracts and to exclude extracts that also contained *B. burgdorferi* sensu lato, we used a quantitative PCR, which differentiates relapsing fever and Lyme disease group species (*2*). Two extracts that met these criteria were Sonom53 from a nymph in Sonoma County, California (38.328758, –122.625286), and SMA107 from an adult male tick in San Mateo County, California (37.466999, –122.283532). We amplified DNA by PCR for 1,307 bp of the 16S ribosomal RNA gene (*8*) and variable lengths of the IGS (*9*). In addition, we performed PCR amplification and sequencing of partial sequences of 8 chromosomal genes used for multilocus sequence typing (MLST): *clpA*, *clpX*, *nifS*, *pepX*, *pyrG*, *recG*, *rplB*, and *uvrA* (*10*). The primers (and annealing temperatures for 35 cycles) were as given (http://pubmlst.org/borrelia), except for these modifications: *clpA* (53°C); *clpX* forward 5′-CCGTTGCTATTTGTTTTGAATGCTCT-3′ (55°C); *pepX* forward 5′-TTAAAACTTGATGATAAATGGTCATTA-3′ and reverse 5′-TTAAAACTTGATGATAAATGGTCATTA-3′ (52°C); *pyrG* forward 5′-CTTTTAGTAATTGAGATTGGTGGT-3′ and reverse 5′-CAGCATCAAGTATTCCACAAAC-3′ (55°C); *recG* forward 5′-CTAGCATTCCTTTAGTTGAGGC-3′ and reverse 5′-TTSTGTTAAAGGTTCCTTATAAAG-3′ (52°C); *rplB* forward 5′-ATTAAAACTTATAGGCCAAAAAC-3′ and reverse 5′-GGCTGACCCCAAGGAGAT-3′ (55°C); and *uvrA* forward 5′-GCTTAAATTTTTAATTGATGTTGGA-3′ and reverse 5′-CAAGGAACAAAAATRTCAGGC-3′ (52°C). On a Bio-Rad T100 thermal cycler (Hercules, CA, USA) and with Apex Master mix (Genesee Scientific, San Diego, CA, USA), PCR extension at 72°C was 1.5 min for *clpX* and 1.0 min for others; final elongation was for 5 min at 72°C. Products were sequenced over both strands at GENEWIZ (San Diego, CA, USA) by the Sanger method either directly or after cloning into a plasmid vector. Resultant sequences were aligned with homologous sequences (Figure). Alignments and distance neighbor-joining and maximum-likelihood phylograms were generated with Seaview4 (http://doua.prabi.fr/software/seaview). The equal length MLST sequences, as specified (*10*), for each locus were concatenated.

We determined a rooted neighbor-joining phylogram of 16S ribosomal RNA gene sequences of *B. miyamotoi* from different *Ixodes* species and that of *Amblyomma americanum* tickborne *B. lonestari* (Figure, panel A). Other species of the relapsing fever group served as an outgroup. *B. miyamotoi* sequences from *I. pacificus* ticks in 2 San Francisco Bay area counties clustered with sequences from *I. scapularis*–borne organisms rather than with *I. persulcatus*–borne organisms in Asia or an *I. ricinus*–borne isolate in Europe. This analysis confirmed that the organism in *I. pacificus* was *B. miyamotoi*. An unrooted phylogram of 4,776 nt of concatenated MLST sequences originating in *I. pacificus*, *I. scapularis*, *I. persulcatus*, or *I. ricinus* ticks had similar topology and differentiated the different strains (Figure, panel B). The *B. miyamotoi* organisms from 2 counties differed at 1 position, a synonymous transition in *pyrG*, among the MLST loci. IGS

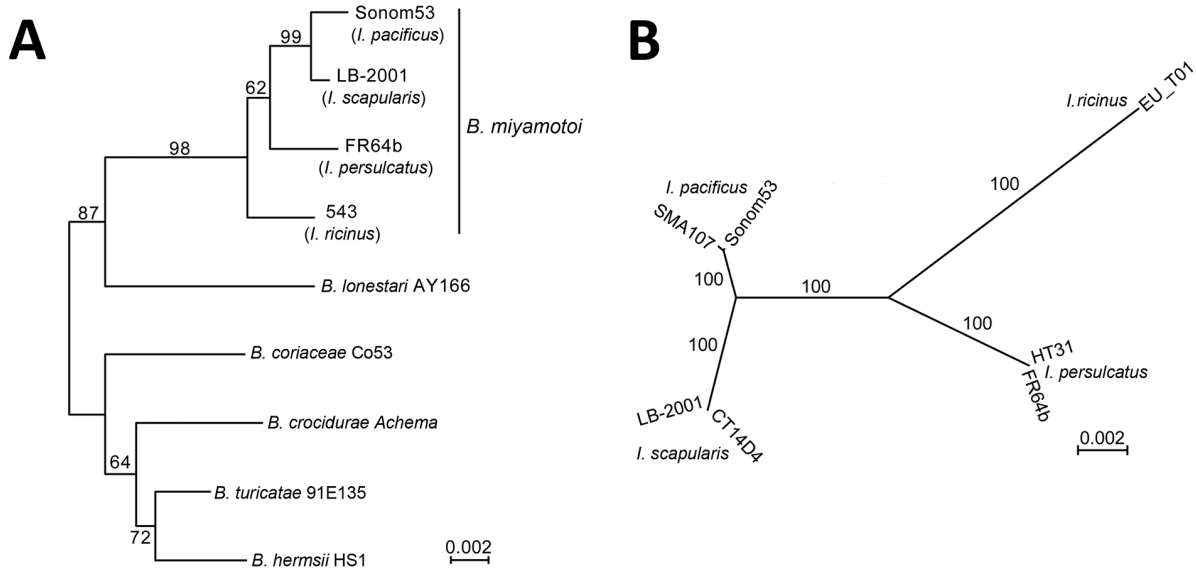

Figure. Phylograms of 16S ribosomal RNA sequences (A) and of multilocus sequence typing (MLST) genes (B) of *Borrelia miyamotoi* strains from *Ixodes* ticks collected in California, USA, and selected other *Borrelia* species. A) Rooted neighbor-joining distance phylogram of observed differences. Percentage support for clades was evaluated by 1,000 bootstrap replications, and values are indicated along branches. The GenBank accession number for the partial 16S ribosomal RNA gene of Sonom53 is KU196080. GenBank accession numbers for 16S ribosomal RNA genes of other *B. miyamotoi* strains are NR_121757 (LB-2001), and KJ847049 (543), and AY604976 (FR64b). GenBank accession numbers for corresponding sequences of designated strains of other species are AY166715 for *B. lonestari* and, for the 4 species constituting the outgroup, AF210134 for *B. coriaceae*, GU350713 for *B. crocidurae*, NR_102958 for *B. turicatae*, and NR_102957 for *B. hermsii*. The tick species sources of the *B. miyamotoi* organisms are indicated. B) Unrooted maximum-likelihood phylogram for 8 concatenated, codon-aligned MLST genes. The model of nucleotide substitution was HKY85 and the empirically estimated γ shape parameter was 0.01. Percentage support for clades was evaluated by 100 bootstrap replications by using full-heuristic search, and values are indicated along branches. GenBank accession numbers for Sonom53 partial sequences of *clpA*, *clpX*, *nifS*, *pepX*, *pyrG*, *recG*, *rplB*, and *uvrA* genes are KU23498–KU234405. The partial sequence of SMA107 *pyrG* is KU307254. The corresponding sequences for FR64b, LB-2001, and CT14D4 were obtained from the complete chromosomes (GenBank accession nos. CP004217, CP006647, and CP010308, respectively). The MLST sequences for strains EU-T01 and HT31 were obtained from the Borrelia MLST Database (http://pubmlst.org/borrelia/), where they have identification numbers of 1279 and 1275, respectively. Scale bar indicates nucleotide substitutions per site.

sequences of the 2 organisms were the same (GenBank accession no. KU184505) and identical to the IGS of other *B. miyamotoi* in *I. pacificus* ticks (e.g., GenBank accession no. KF957669). As observed previously (*4*,*9*), they were distinct from strains associated with other *Ixodes* species.

In conclusion, we identified differences in several genetic loci between *B. miyamotoi* in *I. pacificus* ticks and organism strains associated with other *Ixodes* species. However, we found a close phylogenetic relationship between organisms from the far-western and the northeastern United States.

Acknowledgments

We thank Parth Sitlani for technical assistance.

This research was supported in part by grant R21 AI100236 from the National Institute of Allergy and Infectious Diseases, National Institutes of Health, to A.G.B. and by a generous gift from Tom Eames and Geneva Anderson to R.S.L.

References

1. Krause PJ, Fish D, Narasimhan S, Barbour AG. *Borrelia miyamotoi* infection in nature and in humans. Clin Microbiol Infect. 2015;21:631–9. http://dx.doi.org/10.1016/j.cmi.2015.02.006
2. Barbour AG, Bunikis J, Travinsky B, Hoen AG, Diuk-Wasser MA, Fish D, et al. Niche partitioning of *Borrelia burgdorferi* and *Borrelia miyamotoi* in the same tick vector and mammalian reservoir species. Am J Trop Med Hyg. 2009;81:1120–31. http://dx.doi.org/10.4269/ajtmh.2009.09-0208
3. Mun J, Eisen RJ, Eisen L, Lane RS. Detection of a *Borrelia miyamotoi* sensu lato relapsing-fever group spirochete from *Ixodes pacificus* in California. J Med Entomol. 2006;43:120–3. http://dx.doi.org/10.1603/0022-2585(2006)043[0120:DOABMS]2.0.CO;2
4. Fedorova N, Kleinjan JE, James D, Hui LT, Peeters H, Lane RS. Remarkable diversity of tick or mammalian-associated borreliae in the metropolitan San Francisco Bay area, California. Ticks Tick Borne Dis. 2014;5:951–61. http://dx.doi.org/10.1016/j.ttbdis.2014.07.015
5. Padgett K, Bonilla D, Kjemtrup A, Vilcins IM, Yoshimizu MH, Hui L, et al. Large scale spatial risk and comparative prevalence of *Borrelia miyamotoi* and *Borrelia burgdorferi* sensu lato in *Ixodes pacificus*. PLoS ONE. 2014;9:e110853. http://dx.doi.org/10.1371/journal.pone.0110853

6. Salkeld DJ, Nieto NC, Carbajales-Dale P, Carbajales-Dale M, Cinkovich SS, Lambin EF. Disease risk and landscape attributes of tick-borne *Borrelia* pathogens in the San Francisco Bay area, California. PLoS ONE. 2015;10:e0134812. http://dx.doi.org/10.1371/journal.pone.0134812
7. Barbour AG. Phylogeny of a relapsing fever *Borrelia* species transmitted by the hard tick *Ixodes scapularis*. Infect Genet Evol. 2014;27:551–8. http://dx.doi.org/10.1016/j.meegid.2014.04.022
8. Barbour AG, Maupin GO, Teltow GJ, Carter CJ, Piesman J. Identification of an uncultivable *Borrelia* species in the hard tick *Amblyomma americanum*: possible agent of a Lyme disease–like illness. J Infect Dis. 1996;173:403–9. http://dx.doi.org/10.1093/infdis/173.2.403
9. Bunikis J, Tsao J, Garpmo U, Berglund J, Fish D, Barbour AG. Typing of *Borrelia* relapsing fever group strains. Emerg Infect Dis. 2004;10:1661–4. http://dx.doi.org/10.3201/eid1009.040236
10. Margos G, Gatewood AG, Aanensen DM, Hanincova K, Terekhova D, Vollmer SA, et al. MLST of housekeeping genes captures geographic population structure and suggests a European origin of *Borrelia burgdorferi.* Proc Natl Acad Sci U S A. 2008;105:8730–5. http://dx.doi.org/10.1073/pnas.0800323105

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Xenopsylla brasiliensis Fleas in Plague Focus Areas, Madagascar

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DOI: http://dx.doi.org/10.3201/eid2212.160318

To the Editor: Plague is a life-threatening infectious disease caused by the gram-negative bacterium *Yersinia pestis* (*1*). *Y. pestis* primarily infects rodents but can also cause outbreaks of plague in humans. The infection is usually transmitted within murine populations and then to humans by bites from infected fleas. The oriental rat flea, *Xenopsylla cheopis*, is considered the most efficient plague vector (*1*). Plague remains a major public health threat, causing annual epidemics, especially in Madagascar.

From November 2013 through January 2014, Madagascar reported 427 suspected cases and 45 confirmed cases of plague (both bubonic and pneumonic) in 4 districts. We report here on the flea species associated with rodents and those collected from human dwellings in the Mandritsara District where plague occurred (online Technical Appendix Figure, http://wwwnc.cdc.gov/EID/article/22/10/16-0318-Techapp1.pdf). Four villages in the district were investigated 1 month after the end of the human plague epidemic and after an insecticide-based vector control intervention had taken place. Fleas were collected, either from rats or by using candle traps set inside houses, and preserved in 70% ethanol (online Technical Appendix Table). Rats were trapped alive inside houses and in the cultivated lands.

During the survey, 180 rodents were trapped; they belonged to species *Rattus rattus* (93.3%, n = 168), *Mus musculus* (5.6%, n = 10), and *Suncus murinus* (1.1%, n = 2). A total of 50 fleas were collected from these rodents. The fleas belonged to 4 species: *Sinopsyllus fonquerniei* (n = 26), *Xenopsylla brasiliensis* (n = 14), *X. cheopis* (n = 9), and *Echidnophaga gallinacea* (n = 1) (Table). The first 3 are known to be *Y. pestis* vectors. Of fleas caught in candle traps placed inside houses, ≈98% were the human flea *Pulex irritans*, whose role in plague outbreaks is unknown (*2,3*).

Although *X. cheopis* and *S. fonquerniei* fleas are common *Y. pestis* vectors in Madagascar (*1*), the major finding of this study was the discovery of *X. brasiliensis* fleas, which may be involved in plague transmission in Madagascar. Fleas were identified to the species under binocular magnification by using systematic keys (*4,5*). Each flea specimen was identified independently by 2 different technicians. The morphologic identification of *X. brasiliensis* (Baker, 1904) was also confirmed by Jean-Claude Beaucournu (*6*). Specimens of *X. brasiliensis* fleas identified in this study exhibit the morphologic characteristics of the species, which distinguish it from *X. cheopis* fleas, as follows: antepygidial bristle of male is marginal, inserted on the long pedestal, process 1 of the clasper with 8 or 9 bristles (which are stout, straight, spiniform, and 1 angled) and the process 2 of the clasper with the tip turned up (*5*). Compared with females of other *Xenopsylla* spp., *X. brasiliensis* females have a distinct spermathecal shape with a very swollen bulga, which is larger than the base of the hilla (*4*). Moreover, DNA of *X. brasiliensis, P. irritans,* and *X. cheopis* fleas collected during this study was extracted and amplified by using primers targeting the D3 segment of the 28S ribosomal RNA-encoding gene (*7*) and sequenced. *X. brasiliensis* sequences isolated showed 100% nucleotide similarity with those from Mauritius (*4*) and were different from *X. cheopis* and *P. irritans* sequences. All sequences are available in GenBank (accession nos. KU759935–KU759954).