coeruleoabla), and 6 white-beaked dolphins (*Lagenorhynchus aIbirostris*). Serum samples from 145 bottlenose dolphins (*Tursiops truncates*) from the collection of the Dolphinarium Harderwijk (Harderwijk, the Netherlands) were also tested. DRV-neutralizing antibodies were detected in serum samples from 1 bottlenose dolphin (7%), 5 striped dolphins (55%), 1 white-beaked dolphin (17%), and 3 harbor porpoises (4%). These results suggested that DRV or closely related viruses continue to infect members of cetacean species (*6*).

Although rhabdovirus evolutionary pathways are complicated (*9*), our analysis suggests that DRV is a possible derivative of fish rhabdoviruses. DRV might have originated from an unidentified fish rhabdovirus and might cycle between fish and marine mammals, similar to that suggested for cycling of vesicular stomatitis virus between arthropods and terrestrial mammals (*10*). Future analyses of sequences from other marine mammal rhabdovirus sequences might support the validity of our phylogenetic analysis and result in creation of a new group containing marine mammal rhabdoviruses.

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References

1. King AM, Adams MJ, Carstens EB, Lefkowitz EJ, editors. Virus taxonomy: classification and nomenclature of viruses: ninth report of the International Committee on Taxonomy of Viruses. London; Waltham (MA): Academic Press; 2012.
2. Hoffmann B, Beer M, Schütze H, Mettenleiter TC. Fish rhabdoviruses: molecular epidemiology and evolution. Curr Top Microbiol Immunol. 2005;292:81–117. http://dx.doi.org/10.1007/3-540-27485-5_5
3. Crane M, Hyatt A. Viruses of fish: an overview of significant pathogens. Viruses. 2011;3:2025–46. http://dx.doi.org/10.3390/v3112025
4. van Beurden SJ, Engelsma MY, Roozenburg I, Voorbergen-Laarman MA, van Tulden PW, Kerkhoff S, et al. Viral diseases of wild and farmed European eel *Anguilla anguilla* with particular reference to the Netherlands. Dis Aquat Organ. 2012;101:69–86. http://dx.doi.org/10.3354/dao02501
5. Osterhaus AD, Broeders HW, Teppema JS, Kuiken T, House JA, Vos HW, et al. Isolation of a virus with rhabdovirus morphology from a white-beaked dolphin (*Lagenorhynchus albirostris*). Arch Virol. 1993;133:189–93. http://dx.doi.org/10.1007/BF01309754
6. van Leeuwen M, Williams MM, Koraka P, Simon JH, Smits SL, Osterhaus AD. Human picobirnaviruses identified by molecular screening of diarrhea samples. J Clin Microbiol. 2010;48:1787–94. http://dx.doi.org/10.1128/JCM.02452-09
7. Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S. MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. Mol Biol Evol. 2011;28:2731–9. http://dx.doi.org/10.1093/molbev/msr121
8. Talavera G, Castresana J. Improvement of phylogenies after removing divergent and ambiguously aligned blocks from protein sequence alignments. Syst Biol. 2007;56:564–77. http://dx.doi.org/10.1080/10635150701472164
9. Kuzmin IV, Novella IS, Dietzgen RG, Padhi A, Rupprecht CE. The rhabdoviruses: biodiversity, phylogenetics, and evolution. Infect Genet Evol. 2009;9:541–53. http://dx.doi.org/10.1016/j.meegid.2009.02.005
10 Novella IS, Ebendick-Corpus BE, Zárate S, Miller EL. Emergence of mammalian cell-adapted vesicular stomatitis virus from persistent infections of insect vector cells. J Virol. 2007;81:6664–8. http://dx.doi.org/10.1128/JVI.02365-06

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Genetic and Ecologic Variability among *Anaplasma phagocytophilum* Strains, Northern Italy

To the Editor: The tick-borne pathogen *Anaplasma phagocytophilum* is an increasing potential public health threat across Europe. Its intraspecific genetic variability is associated with different reservoir host and vector tick species (*1–4*); however, the roles of various vertebrates as competent reservoirs of *A. phagocytophilum* in Europe need clarification (*1*). During March 2011–June 2013, we studied the prevalence and genetic variability of *A. phagocytophilum* in 821 questing *Ixodes ricinus* ticks (155 adults [A], 666 nymphs [N] collected by standard blanket dragging) and 284 engorged ixodid ticks (61A, 191N, 21 larvae [L]) collected from humans, dogs, sheep, hunted wild ungulates, live-trapped birds, and rodents. Blood samples from 1,295 rodents (yellow-necked mice [*Apodemus flavicollis*]), bank voles [*Myodes glareolus*], and harvest mice [*Moscardinus avellanarius*]) were also analyzed. All animal-handling procedures and ethical issues

were approved by the Provincial Wildlife Management Committee (authorization n. 595 issued on 04.05.2011). The study site, Valle dei Laghi (northeastern Italian Alps), is a well-studied focus of emerging tick-borne pathogens in northern Italy (*4*).

Tick species were identified morphologically and by molecular analyses by using 16SrRNA sequences. *A. phagocytophilum* was detected in questing and feeding *I. ricinus* ticks by using a nested PCR amplification of the partial 16S rRNA gene (546-bp fragment) as described (*4*,*5*) and in rodent blood by using a real time-PCR assay targeting the *msp2* gene (77 bp) (*6*). All positive samples were confirmed by using Sanger sequencing.

Overall prevalence of *A. phagocytophilum* in questing *I. ricinus* ticks was 1.8% (6A, 9N of 821) (online Technical Appendix Table, http://wwwnc.cdc.gov/EID/article/20/6/13-1023-Techapp1.pdf). Among engorged ticks, only *I. ricinus* ticks were found positive for *A. phagocytophilum*, although tick species such as *I. hexagounus* (20 ticks from dogs and birds), *I. trianguliceps* (11 from rodents), and *I. turdus* (1 from a bird) were also analyzed. Infection prevalence in ticks from various hosts was: 4.3% (5N/115) in ticks from humans, 9.1% (1N/30) in ticks from dogs, 14.3% (4A, 1N, 2L/49) in ticks from wild ungulates, 7.7% (1A/30) in ticks from sheep, 10.7% (3N/28) in ticks from birds, and 6.1% (3N/49) in ticks from rodents (online Technical Appendix Table,). Prevalence in rodent blood samples (*A. flavicolis* mice, *M. avellanarius* mice, *M. glareolus* bank voles) was 0.3% (4/1,295); only bank voles had positive results. None of the feeding *I. ricinus* larvae collected from rodents were infected with *A. phagocytophilum*.

We amplified and sequenced 2 genetic loci, *groEL* and *msp4*, from samples that were positive for *A. phagocytophilum*, which are known to be useful for phylogenetic studies (*4*,*7*,*8*). MrBayes v3.1.2 (http://sourceforge.net/projects/mrbayes/files/mrbayes/3.2.1/) was used to construct Bayesian phylogenetic trees for each gene (*9*). We deposited 54 new *A. phagocytophilum* sequences in GenBank with accession numbers KF031380–KF031433. Fourteen and 9 unique *groEL* and *msp4 A. phagocytophilum* genotypes, respectively, were found to circulate in this alpine valley.

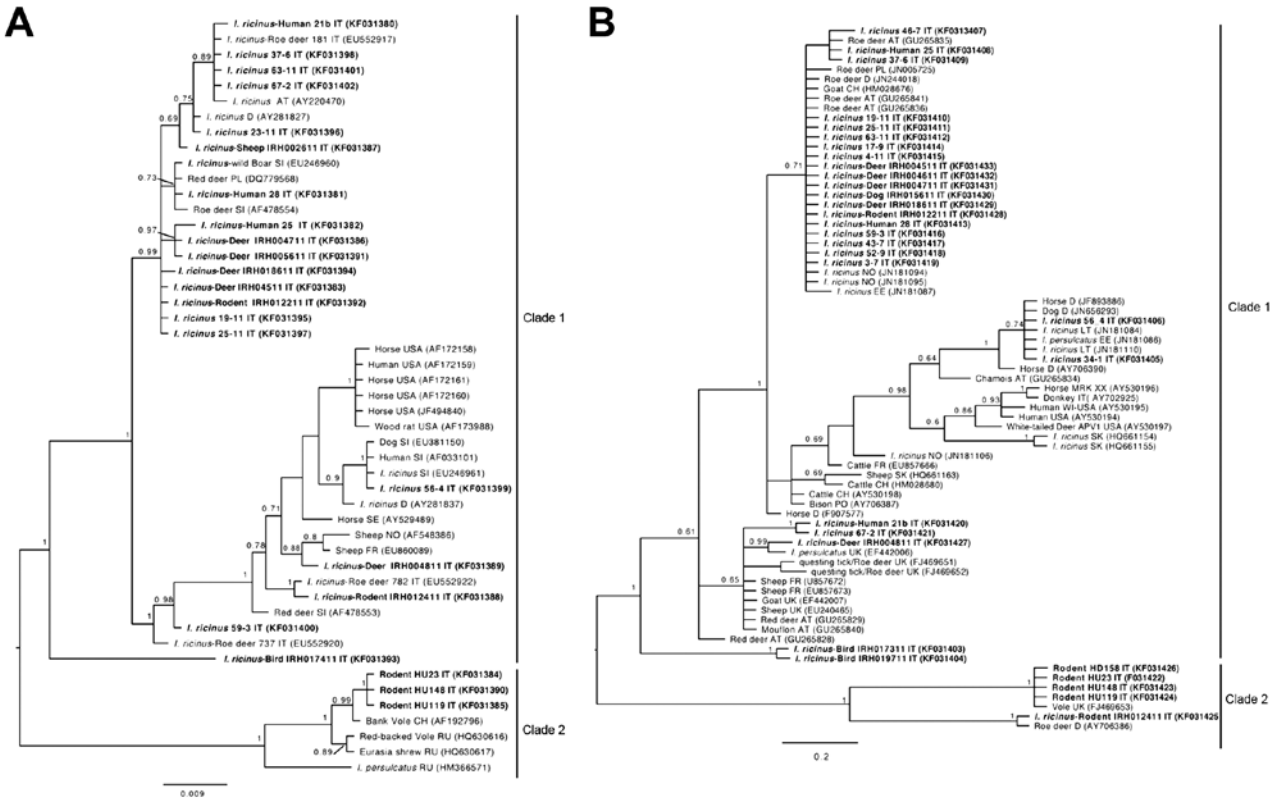

Figure. Phylogenetic trees expressing 50% majority rule consensus constructed by using Bayesian analysis for *Anasplasma phagocytophilum groEL* gene partial sequences (1,119 bp) (A) and 73 *msp4* gene partial sequences (300 bp) (B). Markov chains were run for 1,000,000 generations. The first 1,000 trees were discarded, and the remaining trees were used to construct the tree. Posterior probabilities of >0.60 are indicated above branches. New sequences are shown in boldface. Each *A. phagocytophilum* sequence is indicated with source: questing tick (e.g., *Ixodes ricinus*), engorged tick (e.g., I. *ricinus*-Human), or host blood (e.g., red deer). Two-letter country codes are given, and GenBank accession numbers are shown in parentheses. Scale bars indicate nucleotide substitutions per site.

The phylogenetic trees for *groEL* (Figure, panel A) and *msp4* (Figure, panel B) loci have similar topologies with strong support for 2 main clades (Figure, panels A and B), each with different host and vector association. The first clade (clade 1) contained sequences from questing *I. ricinus* ticks and engorged ticks collected from humans, dogs, wild ungulates, rodents, sheep, and birds. Our findings suggest that humans are exposed to several *A. phagocytophilum* genotypes exclusively from clade 1 (Figure, panels A and B). Our 3 unique *A. phagocytophilum* sequences were from 3 *I. ricinus* nymphs that fed on the same human clustered within this clade, but no clinical symptoms were observed.

The second clade (clade 2) includes sequences from rodents, specifically, bank voles (*M. glareolus*), other voles and shrews. Among tick species we found *I. persulcatus* to belong to this clade (Figure, panels A and B). We have found no evidence of circulation of this genotype in other hosts or in questing or engorged *I. ricinus* ticks in previously published data or in this study (Figure, panels A and B, clade 2). This finding suggests that the *A. phagocytophilum* genotype associated with mice, voles, and shrews in Europe may be maintained in enzootic cycles by another tick vector, such as *I. trianguliceps*, as observed in the UK for the field vole (*Microtus agrestis*) (*8*). This so-called ecologic strain probably does not represent an immediate threat to humans in northern Italy, unlike the rodent strain reported in the USA, since it occurs in very low prevalence, and because *I. trianguliceps* is an endophilic tick species that is unlikely to come into contact with humans.

In 1 questing *I. ricinus* tick at the nymphal stage, we detected a *groEL* sequence (KF031399) identical to a sequence isolated from humans with human granulocytic anaplasmosis in Europe (AF033101). The *msp4* sequence for the same sample (KF031406) belonged to clade 1, and contained sequences of a strain found in 96 infected persons in the United States. This suggests that ≥1 human pathogenic strain now circulates in the investigated area. However, we did not find this strain in any of the host-fed ticks analyzed, so the host responsible for maintaining the circulation of this pathogenic strain must be identified before any recommendation for preventive measures can be provided.

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References

1. Stuen S, Granquist EG, Silaghi C. *Anaplasma phagocytophilum*—a widespread multi-host pathogen with highly adaptive strategies. Front Cell Infect Microbiol. 2013;3:31. http://dx.doi.org/10.3389/fcimb.2013.00031
2. Keesing F, Hersh MH, Tibbetts M, McHenry DJ, Duerr S, Brunner J, et al. Reservoir competence of vertebrate hosts for *Anaplasma phagocytophilum*. Emerg Infect Dis. 2012;18:2013–6. http://dx.doi.org/10.3201/eid1812.120919
3. Mantelli B, Pecchioli E, Hauffe HC, Rosa R, Rizzoli A. Prevalence of *Borrelia burgdorferi* s.l. and *Anaplasma phagocytophilum* in the wood tick *Ixodes ricinus* in the Province of Trento, Italy. Eur J Clin Microbiol Infect Dis. 2006;25:737–9. http://dx.doi.org/10.1007/s10096-006-0208-x
4. Carpi G, Bertolotti L, Pecchioli E, Cagnacci F, Rizzoli A. *Anaplasma phagocytophilum* groEL gene heterogeneity in *Ixodes ricinus* larvae feeding on roe deer in Northeastern Italy. Vector Borne Zoonotic Dis. 2009;9:179–84. http://dx.doi.org/10.1089/vbz.2008.0068
5. Massung RF, Slater K, Owens JH, Nicholson WL, Mather TN, Solberg VB, et al. Nested PCR assay for detection of granulocytic ehrlichiae. J Clin Microbiol. 1998;36:1090–5.
6. Courtney JW, Kostelnik LM, Zeidner NS, Massung RF. Multiplex real-time PCR for detection of *Anaplasma phagocytophilum* and *Borrelia burgdorferi*. J Clin Microbiol. 2004;42:3164–8. http://dx.doi.org/10.1128/JCM.42.7.3164-3168.2004
7. de la Fuente J, Massung RF, Wong SJ, Chu FK, Lutz H, Meli M, et al. Sequence analysis of the *msp4* gene of *Anaplasma phagocytophilum* strains. J Clin Microbiol. 2005;43:1309–17. http://dx.doi.org/10.1128/JCM.43.3.1309-1317.2005
8. Bown KJ, Lambin X, Ogden NH, Begon M, Telford G, Woldehiwet Z, et al. Delineating *Anaplasma phagocytophilum* ecotypes in coexisting, discrete enzootic cycles. Emerg Infect Dis. 2009;15:1948–54. http://dx.doi.org/10.3201/eid1512.090178

9. Ronquist F, Huelsenbeck JP. MrBayes 3: Bayesian phylogenetic inference under mixed models. Bioinformatics. 2003;19:1572–4. http://dx.doi.org/10.1093/bioinformatics/btg180

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Zika Virus, French Polynesia, South Pacific, 2013

To the Editor: Isolated in 1947 from a rhesus monkey in Zika forest, Uganda, Zika virus (ZIKV) is a mosquito-borne flavivirus (*1*). For half a century, ZIKV was described only as causing sporadic human infections in Africa and Asia, which was mostly confirmed by serologic methods (*2*). In 2007, the first ZIKV outbreak reported outside Africa and Asia was retrospectively documented from biological samples of patients on Yap Island, Federated States of Micronesia, North Pacific, who had received an incorrect diagnosis of dengue virus (DENV) (*3*,*4*). We report here the early investigations that led to identification of ZIKV as the causative agent of an outbreak that started in October 2013 in French Polynesia.

French Polynesia is a French overseas territory located in the South Pacific. The ≈270,000 inhabitants live on 67 islands distributed into 5 archipelagoes (Society, Marquesas, Tuamotu, Gambier, and Austral Islands). Surveillance for acute febrile illnesses is coordinated by the Department of Health with the contribution of a sentinel network of public and private practitioners, the main public hospital (Centre Hospitalier du Taaone), and the public health and research institute (Institut Louis Malardé [ILM]). As part of this syndromic surveillance system, ILM has implemented protocols for detecting arboviruses that are known to cause outbreaks in French Polynesia, such as DENV, or that pose a risk for causing epidemics because of the presence of potential mosquito vectors. In addition, ILM provides DENV serotype identification for other Pacific island countries, including Yap State, as part of the regional surveillance of dengue (*5*). For that reason, a ZIKV reverse transcription PCR (RT-PCR) protocol by Lanciotti et al. (*3*) was implemented at ILM.

In October 2013 (week 41), a 53-year-old women (patient 1) and 2 other members of the household—her 52-year-old husband (patient 2) and her 42-year-old son-in-law (patient 3)—experienced a mild dengue-like illness consisting of low fever (<38°C), asthenia, wrist and fingers arthralgia, headache, and rash. Patients 2 and 3 also had conjunctivitis. Patient 1 had swollen ankles and aphthous ulcers. For all 3 patients, results were negative for DENV by RT-PCR and nonstructural protein 1 (NS1) antigen tests (*5*), for West-Nile virus by RT-PCR, and for chikungunya virus by RT-PCR; results of RT-PCR for ZIKV were equivocal for patients 1 and 2. During week 43, a 57-year-old patient (patient 4) reported similar symptoms; results of RT-PCR for DENV were negative, but results of RT-PCR for ZIKV were positive. ZIKV infection was then confirmed by sequencing of the genomic position 858–1138 encompassing the prM/E protein coding regions of ZIKV

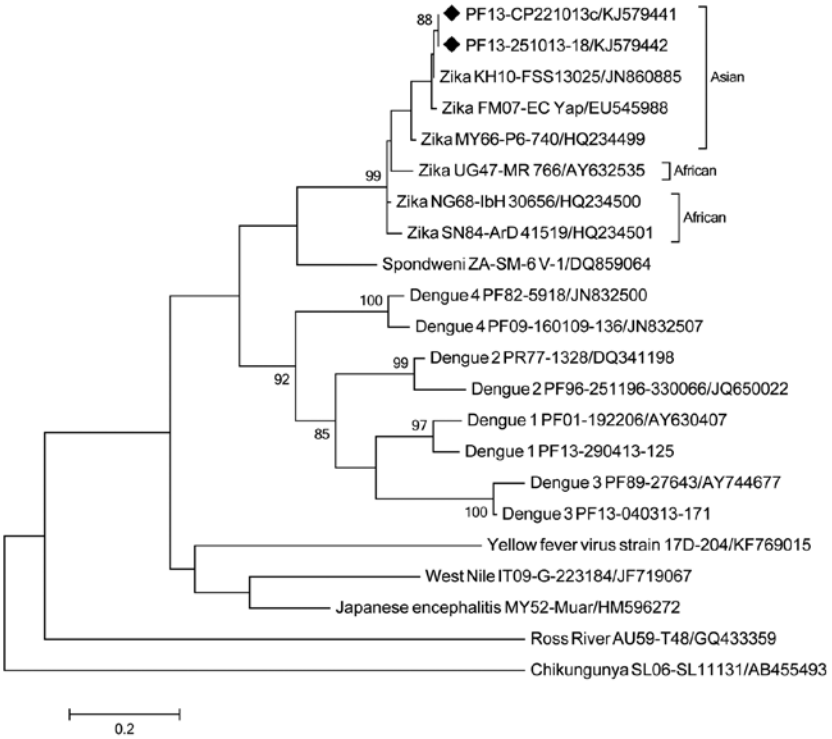

Figure. Phylogenetic analysis of partial M/E genes of 2 ZIKV strains, French Polynesia, 2013. The evolutionary history was inferred by using the maximum-likelihood method based on the Kimura 2-parameter model. The percentage of trees in which the associated taxa clustered is shown for values >85 next to the branches (1,000 replicates). Evolutionary analyses were conducted in MEGA5 (http://megasoftware.net/). Strains are labeled by country of origin and date-strain name/GenBank accession number. The 2 ZIKV strains collected in French Polynesia are marked with a black diamond. ZIKV, Zika virus. Scale bar indicates nucleotide substitutions per site.

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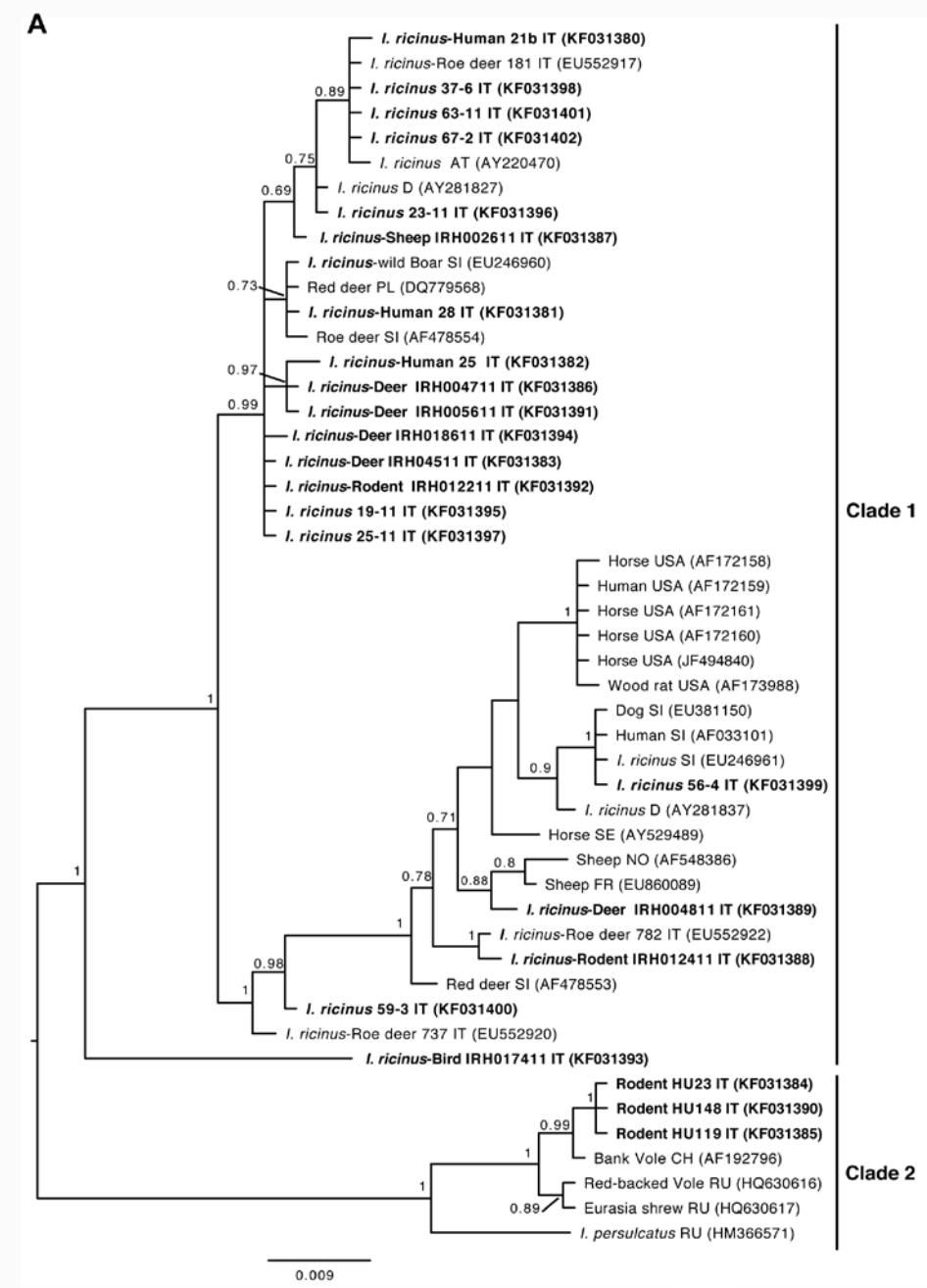
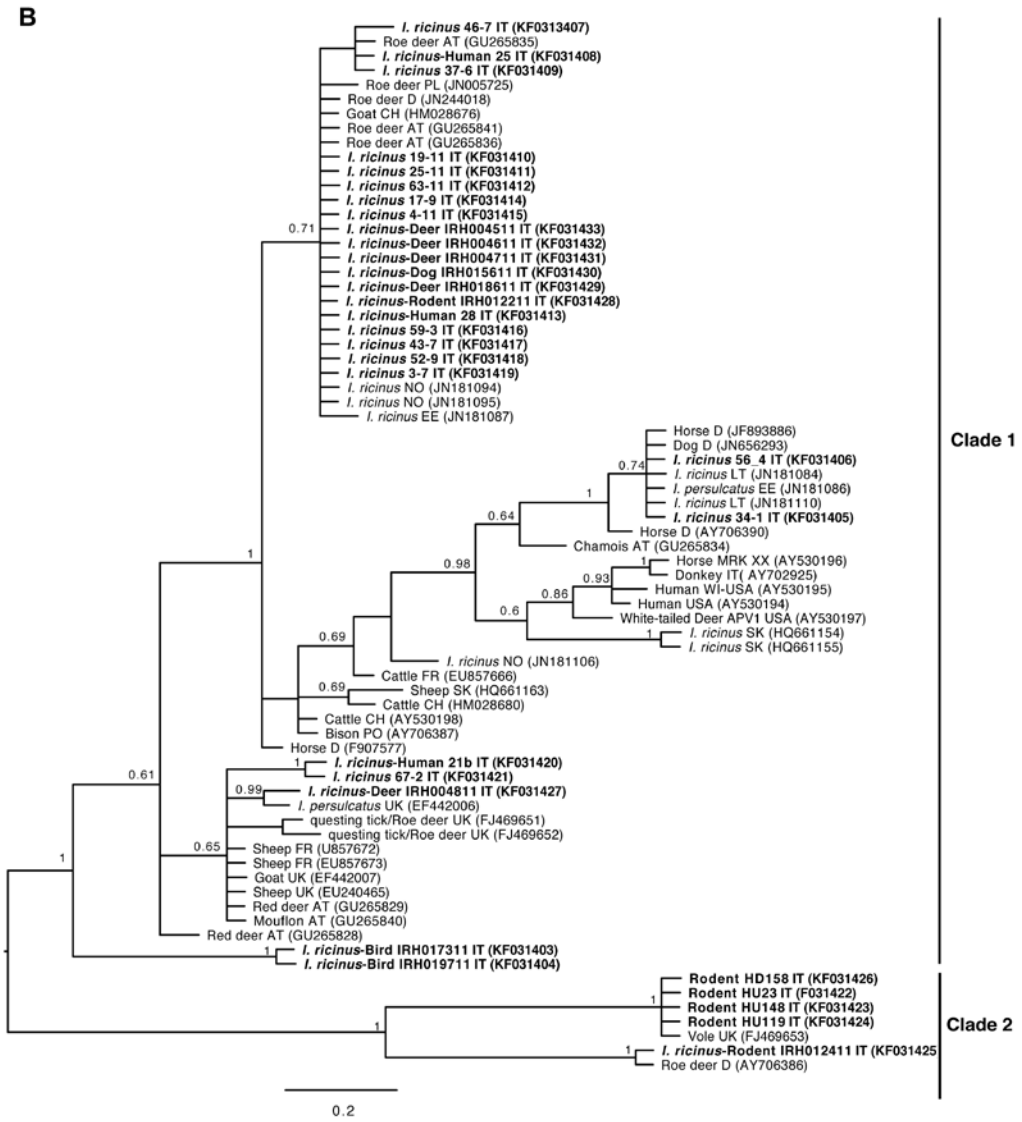
Genetic and Ecologic Variability among *Anaplasma phagocytophilum* Strains, Northern Italy





Technical Appendix Figure. A) 50% majority rule consensus phylogenetic trees constructed by using Bayesian analysis of *Anasplasma phagocytophilum groEL* gene partial sequences (1,119 bp); B) 73 *msp4* gene partial sequences (300 bp). Markov chains were run for 1,000,000 generations. The first 1,000 trees were discarded and the remaining trees were used to construct the tree. Posterior probabilities of >0.60 are indicated above branches. New sequences are shown in boldface. Each *A. phagocytophilum* sequence is indicated with source: questing tick (e.g., *I. ricinus*), engorged tick (e.g., *I. ricinus*-Human), or host blood (e.g., red deer), international organization for standardization α-2 country codes and GenBank accession numbers are in parentheses. Scale bars indicate nucleotide substitutions per site.