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Unique strains of *Anaplasma phagocytophilum* segregate among diverse questing and non-questing *Ixodes* tick species in the western United States

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Abstract

The emerging tick-borne pathogen *Anaplasma phagocytophilum* infects humans, domestic animals, and wildlife throughout the Holarctic. In the western U.S., the ecology of *A. phagocytophilum* is particularly complex, with multiple pathogen strains, tick vectors, and reservoir hosts. A recent phylogenetic analysis of *A. phagocytophilum* strains isolated from various small mammal hosts in California documented distinct clustering of woodrat strains separate from sciurid (chipmunk and squirrel) strains. Here, we identified strains of *A. phagocytophilum* in various *Ixodes* tick species in California and related these genotypes to those found among reservoir and clinical hosts from the same areas. The sequences from all of the nidicolous (nest-dwelling) *Ixodes* ticks grouped within a clade that also contained all of the woodrat-origin *A. phagocytophilum* strains. Two of the *I. pacificus* sequences were also grouped within this woodrat clade, while the remaining five belonged to a less genetically diverse clade that included several sciurid-origin strains as well as a dog, a horse, and a human strain. By comparing *A. phagocytophilum* strains from multiple sources concurrently, we were able to gain a clearer picture of how *A. phagocytophilum* strains in the western U.S. are partitioned, which hosts and vectors are most likely to be infected with a particular strain, and which tick species and reservoir hosts pose the greatest health risk to humans and domestic animals.

Keywords

Anaplasma phagocytophilum; *Ixodes* spp.; Nidicolous ticks; *Ixodes pacificus*; *ank* gene; Phylogeny

Introduction

Anaplasma phagocytophilum, the causative agent of granulocytic anaplasmosis (GA) is a tick-transmitted, intra-leukocytic rickettsial parasite of humans and other animals. Reservoir

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hosts include small mammals, such as white-footed mice (*Peromyscus leucopus*) in the eastern U.S. (Telford et al., 1996) and woodrats (*Neotoma fuscipes*), squirrels (*Sciurus* spp.), and chipmunks (*Tamias* spp.) in the western U.S. (Nicholson et al., 1999; Nieto and Foley, 2008, 2009). The tick vectors for *A. phagocytophilum* are most frequently reported to be the questing ticks of the *Ixodes ricinus* group, including *I. pacificus* in the western U.S. and *I. scapularis* in the eastern U.S. (Foley et al., 2004). While these tick species likely serve as the primary bridge vectors transmitting GA to humans and domestic animals, other nidicolous (i.e. primarily nest dwelling) *Ixodes* ticks including *I. spinipalpis*, *I. ochotonae*, and *I. trianguliceps* harbor *A. phagocytophilum* and likely help maintain enzootic cycles of GA (Zeidner et al., 2000; Bown et al., 2003; Foley et al., 2011).

Although *A. phagocytophilum* was originally classified as 3 distinct organisms – *Ehrlichia equi, Ehrlichia phagocytophila*, and the agent of human granulocytic ehrlichiosis – morphological, phenotypic, and genetic evidence led to the reclassification of these 3 organisms as the modern *A. phagocytophilum* in 2001 (Dumler et al., 2001). Despite this reorganization, phenotypic and preliminary genetic data strongly support the presence of multiple distinct strains. For example, strains in Europe commonly cause clinical disease in small hoofstock, but North American strains are neither particularly infectious nor virulent based on data from experimental infections of sheep or cattle (Pusterla et al., 1997; Stuen, 2007; Gorman et al., 2012). Similarly, in England, 2 genetically distinct subpopulations of *A. phagocytophilum* coexist in separate enzootic cycles, one involving deer and *I. ricinus* ticks and the other involving field voles and *I. trianguliceps* (Bown et al., 2009). The North American strain designated Ap-Variant 1 occurs in ticks and deer and is infectious to goats, but not rodents (Massung et al., 2003, 2007). The California strain DU1, originating from a woodrat, can infect rodent species, but not horses (Nieto et al., 2010). Strain MRK, which was isolated from a horse, reproducibly induces severe clinical disease in horses indistinguishable from that induced when human-origin *A. phagocytophilum* is inoculated into horses and is also infectious to small mammals (Pusterla et al., 1999; Foley et al., 2009b). These clinical and epidemiological distinctions are associated with genotypic segregation into specific clades based on analyses of the 16S rRNA, *msp4*, *msp2*, and *ank* genes (Massung et al., 2003; de la Fuente et al., 2005; Bown et al., 2009; Rejmanek et al., 2011).

In the western U.S., the ecology of *A. phagocytophilum* is particularly complex, with multiple pathogen strains, tick vectors, and reservoir hosts (Foley et al., 2004). A recent phylogenetic analysis of *ank*, *groESL*, and the 23S-5S rRNA genes from 28 Californian rodent strains, the MRK horse strain, and a California dog strain documented distinct clustering of woodrat strains separate from a genetically uniform group consisting of sciurid (chipmunk and squirrel), horse, and dog strains (Rejmanek et al., 2011).

One question that has not been addressed in previous studies of *A. phagocytophilum* is whether particular *A. phagocytophilum* genotypes are associated with certain host-specialist ticks. California is home to numerous nidicolous *Ixodes* spp., many of which infest some of the same small mammal species as *I. pacificus* (Furman and Loomis, 1984). A recent survey of ticks on small mammals from numerous sites across California revealed that 66% of tick-infested small mammals hosted nidicolous *Ixodes* species, while the remaining 34% were infested with *I. pacificus* (Foley et al., 2011). In the current study, our goal was to identify strains of *A. phagocytophilum* in various *Ixodes* tick species in California and relate these genotypes to those found among reservoir and clinical hosts from the same areas. For our analysis, we focused on a single gene (*ank*), which was chosen because it exhibited a high level of polymorphism even among closely related *A. phagocytophilum* strains and was largely concordant with phylogenetic results from analysis of other gene regions (Rejmanek et al., 2011).

Materials and methods

Sample collection

DNA samples for molecular characterization were obtained from *I. pacificus, I. spinipalpis, I. angustus, I. ochotonae*, and *I. woodi* from April 2006 to September 2011. Ticks were collected in central and northern California at the following sites: Soquel Demonstration Forest (SD, Santa Cruz County; 37° 03.25', 121° 50.68'), Henry Cowell State Park (HC, Santa Cruz County; 37° 08.705', 122° 11.12'), Samuel P. Taylor State Park (SPT, Marin County; 38° 01.232', 122° 40.774'), Hendy Woods State Park (HW, Mendocino County; 39° 04.25', 123° 28.238'), Humboldt Redwoods State Park (HR, Humboldt County; 40° 17.770', 123° 59.178'), and Archer Taylor Preserve (ATP, Napa County; 38° 20.54', 122° 25.34'). Two ticks were obtained from the Green Diamond Resource Company (GD), found on a dusky-footed woodrat in an unspecified site of Humboldt County.

Ticks were collected from small mammals trapped and sampled as described previously (Foley et al., 2011). Ticks were found on dusky-footed woodrats, deer mice, a chipmunk (*Tamias ochrogenys*), an eastern grey squirrel (*Sciurus carolinensis*), and a human. Trapping and sample collection were carried out as previously described (Foley et al., 2011).To obtain questing ticks, flagging was performed over herbaceous and shrubby vegetation as well as duff and leaf litter using a 1-m^2 white cotton flag.

Nucleic acid extraction, PCR, and sequencing

DNA was extracted from ticks following a modified protocol described previously (Humair et al., 2007). Briefly, individual ticks were placed in microcentrifuge tubes, cooled in liquid nitrogen for 3 min, and crushed using a microcentrifuge pestle. Next, 100 µl of 0.7 M NH_4OH was added, and the tubes were placed on a 100°C heat block for 15 min. Tubes were then cooled on ice for 30 s followed by an additional 15 min of heating at 100°C with open lids in order to evaporate the ammonia.

All DNA samples were initially screened for the presence of *A. phagocytophilum* DNA using a highly sensitive real-time TaqMan PCR assay targeting the msp2/p44 gene (Drazenovich et al., 2006). Results of real-time PCR were considered positive if they had a cycle threshold (CT) value <40 and a characteristic amplification curve. Nested conventional PCR assays targeting a section of the *ank* gene were then performed on all positive samples. Amplification of the ank gene was performed as described by Massung et al. (2007) using external primers ANK-F1 (5'-GAAGAAATTACAACTCCTGAAG-3') and ANK-R1 (5'-CAGCCAGATGCAGTAACGTG-3'), followed by internal primers ANK-F2 (5'-TTGACCGCTGAAGCACTAAC-3') and ANK-R2 (5'-ACCATTTGCTTCTTGAGGAG-3'), yielding an approximately 600-bp amplicon. Amplified DNA was visualized on a 1% agarose gel stained with GelStar nucleic acid stain (Lonza, Rockland, ME). Bands of the expected size were excised and cleaned with a Qiagen (Valencia, CA) gel extraction kit. Gel-extracted amplicons were cloned into the pGEM-T Easy vector (Promega, Madison, WI), transformed into *Escherichia coli* DH5α cells, and plated onto LB agar containing 100 µg/ml ampicillin. Individual colonies were grown overnight in LB broth containing 100 ug/ml ampicillin, and plasmids were purified using a Quantum Prep plasmid miniprep kit (BioRad, Hercules, CA). Following *Eco*RI digestion, plasmids were assessed for appropriate insert size. Gel-extracted or cloned PCR products were sequenced in both forward and reverse directions on an ABI 3730 sequencer (Davis Sequencing). Consensus sequences were initially aligned using the CLUSTAL_X sequence alignment program (Larkin et al., 2007) and trimmed to a final length of 567 bp. All unique sequences were deposited in GenBank and issued the following accession numbers: KC249918–KC249925.

Phylogenetic analysis

In addition to the *ank* sequences acquired in the current study, 31 previously reported *A. phagocytophilum ank* sequences were used in the phylogenetic analysis. These included sequences from 22 *A. phagocytophilum* strains isolated from small mammal hosts (woodrats, chipmunks, and grey squirrels) sampled in the same areas and during the same time frame as most of the ticks (GenBank accession numbers JF303732–JF303741 and JF776828) (Rejmanek et al., 2011). Additional strains included HZ_NY (the fully sequenced human-origin strain from New York State) (Dunning Hotopp et al., 2006), 2 other human isolates from New York (NY_2 and NY_3), Webster_WI and WI_2 (human isolates from Wisconsin), Mn_Dog (a dog isolate from Minnesota), RI_1 (isolated from an *I. scapularis* tick in Rhode Island), MRK_CA (originally isolated from an infected horse in Shasta County, CA), Dog_CA, (a strain isolated from an infected dog from Tuolumne County, CA), and CAHU_HGE2, a strain isolated from an infected human from southern Humboldt County, CA (GenBank accession numbers: CP000235 and AF172153, AF100884, AF100885, GU236811, AF100890, AF100894, DQ320648, AF153716, JF303732, and AF172153, respectively).

All DNA sequences were aligned using MUSCLE v3.8 (Edgar, 2004), then translated into amino acid codons, and adjusted by eye to ensure that the sequences were in frame and did not contain stop codons. Identical sequences were excluded from the analysis, then reincluded as polytomies for the phylogeny figures. To examine the effect of missing data, 2 separate data matrices were used: one in which all sequence data in hand were included for each accession, and one in which all sequences were trimmed to the length of the shortest sequence read (the Californian human strain, CAHU_HGE2). The longer alignment consisted of 567 aligned sites and the shorter alignment of 222 aligned sites. The analysis pipeline described below was carried out for both alignments, but, because both the final models selected and the majority-rule consensus trees for each alignment were effectively the same, we will only present results from the longer alignment below.

Preliminary analyses were performed using the GTR model of nucleotide substitution, with a discrete gamma distribution used to accommodate among-site variation in the rate of nucleotide substitution (i.e., GTR+G). We accommodated process heterogeneity by partitioning the alignment by codon position: a separate GTR+G model was applied to each subset of nucleotides comprising the 3 codon positions.

We approximated the joint posterior probability distribution of trees and other parameters of the GTR+G substitution model using the Metropolis Coupled Markov Chain Monte Carlo (MC^3) samplers implemented in MrBayes version 3.2 (Ronquist and Huelsenbeck, 2003). Preliminary analysis consisted of 4 independent MC^3 analyses run for 1×10^7 generations, each with 4 incrementally heated chains (with the temp parameter set to 0.2), where the cold chain was thinned by sampling every 1×10^3 cycles. For the purposes of diagnosing MCMC performance (see below), we also estimated the joint prior probability density by running the MCMC samplers in MrBayes on an ‘empty’ alignment consisting of entirely missing data (e.g., a matrix of sequences where each sequence is a string of question marks equal in length to the corresponding sequence in the original data matrix).

We assessed performance of the MCMC analyses by scrutinizing the samples from each of the 4 independent chains using Tracer 1.4 (http://beast.bio.ed.ac.uk/Tracer). We assessed convergence by comparing the marginal posterior probability density from each chain for each of the model parameters to ensure that estimates from each of the 4 independent chains were effectively identical. We assessed mixing of the chains over each parameter using 3 diagnostics: (1) the rate at which proposed updates to each parameter were accepted (with a target window of 20–70%); (2) by examining the form of the marginal posterior probability

densities (to ensure that the density for each parameter was smooth and unimodal); and (3) by comparing the marginal posterior probability density for each parameter to its corresponding prior probability density (to assess the degree of information in the data for each parameter).

We identified weak parameters as those with low ESS values (i.e., <200) and for which the inferred marginal posterior and prior probability densities were similar. We eliminated any weak parameters by collapsing to submodels of the GTR family as necessary, and then running new analyses under these simplified models (using the same details as described above). Specifically, this process resulted in the selection of the following substitution model for final analysis: a separate GTR model was applied to each of 3 partitions (by codon position) with unlinked stationary frequencies, instantaneous rate matrices, and rate priors.

For the phylogenies presented below, all final MrBayes analyses were carried out with 4 independent runs and 4 chains per run. Each analysis ran for 5×10^7 generations, sampled every 1×10^3 generations, with a burn-in of 1×10^4 sampled generations. To summarize the posterior distribution of trees, we present only the majority-rule consensus tree (Holder et al., 2008). Alignments, trace files, and tree posteriors are available upon request.

Results

Six-hundred and sixty-two ticks including 99 *I. angustus*, 1 *I. auritulus*, 17 *I. ochotonae*, 492 *I. pacificus*, 1 *I. sculptus*, 31 *I. spinipalpis*, and 21 *I. woodi*, were collected at 7 different study sites throughout central and northern California from April 2005 to September 2011. Twenty-five (3.8%) were positive for *A. phagocytophilum* DNA by real-time PCR, and partial *A. phagocytophilum ank* gene sequences were successfully amplified and cloned from 13 of them including 6 nidicolous ticks (1 *I. angustus*, 1 *I. ochotonae*, 2 *I. spinipalpis*, and 2 *I. woodi*) and 7 *I. pacificus* ticks. Additional information about each *A. phagocytophilum* strain including the tick life stage it was isolated from, the study site, the source of each tick (i.e. flag or animal host), and the *A. phagocytophilum* infection status of the animal host each tick was removed from, are presented in Table 1. Interestingly, 2 of the hosts (a woodrat and chipmunk) from which positive ticks were removed did not have detectable *A. phagocytophilum* infections, suggesting that those ticks were infected during a previous blood meal.

The phylogenetic relationships among all of the tick-derived *A. phagocytophilum ank* sequences along with sequences from small mammals and clinical hosts from many of the same locations in the current study as well as several sequences from the upper Midwest and East Coast are presented in Fig. 1. The sequences from all of the nidicolous ticks grouped within a clade that also contained all of the woodrat-origin *A. phagocytophilum* strains. Two of the *I. pacificus* sequences were also grouped within this woodrat clade, while the remaining 5 belonged to a clade that included several squirrel- and chipmunk-origin strains as well as dog, horse, and human strains. This sciurid/large mammal clade showed very little genetic divergence, with only 3 single nucleotide polymorphisms (SNPs) among all 17 sequences. In contrast, within the woodrat clade, there was a much higher level of polymorphism. For the most part, all of the tick and woodrat strains within this clade were grouped by geographic location. The exception was a strain isolated from an *I. pacificus* tick (Ip_755_SD) from Soquel Demonstration forest (SD), one of the southernmost sampling sites. Its *ank* sequence phylogenetically grouped most closely with tick and woodrat strains from Hendy Woods (HW) and Humboldt Redwoods (HR). The 3 New York-origin human strains (HZ_NY, NY_2, and NY_3) along with the Rhode Island tick strain (RI_1) formed their own separate clade.

Discussion

The complex ecology of *A. phagocytophilum* in the far-western U.S. features multiple reservoir host species, multiple bacterial strains (Foley et al., 2009b; Rejmanek et al., 2011), and differences in dynamics of hosts and vectors across diverse landscapes (Foley et al., 2009a, 2011). There is also a large diversity of *Ixodes* tick species. Data in the present study revealed that, in addition to *I. pacificus*, at least 4 nidicolous tick species (*I. angustus, I. ochotonae, I. spinipalpis*, and *I. woodi*) may serve as vectors for *A. phagocytophilum* in California. Importantly, all of the nidicolous ticks in this study were infected with *A. phagocytophilum* strains characteristic of woodrat infections (Rejmanek et al., 2011). In contrast, all but 2 *I. pacificus* ticks were infected with a phylogenetically distinct strain previously detected in squirrel and chipmunk reservoir hosts, humans, dogs, and horses.

The detection of both human/sciurid strains and woodrat strains in ticks and small mammals in many of the same locations indicates the presence of at least 2 coexisting, yet discrete, enzootic cycles of *A. phagocytophilum* in California. A similar scenario has been reported by Bown et al. (2009) in the United Kingdom. In their system, a unique strain of *A. phagocytophilum* was detected only in field voles (*Microtus agrestis*) and their associated nidicolous tick, *I. trianguliceps*, whereas in the same habitats, a genetically divergent strain of *A. phagocytophilum* was detected exclusively in roe deer (*Capreolus capreolus*) and the questing tick *I. ricinus*. Interestingly, none of the *I. ricinus* ticks tested in their study were infected with the vole-associated *A. phagocytophilum* strain even though blood meal analysis showed that over half of the questing *I. ricinus* nymphs had fed on voles as larvae. This led the authors to conclude that *I. ricinus* was not functioning as a bridge vector of the vole-associated *A. phagocytophilu*m strain to other animals including deer, livestock, and humans. By contrast, 2 out of 7 *I. pacificus* ticks tested in our study were infected with woodrat strains, demonstrating that *I. pacificus* in the western U.S. may function as a bridge vector of multiple *A. phagocytophilum* strains. And while at least one woodrat strain appeared to be minimally infectious to horses and likely humans as well (Foley et al., 2009b), *I. pacificus* is likely able to vector multiple strains of *A. phagocytophilum* in the wild, and some woodrat strains may have broader host tropism than DU1. Intriguingly, the one *A. phagocytophilum*-positive *I. pacificus* in this study that was found on a person was infected with a woodrat-like strain.

The phylogenetic tree indicates that the clade comprising woodrat strains is genetically much more variable than the human/sciurid clade, even though the strains comprising both clades come from ticks and animals sampled at numerous sites spanning a similarly large geographic range across central and northern California (approximately 500 km north to south). One possible explanation for the extensive genetic variability within the woodrat clade is that nidicolous ticks tend to be closely associated with their small mammal hosts and are therefore limited in their ability to disperse over long distances. This would allow geographically distant strains to accumulate mutations and diverge overtime. The observation of geographically clustered groups within the woodrat clade lends support to this hypothesis. In contrast, *I. pacificus* is a questing tick that often feeds on larger mammals such as deer, coyotes, and bobcats, which can carry the tick across much greater distances (Furman and Loomis, 1984). *I. pacificus* has also been found on numerous domestic and migratory bird species allowing even greater dispersal potential (Furman and Loomis, 1984; Wright et al., 2011). It is conceivable that an *A. phagocytophilum* strain readily vectored by *I. pacificus* could spread across a large geographical area in a short enough time span that only limited genetic variation would be detected among distantly sampled isolates. Interestingly, sequences from the 3 Midwestern human and dog-origin strains included in our analysis were almost identical to the other strains within the human/sciurid clade suggesting a fairly recent evolutionary history between the California and Midwestern

strains. In contrast, sequences comprising the 4 East Coast human and tick-origin strains were markedly different from all the other strains tested.

While our data support the conclusion that there are at least 2 discrete and coexisting enzootic cycles of *A. phagocytophilum* in the western U.S., there are still many unanswered questions regarding the different strains involved in these cycles. For one, only a few woodrat strains (including DU1) have ever been experimentally tested in the laboratory (Nieto et al., 2010; Rejmanek et al., 2012). Given the considerable genetic diversity observed among these strains, it may be premature to assign similar phenotypic properties to all of them. Another important question is whether any of these strains can provide cross-immune protection against each other. Although experimental work in sheep suggests that superinfection with multiple *A. phagocytophilum* strains is possible, it has yet to be demonstrated in other species (Stuen et al., 2009). In the current study, we found no evidence of superinfection. In fact, our data suggest a high level of host tropism among reservoir hosts, that is, woodrat *A. phagocytophilum* strains were only detected in woodrats, while sciurid strains were only detected in sciurids. To some extent, host tropism was also evident among nidicolous ticks, which were only infected with woodrat *A. phagocytophilum* strains. This is interesting because except for *I. woodi*, which almost exclusively feeds on woodrats, the other nidicolous ticks targeted in this study have a fairly broad host range, which includes among other hosts, squirrels and chipmunks (Furman and Loomis, 1984; Foley et al., 2011). However, this finding should be interpreted cautiously since all of the *A. phagocytophilum*-positive nidicolous ticks except one, were removed from woodrats. The one nidicolous tick not removed from a woodrat (an adult *I. ochotonae*) was found on a deer mouse, a poor host for *A. phagocytophilum* in California (Foley et al., 2008; Rejmanek et al., 2011), indicating that the tick was most likely infected during a previous blood meal, presumably on a woodrat. Determining the extent of host tropism among the reservoir hosts and their associated nidicolous ticks in our system and testing whether different strains can provide cross-immune protection will require future laboratory work as well as additional testing of nidicolous ticks from other hosts.

In order to most effectively understand the ecology and genetic variability of *A. phagocytophilum* in the western U.S., we strategically focused on strains from multiple sources, including reservoir hosts, clinical hosts, and numerous tick vectors throughout central and northern California. Only by comparing strains from all of these different sources concurrently were we able to gain a clearer picture of how *A. phagocytophilum* strains are partitioned, which hosts and vectors are most likely to be infected with a particular strain, and which tick species and reservoir hosts pose the greatest health risk to humans and domestic animals. Furthermore, based on these findings, we would urge vector control agencies charged with the task of *A. phagocytophilum* detection to start genotyping PCR-positive ticks whenever possible in order to more accurately assess the potential risk to humans.

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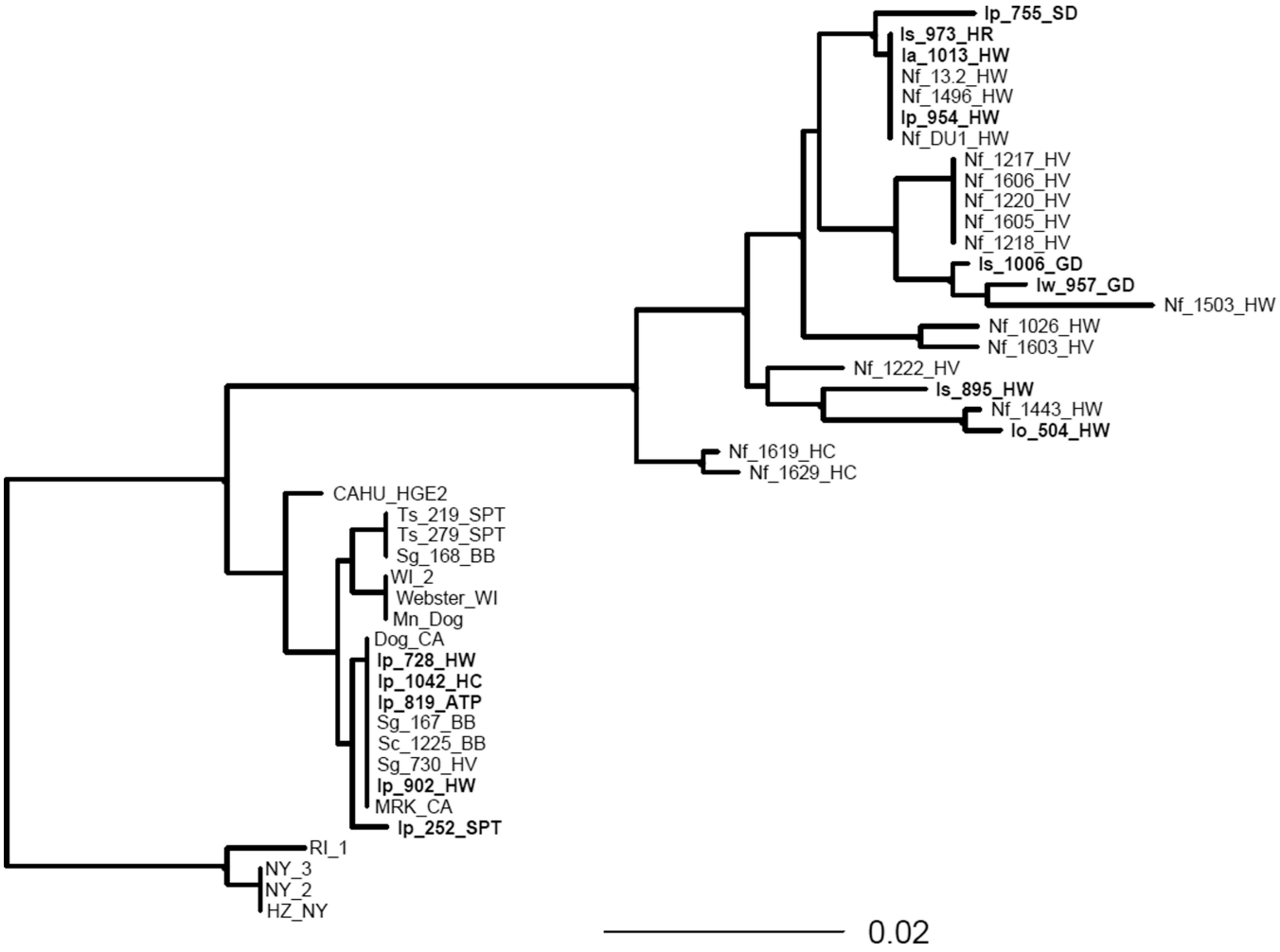

Fig. 1.

Majority-rule consensus phylogram inferred from an alignment of *Anaplasma phagocytophilum ank* sequences. Bayesian analyses of phylogeny was performed using MrBayes v. 3.1.2. Identical sequences were excluded from analysis and reincluded for this figure. Black circles indicate >90% nodal support based on Bayesian posterior probabilities. Branch lengths are in units of expected number of nucleotide substitutions per site. *Ank* sequences derived from ticks in this study are presented in bold text. HZ_NY, NY_2, and NY_3 = human strains from New York State; RI_1 = tick strain from Rhode Island; Webster_WI and WI2 = human strains from Wisconsin; Mn_Dog = dog strain from Minnesota; CAHU_HGE2 = human strain from California; MRK_CA = horse strain from California; Dog_CA = dog strain from California; Ip = *Ixodes pacificus*; Ia = *Ixodes angustus*; Io = *Ixodes ochotonae*; Is = *Ixodes spinipalpis*; Iw = *Ixodes woodi*; Sg = *Sciurus griseus*; Sc = *Sciurus carolinensis*; Ts = *Tamias sonomae*; Nf = *Neotoma fuscipes*; HV = Hoopa Valley; BB = Big Basin; SPT = Samuel P. Taylor; HW = Hendy Woods; HC = Henry Cowell; APT = Archer Taylor preserve; GD = Green Diamond Resource Company.

Table 1

Description of tick *A. phagocytophilum* strains analyzed in this study.

Strain ID	Tick species	Life stage	Study Site	Tick source	Host infection status*
Ia_1013_HW	*Ixodes angustus*	Nymph	Hendy Woods	*Neotoma fuscipes*	Positive
Io_504_HW	*Ixodes ochotonae*	Adult	Hendy Woods	*Peromyscus sp.*	NA
Ip_252_SPT	*Ixodes pacificus*	Nymph	Samuel P. Taylor	flag	NA
Ip_728_HW	*Ixodes pacificus*	Adult	Hendy Woods	flag	NA
Ip_755_SD	*Ixodes pacificus*	Adult	Soquel D. Forest	*Homo sapiens*	NA
Ip_819_ATP	*Ixodes pacificus*	Adult	Archer Taylor Preserve	flag	NA
Ip_902_HW	*Ixodes pacificus*	Adult	Hendy Woods	flag	NA
Ip_954_HW	*Ixodes pacificus*	Adult	Hendy Woods	*Tamias ochrogenys*	Negative
Ip_1042_HC	*Ixodes pacificus*	Nymph	Henry Cowell	*Neotoma fuscipes*	Negative
Is_895_HW	*Ixodes spinipalpis*	Adult	Hendy Woods	*Neotoma fuscipes*	NA
Is_973_HR	*Ixodes spinipalpis*	Adult	Humboldt Redwoods	*Neotoma fuscipes*	Positive
Iw_957_GD	*Ixodes woodi*	Adult	Green Diamond	*Neotoma fuscipes*	Positive
Iw_1006_GD	*Ixodes woodi*	Adult	Green Diamond	*Neotoma fuscipes*	Positive

*Based on real-time PCR detection of *A. phagocytophilum* in blood of hosts that ticks were removed from.

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