Full Title: *Rickettsia* infection rate along an altitudinal gradient as influenced by population genetic structure of Ixodid ticks

Short Title: Tick population genetic structure and Rickettsia infection rate along an altitudinal gradient

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Abstract

Ixodid ticks, such as *Ixodes ovatus* and *Haemaphysalis flava,* are important vectors of tick-borne diseases in Japan, such as Japanese spotted fever caused by *Rickettsia japonica*. This study describes the population genetic structure and gene flow of *I. ovatus* and *H. flava* and their *Rickettsia* infection rates along an altitudinal gradient. A total of 346 adult *I. ovatus* and 243 *H. flava* were analyzed for the presence of *Rickettsia* by nested PCR targeting the 17kDA, *gltA, rOmpA,* and *rOmpB* genes. Population genetic structure was performed using the mitochondrial cytochrome oxidase 1 (*cox1*) marker. The *Rickettsia* infection rates were 13.26% in *I. ovatus* and 6.17% in *H. flava.* For *I. ovatus,* the global F_{ST} value revealed significant genetic differentiation among the different populations, whereas *H. flava* showed non-significant genetic differentiation. The *cox1 I. ovatus* cluster dendrogram showed two cluster groups, while the haplotype network and phylogenetic tree showed three genetic groups. The two cluster groups and the three genetic groups found in *I. ovatus* showed a significant difference in their *Rickettsia* infection rates and mean altitude per group. No significant differences were found in the mean altitude or *Rickettsia* infection rates of *H. flava*. Our results suggest that low gene flow in the *I. ovatus* populations has caused spatially heterogenous *Rickettsia* infection rates along the altitudinal gradient. This information can be used in understanding the relationship between the tick vector, its pathogen, and environmental factors, such as altitude, and for the control of tick-borne diseases in Japan.

Keywords: environment, haplotype, hosts, pathogen, dispersal, vector

41 ## 1. Introduction

42 Tick-borne diseases are a significant public health concern in Japan and are transmitted by
43 a diverse range of tick species, such as *Ixodes ovatus* [1] that potentially transmit *Borrelia sp.*
44 causing *Lyme* disease [2] and *Haemaphysalis flava* [3], which transmits *Rickettsia japonica* and is a
45 suspected vector of severe fever with thrombocytopenia syndrome virus [4-6]. Since ticks are
46 small, their dispersal is closely related to the movement of their hosts [7-8]. The dynamics of tick-
47 borne pathogens are influenced by the habitat distribution and dispersal behaviors of vectors and
48 hosts along environmental gradients [9]. Therefore, understanding the complex interaction
49 between these factors is important in understanding the spread of tick-borne diseases in Japan.

50 Tick population genetic analysis provides data that help identify the dispersal pattern of
51 ticks based on gene flow between local populations [10]. Tick dispersal of many three-host Ixodid
52 ticks depends on the movements of their vertebrate hosts, which influences each tick's potential
53 to spread its pathogen [7-8]. For example, contrasting patterns in the population genetic
54 structures of *I. ovatus* and *H. flava* in the Niigata Prefecture of Japan suggest that host mobility
55 during the immature stages of tick development may influence the genetic structure of adult ticks
56 by affecting survivability into their adult stages [11-12]. *I. ovatus* populations had greater genetic
57 divergence possibly due to the limited dispersal of their small mammalian hosts during the
58 immature development stage; *H. flava* populations showed a more homogenized structure
59 possibly due to the larger mobility of their large mammalian hosts and avian-mediated dispersal
60 [11]. Other studies have also revealed low gene flow in ticks with low-mobility hosts (e.g., small
61 mammals) and higher gene flow in ticks with highly mobile hosts (e.g., large mammals and birds)
62 [10; 13-16].

63 The spatial distribution and movement of the vector (i.e., ticks) may determine the spatial
64 distribution of the pathogen's (i.e., *Rickettsia*) infection rate [17]. Previous studies have shown
65 that the pathogen infection rate can be influenced by many factors, such as the vector's genetic
66 diversity, gene flow, and spatial structure [5-6; 11; 18-25]. For example, previous studies have
67 shown that strong gene flow between local vector populations tends to reduce the spatial
68 heterogeneity of pathogen infection rates between populations [26-27]. Thus, the spatial
69 distribution and movement of the vector may affect the spatial distribution of the pathogen. To
70 our knowledge, no previous studies have examined the relationship between the spatial
71 heterogeneity of *Rickettsia* infection rates and population genetic structure in ticks.

72 Environmental factors may relate to the population genetic structure of ticks [28-29], with
73 limited gene flow increasing genetic variation between populations along an altitudinal gradient,
74 as reported in several studies on other species [30-32]. Thus, in this study, we expected to see a
75 highly divergent population genetic structure in *I. ovatus* along an altitudinal gradient due to the
76 limited movement of their mammalian hosts along that gradient; whereas *H. flava* should show a
77 less divergent structure along the altitudinal gradient due to the higher mobility of its hosts. To
78 our knowledge, no studies have focused on tick gene flow along an altitudinal gradient.

79 In the study by [11], no significant influence of environmental factors, including altitude,
80 was observed in the genetic structures of *I. ovatus* and *H. flava* based on the mantel test, but the
81 study did not use any other robust analytical methods to thoroughly examine the influence of
82 altitude on tick genetic structure. In another study, major spotted fever group *Rickettsia* (SFGR)
83 prevalence was analyzed in a total of 3,336 immature and adult ticks across the Niigata Prefecture,
84 Japan in the following tick species: *Dermacentor taiwanensis*, *Haemaphysalis flava*,
85 *Haemaphysalis hystricis*, *Haemaphysalis longicornis*, *Haemaphysalis megaspinosa*, *Ixodes*
86 *columnae*, *Ixodes monospinosus*, *Ixodes nipponensis*, *Ixodes ovatus*, and *Ixodes persulcatus* [6].

87 Three SFGR species namely *Rickettsia asiatica, R. helvetica* and *R. monacensis* were detected in *H.*
88 *flava, Haemaphysalis longicornis, Ixodes monospinus, Ixodes nipponensis*, and *Ixodes ovatus*, no
89 spatial distribution of *Rickettsia* infection rates was found among the local populations. To our
90 knowledge, no previous studies have considered the influence of environmental factors on tick
91 population genetic structure or the spatial distribution of *Rickettsia* infection rates along an
92 altitudinal gradient in local tick populations.

93 Here, we determine the relationship between the vector tick population genetic structure
94 and *Rickettsia* infection rates as influenced by an altitudinal gradient. Based on the isolation by
95 environment (IBE) theory, genetic differentiation increases with environmental variation,
96 regardless of geographic distance [33-35]. Thus, we hypothesized that limited gene flow among
97 tick populations along an altitudinal gradient might cause spatially heterogenous *Rickettsia*
98 infection rates in ticks. The present study used cytochrome oxidase 1 (*cox1*) mitochondrial gene
99 sequences and *Rickettsia*-infected and uninfected data for *I. ovatus* (n = 307) and *H. flava* (n = 220)
100 individuals collected in 2016–2017 as reported in [6; 11]. Additionally, we added new *cox1*
101 sequences and *Rickettsia*-infected/uninfected ticks from *I. ovatus* (n= 39)and *H. flava* (n=23)
102 individuals and five other species collected in 2018.

103

104 ## 2. Materials and Method

105 ***Published data of [6;11]***

106 In this study, we used *cox1* sequence data from [11] for *I. ovatus* (n = 307) and *H. flava*
107 (n = 220) ticks collected from April 2016 to November 2017 from 30 sites across the Niigata
108 Prefecture, Japan. *Rickettsia* infection rate data were obtained from [6] from *I. ovatus* (n = 29), *H.*
109 *flava* (n = 2), *I. monospinus* (n = 4), and *I. nipponensis* (n = 2) from 38 sites across Niigata

110 Prefecture. The 38 sites surveyed in the previous study by [6] include the 30 sites that were used
111 in this present study (S1 Table). Please refer to [6;11] for more information about the study sites,
112 collection, sampling identification, DNA extraction, PCR amplification, and sequencing methods
113 used in each respective study.

114 To strengthen our analysis, we also added data collected from April to October 2018 from
115 additional individuals (n=62;) sampled at 30 sites across the Niigata Prefecture, including two sites
116 not previously sampled by [11]. At these sites, ticks were collected 2–14 times from six core sites
117 among the 30 sites, while ticks were collected once at the remaining sites. The altitude at each site
118 ranged from 8 to 1402 meters above sea level (m.a.s.l.), with a mean altitude of 348 m.a.s.l.

119

120 ***Unpublished data from 2018 collection***

121 Ticks collected were stored at 4 °C in microcentrifuge tubes with 70% ethanol. Each
122 collected tick was morphologically identified using a stereo microscope following the identification
123 keys of [36]. Genomic DNA was extracted using Isogenome DNA extraction kits (Nippon Gene Co.
124 Ltd. Tokyo, Japan) following the manufacturer's recommended protocol.

125 In this study, we combined previously published data from [6] with our newly collected
126 data to calculate the *Rickettsia* infection rate, which is the percentage of *Rickettsia*-infected ticks
127 from each obtained population. We analyzed the obtained tick DNA for spotted fever group
128 *Rickettsia* (SFGR) detection and host identification and amplified the mitochondrial gene *cox1* for
129 population genetic analysis. We performed nested PCR targeting the following genes for the
130 detection of *Rickettsia* sp.: 17-kDA antigen gene (17-kDA); citrate synthase gene (gltA); spotted
131 fever group (SFG)-specific outer membrane protein A gene (rOmpA); and outer membrane protein
132 B gene (rOmpB) as described and analyzed in [6]. Briefly, we first amplified the 17-kDa protein. If

133 the results were positive, then PCR was performed to target gltA. Samples that were positive with
134 both 17-kDA and gltA were regarded as positive for SFGR and a nested PCR was performed to
135 target the rOmpA and rOmpB gene, samples that are positive for 17-Kda, gltA, rOmpA, and rOmpB
136 genes were sequenced to identify the *Rickettsia* species. The amplified PCR products were purified
137 using AMPure XP (Beckman Coulter Co., Japan) and sequenced using the Big Dye Terminator Cycle
138 Sequence Kit (Thermo Fisher Scientific).

139 The *cox1* mitochondrial gene was amplified by PCR for *cox1* (658 base pairs) using the
140 primer pairs LCO-1490 (5- AAACTTCAGGGTGACCAAAAAATCA-3') and HCO1–2198 (5–
141 AAACTTCAGGGTGACCAAAAAATCA - 3) for phylogenetic analysis and tick species identification [37].
142 The PCR amplification profile included an initial denaturation of 94 °C for 2 min, followed by
143 denaturation at 94 °C for 30 s, then annealing at 38 °C for 30 s, followed by an extension of 72 °C
144 for 1 min for 30 cycles, and a final extension of 72 °C for 10 min. The obtained PCR products were
145 purified using the QIAquick 96 PCR Purification Kit (Qiagen, Germany) following the
146 manufacturer's instructions and were sequenced by Eurofin Genomics, Inc. (Tokyo, Japan).

147 Each forward and reverse read was assembled using CodonCode Aligner version 1.2.4
148 software (https://www.codoncode.com/aligner/). Low-quality bases were removed in the aligned
149 sequences, and no ambiguous bases were detected. We used the MAFFT alignment online
150 program (https://mafft.cbrc.jp/alignment/server/) to perform multiple alignments using the
151 default settings. The sequences were checked for similarities with the deposited reference
152 sequences from GenBank for sequence quality and tick species confirmation using BLAST
153 (https://blast.ncbi.nlm.nih.gov/Blast.cgi). All final aligned sequences were checked in Mesquite
154 version 3.5 [38]. The protein-coding genes were translated to amino acids to confirm the absence
155 of stop codons.

156

157 ***Population genetic analysis***

158 Multiple sites that are in proximity to each other were combined for population genetic
159 analysis if less than eight individuals were obtained per site, which resulted in 8 populations
160 labeled A to H (S1 Table). Three sites were excluded from the population genetic analysis because
161 of the limited number of obtained individuals (<8) and the lack of a nearby site to combine into a
162 single population.

163 The final *cox1* sequences of the tick species: *I. ovatus* and *H. flava* were individually
164 analyzed using DNASp version 6.12.03 to determine the haplotype diversity per species [39]. The
165 level of genetic divergence between each population was quantified per species using global F_{ST}.
166 Significance was tested using Arlequin software version 3.5.2.2 [40] with 9999 permutations.

167 The genetic relationship between the *I. ovatus* populations was visualized using the
168 unweighted pair group with the arithmetic mean (UPGMA) cluster method using the APE package
169 [41] for the RStudio software (R Development Core Team, 2016). A cluster dendrogram was
170 created using pairwise F_{ST} values genetic distance matrix from GenAlEx.

171

172 ***Haplotype network and phylogenetic analyses***

173 We constructed a haplotype network analysis using PopART program version 1.7
174 (http://popart.otago.ac.nz/index.shtml) on *cox1 I. ovatus* and *H. flava* sequences to assess
175 haplotype relationships using the median-joining network algorithm [42]. Briefly, we constructed a
176 Bayesian phylogenetic tree of *cox1* haplotypes for *I. ovatus* and *H. flava,* respectively, using BEAST
177 version 1.10.14 [43]. We used the Hasegawa-Kishino-Yano substitution model with estimated base
178 frequencies. We employed a strict clock model and used the coalescent prior as the tree prior. A
179 maximum clade credibility tree was acquired using TreeAnnotator version 1.10.14 using trees from

180 BEAUti version1.10.14 with 90% of the trees as the burn-in. We viewed the constructed maximum
181 clade credibility tree using FigTree version 1.4.4.

182

183 ***Statistical analysis***

184 To determine whether there was a significant difference in the *Rickettsia* infection rate
185 between haplotype groups for *I. ovatus* and *H. flava,* we performed a z-score test at $p < 0.05$. To
186 determine whether there were differences in the mean altitude between the haplotype groups,
187 we used the Welch t-test at $p < 0.05$.

188

189 **3. Results**

190 The total number of positive (pos) and negative (neg) ticks for *Rickettsia* infections from
191 the ticks collected in 2018 were: *I. ovatus* (neg = 22, pos = 17); *H. flava* (neg = 10, pos = 13); *I.*
192 *monospinus* (neg = 11, pos = 6); *I. asanumai* (neg = 4, pos=5); *I. nipponensis* (pos = 4); *I. persulcatus*
193 (neg = 4); and *H. japonica* (pos = 2). In this study, the total number of samples from the previously
194 published data [6;11] and the unpublished data from 2018 were: *I. ovatus* (n = 346); *H. flava*
195 (n = 243); *I. monospinus* (n = 19); *I. asanumai* (n = 9); *I. nipponensis* (n = 4); *I. persulcatus* (n = 4);
196 and *H. japonica* (n = 2). **T**he number of adult ticks whose *cox1* was successfully sequenced per
197 species were: *I. ovatus* (346); *H. flava* (243); *I. monospinus* (19); *I. asanumai* (9); *I. nipponensis* (4); *I.*
198 *persulcatus* (4); and *H. japonica* (2) (Table 1).

199 **Table 1**. Summary of the haplotype and *Rickettsia* infection rates among the 7 ixodid tick species
200 obtained in the Niigata Prefecture, Japan

Tick species ***	ns	n	nh	r	Global F_{ST}
1. *Ixodes ovatus*	30	346	59	46 (13.26%)	0.4154*
2. *Haemaphysalis flava*	18	243	66	15 (6.17%)	0.3597
3. *Ixodes monospinus*	6	19	9	6 (31.58%)	0.9034
4. *Ixodes asanumai*	7	9	4	5 (55.55%)	1.0000
5. *Ixodes nipponensis*	2	4	4	4 (100%)	0.3345
6. *Ixodes persulcatus*	3	4	3	0 (0%)	1.0000
7. *Haemaphysalis japonica*	2	2	2	2 (100%)	**
Total		628		78	

Abbreviations: ns no. of sampling sites; n sample size; nh no of haplotypes; r *Rickettsia* infection rate per species; *p < 0.05** not enough samples for analysis***tick species identification is based on molecular identification using the *cox1* marker and BLAST results

We detected SFGR in 78 (12.44%) out of 627 ixodid ticks, with the highest detected in *I. ovatus* (46/346; 13.29%), *H. flava* (15/243; 6.17%), and *I. monospinus* (6/19; 31.58%) as summarized in Table 1. Out of the 46 *Rickettsia*-infected *I. ovatus* ticks, 25 displayed a 100% identity match with *Rickettsia asiatica* in the 17kDA, *gltA,* and *rOmpB* markers [6], while an additional 19 adult *I. ovatus* from the 2018 collection were also positive with *R. asiatica.* Two haplotypes were found in the rOmpB and 17kDA markers, respectively. One haplotype was found in only one individual (17369). Two out of the 15 *Rickettsia*-infected *H. flava* ticks were found to have the same haplotypes in the 17Kda, gltA,and rOmpA markers, and were identified as *Rickettsia sp.* (LC461063). The remaining 13 *Rickettsia*-infected *H. flava* ticks were identified as *Rickettsia sp.* Four out of the six *Rickettsia*-infected *I. monospinus* were identified as *Rickettsia helvetica* with the same haplotype found in the 17kDA, gltA, and rOmpB markers.

Based on the population genetic analysis of the *cox1* sequences, we found a significant global F_{ST} of 0.4154 at $p < 0.05$ for *I. ovatus* (Table 1). In contrast, no significant global F_{ST} values

223 were observed in the *H. flava, I. monospinus, I. asanumai, I. nipponensis,* or *I. persulcatus* samples
224 (Table 1). There were 59 and 66 *cox1* haplotypes found among the 346 *I. ovatus* and 243 *H. flava*
225 individuals, respectively. We found the following number of *cox1* haplotypes in the remaining
226 species: *I. monospinus* (n = 9), *I. asanumai* (n = 4), *I. nipponensis* (n = 4), *I. persulcatus* (n = 3), and
227 *H. japonica* (n = 2).

228 The *cox1* haplotype network of *I. ovatus* (Figure 1) revealed four genetic groups, wherein
229 three genetic groups (1, 2, and 3) were distributed along different altitudinal gradients, as shown
230 in Figure 2. These four genetic groups were concordant with the four clusters found in the *I.*
231 *ovatus* phylogenetic tree (S1 Figure). The habitat distribution of genetic group 3 was limited to
232 high altitude sites only (range = 16–912 m.a.s.l.), whereas genetic groups 1 (255–471 m.a.s.l.) and
233 2 (84–1354 m.a.s.l.) were distributed at lower altitudes (Figure 2). The Welch t-test revealed a
234 significant difference between the mean altitudes of genetic groups 1 and 3 at $p < 0.05$ (Table 2);
235 however, no significant difference was observed between genetic groups 1 and 2 or groups 2 and
236 3. We found a significant difference in the *Rickettsia* infection rates between *I. ovatus* genetic
237 groups 1 and 2 based on the z-score test, but no significant difference between groups 1 and 3 or
238 groups 2 and 3 (Table 2). The mean altitude between the *Rickettsia*-infected (=273.72 m.a.s.l.) and
239 non-infected *I. ovatus* (=369.61 m.a.s.l.) revealed a significant difference based on the Welch t-test
240 at $p < 0.05$ (Figure 3). The UPGMA dendrogram of *I. ovatus* revealed two genetic clusters, 1 and 2
241 using the genetic distance among the seven populations excluding one population due to the
242 limited number of samples (Figure 4).

243 **Figure 1.** Median-joining network of the 59 *cox1* haplotype sequences of *Rickettsia* positive and
244 negative *I. ovatus.* Haplotype groups are indicated by numbers (1 to 4).

245

246 **Figure 2.** The influence of altitude on the habitat distribution of *I. ovatus*. The points indicate the
247 sampling sites of the collected individuals per *I. ovatus* haplotype group (n = 3) in the haplotype
248 network (Figure 1). The map shows the elevation level across the sampling area, with Niigata
249 Prefecture Japan depicted as a color gradation. Group 4, not shown in this figure, with haplotypes
250 54 and 55 was found on Sado island, which is encircled in black in box 3.

251

252 **Figure 3.** The relationship between the mean altitude of *Rickettsia* positive (n = 46) and negative
253 (n = 300) *I. ovatus.* Welch t-tests revealed a significant difference in the mean altitude between
254 *Rickettsia-infected* and non-infected *I. ovatus* at $p < 0.05$. Populations with combined sites are
255 labeled A to H.

256

257 **Figure 4.** An unweighted pair group method with the arithmetic mean (UPGMA) dendrogram of *I.*
258 *ovatus* based on the pairwise genetic distance (F_{ST}) of *cox1* among the 7 populations across Niigata
259 Prefecture, Japan. We excluded one population due to the limited number of samples.

260

261 **Table 2**. The differences in *Rickettsia* infection rates and mean altitude in *I. ovatus* haplotype
262 groups and cluster dendrogram groups. The table shows the distribution of *Rickettsia*-infected and
263 uninfected *I. ovatus* and the mean altitude in each of the haplotype groups as shown in Figure 1 *(I.*
264 *ovatus* haplotype network) and the cluster dendrogram in Figure 4 (*I. ovatus* cluster dendrogram).
265 The z-score test showed a significant difference at $p < 0.05$ between the *Rickettsia* detection rates
266 in haplotype groups 1 and 2 (indicated by [ab)] and in cluster dendrogram groups 1 and 2. The Welch
267 t-test at $p < 0.05$ revealed a significant difference in the mean altitude of haplotype groups 1 and 3

268 and cluster dendrogram groups 1 and 2 (indicated by [ab]). Haplotype group 4 was not included in

269 the analysis due to its low sample size.

Haplotype group	***Rickettsia* infection rate**			**Mean altitude**
	Positive	Negative	Detection rate	
1	1	92	1.07%[ab]	294[ab]
2	28	99	22.04%[ab]	341[a]
3	16	110	12.70%[a]	374[ab]
Dendrogram Group				
1	4	120	3.23%[ab]	30
2	40	227	14.98%[ab]	345

270

271 The *cox1* haplotype network of *H. flava* displayed two genetic groups (S2 Figure)

272 consistent with the *H. flava* phylogenetic tree (S3 Figure). Based on the Welch t-test, we found no

273 significant differences in mean altitude (205 and 165 m.a.s.l.) at $p < 0.05$ between the two *H. flava*

274 genetic groups (S3 Table). No significant difference between the *Rickettsia* infection rates of *H.*

275 *flava* genetic groups 1 and 2 was observed using the z-score test at $p < 0.05$ (S2 Table).

276

277 ## 4. Discussion

278 Our findings support our hypothesis that a genetically structured tick population, such as *I.*

279 *ovatus*, can cause the *Rickettsia* infection rate to be spatially heterogenous due to limited gene

280 flow along an altitudinal gradient. The significant global F_{ST} estimate of 0.4154 among the *I. ovatus*
281 populations revealed genetic differentiation between the populations as supported by the
282 occurrence of two genetic clusters in the cluster dendrogram. Our results were consistent with our
283 previous study [11] which suggested that the low mobility of the host species for immature *I.*
284 *ovatus* contributed to low gene flow in the tick populations. The low *I. ovatus* gene flow along the
285 altitudinal gradient might have caused the spatial heterogeneity of *Rickettsia* infection rates
286 among these populations, which is supported by the significant difference found in *Rickettsia*
287 infection rates between genetic clusters 1 and 2. A similar pattern was observed in the studies of
288 [44-46] which found that the infection rate of *Borrelia burgdorferi*, the causative agent of Lyme
289 disease, decreased in ticks along the altitudinal gradient. Low gene flow can cause infected and
290 uninfected ticks to have limited opportunities to traverse a wider spatial area thus causing a
291 heterogeneous *Rickettsia* infection rate [47-48].

292 We found two genetic groups in the *H. flava* haplotype network, but no significant
293 difference in the *Rickettsia* infection rates between the two groups. These results might be due to
294 the high gene flow observed in the *H. flava* populations, which enable *Rickettsia*-infected and
295 uninfected *H. flava* individuals to traverse between the study sites. The high mobility of the large
296 mammalian hosts used by adult *H. flava* and avian-mediated dispersal during their immature stage
297 probably contributed to their homogenized population genetic structure [11], and the resulting
298 homogenized *Rickettsia* infection rates. Large mammalian hosts and birds have a wide dispersal
299 range that enables the broader movement of *Rickettsia*-infected ticks, as observed in previous
300 studies of *Amblyomma Americanum* [49-51], *H. flava* [11], and *I. ricinus* [52]. Birds are especially
301 good at dispersing over large areas since they can easily traverse landscape barriers such as
302 mountains, fences, glaciers, and oceans that would be difficult for mammals to cross [53].

303 In addition to host mobility, environmental factors can influence the population genetic
304 structure of ticks [54]. Each tick species has preferred environmental conditions that are conducive
305 to completing the tick life cycle, thus influencing the geographical distribution of ticks and the risk
306 areas for tick-borne diseases [55-56]. Altitude may influence the population genetic structure of
307 ticks through the effect of altitude on the distribution and abundance of ticks and/or their hosts
308 [57-59]. Altitudinal differences between populations can affect genetic divergence [60], through,
309 for example, ecological isolation, which causes natural selection against maladapted immigrants
310 and limits gene flow [61-62]. For example, organisms adapted to low altitudinal sites that cannot
311 tolerate the lower temperatures at higher altitudes would not survive if they dispersed to those
312 higher altitude sites, thus restricting gene flow across the altitudinal gradient [60].

313 The difference in mean altitude between *I. ovatus* cluster groups 1 and 2 might be due to
314 adaptive divergence along the altitudinal gradient. Individuals from cluster group 1 were
315 distributed in higher altitude areas of the northern mountainous area of Niigata Prefecture, while
316 cluster group 2 individuals were found in the lower altitude regions of northern Niigata Prefecture.
317 Populations along an altitudinal gradient are prone to differentiating selection pressures, which
318 result in local adaptation [63]. Altitudinal gradients may also cause the varying ambient
319 temperature, precipitation, and humidity levels essential to ticks' development and survival [64].
320 These varying environmental factors may cause ticks to have difficulty dispersing over a wide
321 habitat range. Thus, ticks may need to adapt to extreme habitats, such as extreme altitude or
322 precipitation levels, for survival. For example, *Rhipicephalus compositus* were found in altitudes of
323 1000–2500 m, but optimal conditions were between 1200–2500 m [64-66].

324 The different *Rickettsia* infection rates and altitudinal ranges between the *I. ovatus*
325 phylogenetic groups may be explained by adaptive evolutionary theory, which states that
326 organisms adjust to new or severe changes in their environment to become better suited to their

327 habitat[67-68]. Based on the relationship between the *I. ovatus* phylogenetic groups and their
328 mean attitudes, *I. ovatus* might be undergoing local adaptation along the altitudinal gradient due
329 to the higher genetic differentiation between populations as supported by the significant global F_{ST}
330 (0.4154) found in *I. ovatus*. Based on isolation by environment (IBE), genetic differentiation will
331 increase with increased environmental differences independent of geographic distances [33-
332 34;69]. When environmental conditions differ, the success of immigration in a new habitat is
333 reduced, which may increase the genetic fixation rate due to a lower chance of outcrossing;
334 thereby enhancing genetic isolation [70]. Thus, the lower gene flow along the altitudinal gradient
335 reduced the spatial homogeneity of *Rickettsia* infection rates among the *I. ovatus* tick populations,
336 thus causing the different *Rickettsia* infection rates obtained.

337 In this study, only a few (n < 20) *I. monospinus, I. asanumai, I. nipponensis* and *H. japonica*
338 were collected; however, a high *Rickettsia* infection rate (31.58%–100%) was found despite these
339 limited numbers. In previous studies, high *Rickettsia* infection rates despite a few individuals were
340 also observed in *I. monospinus* in several prefectures in Japan [71-73]. The extent of tick habitat
341 distribution may vary between species, which may have influenced the widely different *Rickettsia*
342 infection rates observed among the species in this study. The high *Rickettsia* infection rate in these
343 ticks is probably due to effective transovarial transmission [74]. Furthermore, *Rickettsial*
344 endosymbionts are inclined to have a high infection rate in some tick species populations because
345 they contribute to the tick microbiome which might imply nutritional symbiosis in the tick species
346 with high *Rickettsia* infection despite the low number of samples tested [75-77]. Additionally, the
347 few collected individuals of these species may be habitat specialists that thrive in a narrow habitat
348 range in contrast to *I. ovatus* and *H. flava*, which may be habitat generalists capable of thriving in a
349 wide habitat range [78]. The narrow habitat range of habitat specialist ticks might have enabled
350 increased interactions between infected and uninfected *Rickettsia* ticks within the small spatial

351 scale compared to the habitat generalist ticks, thus causing their high *Rickettsia* infection rates.
352 This conclusion should be considered with caution, since the limited number of individual ticks
353 collected across the Niigata Prefecture may have caused a bias in the estimated *Rickettsia*
354 infection rates determined in this study. We suggest conducting future research on *Rickettsia*
355 infection rates in a wider sampling range with an increased number of *I. monospinus, I. asanumai,*
356 *I. nipponensis*, and *H. japonica* individuals to further increase our understanding of the association
357 between *Rickettsiae* and their tick vectors.

358 The occurrence of local adaptation in tick populations could affect the future of the tick-
359 borne disease landscape. Environmental factors, such as precipitation, temperature, and altitude,
360 have been shown to drive population differentiation in insects, such as *Anopheles* mosquitoes and
361 *Drosophila* flies [79-81], but studies on the environmental adaptation of ixodid ticks, such as *I.*
362 *ovatus*, and its *Rickettsia* infection susceptibility, have not yet been performed. [7] suggested that
363 environmental conditions that affect bird hosts can also affect the local adaptation of ticks. Few
364 studies have assessed such local adaptation in multiple organisms with varying dispersal abilities
365 [82-86] and are an area in need of future research.

366 One of the limitations of this study is the use of one mitochondrial gene *cox1*, despite this,
367 we were able to determine the relationship between the tick population genetic structure and
368 *Rickettsia* infection rates as influenced by the altitudinal gradient. The mitochondrial *cox1* gene
369 has been widely used for population genetic analysis of many tick species and was proven to be
370 informative in determining the relationship from the subfamily to the population level [87-91].
371 Mitochondrial genes have a mutation rate that is useful in species-level phylogenetics and can be
372 used for wide geographic ranges however its resolution is not fine enough to study species
373 selection [10]. In future studies, we suggest including additional mitochondrial genes and or
374 nuclear genes.

375 Since ticks are blood-sucking ectoparasites, they directly influence their mammalian hosts
376 and the pathogens they transmit [94-96]. The interaction between the vector (tick), host, and
377 pathogen (*Rickettsia*) is essential in understanding and predicting the risk and transmission of tick-
378 borne diseases [97]. Understanding the genetic structure of ticks can serve as an alternative
379 indicator to infer the potential spread of its pathogen [98]. Our study found relationships between
380 (1) the population genetic structure of ticks and the corresponding *Rickettsia* infection rates, (2)
381 altitude and the population genetic structure of ticks, and (3) altitude and *Rickettsia* infection rates.
382 We found that host mobility may influence the genetic structure of ixodid ticks. This information
383 can be used to design more effective tick-borne disease control programs that focus on screening
384 and detecting pathogens found in ticks and their mammalian hosts. For example, patterns of
385 disease transmission from ticks with a high genetic divergence and less mobile hosts, such as *I.*
386 *ovatus*, are likely due to the movement of infected hosts rather than infected ticks. Thus,
387 screening prospective tick hosts for *Rickettsia* infection would be more suitable in this example.
388 We suggest screening the hosts of immature *I. ovatus*, such as small rodents, instead of screening
389 ticks. The *Rickettsia* infection rate in tick genetic groups can predict the spread of tick-borne
390 diseases caused by *Rickettsia,* such as the Japanese spotted fever. We also found that altitude may
391 influence the *Rickettsia* infection rate of *I. ovatus* genetic groups. This information can be used to
392 determine the high-risk areas (e.g., lowland, mountains, etc.) of tick-borne diseases along an
393 altitudinal gradient. Genetically structured arthropod vectors, such as ticks, can have different
394 vector competencies, and environmental factors, such as altitudinal gradients, that can influence
395 the vector's ability to acquire, transmit, and maintain the pathogen infection [99].

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403 **References**

404 1. Neumann M. Eine hysterische Hausepidemie. European Neurology. 1899;5(6):405-10.

405 2. Miyamoto, K., Nakao, M., Fujita, H., Sato, F., 1993. The ixodid ticks on migratory birds in
406 Japan and the isolation of Lyme disease spirochetes from bird-feeding ticks. Med. Entomol.
407 and zool., 44(4), 315-326. https://doi.org/10.7601/mez.44.315

408 3. Neumann H. Zur Behandlung der Pleuritis exsudativa. DMW-Deutsche Medizinische
409 Wochenschrift. 1897 Jan;23(05):73-5.

410 4. Yu XJ, Liang MF, Zhang SY, Liu Y, Li JD, Sun YL, Zhang L, Zhang QF, Popov VL, Li C, Qu J. Fever
411 with thrombocytopenia associated with a novel bunyavirus in China. N. Engl. J. Med. 2011
412 Apr 21;364(16):1523-32. https://doi.org/10.1056/nejmoa1010095

413 5. Yamaji K, Aonuma H, Kanuka H. Distribution of tick-borne diseases in Japan: Past patterns
414 and implications for the future. J. Infect. Chemother.2018 Jul 1;24(7):499-504.
415 https://doi.org/10.1016/j.jiac.2018.03.012

416 6. Arai R, Sato M, Kato M, Aoki J, Nishida A, Watanabe K, Hirokawa C, Ikeda S, Watanabe K,
417 Regilme MA, Sato MO. Spotted fever group rickettsiae (SFGR) detection in ticks following
418 reported human case of Japanese spotted fever in Niigata Prefecture, Japan. Sci. Rep. 2021
419 Jan 28;11(1):2595. https://doi.org/10.1038/s41598-021-81587-9

420 7. McCoy KD, Boulinier T, Tirard C, Michalakis Y. Host specificity of a generalist parasite: genetic
421 evidence of sympatric host races in the seabird tick *Ixodes uriae*. J. Evol. Biol.. 2001 May
422 9;14(3):395-405. https://doi.org/10.1046/j.1420-9101.2001.00290.x

423 8. Oliver JD, Bennett SW, Beati L, Bartholomay LC. Range expansion and increasing *Borrelia*
424 *burgdorferi* infection of the tick *Ixodes scapularis* (Acari: Ixodidae) in Iowa, 1990–2013. J. Med.
425 Entomol. 2017 Nov 7;54(6):1727-34. https://doi.org/10.1093/jme/tjx121

426 9. Agany, D. D., Pietri, J. E., Gnimpieba, E. Z., 2020. Assessment of vector-host-pathogen
427 relationships using data mining and machine learning. Comput. Struct. Biotechnol. J., 18,
428 1704-1721. https://doi.org/10.1016/j.csbj.2020.06.031

429 10. Araya-Anchetta A, Busch JD, Scoles GA, Wagner DM. Thirty years of tick population genetics:
430 a comprehensive review. Infect. Genet. Evol. 2015 Jan 1;29:164-79.
431 https://doi.org/10.1016/j.meegid.2014.11.008

432 11. Regilme MA, Sato M, Tamura T, Arai R, Sato MO, Ikeda S, Gamboa M, Monaghan MT,
433 Watanabe K. Comparative population genetic structure of two ixodid tick species (Acari:
434 Ixodidae)(*Ixodes ovatus* and *Haemaphysalis flava*) in Niigata prefecture, Japan. Infect. Genet.
435 Evol. 2021 Oct 1;94:104999.

436 12. Estrada-Peña AJ. Ticks as vectors: taxonomy, biology and ecology. Rev. Sci. Tech. 2015 Apr
437 1;34(1):53-65. https://doi.org/10.20506/rst.34.1.2345.

438 13. Mixson TR, Lydy SL, Dasch GA, Real LA. Inferring the population structure and demographic
439 history of the tick, *Amblyomma americanum* Linnaeus. J. Vector Ecol. 2006 Jun;31(1):181-92.
440 https://doi.org/10.3376/1081-1710(2006)31[181:itpsad]2.0.co; 2.

441 14. Trout RT, Steelman CD, Szalanski AL. Population genetics of *Amblyomma americanum* (Acari:
442 Ixodidae) collected from Arkansas. J. Med. Entomol. 2014 Dec 1;47(2):152-61.
443 https://doi.org/10.1603/me09106

444 15. Guglielmone AA, Nava S, Mastropaolo M, Mangold AJ. Distribution and genetic variation of
445 *Amblyomma triste* (Acari: Ixodidae) in Argentina. Ticks Tick Borne Dis. 2013 Sep 1;4(5):386-90.
446 https://doi.org/10.1016/j.ttbdis.2013.01.009

447 16. Lampo M, Rangel Y, Matat A. Population genetic structure of a three-host population genetic
448 in *Amblyommma dissimile*. J. Parasitol. 2015;84:1137-42.

449 17. Dougherty ER, Seidel DP, Carlson CJ, Spiegel O, Getz WM. Going through the motions:
450 incorporating movement analyses into disease research. Ecol. Lett. 2018 Apr;21(4):588-604.
451 https://doi.org/10.1111/ele.12917

452 18. Regoes RR, Nowak MA, Bonhoeffer S. Evolution of virulence in a heterogeneous host
453 population. Evol. 2000 Feb;54(1):64-71. https://doi.org/10.1111/j.0014-3820.2000.tb00008.x

454 19. Boots M, Mealor M. Local interactions select for lower pathogen infectivity. Sci. 2007 Mar
455 2;315(5816):1284-6.https://doi.org/10.1126/science.1137126

456 20. Lion S, Gandon S. Evolution of spatially structured host–parasite interactions. Journal of Evol.
457 Biol. 2015 Jan;28(1):10-28. https://doi.org/10.1111/jeb.12551

458 21. Chin KM, Wolfe MS. Selection on *Erysiphe graminis* in pure and mixed stands of barley. Plant
459 Pathol. 1984;33:535-546. doi: 10.1111/j.1365-3059.1984.tb02878.x

460 22. Thrall PH, Burdon JJ. Evolution of virulence in a plant host-pathogen metapopulation. Science.
461 2003;299:1735-1737. doi: 10.1126/science.1080070.

462 23. White PS, Choi A, Pandey R, Menezes A, Penley M, Gibson AK, de Roode J, Morran L. Host
463 heterogeneity mitigates virulence evolution. Biol Lett. 2020;16:20190744. doi:
464 10.1098/rsbl.2019.0744.

465 24. Gibson AK, Baffoe‐Bonnie H, Penley MJ, Lin J, Owens R, Khalid A, Morran LT. The evolution
466 of parasite host range in heterogeneous host populations. J Evol Biol. 2020;33:773-782. doi:
467 10.1111/jeb.13608.

468 25. Bono LM, Gensel CL, Pfennig DW, Burch CL. Competition and the origins of novelty:
469 experimental evolution of niche-width expansion in a virus. Biol Lett. 2013;9:20120616. doi:
470 10.1098/rsbl.2012.0616.

471 26. Streicker DG, Winternitz JC, Satterfield DA, Condori-Condori RE, Broos A, Tello C, Recuenco S,
472 Velasco-Villa A, Altizer S, Valderrama W. Host–pathogen evolutionary signatures reveal
473 dynamics and future invasions of vampire bat rabies. Proc Natl Acad Sci U S A.
474 2016;113:10926-10931. doi: 10.1073/pnas.1606587113.

475 27. Fountain‐Jones NM, Craft ME, Funk WC, Kozakiewicz C, Trumbo DR, Boydston EE, Lyren LM,
476 Crooks K, Lee JS, VandeWoude S, Carver S. Urban landscapes can change virus gene flow and
477 evolution in a fragmentation‐sensitive carnivore. Mol Ecol. 2017;26:6487-6498. doi:
478 10.1111/mec.14375.

479 28. Daniel M, Malý M, Danielová V, Kříž B, Nuttall P. Abiotic predictors and annual seasonal
480 dynamics of *Ixodes ricinus*, the major disease vector of Central Europe. Parasit Vectors.
481 2015;8:1-12. doi: 10.1186/s13071-015-1092-y.

482 29. Eisen RJ, Eisen L, Beard CB. County-scale distribution of *Ixodes scapularis* and *Ixodes pacificus*
483 (Acari: Ixodidae) in the continental United States. J Med Entomol. 2016;53:349-386. doi:
484 10.1093/jme/tjv237.

485 30. Chapman MA, Hiscock SJ, Filatov DA. Genomic divergence during speciation driven by
486 adaptation to altitude. Mol Biol Evol. 2013;30:2553–2567. doi: 10.1093/molbev/mst168.

487 31. Osborne OG, Batstone TE, Hiscock SJ, Filatov DA. Rapid speciation with gene flow following
488 the formation of Mt. Etna. Genome Biol Evol. 2013;5:1704–1715. doi: 10.1093/gbe/evt127.

489 32. Polato NR, Gray MM, Gill BA, Becker CG, Casner KL, Flecker AS, Kondratieff BC, Encalada AC,
490 Poff NL, Funk WC, Zamudio KR. Genetic diversity and gene flow decline with elevation in
491 montane mayflies. Heredity. 2017 Aug;119(2):107-116. doi: 10.1038/hdy.2017.19. PMID:
492 28333156.

493 33. Wang ,J., Summers,K., 2010. Genetic structure is correlated with phenotypic divergence
494 rather than geographic isolation in the highly polymorphic strawberry poison-dart frog. Mol.
495 Ecol., 19, 447–458. https://doi.org/10.1111/j.1365-294x.2009.04465.x

496 34. Bradburd, G.S., Ralph, P.L., Coop, G.M., 2013. Disentangling the effects of geographic and
497 ecological isolation on genetic differentiation. Evolution, 67, 3258–3273.
498 https://doi.org/10.1111/evo.12193

499 35. Sexton,J.P., Hangartner,S.B., Hoffmann,A.A., 2014. Genetic isolation by environment or
500 distance: which pattern of gene flow is most common?. Evol., 68, 1–15.
501 https://doi.org/10.1111/evo.12258

502 36. Yamaguti, N., Tipton, V. J., Keegan, H. L., & Toshioka, S. (1971). Ticks of Japan, Korea, and the
503 Ryukyu islands. Brigham Young University Science Bulletin, Biological Series, 15, 1.

504 37. Vrijenhoek R. DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I
505 from diverse metazoan invertebrates. Mol Mar Biol Biotechnol. 1994 Oct 1;3(5):294-9. PMID:
506 7881515.

507 38. Maddison, W.P., Maddison, D.R., 2011. Mesquite: A modular system for evolutionary analysis.
508 Available at: http://mesquiteproject.org.

509 39. Rozas J, Ferrer-Mata A, Sánchez-DelBarrio JC, Guirao-Rico S, Librado P, Ramos-Onsins SE,
510 Sánchez-Gracia A. DnaSP 6: DNA sequence polymorphism analysis of large datasets. Mol Biol
511 Evol. 2017 Dec 1;34(12):3299-3302. doi: 10.1093/molbev/msx248. PMID: 29036515.

512 40. Excoffier, L., & Lischer, H. E. L. (2010). Arlequin suite ver 3.5: A new series of programs to
513 perform population genetics analyses under Linux and Windows. Molecular Ecology
514 Resources, 10(3), 564-567. https://doi.org/10.1111/j.1755-0998.2010.02847.x

515 41. Paradis, E., Schliep, K., 2018. APE 5.0: an environment for modern phylogenetics and
516 evolutionary analyses in R. Bioinformatics, 35, 526–528.
517 https://doi.org/10.1093/bioinformatics/bty633.

518 42. Bandelt, H.J., Forster, P., Röhl, A., 1999. Median-joining networks for inferring intraspecific
519 phylogenies. Mol. Biol. Evol. 16, 37–48.
520 https://doi.org/10.1093/oxfordjournals.molbev.a026036

521 43. Drummond AJ, Rambaut A. BEAST: Bayesian evolutionary analysis by sampling trees. BMC
522 Evol Biol. 2007 Nov 8;7:214. doi: 10.1186/1471-2148-7-214. PMID: 17996036; PMCID:
523 PMC2222611.

524 44. Burri, C., Cadenas, F.M., Douet, V., Moret, J., Gern, L., 2007. Ixodes ricinus density and
525 infection prevalence of *Borrelia burgdorferi* sensu lato along a north-facing altitudinal
526 gradient in the Rhône Valley (Switzerland). Vector-Borne and Zoonotic Diseases, 7, 50-58.
527 https://doi.org/10.1089/vbz.2006.0569.

528 45. Jouda, F., Perret, J.L., Gern, L., 2004. *Ixodes ricinus* density, and distribution and prevalence of
529 *Borrelia burgdorferi* sensu lato infection along an altitudinal gradient. J. Med. Entomol. 41,
530 162-169. doi: 10.1603/0022-2585-41.2.162.

531 46. Moran-Cadenas F, Schneider H, Lommano E, Burri C, Moret J, Gern L. A comparison of two
532 DNA extraction approaches in the detection of *Borrelia burgdorferi* sensu lato from live
533 Ixodes ricinus ticks by PCR and reverse line blotting. Vector Borne Zoonotic Dis. 2007 Dec
534 1;7(4):555-62. https://doi.org/10.1089/vbz.2006.0596

535 47. Gooding, R.H. Genetic variation in arthropod vectors of disease-causing organisms: Obstacles
536 and opportunities. Clin. Microbiol. Rev. 1996; 9: 301.https://doi.org/10.1128/cmr.9.3.301

537 48. Páez-Triana, L., Muñoz, M., Herrera, G., Moreno-Pérez, D.A., Tafur-Gómez, G.A., Montenegro,
538 D., Patarroyo, M.A., Paniz-Mondolfi, A., Ramírez, J.D., 2021. Genetic diversity and population
539 structure of *Rhipicephalus sanguineus* sensu lato across different regions of Colombia. Parasit
540 Vectors, 14, 1-11. https://doi.org/10.1186/s13071-021-04898-w

541 49. Reichard, M.V., Kocan, A.A., Van Den Bussche, A., Barker, R.W., Wyckoff, J., Ewing, S.A., 2005.
542 Sequence variation of the ribosomal DNA second internal transcribed spacer region in two
543 spatially distinct populations of *Amblyomma americanum* (L.)(Acari: Ixodidae). J. Parasitol. 91,
544 260–264. https://doi.org/10.1645/GE-292R1.

545 50. Trout, R.T., Steelman, C.D., Szalanski, A.L., 2010. Population genetics of *Amblyomma*
546 *americanum* (Acari: Ixodidae) collected from Arkansas. J. Med. Entomol. 47, 152–161.
547 https://doi.org/10.1603/me09106.

548 51. Mixson TR, Lydy SL, Dasch GA, Real LA. Inferring the population structure and demographic
549 history of the tick, *Amblyomma americanum* Linnaeus. J Vector Ecol. 2006 Jun;31(1):181-92.
550 doi: 10.3376/1081-1710(2006)31[181:itpsad]2.0.co; 2.

551 52. Casati, S., Bernasconi, M.V., Gern, L., Piffaretti, J.C., 2008. Assessment of intraspecific mtDNA
552 variability of European *Ixodes ricinus* sensu stricto (Acari: Ixodidae). Infect. Genet. Evol. 8,
553 152–158. https://doi.org/10.1016/j.meegid.2007.11.007

554 53. Hasle, G., 2013. Transport of ixodid ticks and tick-borne pathogens by migratory birds. Front.
555 Cell. Infect. Microbiol., 3, 48. https://doi.org/10.3389/fcimb.2013.00048

556 54. Eremeeva, M.E. and Dasch, G.A., 2015. Challenges posed by tick-borne rickettsiae: eco-
557 epidemiology and public health implications. Front. Public Health. 3, 55. doi:
558 10.3389/fpubh.2015.00055.

559 55. Gage, K.L., Burkot, T.R., Eisen, R.J., Hayes, E.B., 2008. Climate and vector-borne diseases. Am.
560 J. Prev. Med. 35, 436-50. doi: 10.1016/j.amepre.2008.08.030.

561 56. Parola, P., Raoult, D., 2001. Tick-borne bacterial diseases emerging in Europe. Clin. Microbiol.
562 Infect. 7,80-83. https://doi.org/10.1046/j.1469-0691.2001.00200.x

563 57. Van Treuren W, Ponnusamy L, Brinkerhoff RJ, Gonzalez A, Parobek CM, Juliano JJ, et al.
564 Variation in the microbiota of Ixodes ticks with regard to geography, species, and sex. Appl
565 Environ Microbiol. 2015;81(18):6200-9. https://doi.org/10.1128/AEM.01562-15.

566 58. Randolph SE, Miklisová D, Lysy J, Rogers DJ, Labuda M. Incidence from coincidence: patterns
567 of tick infestations on rodents facilitate transmission of tick-borne encephalitis virus.
568 Parasitology. 1999;118:177–186. https://doi.org/10.1017/s0031182098003643.

569 59. MacDonald AJ, Hyon DW, Iii JBB, Connor KEO, Swei A, Briggs CJ. Lyme disease risk in southern
570 California: abiotic and environmental drivers of *Ixodes pacificus* (Acari: Ixodidae) density and
571 infection prevalence with *Borrelia burgdorferi*. Parasit Vectors. 2017 Jan;10(1):7.
572 https://doi.org/10.1186/s13071-016-1938-y.

573 60. Funk WC, Murphy MA, Hoke KL, Muths E, Amburgey SM, Lemmon EM, Lemmon AR.
574 Elevational speciation in action? Restricted gene flow associated with adaptive divergence
575 across an altitudinal gradient. J Evol Biol. 2016 Feb;29(2):241-252.
576 https://doi.org/10.1111/jeb.12760.

577 61. Coyne JA, Orr HA. Speciation. Sunderland, MA: Sinauer Associates; 2004.

578 62. Nosil P. Ecological Speciation. Oxford University Press; 2012.

579 63. Lim MC, Bi K, Witt CC, Graham CH, Dávalos LM. Pervasive genomic signatures of local
580 adaptation to altitude across highland specialist Andean hummingbird populations. J Hered.
581 2021 Mar 1;112(2):229-240. https://doi.org/10.1093/jhered/esab008.

582 64. Juvenal N, Edward M. Seasonal dynamics and distribution of ticks in Rwanda: implications for
583 tick control strategy in Rwanda. J Anim Vet Adv. 2010;9(1):21-25.

584 65. Nzige T. Ecologie des tiques du bétail. Rapport de stage, Higher Institute of Agriculture and
585 Animal Husbandry (ISAE-Busogo) Publications, Musanze, Rwanda. 1978.

586 66. Morel P. Maladies à tiques du bétail en Afrique In: Précis de parasitologie vétérinaire
587 tropicale. Ministére de la Coopération et du Développement, République Française, Norval,
588 R.A.I., Donachie, pp: 471-717. 1981.

589 67. Kawecki TJ, Ebert D. Conceptual issues in local adaptation. Ecol Lett. 2004 Dec;7(12):1225-
590 1241. https://doi.org/10.1111/j.1461-0248.2004.00684.x.

591 68. Gregory TR. Understanding natural selection: essential concepts and common
592 misconceptions. Evol Educ Outreach. 2009 Jun;2(2):156-175.
593 https://doi.org/10.1007/s12052-009-0128-1.

594 69. Wang IJ, Bradburd GS. Isolation by environment. Mol. Ecol. 2014 Dec;23(23):5649-5662.
595 https://doi.org/10.1111/mec.12938.

596 70. Jiang S, Luo MX, Gao RH, Zhang W, Yang YZ, Li YJ, Liao PC. Isolation-by-environment as a
597 driver of genetic differentiation among populations of the only broad-leaved evergreen shrub
598 *Ammopiptanthus mongolicus* in Asian temperate deserts. Sci Rep. 2019 Jul 30;9(1):1-14.
599 https://doi.org/10.1038/s41598-019-48472-y.

600 71. Yamauchi T, Takeno K. Record of *Haemaphysalis flava* Neumann (Acari,Ixodidae) from
601 *Zoothera dauma* (Latham) (Aves) in Kyushu, Japan. Esakia. 2000;40:99-101.
602 https://doi.org/10.5109/2647

603 72. Ishiguro F, Takada N, Fujita H, Noji Y, Yano Y, Iwasaki H. Survey of the vectorial competence
604 of ticks in an endemic area of spotted fever group rickettsioses in Fukui Prefecture, Japan.
605 Microbiol Immunol. 2008;52:305-9. https://doi.org/10.1111/j.1348-0421.2008.00042.x

606 73. Sato M, Ikeda S, Arai R, Kato M, Aoki J, Nishida A, et al. Diversity and distribution of ticks in
607 Niigata prefecture, Japan (2016–2018): Changes since 1950. Ticks Tick Borne Dis.
608 2021;12:101683. https://doi.org/10.1016/j.ttbdis.2021.101683.

609 74. Parola, P., Paddock, C.D., Socolovschi, C., Labruna, M.B., Mediannikov, O., Kernif, T., Abdad,
610 M.Y., Stenos, J., Bitam, I., Fournier, P.E., Raoult, D., 2013. Update on tick-borne rickettsioses
611 around the world: a geographic approach. Clin. Microbiol. Rev., 26, 657-702. doi:
612 10.1128/cmr.00032-13.

613 75. Gillespie JJ, Joardar V, Williams KP, Driscoll T, Hostetler JB, Nordberg E, Shukla M, Walenz B,
614 Hill CA, Nene VM, Azad AF. A *Rickettsia* genome overrun by mobile genetic elements provides
615 insight into the acquisition of genes characteristic of an obligate intracellular lifestyle. J
616 Bacteriol. 2012 Jan 15;194(2):376-94. https://doi.org/10.1128/JB.06244-11

617 76. Clay K, Klyachko O, Grindle N, Civitello D, Oleske D, Fuqua C. Microbial communities and
618 interactions in the lone star tick, *Amblyomma americanum*. Mol Ecol. 2008 Oct;17(19):4371-
619 4381.

620 77. Budachetri K, Browning RE, Adamson SW, Dowd SE, Chao CC, Ching WM, Karim S. An insight
621 into the microbiome of the *Amblyomma maculatum* (Acari: Ixodidae). J Med Entomol. 2014
622 Jan;51(1):119-29. doi: 10.1603/me13139. PMID: 24605422.

623 78. Richmond, C.E., Breitburg, D.L., Rose, K.A., 2005. The role of environmental generalist species
624 in ecosystem function. Ecol. Model. 188, 279-295. doi: 10.1016/j.ecolmodel.2005.03.002.

625 79. Cheng, C., White, B.J., Kamdem, C., Mockaitis, K., Costantini, C., Hahn, M.W., Besansky, N.J.,
626 2012. Ecological genomics of *Anopheles gambiae* along a latitudinal cline: a population-
627 resequencing approach. Genetics, 190(4), 1417-1432.
628 https://doi.org/10.1534/genetics.112.139899

629 80. Kapun, M., Barrón, M.G., Staubach, F., Obbard, D.J., Wiberg, R.A.W., Vieira, J., Goubert, C.,
630 Rota-Stabelli, O., Kankare, M., Bogaerts-Márquez, M., Haudry, A. (2020). Genomic analysis of
631 European *Drosophila melanogaster* populations reveals longitudinal structure, continent-
632 wide selection, and previously unknown DNA viruses. Mol. Biol. Evol., 37, 2661-2678.
633 https://doi.org/10.1093/molbev/msaa120

634 81. Love RR, Steele AM, Coulibaly MB, Traore SF, Emrich SJ, Fontaine MC, Besansky NJ.
635 Chromosomal inversions and ecotypic differentiation in *Anopheles gambiae*: the perspective
636 from whole-genome sequencing. Mol Ecol. 2016 Dec;25(23):5889-906.
637 https://doi.org/10.1111/mec.13888

638 82. Exposito-Alonso M, Vasseur F, Ding W, Wang G, Burbano HA, Weigel D. Genomic basis and
639 evolutionary potential for extreme drought adaptation in *Arabidopsis thaliana*. Nat Ecol Evol.
640 2018 Feb;2(2):352-8., https://doi.org/10.1038/s41559-017-0423-0

641 83. Hancock AM, Brachi B, Faure N, Horton MW, Jarymowycz LB, Sperone FG, Toomajian C, Roux
642 F, Bergelson J. Adaptation to climate across the *Arabidopsis thaliana* genome. Science. 2011
643 Oct 7;334(6052):83-6.https://doi.org/10.1126/science.1209244

644 84. Zhen Y, Harrigan RJ, Ruegg KC, Anderson EC, Ng TC, Lao S, Lohmueller KE, Smith TB. Genomic
645 divergence across ecological gradients in the Central African rainforest songbird (*A ndropadus*
646 *virens*). Mol. Ecol. 2017 Oct;26(19):4966-77.https://doi.org/10.1111/mec.14270

647 85. Ahrens CW, Byrne M, Rymer PD. Standing genomic variation within coding and regulatory
648 regions contributes to the adaptive capacity to climate in a foundation tree species. Mol Ecol.
649 2019 May;28(10):2502-16. https://doi.org/10.1111/mec.15092

650 86. Miller AD, Hoffmann AA, Tan MH, Young M, Ahrens C, Cocomazzo M, Rattray A, Ierodiaconou
651 DA, Treml E, Sherman CD. Local and regional scale habitat heterogeneity contribute to
652 genetic adaptation in a commercially important marine mollusc (*Haliotis rubra*) from
653 southeastern Australia. Mol. Ecol. 2019 Jun;28(12):3053-72.
654 https://doi.org/10.1111/mec.15128

655 87. Crosbie PR, Boyce WM, Rodwell TC. DNA sequence variation in *Dermacentor hunteri* and
656 estimated phylogenies of *Dermacentor* spp.(Acari: Ixodidae) in the New World. J. Med.
657 Entomol. 1998 May 1;35(3):277-88. https://doi.org/10.1093/jmedent/35.3.277

658 88. Norris DE, Klompen JS, Keirans JE, Black IV WC. Population genetics of *Ixodes scapularis*
659 (Acari: Ixodidae) based on mitochondrial 16S and 12S genes. J. Med. Entomol.1996 Jan
660 1;33(1):78-89. https://doi.org/10.1093/jmedent/33.1.78

661 89. Qiu WG, Dykhuizen DE, Acosta MS, Luft BJ. Geographic uniformity of the Lyme disease
662 spirochete (*Borrelia burgdorferi*) and its shared history with tick vector (*Ixodes scapularis*) in
663 the northeastern United States. Genetics. 2002 Mar 1;160(3):833-49.
664 https://doi.org/10.1093/genetics/160.3.833

665 90. Rich SM, Caporale DA, Telford 3rd SR, Kocher TD, Hartl DL, Spielman A. Distribution of the
666 *Ixodes ricinus*-like ticks of eastern North America. Proceedings of the National Academy of
667 Sciences. 1995 Jul 3;92(14):6284-8. https://doi.org/10.1073/pnas.92.14.6284

668 91. de la Fuente J, Almazán C, Van Den Bussche RA, Bowman J, Yoshioka JH, Kocan KM.
669 Characterization of genetic diversity in *Dermacentor andersoni* (Acari: Ixodidae) with body
670 size and weight polymorphism. Exp. Parasitol. 2005 Jan 1;109(1):16-26.
671 https://doi.org/10.1016/j.exppara.2004.10.004

672 92. Caporale DA, Rich SM, Spielman A, Telford SR, Kocher TD. Discriminating between *Ixodes*
673 ticks by means of mitochondrial DNA sequences. Mol. Phylogenet. Evol. 1995 Dec 1;4(4):361-
674 5. https://doi.org/10.1006/mpev.1995.1033

675 93. Mitani H, Takahashi M, Masuyama M, Fukunaga M. *Ixodes philipi* (Acari: Ixodidae):
676 phylogenetic status inferred from mitochondrial cytochrome oxidase subunit I gene sequence
677 comparison. J. Parasitol. Res. 2007 Jun;93(3):719-22. https://doi.org/10.1645/ge-953r.1

678 94. Anderson JF, Magnarelli LA. Biology of ticks. Infectious disease clinics of North America. 2008
679 Jun 1;22(2):195-215.

680 95. Hill CA, Wikel SK. The *Ixodes scapularis* Genome Project: an opportunity for advancing tick
681 research. Trends Parasitol.. 2005 Apr 1;21(4):151-3.https://doi.org/10.1016/j.pt.2005.02.004

682 96. Van Zee JP, Geraci NS, Guerrero FD, Wikel SK, Stuart JJ, Nene VM, Hill CA. Tick genomics: the
683 *Ixodes* genome project and beyond. Int. J. Parasitol. 2007 Oct 1;37(12):1297-305.
684 https://doi.org/10.1016/j.ijpara.2007.05.011

685 97. Jongejan F, Nene V, de la Fuente J, Pain A, Willadsen P. Advances in the genomics of ticks and
686 tick-borne pathogens. Trends Parasitol. 2007 Sep 1;23(9):391-6.
687 https://doi.org/10.1016/j.pt.2007.07.004

688 98. Gray JS. Biology of Ixodes species ticks in relation to tick-borne zoonoses. Wiener Klinische
689 Wochenschrift. 2002 Jul 1;114(13-14):473-8.

690 99. Parola P, Paddock CD. Travel and tick-borne diseases: Lyme disease and beyond. Travel Med
691 Infect Dis. 2018;26:1-2. https://doi.org/10.1016/j.tmaid.2018.09.010

692

693 **Supporting information**

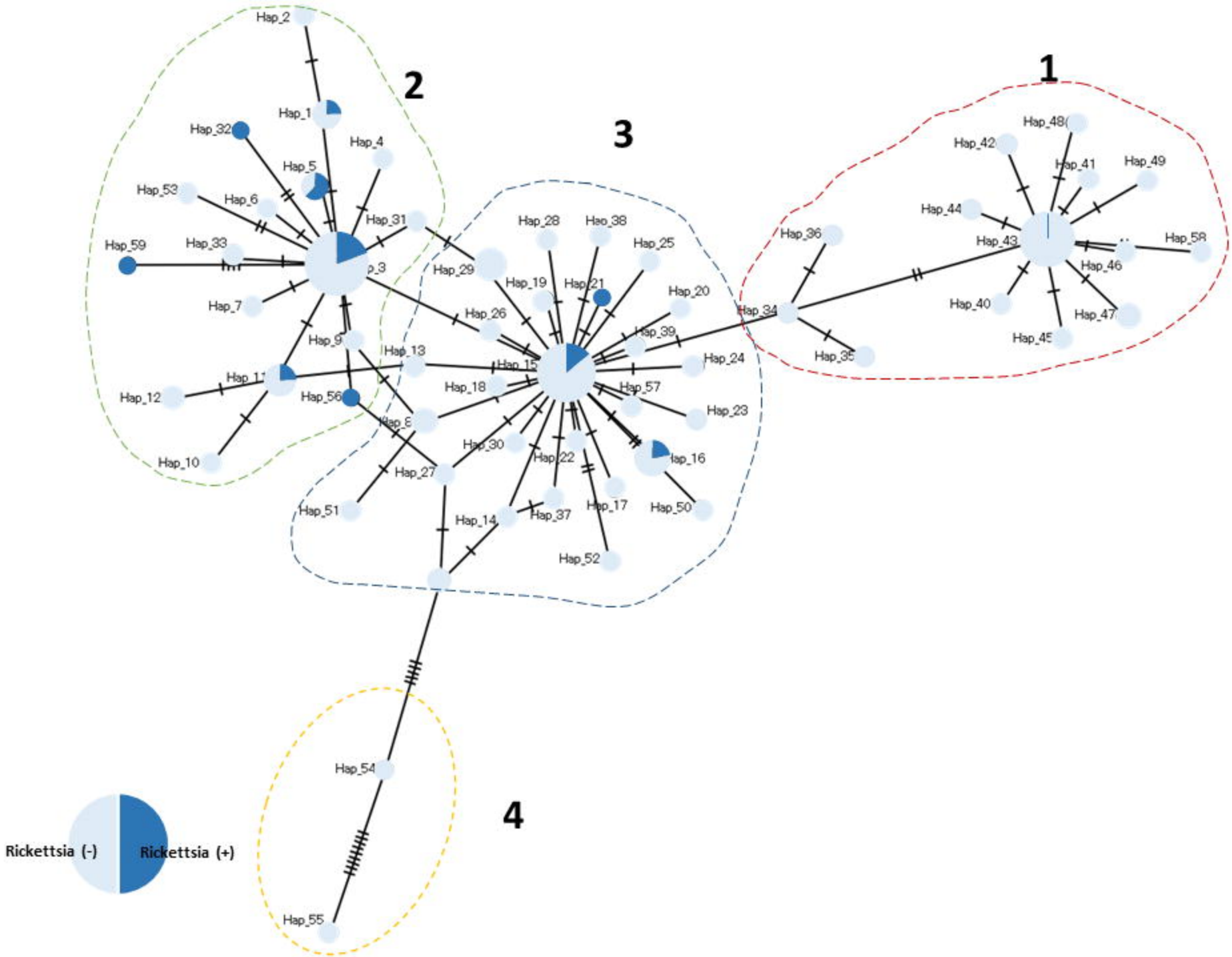
694 **S1 Table.** Summary of *Ixodes ovatus* and *Haemaphysalis flava* collected from the different
695 locations of Niigata Prefecture and its corresponding sample number and number of *Rickettsia*
696 infection per site

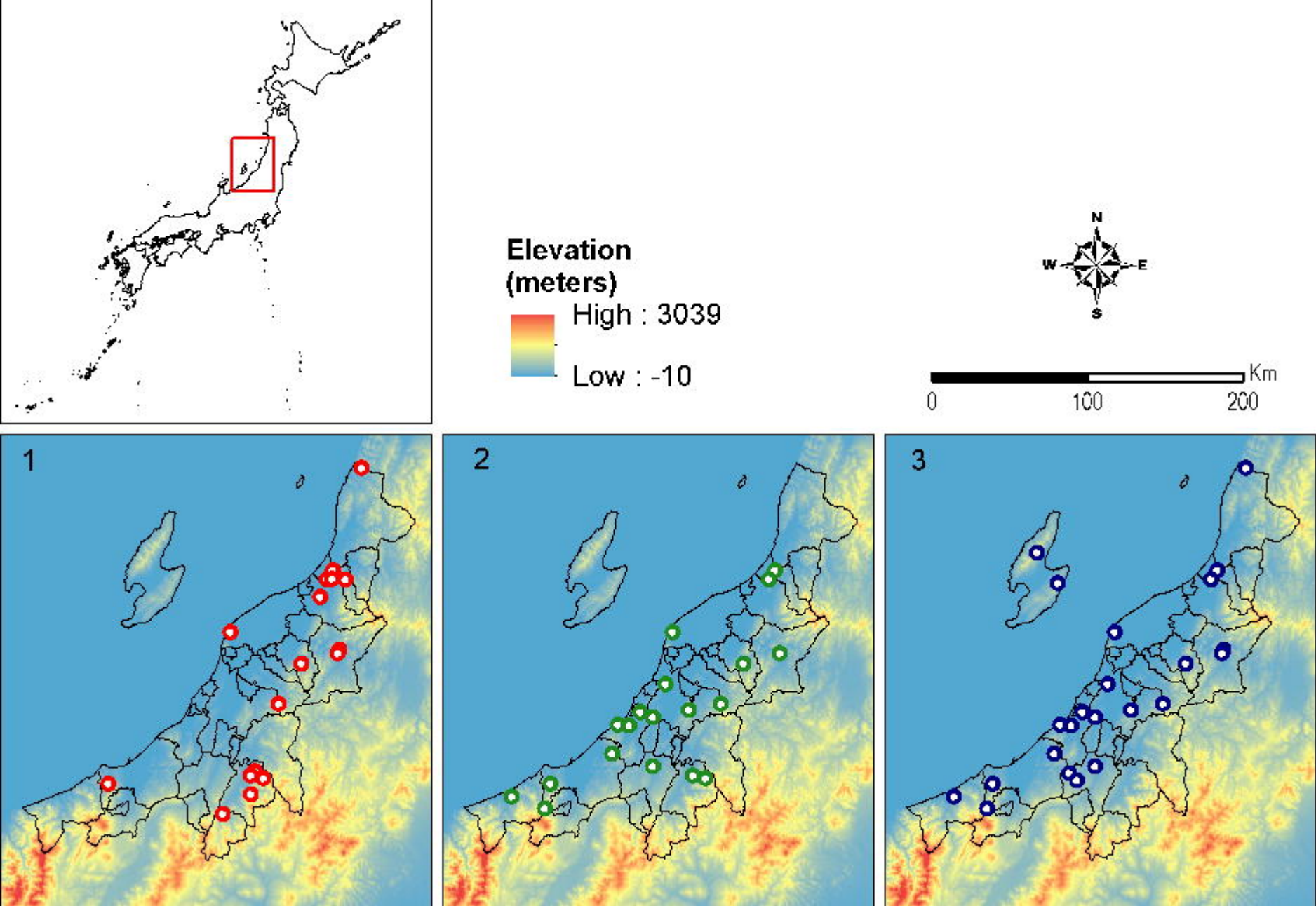
697 **S2 Table.** The difference in *Rickettsia* infection rates in *H. flava* haplotype groups and a table
698 showing the distribution of *Rickettsia*-infected and uninfected *H. flava* in each genetic groups as
699 shown in S2 Figure *(H. flava* median-joining network). The results of the z-score test for two
700 populations proportions at $p < 0.05$ showed no significant difference between the *Rickettsia*
701 detection rate in haplotype groups 1 and 2. The Welch t-test at $p < 0.05$ revealed no significant
702 difference in the mean altitude of the two groups.

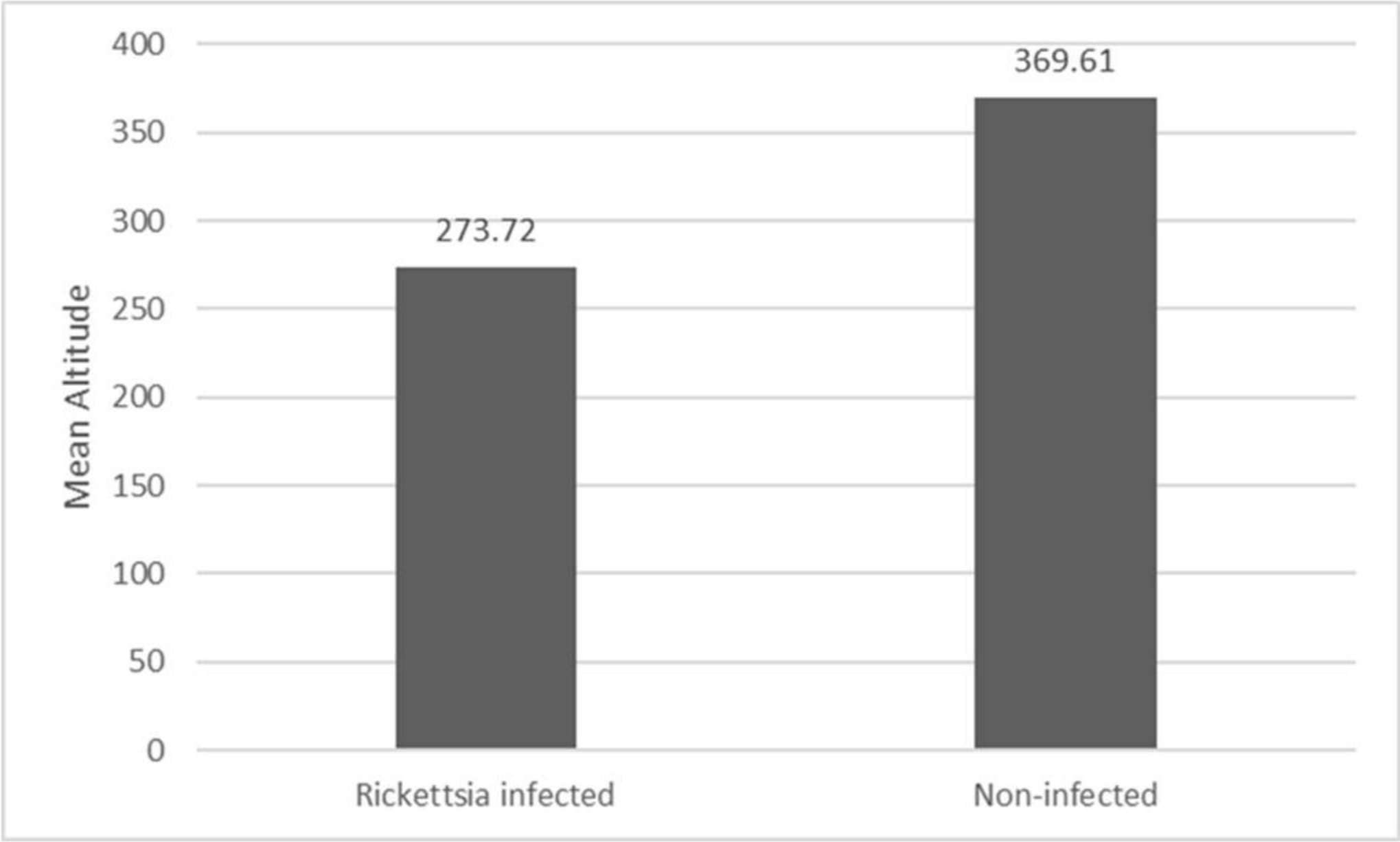
703 **S1 Fig.** Phylogenetic tree from the BEAST analysis of 59 haplotype *cox1* sequences of *I. ovatus.* The
704 blue-labeled haplotypes indicate the presence of *Rickettsia* infection. The red parentheses provide
705 the number of *Rickettsia*-positive individuals per haplotype. The black labeled haplotypes are
706 negative for *Rickettsia* infection.

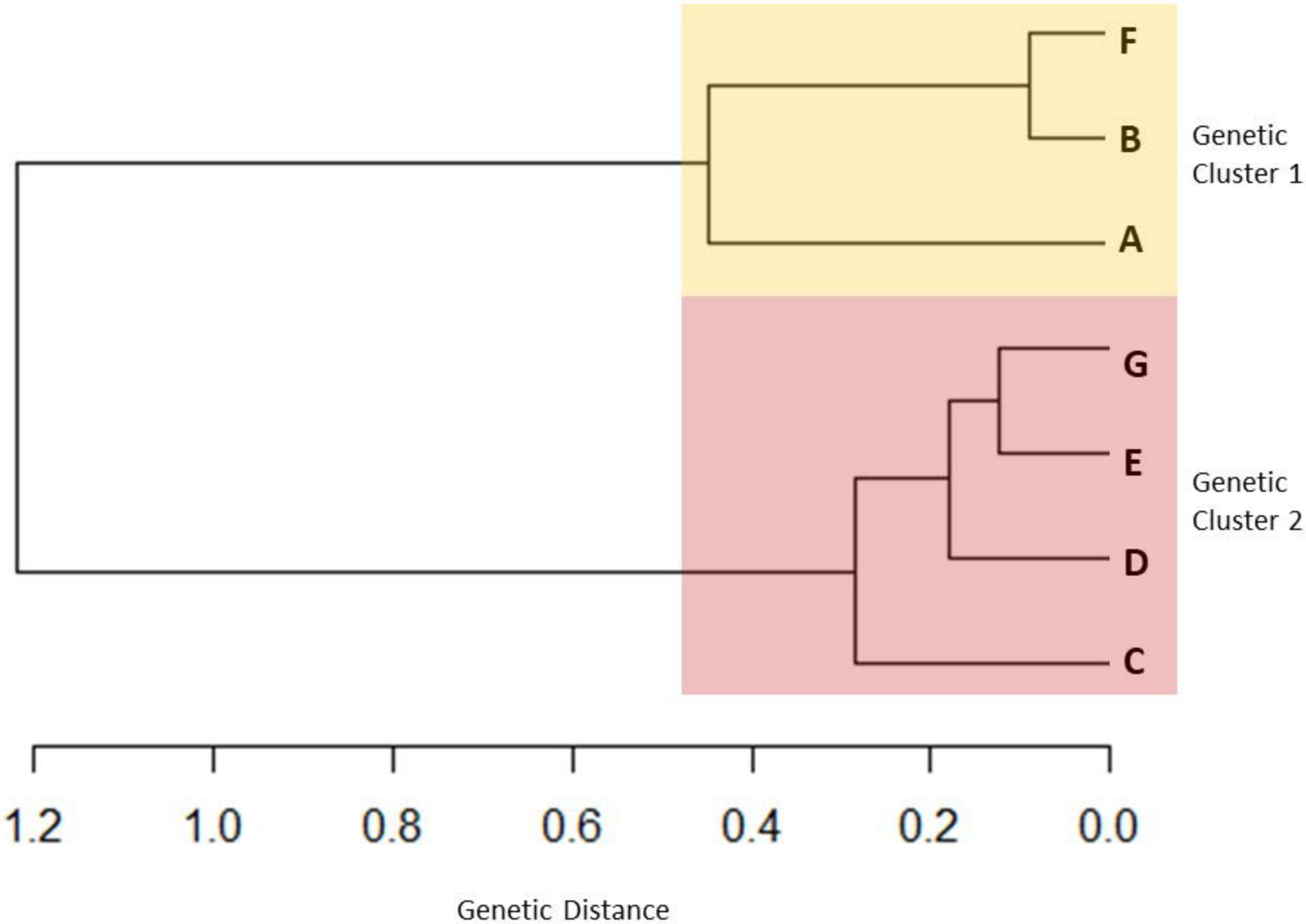
707 **S2 Fig.** Median-joining network of the 66 *cox1* haplotype sequences of *Rickettsia* positive and
708 negative *H. flava.* Haplotype groups are indicated as 1 and 2.

709 **S3 Fig.** Phylogenetic tree from the BEAST analysis of 66 haplotype *cox1* sequences of *H. flava.* The
710 blue-labeled haplotypes indicate *Rickettsia* infection in individual samples. The parentheses in red
711 provide the number of *Rickettsia*-infected ticks. The black-labeled haplotypes are negative for
712 *Rickettsia* infection.


1
2
3
4
Hap_2
Hap_1
Hap_32
Hap_4
Hap_5
Hap_53
Hap_6
Hap_31
Hap_59
Hap_33
Hap_7
Hap_9
Hap_13
Hap_11
Hap_12
Hap_56
Hap_10
Hap_28
Hap_38
Hap_25
Hap_29
Hap_19
Hap_21
Hap_20
Hap_26
Hap_39
Hap_24
Hap_15
Hap_18
Hap_57
Hap_23
Hap_30
Hap_22
Hap_16
Hap_27
Hap_51
Hap_14
Hap_37
Hap_17
Hap_50
Hap_52
Hap_48
Hap_42
Hap_41
Hap_49
Hap_44
Hap_43
Hap_58
Hap_36
Hap_46
Hap_34
Hap_40
Hap_47
Hap_45
Hap_35
Hap_54
Hap_55
Rickettsia (-)
Rickettsia (+)


Elevation
(meters)
High : 3039
Low : -10
N
W
E
S
Km
0
100
200
1
2
3


400
350
300
250
200
150
100
50
0
Mean Altitude
273.72
369.61
Rickettsia infected
Non-infected


F
B
A
G
E
D
C
Genetic Cluster 1
Genetic Cluster 2
1.2
1.0
0.8
0.6
0.4
0.2
0.0
Genetic Distance