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Distribution pattern and diversity of *Borrelia* spp. detected from ticks in Niigata prefecture, Japan

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Abstract

Objective Borreliosis is a tickborne disease caused by several species of *Borrelia* spirochete. In Japan, autochthonous cases are increasing in Hokkaido, and in the central Honshu, where Niigata is located. This study aimed to reckon the presence of *Borrelia* spp. in ticks and its epidemiological significance in Niigata prefecture.

Results From 41 sites of Niigata, 1,939 DNA samples from ticks were tested for the presence of *Borrelia* spp. by PCR. The spirochete was detected in 55 samples, resulting in a prevalence of 2.83% (55/1,939) overall. The DNA sequencing analysis revealed 3 species of *Borrelia* in Niigata prefecture, *B. japonica* 76.4% (42/55), *B. miyamotoi* 3.6% (2/55) and an unidentified *Borrelia* sp. 20% (11/55). *Borrelia japonica* was detected from adults of *Ixodes ovatus*, predominantly in females. Higher prevalence of *B. japonica* was found in Joetsu area, border with Nagano and Toyama prefectures. *B. miyamotoi* was detected in Chuetsu region, the central area of Niigata in adult females of *I. ovatus*. One type of *Borrelia*, identified to genus level, was detected in larvae, nymph and adult stages of *Haemaphysalis* spp. ticks mainly in Kaetsu, the northern region of the prefecture.

Keywords Borreliosis, Host–pathogen interactions, *Borrelia miyamotoi*, *Ixodes ovatus*, *Haemaphysalis*, Climate change

Introduction

Common tick-borne diseases in Japan include Japanese spotted fever, Lyme borreliosis and Severe fever with thrombocytopenia syndrome (SFTS). According to the Japanese reportable diseases list, these tick-borne infections are classified in Category 4, with mandatory report by the responsible physician [1].

Lyme borreliosis (LD), described as Lyme arthritis in the early 1970s in USA is an infectious disease caused by the spirochete *Borrelia burgdorferi*, transmitted by hard-ticks *Ixodes ricinus* complex in North America. Early infection signals consists of localized erythema migrans, with systemic symptoms occurring after the onset. The evolution of the symptoms follows within days or weeks, affecting the nervous system, heart, or joints. Within weeks or months, late or persistent infection characterizes the third stage with severe chronic fatigue [2].

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In Europe, *I. ricinus* is the most important vector of Lyme borreliosis. In questing *I. ricinus*, *B. burgdorferi* sensu lato (s.l.) average prevalence was reported as 15.6% across Europe [3]. In Europe, and incidence of Lyme borreliosis caused by *B. burgdorferi* s.l. complex estimates ranging from 85,000 to more than 200,000 cases per year [3]. In Asia, surveys in ticks showed prevalences ranging from 2.8 to 46.2% in 9 Countries (China, Japan, Malaysia, Mongolia, Pakistan, Russia (Siberia), South Korea, Thailand and Turkey), found mainly in *Ixodes* spp. and *Haemaphysalis* spp. and other Ixodidae in minor proportions as *Dermacentor* spp. (Mongolia), *Rhipicephalus* spp., *Amblyomma* spp. and *Hyalomma* spp. (Turkey and Pakistan) [4].

In Japan, the etiological agents of Lyme borreliosis are *B. bavariensis*, *B. garinii*, and *B. burgdorferi* s.l. which are mainly transmitted by the hard tick *Ixodes persulcatus* [5, 6]. *Ixodes persulcatus* is present in different areas of Japan; it is endemic in all Hokkaido prefecture area, mountainous areas of Honshu (the main Island of Japan) in altitudes higher than 900 m in north,1200 m in central and 1500 m in west, and part of Shikoku and Kyushu (in areas with altitudes higher than 1700 m) [7].

Compared to European and North American countries, Lyme borreliosis case reports in Japan are low. However, reports of Lyme borreliosis cases in Japan are increasing gradually from 48 cases (2001–2005) to 84 cases (2016–2020) [6]. Most Lyme borreliosis cases are from Hokkaido Prefecture and are autochthonous. In reports from other areas of Japan, the tick bite occurred in Hokkaido or foreign countries (mostly in the USA), with the cases reported in the patient's resident area [6].

The etiologic agents of tick-borne diseases are associated with specific tick species, determining pathogens' positivity in ticks in a specified region can help predict occurrence of potential tick-borne diseases [8–10]. This study aimed to reveal the epidemiology of LD *Borrelia*e from ticks collected in Niigata Prefecture for a baseline understanding.

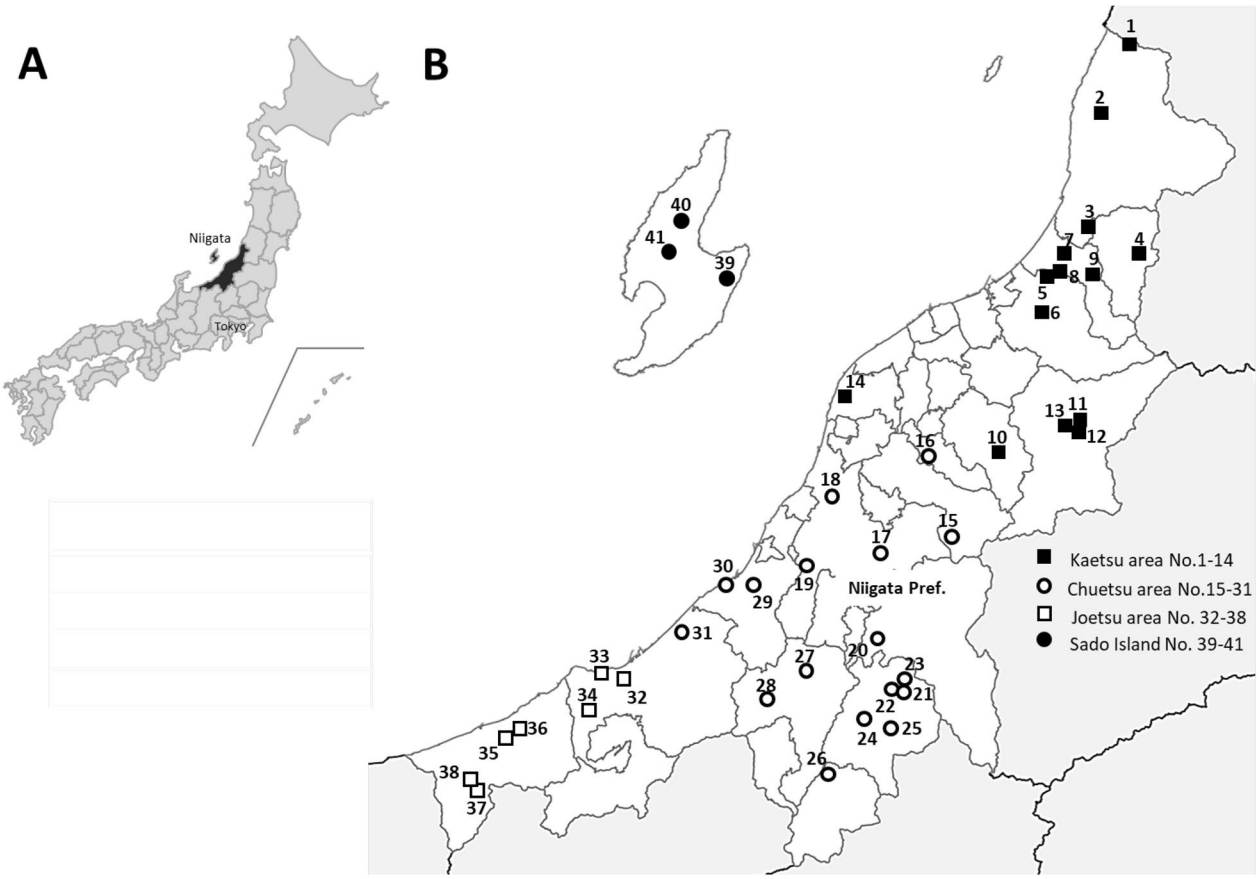

Fig. 1 Localization of Niigata prefecture in Japan (**A**). Map of Niigata prefecture with the 41 sites surveyed, in Niigata prefecture from April 2016 to October 2018 (**B**). Black squares (correspond to the sites 1 to 14 in Kaetsu region, open circles correspond to sites 15 to 31 in Chuetsu region, open squares 32 to 38 in Joetsu region, and black circles correspond to the sites 39 to 41 in Sado island [8]

Table 1 Detected *Borrelia* spp. in ticks collected in Kaetsu, Chuetsu, Joetsu and Sado geographical regions of Niigata prefecture, Japan (2016–2018)

Area	Collection Site (N°)	Tick collection Date (Y/M/D)	Host tick sp. / Stage (Sex)	Detected *Borrelia* sp.	*fla*B Sequences Access N°s
Kaetsu	9	2016.06.10	*I. ovatus* / Adult (F)	*B. japonica*	LC684850
	10	2016.06.08	*I. ovatus* / Adult (M)	*B. japonica*	LC684849
	11	2018.06.08	*H. longicornis*/ nymph	*Borrelia sp.*	LC684892
	12	2016.05.10	*I. ovatus* / Adult (F)	*B. japonica*	LC684848
	14	2016.11.21	*H. flava* / Adult (M)	*Borrelia sp.*	LC684856
	14	2017.10.05	*H. flava*/Adult (M)	*Borrelia sp.*	LC684881
	14	2017.10.05	*H. flava*/ nymph	*Borrelia sp.*	LC684882
	14	2017.10.27	*H. longicornis* / nymph	*Borrelia sp.*	LC684883
	14	2017.11.22	*H. longicornis* / nymph	*Borrelia sp.*	LC684884
	14	2018.04.13	*H. flava*/nymph	*Borrelia sp.*	LC684885
	14	2018.05.29	*H. flava*/nymph	*Borrelia sp.*	LC684886
Chuetsu	15	2016.06.28	*I. ovatus* / Adult (F)	*B. japonica*	LC684853
	15	2016.06.28	*I. ovatus* / Adult (F)	*B. japonica*	LC684854
	15	2017.05.02	*I. ovatus*/Adult (F)	*B. japonica*	LC684859
	19	2017.05.19	*I. ovatus*/Adult (M)	*B. japonica*	LC684861
	19	2017.05.19	*I. ovatus*/Adult (F)	*B. japonica*	LC684862
	19	2017.05.19	*I. ovatus*/Adult (F)	*B. miyamotoi*	LC684863
	19	2017.05.19	*I. ovatus*/Adult (F)	*B. japonica*	LC684864
	19	2017.05.19	*I. ovatus*/Adult (F)	*B. japonica*	LC684865
	21	2016.07.20	*I. ovatus* / Adult (F)	*B. japonica*	LC684855
	21	2017.07.11	*I. ovatus*/Adult (F)	*B. japonica*	LC684880
	22	2017.05.16	*I. ovatus*/Adult (F)	*B. japonica*	LC684860
	26	2016.06.15	*I. ovatus* / Adult (M)	*B. japonica*	LC684851
	26	2016.06.15	*I. ovatus* / Adult(F)	*B. japonica*	LC684852
	27	2017.06.16	*I. ovatus*/Adult (M)	*B. japonica*	LC684870
	27	2017.06.16	*I. ovatus*/Adult (F)	*B. japonica*	LC684871
	27	2017.06.16	*I. ovatus*/Adult (F)	*B. japonica*	LC684872
	27	2017.06.16	*I. ovatus*/Adult (F)	*B. miyamotoi*	LC684873
	27	2017.06.16	*I. ovatus*/Adult (F)	*B. japonica*	LC684874
	29	2017.04.28	*I. ovatus*/Adult (F)	*B. japonica*	LC684857
	29	2018.06.05	*H. flava*/ Adult (M)	*Borrelia sp.*	LC684887
	29	2018.06.05	*I. ovatus*/ Adult (M)	*B. japonica*	LC684888
	31	2017.04.28	*I. ovatus*/Adult (F)	*B. japonica*	LC684858

Table 1 (continued)

Area	Collection Site (N°)	Tick collection Date (Y/M/D)	Host tick sp. / Stage (Sex)	Detected *Borrelia* sp.	*fla*B Sequences Access N°s
Joetsu	32	2018.06.05	*I. ovatus*/ Adult (M)	*B. japonica*	LC684889
	32	2018.06.05	*I. ovatus*/ Adult (M)	*B. japonica*	LC684890
	32	2018.06.05	*I. ovatus* /Adult (F)	*B. japonica*	LC684891
	34	2017.06.06	*I. ovatus*/Adult (F)	*B. japonica*	LC684866
	34	2017.06.06	*I. ovatus*/Adult (F)	*B. japonica*	LC684867
	34	2017.06.06	*I. ovatus*/Adult (F)	*B. japonica*	LC684868
	34	2017.06.06	*I. ovatus*/Adult (F)	*B. japonica*	LC684869
	34	2018.07.24	*I. ovatus*/ Adult (M)	*B. japonica*	LC684895
	34	2018.07.24	*I. ovatus*/ Adult (F)	*B. japonica*	LC684896
	34	2018.07.24	*H. flava*/ larvae	*Borrelia sp.*	LC684897
	34	2018.07.24	*I. ovatus* /Adult (M)	*B. japonica*	LC684898
	34	2018.07.24	*I. ovatus* /Adult (M)	*B. japonica*	LC697039
	34	2018.07.24	*I. ovatus* / Adult (M)	*B. japonica*	LC697040
	34	2018.07.24	*I. ovatus* /Adult (F)	*B. japonica*	LC684899
	34	2018.07.24	*I. ovatus* Adult (F)	*B. japonica*	LC684900
	36	2017.06.20	*I. ovatus*/Adult (M)	*B. japonica*	LC684875
	36	2017.06.20	*I. ovatus*/Adult (F)	*B. japonica*	LC684876
	36	2017.06.20	*I. ovatus*/Adult (F)	*B. japonica*	LC684877
	36	2017.06.20	*I. ovatus*/Adult (F)	*B. japonica*	LC684878
	36	2017.06.20	*H. longicornis*/nymph	*Borrelia sp.*	LC684879
Sado	39	2018.07.13	*I. ovatus*/ Adult (F)	*B. japonica*	LC684893
	39	2018.07.13	*I. ovatus*/ Adult (F)	*B. japonica*	LC684894

Main text

Materials and methods

Study area

The study was conducted in Niigata prefecture, located on the coast of the Sea of Japan, has an area of 12,583.96km^2 from N37° 021.26823 to N38° 32.88925'latitude, slightly north of the center of Honshu. Niigata prefecture consists of four regions: Kaetsu, Chuetsu, Joetsu, and Sado Island.

Field surveys were conducted in 41 sites, from north to south, in Niigata prefecture from April 2016 to October 2018 as previously described [9]. Briefly, questing ticks were collected by flagging method in a total of 105 field surveys, with site No. 14 as a fixed point for monthly tick collection (Fig. 1). Live ticks were kept in cooler boxes and returned to the laboratory for species identification and further processing.

Identification of ticks

Collected ticks were identified morphologically under stereoscope based on the keys by Yamaguti et al. [11] and separated by species, sex and stage, collection day, and collection sites. After morphological identification, the ticks were separated in micro tubes and stored at − 80 °C until further processing. The identification of ticks with insufficient morphologic characteristics was confirmed by DNA sequencing of the mitochondrial 16S rDNA gene, as previously described [12] (data not shown).

Table 2 *B. japonica* detection in *I. ovatus* DNA samples by tick collection sites in Niigata prefecture, Japan (2016–2018)

Collection Site Nº	*I. ovatus*						Infection rate (%)
	Male		Female		Nimph	Larvae	
	infected	collected	infected	collected			
Kaetsu							
1		1		3			
2		1		2			
3		1		6			
4		0		0			
5		4		5			
6		3		7			
7		10		7			
8		3		1			
9		1	1	3			
10	1	3		1			
11		46		53			
12		15	1	19			
13		0		0			
14		33		46			
	1/121(0.8)		2/153(1.3)				3/274 (1.09)
Chuetsu							
15		13	3	7			
16		3		4			
17		13		10			
18		8		6			
19	1	15	3	20			
20							
21		10	2	5			
22		2	1	3			
23		5		7			
24							
25				2			
26	1	2	1	9			
27	1	16	3	19			
28		1		1			
29	1	18	1	22	5		
30		3		2	2		
31		4	1	6			
	4/113 (3.5)		15/123 (12.2)		7		19/236 (8.05)
Joetsu							
32	2	16	1	28			
33							
34	4	25	7	26			
35		4		2			
36	1	5	3	6			
37							
38							
	7/50 (14.0)		11/62 (17.7)		0	0	18/112 (16.07)
Sado							
39		7	2	29			
40				2			
41		4		4			
	0/11 (0)		2/35 (5.7)		0	0	2/46 (4.35)
Total	12/295 (4.1)		30/373 (8.0)		7*	675	42/668 (6.3)

* Samples from larvae and nymph stage were not included in calculations

DNA extraction, PCR and sequencing analyses

The number of DNA samples used in this study totalized 1,939; comprised of 386 for 2016 year, 926 for 2017 year and 627 for 2018 year. The DNA extraction, PCR and sequencing analyses were performed as described by Arai et al. [10]. To detect *Borrelia* spp., a nested-PCR for the genus-common flagellin gene (*fla*B) was targeted [13]. The PCR primers used were; BflaPAD: GAT CA(G/A) GC(T/A) CAA (C/T)AT AAC CA(A/T) ATG CA and BflaPDU: AGA TTC AAG TCT GTT TTG GAA AGC for 1st PCR, BflaPBU: GCT GAA GAG CTT GGA ATG CAA CC and BflaPCR: TGA TCA GTT ATC ATT CTA ATA GCA for 2nd PCR. PCR cycle consists of 95℃ for 10 s, 50 ℃ for 30 s, 72℃ for 30 s (30 cycles) and 72 ℃ for 2 min for post elongation.

PCR amplicons were purified using AMPure XP (Beckman Coulter Co., Japan) and directly sequenced using a Big Dye Terminator Cycle Sequence Kit (Applied Biosystems, USA) and Applied Biosystems 3500 Genetic Analyzer. The analyses of the obtained sequences were carried out using Molecular Evolutionary Genetics Analysis (MEGA software version 11). The obtained sequences from this study and from DDBJ/EMBL/GenBank databases were aligned by Clustal W 2.0. The neighbor-joining phylogenetic tree construction and bootstrap analysis (1000 replicates) were performed according to the Kimura 2-parameter distances method [10].

Results

Abundance and diversity of *Borrelia* spp

A total of 4,806 ticks of 12 species namely *Dermacentor taiwanensis* (36 Adults, 1 Nymph), *Haemaphysalis flava* (405 Adults, 1479 Nymphs, 547 Larvae), *Haemaphysalis hystricis* (2 Adults, 1 Nymph), *Haemaphysalis japonica* (4 Adults, 3 Nymphs), *Haemaphysalis longicornis* (53 Adults, 892 Nymphs, 461 Larvae), *Haemaphysalis megaspinosa* (3 Adults, 77 Larvae), *Ixodes ovatus* (668 Adults, 7 Nymphs), *Ixodes nipponensis* (37 Adults, 13 Nymphs, 2 Larvae), *Ixodes persulcatus* (8 Adults, 1Nymph), *Ixodes monospinosus* (55 Adults, 5 Nymphs, 5 Larvae), *Ixodes columnae* (2 Larvae), and *Ixodes turdus* (11 Nymph, 3 Larvae), *Ixodes* spp. (N = 1), *Haemaphysalis* spp. (N = 24) were collected from all 41 sites of collection, covering all the 4 regions of Niigata prefecture [9]. From all the species of ticks, a total of 1,939 DNA samples were extracted, purified and tested for the presence of *Borrelia* spp. by PCR. The spirochete was detected in 55 samples, resulting in a prevalence of 2.83% (55/1,939) overall. The DNA sequencing revealed 3 species of *Borrelia* in Niigata prefecture, *B. japonica* 76.4% (42/55), *B. miyamotoi* 3.6% (2/55) and an unidentified *Borrelia* sp. 20% (11/55) (Table 1).

Distribution and niches of *Borrelia* spp. in Niigata prefecture

The main etiological agent of Lyme disease found in this study was *B. japonica*. The spirochete was present in *I. ovatus* of all the regions studied with an overall infection rate of 6.3% (42/668) of the adults of *I. ovatus*. With a remarkably high rate, double of detected *B. japonica* was in female ticks 30/373 than in males 12/295 corresponding to 8.0% and 4.1% respectively (Table 2).

The distribution per region showed *B. japonica* most present in Joetsu area, with a detection rate of *B. japonica* in *I. ovatus* of 16.07% (18/112), followed by Chuetsu 8.05% (19/236), Sado 4.34% (2/46) and Kaetsu with 1.09% (3/274) (Table 2).

Interestingly, the *Borrelia* sp. identified only to genus level, were detected in larvae, nymph and adult stages of *Haemaphysalis* spp. ticks, distributed mainly in Kaetsu, with 72.72% (8/11) of the detected samples, followed by Chuetsu with 18.18% (2/11) and 9.09% (1/11) in Joetsu region, despite the ticks were fairly distributed in the 4 regions of the prefecture (Table 1).

B. miyamotoi was detected in 2 samples from 2017 of Chuetsu region. Both *B. miyamotoi* infected ticks were detected in adult females of *I. ovatus*.

The distribution of *B. japonica*, *B. miyamotoi* and *Borrelia* sp., the 3 *Borrelia* species found in this study, presented a clear host preference to determined tick species; *Ixodes ovatus*, *Haemaphysalis flava* and *H. longicornis* (Table 1).

Phylogenetic analysis

A total of 55 sequences of 276 to 300 bp of *flaB* were used for characterization of the *Borrelia* spp. from Niigata prefecture from 2016 to 2018, as presented in Fig. 2. The phylogenetic tree showed the sequences obtained in this study were found in 3 clusters: *B. japonica*, *B. miyamotoi* and *Borrelia* sp. (Fig. 2).

The *B. japonica* obtained in this study was almost genetically identical, with a predominant genotype present in 97.6% (41/42) of the identified samples. One sequence (LC684849) from Kaetsu presented a pairwise divergence of 0.00675 compared to the other sequences with 2 divergent bases. The 2 *B. miyamotoi* from this

(See figure on next page.)

Fig. 2 Phylogenetic tree constructed by *flaB* sequences of *Borrelia* spp. from ticks collected in Niigata Prefecture during 2016 to 2018 (bold). The ticks species of the obtained *Borrelia* spp. are indicated by color as blue for *Ixodes ovatus*, green for *Haemaphysalis longicornis* and brown for *Haemaphysalis flava*. The tree was constructed based on the Neighbor-joining method with Kimura-2 parameter under pair-wise deletion option. Bootstrap test (1000 replicates) was calculated. The phylogenetic branches which were supported in > 80% by the bootstrap analysis were indicated

study presented 100% of identity and clustered with other *B. miyamotoi* from the database. Within the 11 samples identified as *Borrelia* sp. 4 genotypes were observed with a median overall difference of pairwise distance of 0.005, the most divergent genotype was the sequence LC684879 from Joetsu, presenting 4 divergent bases and with 0.011 to 0.018 pairwise differences compared to the other sequences (Fig. 2).

Discussion

The carrier rate of *Borrelia* spp. from ticks found in this study was of 2.83% overall, it is lower than the average range in Japan of 6.7–22% [1]. This could explain the difference in Lyme case reports in Niigata of 2 cases compared to Hokkaido 15 cases from 2006 to 2010 [6]. After Hokkaido the central area of Honshu, where Niigata is located has the higher number of borreliosis cases reported [6]. Environmental changes such as climate, land cover, and vegetation have direct influence on hosts of the ticks harboring the spirochete which change their areas to find suitable habitats accordingly and diffusing the etiological agent to non-endemic areas.

From 1901 to 2022, the frequency of extremely high monthly temperatures has increased while extremely low monthly temperatures have decreased, with an accentuated increase on the frequency of extremely high monthly temperatures since 1990. Niigata showed a significant increase of 2.1 °C/Century [14].

We have reported the changes in diversity and distribution of ticks in Niigata from the last wide tick survey report occurred in 1959 [8]. From 1956 to 1957, the collected number of *I. persulcatus* was 1987 (17.4%) of a total of 11,404 collected ticks in Niigata prefecture [15]. However, even while some collection sites were the same, *I. persulcatus* collection number decreased from 17.4% to 0.2% (9 of 4806) [9]. The diversity of tick species is directly related to the tick-borne etiologic agents in a determined area. The *Borrelia* spp. identified in this study, presented a peculiar species-specific infecting behavior; *Borrelia japonica* and *B. miyamotoi* were found only in *I. ovatus* in this study and not in *I. persulcatus* as observed in other works [5, 6], showing a type of adaptation to a different host species. This is an important finding due to the enzootic nature of borreliosis transmission, indicating their animal hosts might carry the Lyme/relapsing fever (RF) disease agents in Niigata and possibly spread the disease through a different tick species. Hares (Leporidae) were described as the preferential hosts of *I. ovatus* [11] besides small mammals during immature stages [12]. Considering Lagomorpha as susceptible hosts of spirochetes, these hares could act as natural reservoirs of *B. japonica* and *B. miyamotoi* in the region. However, more studies are necessary to confirm the importance of hares in the eco-epidemiology of these pathogens.

Despite Lyme disease in Japan is caused mainly by *Borrelia garinii*, there was a reported case of borreliosis caused by *B. japonica* [16]. The transmission of the spirochete by ixodid ticks requires more than 48 h of feeding in their hosts [16] which makes the female ticks as the main vector for the disease. The risk of occurrence of Lyme disease by *B. japonica* in Niigata is real, once the proportion of female ticks infected by *B. japonica* was 2.5 times higher than in males and agent found in all mainland areas surveyed in this study.

Borrelia miyamotoi was detected in adult female ticks in Chuetsu region of Niigata. Interestingly, *B. miyamotoi* usually isolated mainly from *I. persulcatus* in other studies [17–19], was found only in *I. ovatus* showing a type of host adaptation. This *Borrelia* species confirmed to be pathogenic in 2011, causes *B. miyamotoi* disease (BMD), a relapsing fever like illness characterized by fever, headache, chills, fatigue, and myalgia [20]. It is considered an emerging infectious pathogen [21]. Thus, there is a need to consider the occult occurrence of BMD in Niigata in undiagnosed and subclinical cases.

The *flaB* sequences analysis identified *B. japonica* and *B. miyamotoi* in Niigata prefecture. Moreover, an unidentified *Borrelia* sp. was revealed with a relatively high occurrence of 20% among all species of *Borrelia* detected. Interestingly, it was detected only in *Haemaphysalis* spp. ticks mainly in Kaetsu region with no stage specificity. *H. longicornis* and *H. flava*, found in this study harboring *Borrelia* sp. have a diverse host preference, from small mammals to birds, and it is difficult to find a probable *Borrelia* species found in this study with the genetic material analyzed. Despite presenting a high genetic similarity of a relapsing fever borreliae detected in the *Haemaphysalis* spp. ticks from Yamaguchi and

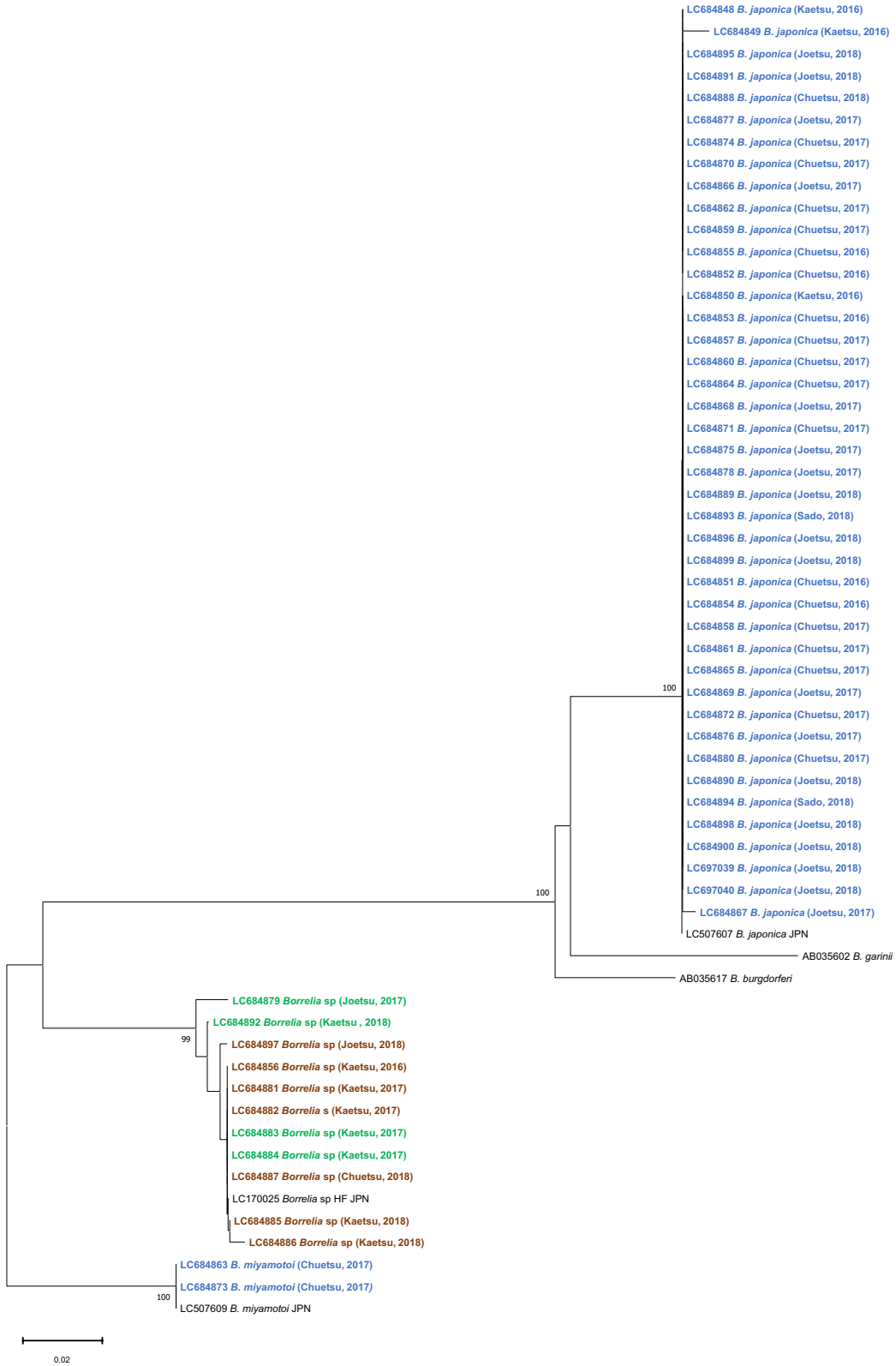

Fig. 2 (See legend on previous page.)

Wakayama prefectures [22], further studies, including bacterial culture and characterization are necessary to identify this *Borrelia* at species level and reckon if it is an endosymbiont and/or the possibility of human infection.

Limitations

Due to climatic reasons, no tick collections were conducted in the winter season, or in conditions when there was considerable quantity of snow in the collection site.

The identification was done only by DNA sequencing, no culture was performed. Therefore, the *Borrelia* sp. detected in *Haemaphysalis* spp. ticks could not be assigned to a known spirochete species based on the existing DNA database.

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Author contributions
Conceptualization: MOS, MS, TT Methodology: SI, MS, RA, JA, CH, KW, MAFR, MOS, TT Formal analysis: KW, MAFR, MOS Investigation: SI, MS, RA, JA, CH, KW, MAFR, MOS, TT Resources: MS, KW, TT Data Curation: MOS, MS Writing—Original: SI, MOS, MS Draft Writing—Review & Editing: all authors Visualization: TT Supervision: MOS, MS, TT, KW Project administration: MS, TT Funding acquisition: M.S.

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Availability of data and materials
The data that support the findings of this study are available from the corresponding author upon request. All DNA sequence data used in this study is deposited and available in National Library of Medicine (NLM), of the National Center for Biotechnology Information (NCBI) at the National Institutes of Health, with the following accession numbers: *Borrelia* sp.: LC684856, LC684887, LC684897, LC684882, LC684881, LC684885, LC684886, LC684883, LC684884, LC684892, LC684879, LC170025; *B. japonica*: LC684853, LC684854, LC684855, LC684850, LC684848, LC684851, LC697040, LC684849, LC684852, LC684891, LC684899, LC684898, LC697039, LC684900, LC684896, LC684893, LC684894, LC684888, LC684889, LC684890, LC684895, LC684859, LC684862, LC684864, LC684865, LC684880, LC684860, LC684871, LC684872, LC684874, LC684857, LC684858, LC684866, LC684867, LC684868, LC684869, LC684876, LC684877, LC684878, LC684861, LC684870, LC684875, LC507607; *B. miyamotoi*: LC684863, LC684873, LC507609; *B. garinii*: AB035602; *B. burgdorferi*: AB035617.

Declarations

Ethics approval and consent to participate
No ethical permissions were necessary for this study. The ticks were collected from the environment of public places and no capture or handling of hosts were performed.

Consent for publication
Not Applicable.

Competing interests
The authors declare no competing interests.

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