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Diversity within *Borrelia burgdorferi* sensu lato genospecies in Switzerland by *recA* gene sequence

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Abstract

A total of 874 *Ixodes ricinus* ticks were collected in Switzerland to investigate the genetic diversity of the *Borrelia* population. We integrated to the RT-PCR method the DNA sequence analysis of a 162-bp fragment of the *recA* gene. Five genospecies were detected: *Borrelia afzelii*, *Borrelia burgdorferi* s.s., *Borrelia garinii*, *Borrelia valaisiana*, and *Borrelia lusitaniae*. A heterogeneous distribution was observed within the *B. burgdorferi* s.l. genospecies. The most prevalent and diverse genospecies found in Switzerland was *Borrelia afzelii*, which might suggest a rapid evolution of this genospecies.

Keywords: *recA*; *Ixodes ricinus*; *Borrelia burgdorferi* sensu lato; Switzerland

1. Introduction

Borrelia burgdorferi sensu lato (s.l.) is a bacterial species complex consisting of at least 11 genospecies: *B. burgdorferi* sensu stricto (s.s.), *Borrelia afzelli*, *Borrelia garinii*, *Borrelia valaisiana*, *Borrelia lusitaniae*, *Borrelia bissettii*, *Borrelia japonica*, *Borrelia turdi*, *Borrelia tanukii*, *Borrelia andersoni*, and *Borrelia sinica* [1]. *Ixodes* ticks are the most important vectors of *B. burgdorferi* s.l. in the world. In Europe, six different *B. burgdorferi* genospecies have been recorded, four of which (*B. afzelli*, *B. garinii*, *B. burgdorferi* s.s., and *B. valaisiana*) are distributed throughout the continent [2]. *B. burgdorferi* s.s., *B. lusitaniae* and *B. bissettii* are relatively rare in Europe [3]. *B. lusitaniae* has also been found recently in Switzerland [4,5]. To better understand the ecology and the distribution of tick-borne spirochetes, knowledge of their genetic diversity is required. The genetic diversity of *B. burgdorferi* s.l. may be analysed at two different taxonomic levels: (i) between the recognised genospecies and (ii) within each of them. At the genospecies level, diversity is clinically important. Lyme disease is principally caused by 3 of the 11 described genospecies, i.e., *B. burgdorferi* s.s., *B. garinii*, and *B. afzelii*. Occasionally, *B. valaisiana*, *B. lusitaniae*, and *B. bissettii* have been detected in pathological situations, mostly erythema migrans lesions [6–8]. The association of some of the various clinical manifestations of the disease to the three recognised pathogenic genospecies has been suggested in the past [9]. Recently, Baranton et al. [10] described the association between genetic groups within single genospecies and different aspects of pathogenicity. Only two genetic groups of *B. afzelii* and four of *B. garinii* seem to cause invasive diseases.

Borrelia burgdorferi is maintained in nature by complex zoonotic transmission cycles, in which *Ixodes* ticks

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are the vectors and vertebrates, involving more than 50 avian and mammalian wildlife species, are the reservoir hosts [11]. It has been hypothesised that the genetic diversification of the genospecies is driven mainly by the host [12], in particular by the sensitivity or resistance to the complement system of a particular host. Thus, complement might be a key factor of the *B. burgdorferi* s.l. evolution.

In order to enhance our vision on the genetic diversity of *B. burgdorferi* s.l., there is the need to develop a rapid and accurate method able to identify and differentiate the genospecies not only at the species level but also within them.

In this study, we integrated an RT-PCR method based on the *recA* gene and designed to identify *B. burgdorferi* s.l. organisms [13], to the DNA sequence analysis of the short 162 bp amplified fragment. We applied this approach to a large-scale survey of ticks collected on vegetation and from animals to investigate the genetic diversity of the *Borrelia* population in Switzerland.

2. Materials and methods

2.1. Bacterial strains used as reference

A total of 33 *B. burgdorferi* s.l. strains were used as reference strains (Table 1).

2.2. DNA extraction and PCR of Borrelia reference strains

DNA was extracted from 200 μl of BSK-H liquid culture previously conserved at −80 °C with Instagene DNA extraction matrix (Biorad, Reinach, Switzerland), according to the manufacturer's instructions. A standard PCR was performed with 5 μl of the extracted DNA, 0.5 μM of each primer, 1 U Taq Polymerase (Qiagen AG, Basel, Switzerland) in a total volume of 50 μl (buffer provided by the manufacturer). Primers were those reported by Morrison et al. [14], nTM17.F (5′-GTG GAT CTA TTG TAT TAG ATG AGG CTC TCG-3′) and nTM17.R (5′-GCC AAA GTT CTG CAA CAT TAA CAC CTA AAG-3′) amplifying a fragment of 162-bp of the *recA* gene. The reaction mixtures were subjected to an initial denaturation step of 10 min at 94 °C, followed by 35 cycles of denaturation at 94 °C for 1 min, annealing at 50 °C for 1 min and elongation at 72 °C for 2 min. The elongation was completed by a further 5 min step at 72 °C. The PCRs were performed in a DNA Thermal Cycler (Perkin–Elmer Applied Biosystems, Rotkreuz, Switzerland).

2.3. Tick sampling

In spring and in autumn 2002, *I. ricinus* ticks were collected in three Swiss Cantons (Neuchâtel, Valais and Ticino). Sampling of questing *I. ricinus* was made by pulling a 1-m^2 white terry flag over the vegetation. In Canton Neuchâtel, an extended area of 1049 m^2 surface was flagged. In Canton Valais a set of three areas were chosen as sampling locations: Mt. d'Orge (Sion), Gueroz (Val Trient) and Finges. In Ticino (South Switzerland), ticks were collected from the animal hosts (dogs, cows, cats, goats, asses and humans). Collected ticks were immediately stored in 100% ethanol and conserved at 4 °C until taxonomical identification (performed on the basis of their morphological characteristics) and DNA extraction.

2.4. DNA extraction and PCR of the collected ticks

DNA was extracted from minced ticks by using Dneasy Tissue kit (Qiagen AG, Basel, Switzerland) according to the manufacturer's instructions. The PCR to detect *B. burgdorferi* s.l. in *Ixodes* ticks was performed using a fluorescence temperature cycler (LightCycler, Roche, Switzerland). We used the primers nTM17.F and nTM17.R described by Morrison et al. [14], and the PCR conditions of Pietila et al. [13]. The melting curve technique was used to determine the specific *Borrelia* PCR products. Five *B. burgdorferi* s.l. genospecies (*B. afzelii*, *B. garinii*, *B. burgdorferi* s.s., *B. valaisiana*, and *B. lusitaniae*) were used as positive controls and were included in each run. All unspecific products melted at temperature below 80 °C.

2.5. DNA sequencing

After purification (Amicon Microcon, Millipore, Milan, Italy), the PCR positive samples were sequenced by using an ABI Prism Big Dye Terminator Cycle Sequencing Kit (Perkin–Elmer Applied Biosystems, Rotkreuz, Switzerland) on an ABI Prism 310 Genetic Analyser (Perkin–Elmer Applied Biosystems), according to the manufacturer's instructions. DNA sequencing for *Borrelia* (*recA* gene, 162 bp) was performed in both directions.

2.6. DNA sequence analysis

The *recA* sequences were handled and stored with the Lasergene program Editseq (DNAstar Inc., Madison, WI) and aligned with Megalign (DNAstar Inc.). Phylogenetic analyses of the *recA* sequences were performed by using two different methods: (i) the neighbor-joining (NJ) method with Kimura 2 parameters distances (performed using MEGA Molecular Evolutionary Genetics Analysis version 2.1 [15]) and (ii) the maximum parsimony (MP) method, using heuristic search with stepwise addition (using PAUP 4.0, [16]). The reliability of internal branches was assessed by bootstrapping with 1000 (NJ) and 100 (MP) pseudoreplicates.

Table 1
Borrelia strains used as reference in this study

Strain	Genospecies	Geographic origin	Accession No.
B31	*B. burgdorferi* sensu stricto	United States	AY586362
NY1387	*B. burgdorferi* sensu stricto	United States	AY586363
A44S	*B. burgdorferi* sensu stricto	Holland	AY586364
P1G	*B. burgdorferi* sensu stricto	Switzerland	AY586365
IP1	*B. burgdorferi* sensu stricto	France	AY586366
IP2	*B. burgdorferi* sensu stricto	France	AY586367
IP3	*B. burgdorferi* sensu stricto	France	AY586368
VS219	*B. burgdorferi* sensu stricto	Switzerland	AY586369
SIKA2	*B. garinii*	Japan	AY586370
SIKA1	*B. garinii*	Japan	AY586371
VSPB	*B. garinii*	Switzerland	AY586372
P/Bi	*B. garinii*	Germany	AY586373
VS102	*B. garinii*	Switzerland	AY586374
NT29	*B. garinii*	Japan	AY586375
Ip89	*B. garinii*	Russia	AY586376
A19S	*B. garinii*	Holland	AY586377
Poti B1	*B. lusitaniae*	Portugal	AY586378
Poti B2	*B. lusitaniae*	Portugal	AY586379
Poti B3	*B. lusitaniae*	Portugal	AY586380
VS116	*B. valaisiana*	Switzerland	AY586381
UK	*B. valaisiana*	England	AY586382
CA2	*B. burgdorferi* sensu lato	United States	AY586383
VS461	*B. afzelii*	Switzerland	AY586384
DK8	*B. afzelii*	Denmark	AY586383
A26S	*B. afzelii*	Holland	AY586384
ECM1	*B. afzelii*	Sweden	AY586385
BO23	*B. afzelii*	Germany	AY586386
HO14	*B. japonica*	Japan	AY586387
COW611A	*B. japonica*	Japan	AY586388
DN127	*B. bissettii*	United States	AY586389
CA128	*B. bissettii*	United States	AY586390
CA55	*B. bissettii*	United States	AY586391
19952	*B. andersoni*	United States	AY586392

2.7. Nucleotide sequence accession numbers

The *recA* gene sequences (162 bp) of the *B. burgdorferi* s.l. used as reference strains determined in this study have been deposited in GenBank (Table 1).

3. Results

3.1. Borrelia reference strains

3.1.1. Sequence characteristics

Using the primers nTM17.F and nTM17.R [14], *recA* fragments of 162 bp were successfully amplified and sequenced from 33 reference strains of *Borrelia* (Table 1). No insertions or deletions were observed. Thirty-eight of 162 nucleotide sites (23.5%) were variable and 32 (19.7%) were parsimony informative. Within the *B. garinii* genospecies a variability of 5.0% was observed, and within the *B. burgdorferi* s.s. and *B. afzelii* genospecies the observed variability was 1.9% and 1.3%, respectively. The deduced amino acid sequences of the amplified *recA* DNA fragments comprised 54 amino acid residues. The amino acid sequences among the *Borrelia* species were highly conserved.

3.1.2. Phylogenetic analysis

Fig. 1 shows the NJ tree generated from the alignment of the 162-bp of the *recA* nucleotide sequences of the 33 *Borrelia* strains. Despite the limited size of the sequenced fragment, the strains belonging to the same genospecies clustered together, usually with high bootstrap support (82–100%). However, the *B. burgdorferi* s.s. clade was supported by a bootstrap value lower than 50%. In addition, *B. garinii* and *B. valaisiana* grouped together with a bootstrap support slightly higher than 50%. The topology of the tree obtained by MP analysis is generally congruent with those inferred by NJ method (data not shown). The most important difference is represented by the *B. burgdorferi* s.s. strains, which did not form a clade and the position of which remained unresolved within the MP tree.

3.2. Borrelia DNA in ticks

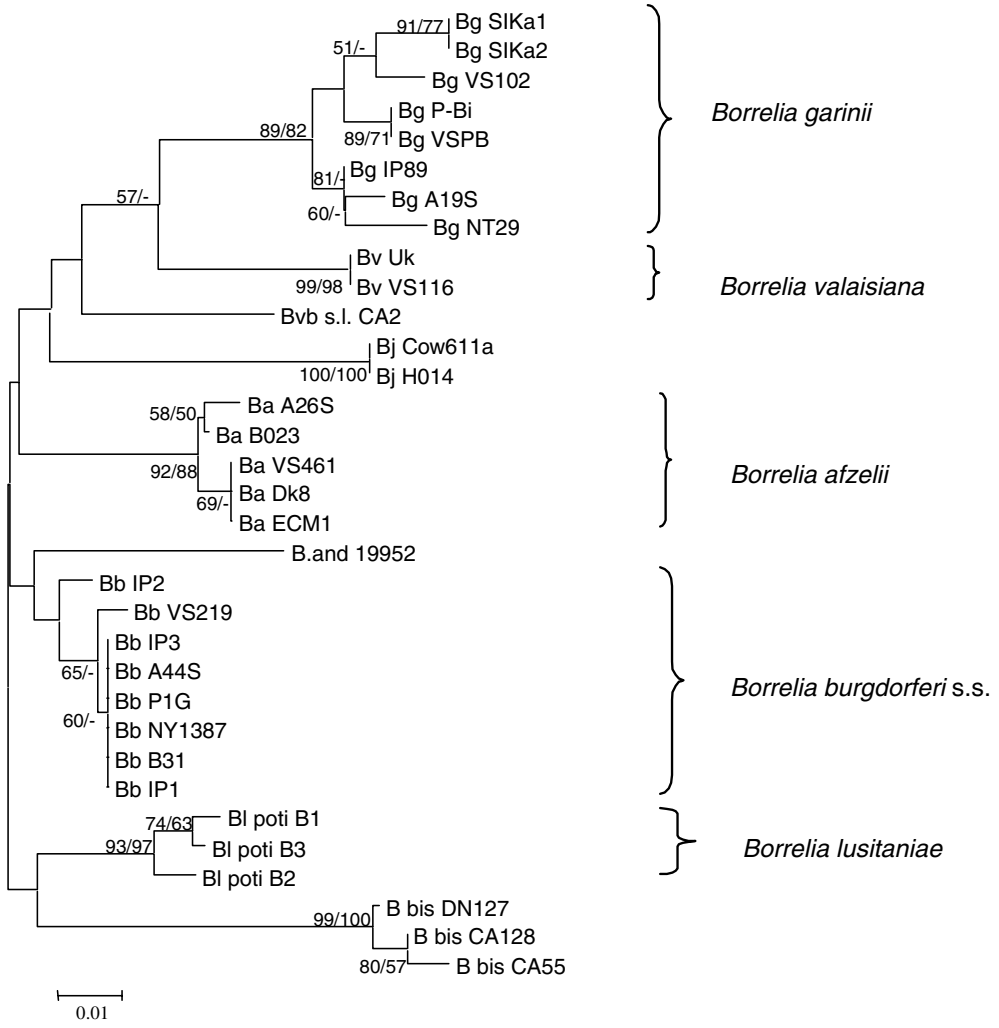

Fig. 1. Neighbour-joining tree of 1000 bootstrap pseudo-replicates with Kimura 2 parameters distances from *recA* sequences (162 bp) for 33 *Borrelia* strains. Numbers above branches indicate bootstrap support values higher than 50% for NJ/MP.

3.2.1. Borrelia *detected by PCR in the collected ticks*

Presence and identity of *Borrelia* in ticks were tested by LightCycler PCR and DNA sequence analysis of the *recA* gene (162 bp). A total of 874 ticks were tested for the presence of *B. burgdorferi* s.l. (Table 2): 580 ticks were collected on vegetation in Cantons Neuchâtel and Valais, the rest (294 specimens) from hosts (148 dogs, 70 cows, 45 cats, 15 cats or dogs, 6 goats, 2 unknown hosts, 1 ass, and 7 humans) in Canton Ticino. 196 ticks (22.4%) showed the presence of *B. burgdorferi* s.l. genome (Table 3): 96 ticks from Neuchâtel (32.6%, 45/155 nymphs and 51/139 adults), 44 from Ticino (15.0%, 44/284 adults) and 56 from Valais (19.6%, 17/82 nymphs and 39/204 adults) (Table 2).

3.2.2. Phylogenetic analysis

To allow the precise identification of the *B. burgdorferi* s.l. genospecies detected in ticks, the sequences of the *recA* amplified fragments were compared with the 33 *Borrelia* strains used as reference. A phylogenetic tree was constructed by the NJ method with Kimura 2 parameters (Fig. 2).

Different *Borrelia* genospecies were recorded in each region studied (Table 2). In the Neuchâtel forest, five genospecies were present. *B. afzelii* (30.2%) and *B. garinii* (30.2%) were dominant, while *B. burgdorferi* s.s. (10.4%), *B. valaisiana* (17.7%), and *B. lusitaniae* strains Poti B2 (11.5%) were less abundant. In the other regions, Ticino and Valais, four *Borrelia* genospecies were found in *I. ricinus* ticks: *B. afzelii* was the dominant species (79.5% and 75%, respectively). In addition, in Canton Ticino, spirochetes identical to *B. lusitaniae* Poti B3 (15.9%) were detected, but *B. burgdorferi* s.s was absent. Conversely, in Canton Valais, *B. burgdorferi* s.s (16.1%) was present, but not *B. lusitaniae*. The other genospecies seem to be rare (2.3% for *B. garinii* and *B. valaisiana* in Ticino; and 7.1% for *B. garinii* and 1.8% for *B. valaisiana* in Valais).

Table 2
Distribution of *Borrelia* genospecies isolated from the *I. ricinus* collected from three areas in Switzerland

Areas	Total number of ticks examined	Infection rate[a]			Number of ticks positive for genospecies (% of positive ticks)											
		Total	Nymph stage	Adult stage	*B. afzelii*			*B. garinii*			*B. burgdorferi* s.s.			*B. valaisiana*			*B. lusitaniae*		
					Total	N	A	Total	N	A	Total	N	A	Total	N	A	Total	N	A
Ticino	294	44/294	0/9	44/284	35	–	35	1	–	1	–	–	–	1	–	1	7	–	7
	(1L[b], 9N, 284A)	(15.0)	(0)	(15.5)	(79.5)		(79.5)	(2.3)		(2.3)				(2.3)		(2.3)	(15.9)		(15.9)
Neuchâtel	294	96/294	45/155	51/139	29	12	17	29	12	17	10	5	5	17	7	10	11	9	2
	(155N, 139A)	(32.6)	(29.0)	(36.7)	(30.2)	(26.6)	(33.3)	(30.2)	(26.6)	(33.3)	(10.4)	(11.1)	(9.8)	(17.7)	(15.5)	(19.6)	(11.5)	(20.0)	(3.9)
Valais	286	56/286	17/82	39/204	42	14	28	4	–	4	9	3	6	1	–	1	–	–	–
	(82N, 204A)	(19.6)	(20.7)	(19.1)	(75.0)	(82.3)	(71.8)	(7.1)		(10.2)	(16.1)	(17.6)	(15.4)	(1.8)		(2.6)			

A, Adults; N, Nymphs and L, Larvae.
[a] Number of ticks infected/number of ticks examined (%).
[b] Tested negative for *Borrelia*.

The overall variability at the nucleotide level of the 162-bp amplified fragment was 23.5%, whereas the variability at the amino acid level was 11.1%.

Genetic variability was detected within the single *Borrelia* genospecies in all the three areas studied. The variability within the *B. afzelli* genospecies was similar for Neuchâtel and Ticino (9.2% and 10.5%, respectively) but lower (5%) in Valais. Within the *B. garinii* genospecies the variability in Neuchâtel was 6.9%. Genetic variabilities for the other genospecies are not estimated because of the low number of samples.

4. Discussion

4.1. Validity of the genetic marker recA

The DNA sequence analysis on the *recA* gene performed in this study allows the characterisation and identification of *B. burgdorferi* s.l. strains. A short genetic marker as the 162-bp fragment of the *recA* gene may be used for rapid and accurate screening of sequence variations. The 162 nucleotides of the *recA* gene are not only sufficient for genospecies determination, but also allow identification of single strains within each genospecies (Fig. 1). The variability of this fragment is relatively high (23.5%) compared to other genetic markers used in the past such as 16S rDNA (10% [17]), *rpoB* gene (8.8% [18]), and *hbb* gene (16.2% [19]). Only the *fla* gene [20] has shown a slightly higher variability (26.3%) than *recA*, but with a longer fragment sequenced (580 bp).

However, the *recA* fragment variability encountered at the nucleotide level was not paralleled by a similar variability at the amino acid level (11.1%). This is probably due to the function of the recA protein which has to be conserved during evolution.

4.2. Validity of the RT-PCR method

The real-time PCR analysis of the *recA* gene is a rapid detection method of *B. burgdorferi* s.l. It shows the same sensitivity as a nested PCR [13], but with less contamination problems. Moreover, when considering the melting curves of the *recA* specific products, detection sensitivity approaches one bacterial genome per sample [14]. Thus, the Light-Cycler PCR is suitable for the detection of *B. burgdorferi* s.l. in clinical material as well as in infected ticks, where concentration of *Borrelia* spirochetes may be extremely low.

In the Light-Cycler PCR technique, detailed analysis of the melting curves shows species-specific peaks. However, due to the short distance separating them, it is often difficult to distinguish one from the other. This is why we decided to perform direct sequencing of the amplified products. This approach allows not only the detection of *Borrelia* strains in the ticks, but also their clear-cut and precise identification at the species level.

4.3. B. burgdorferi s.l. detected in the collected

Table 3
Description of the positive samples detected by PCR

Samples	*Borrelia* sp.	Region	Origin	Host
3	*B. garinii*	Ticino	Giornico	Dog
3 2f/3 3f/3 10M	*B. afzelii*	Ticino	Giornico	Dog
3 16M	*B. afzelii*	Ticino	Giornico	Dog
8	*B. afzelii*	Ticino	Giornico	Cat
14	*B. lusitaniae*	Ticino	Lottigna	Dog
42	*B. afzelii*	Ticino	Valle di Muggio	Dog
50	*B. afzelii*	Ticino	Alpe Müreccl/Isone	Cow
54	*B. afzelii*	Ticino	Valle di Blenio	Dog
59/60	*B. afzelii*	Ticino	Osogna	Cat and dog
63 2f	*B. valaisiana*	Ticino	Lamone	Cat
84 2f	*B. afzelii*	Ticino	Iragna	Goat
90 1f/90 9f/90 10f/90 11f/90 12f/90 13f	*B. afzelii*	Ticino	Freggio	Cow
92 3f/92 4f/92 5f/92 6f/92 7f/92 8f/92 9f/92 10f/92 11f/92 12f	*B. afzelii*	Ticino	Freggio	Cow
101	*B. lusitaniae*	Ticino	Breganzona	Dog
104 2f	*B. lusitaniae*	Ticino	Carena	Cat
121 2m	*B. lusitaniae*	Ticino	Lodrino	Dog
125	*B. afzelii*	Ticino	Arogno	Cat
135 2f	*B. lusitaniae*	Ticino	Lugano	Cat
162	*B. afzelii*	Ticino	Rivera	Dog
166 2m	*B. lusitaniae*	Ticino	Monti di Cavigliano	Dog
171	*B. afzelii*	Grigioni	Grono	Dog
234 2f	*B. afzelii*	Ticino	Rodi-Fiesso	Dog
265	*B. afzelii*	Ticino	Airolo	Dog
579	*B. afzelii*	Ticino	Melano	Cat
602	*B. afzelii*	Ticino	Vacallo	Human
603	*B. afzelii*	Ticino	Porza	Dog
607	*B. lusitaniae*	Ticino	Giornico	Dog
273/276/287/288/308/312/317/322/324/329/349/365/382/393/397/ 398/403/404/412/421/430/438/485/490/516/534/537/546/612	*B. afzelii*	Neuchâtel	Forest	Vegetation
278/283/290/316/352/390/399/425/435/453/460/465/493/510/512/ 523/624	*B. valaisiana*	Neuchâtel	Forest	Vegetation
282/285/291/297/298/321/326/330/331/341/343/353/367/368/420/ 431/440/ 450/459/473/474/481/494/526/528/535/538/625/626	*B. garinii*	Neuchâtel	Forest	Vegetation
332/414/419/422/439/470/487/495/513/545/618	*B. lusitaniae*	Neuchâtel	Forest	Vegetation
336/345/378/402/413/443/445/501/519/621	*B. burgdorferi s.s.*	Neuchâtel	Forest	Vegetation
684/685/698/705/708/710/713/714/717/721/729/732/740/743/746/ 747/ 753/754/755/758/760/765/795/825/837/838/848/	*B. afzelii*	Valais	Mt d'Orge (Sion)	Vegetation
691	*B. valaisiana*	Valais	Mt d'Orge (Sion)	Vegetation
694/706/709	*B. garinii*	Valais	Mt d'Orge (Sion)	Vegetation
704		Valais	Mt d'Orge (Sion)	Vegetation
716/738/745/759/809/842/860	*B. burgdorferi s.s.*	Valais	Mt d'Orge (Sion)	Vegetation
886/893/895/897/900/904/905/906/915/922/924/935/937/941/951	*B. afzelii*	Valais	Val Trient	Vegetation
891/956	*B. burgdorferi s.s.*	Valais	Val Trient	Vegetation

ticks from the three Swiss regions

In the present study, five different genospecies of the *B. burgdorferi* s.l. complex, e.g., *B. afzelii*, *B. burgdorferi* s.s., *B. garinii*, *B. valaisiana*, and *B. lusitaniae* were detected in Switzerland. The tick infection rates were 32.6% for Neuchâtel, 19.6% for Valais and 15.0% for Ticino. Considering the three regions together, the most prevalent genospecies in Switzerland was *B. afzelii*.

This finding is in agreement with several studies, which described the high prevalence of *B. afzelli* in *I. ricinus* ticks in North and Central Europe countries [21–23]. However, it is in contrast with other reports showing that *B. garinii* is the most common genospecies in a number of countries of Central Europe and South Western Europe [2,24]. In addition, according to Kirsten et al. [25], *B. valaisiana* is the most abundant species in Ireland, and *B. lusitaniae* in Tunisia [26], in Morocco [27] as well as in Portugal [28]. Summarising these different studies, *B. afzelii* and *B. garinii* appear be the most abundant genospecies in Europe, while the presence of the other genospecies differs according to the study area and are in any case less abundant.

Considering, the situation in the three Swiss regions analysed, in Neuchâtel the five genospecies were recorded, which confirms previous results [5]. In the Canton Ticino, it has been possible for the first time to show the presence of *B. afzelii* strains. In a previous report [29], *B. valaisiana* (VS116) was detected and identified by PCR in ticks collected from animals, while Jo-

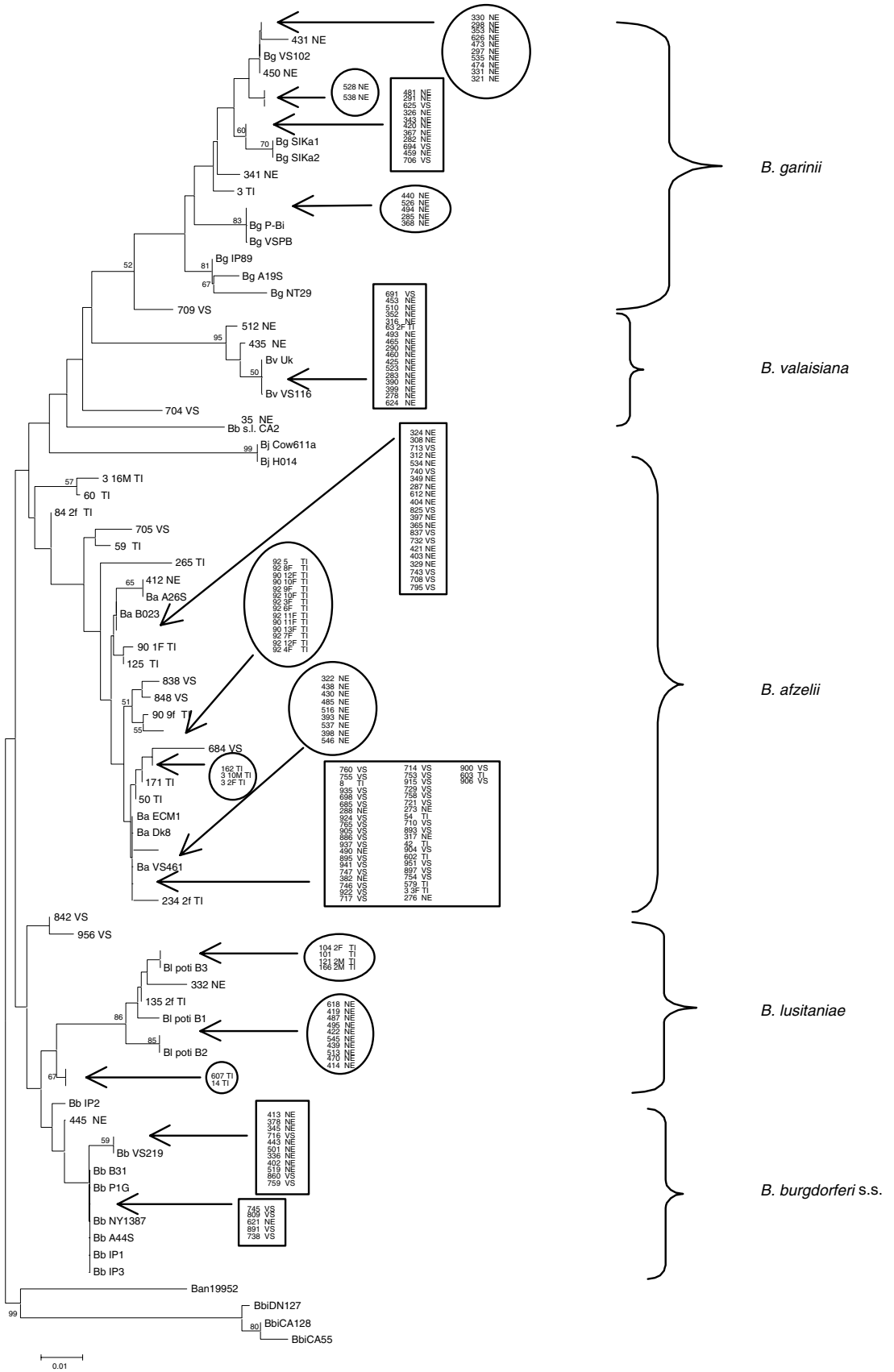

Fig. 2. Neighbour-joining tree of 1000 bootstrap pseudo-replicates with Kimura 2 parameters distances from *recA* sequences for 229 *Borrelia* sp. samples. Bootstrap proportions are provided when greater than 50%. Numbers refer to *Borrelia* identified in ticks collected in three regions of Switzerland: Neuchâtel (NE), Ticino (TI) and Valais (VS). The circle (“○”) shows the groups formed in according to the geographical origin and the square (“□”) shows the mixed groups.

uda et al. [4] reported *B. garinii*, *B. lusitaniae*, and *B. valaisiana* in ticks collected from vegetation. *B. burgdorferi* s.s. seems to be very rare or even absent in Ticino. Concerning the Valais region, *B. lusitaniae* was not detected. A previous study [30] already showed the presence of *B. burgdorferi* s.s., *B. afzelii*, *B. garinii* and *B. valaisiana*, but with different infection rates.

Interestingly, *B. lusitaniae* Poti B2 strains were reported in Neuchâtel and *B. lusitaniae* Poti B3 in Ticino. In the past, the *B. lusitaniae* Poti B2 has been found in various European countries [2], but in contrast the *B. lusitaniae* Poti B3 was noted only in Spain [28]. The dynamics of the two *B. lusitaniae* strains distribution seems to be different.

4.4. Phylogenetic relationships

In the phylogenetic tree of Fig. 2, we identified groups formed according to the geographical origin of the vector ticks as local *Borrelia* populations; alternatively, we found mixed groups formed by *Borrelia* ssp. originating from samples coming from two or all the three regions considered. For instance, strains corresponding to *B. lusitaniae* Poti B2 are characteristic of the Neuchâtel forest while *B. lusitaniae* Poti B3 strains were recorded in various localities for the Canton Ticino (Table 3). On the other hand, *B. valaisiana* strains are indistinguishable in all three regions considered.

From the data presented, we observe that each area studied has its particular *Borrelia* population. Each population probably has its own internal dynamics that results in a heterogeneous distribution of the *B. burgdorferi* s.l. population in Switzerland. This distribution reflects the situation in Europe, where the prevalence of *B. burgdorferi* s.l. in the vector *I. ricinus* ticks differs considerably.

The heterogeneous structure and distribution of *B. burgdorferi* s.l. has been associated with the diversity of the reservoir hosts [4]. *B. burgdorferi* s.l. may be maintained in nature through distinct transmission cycles, involving small mammals and/or birds [31]. According to Kurtenbach [12], the vertebrate hosts rather than tick species are the key to the Lyme borreliosis spirochete diversity. Local host communities probably contribute significantly to the population structure of the *B. burgdorferi* s.l. populations.

At the local level, genetic factors such as mutations generate new forms of *Borrelia*, many of which remain on the same place (host population closed). At the European level, the circulation of the different forms of *Borrelia* is probably due to host movements or migrations (rodents moving only short distances, highly mobile and birds migrating long distance).

The heterogeneity of the *B. burgdorferi* s.l. genospecies in Switzerland, and also in Europe, seems to infer continuous evolution. Our data showed that *B. afzelii* is the most frequent and heterogeneous genospecies in Switzerland. This is surprising since *B. afzelii* is known to include only one ospA serotype, whereas *B. garinii* shows different serotypes, a number of which might be related to neuroborreliosis [32]. The diversity of *B. burgdorferi* s.l. in Eurasia is much greater than in North America, where we observe an apparent homogeneity with *B. burgdorferi* s.s. as the predominant genospecies. In addition, it is interesting to note the presence in Europe of other genospecies such as the *Borrelia* isolate I-77 [33]. The whole Lyme borreliosis spirochete complex might originate from Eurasia and is close to *B. garinii*, which has shown the greatest genetic heterogeneity [34]. However, in the present study based on *recA* diversity, *B. afzelii* has been found to be more heterogeneous than *B. garinii*, which might suggest a rapid evolution of this genospecies.

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