

*Microbiologia di *Borrelia burgdorferi* Sensu Lato e ceppi isolati da zecche e tessuti umani a Trieste.*

Marina Cinco

*Laboratorio Spirochete,
Dipartimento Scienze della Vita,
Università di Trieste.*

Segnalazioni precedenti di sintomatologie Lyme compatibili

- 1883 Buchwald, Germania (Acrodermatite Cronica Atrofica)
- 1909 Afzelius, Svezia (Erythema Migrans)
- 1922 Garin, Francia (Meningite)
- 1943 Bäfverstedt, Svezia (lymphadenosis benigna cutis)
- 1975 Polly Murray & Judith Mensch, USA ←
- 1975 Steere, USA
- 1982 Burgdorfer & Barbour, USA
- 1983 Genova
- 1986 Trieste



Borrelia burgdorferi

1983

Genere Borrelia:

microorganismi elicoidali (0,2-0,3 micron), flagelli periplasmici che si sovrappongono nel mezzo della cellula. Flessibili e mobili mediante movimenti di rotazione avanti/indietro. Sono microaerofili ed associati ad ospiti. Sono veicolati da zecche argasidi, oppure Ixodidi o pidocchi del corpo.

Il genoma è frammentato in un cromosoma lineare e plasmidi lineari e circolari. Il contenuto G/C del genoma è 27-32 .

I membri di questo genere sono agenti delle febbri ricorrenti ,o della malattia di Lyme.

Il ceppo tipo è Borrelia anserina (Bergey's manual 1925).

Borrelie febbre ricorrente

Borrelia

Malattia

Vettore

B.recurrentis *Febbre ricorrente*
B. turicatae *epidemica*
B. hermsii

Pidocchio



B. parkeri *Febbre ricorrente*
B. caucasica *endemica*
B. hispanica
*B.myamotoi**
altre

Zecche
(Ornithodoros)



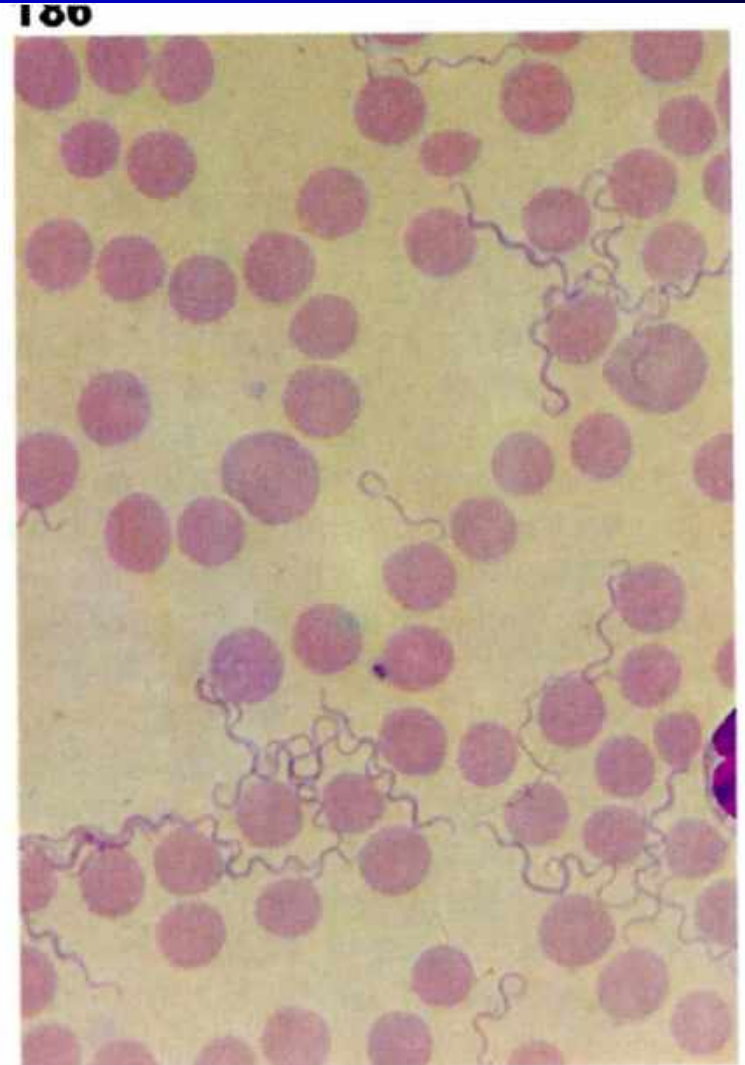
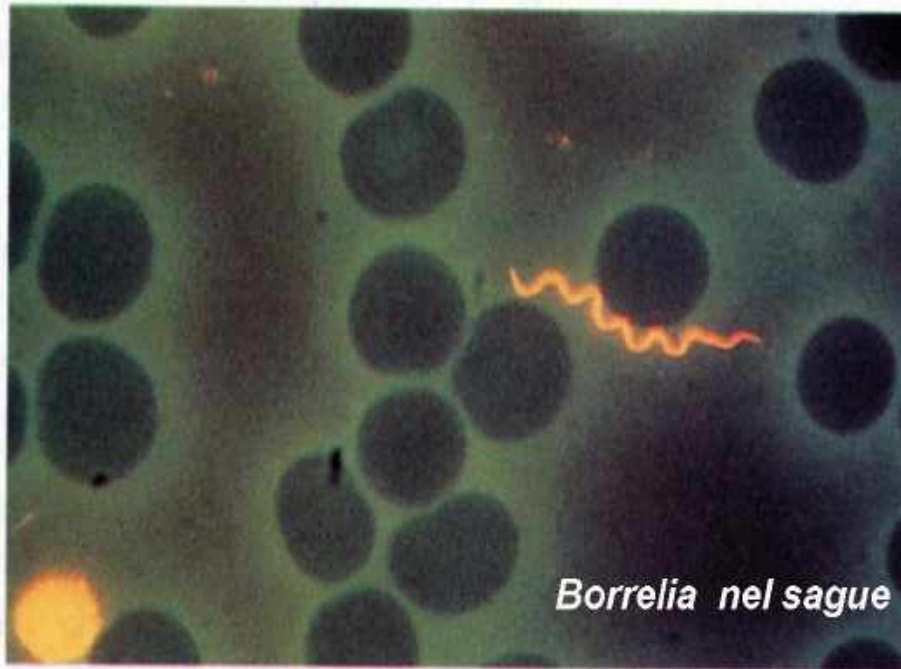
Borrelia burgdorferi *Morbo di Lyme* *Zecche*
sensu lato *(Ixodidi)*

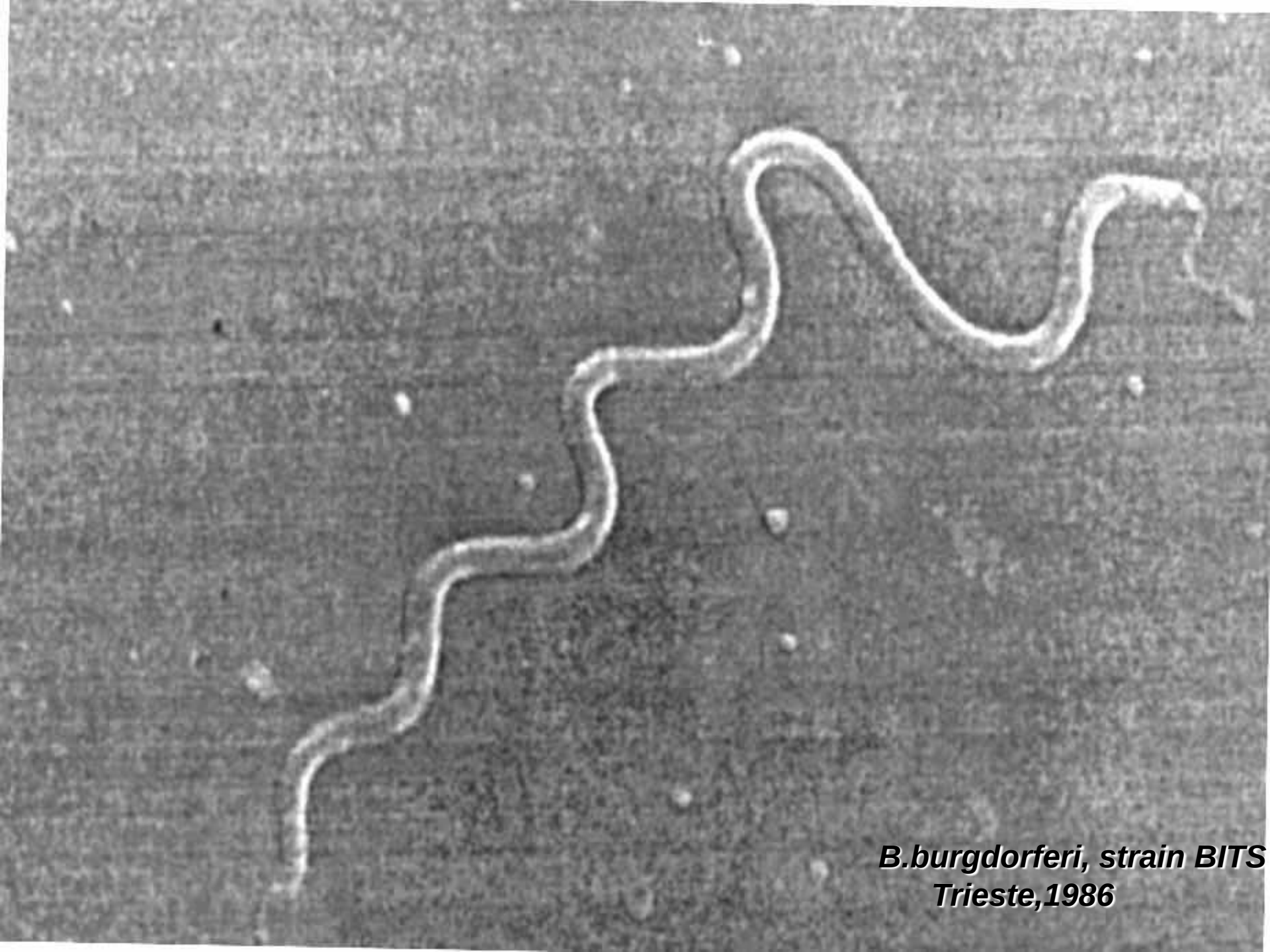
Borrelia burgdorferi

1983

Coltivabile !!!
Barbour's medium

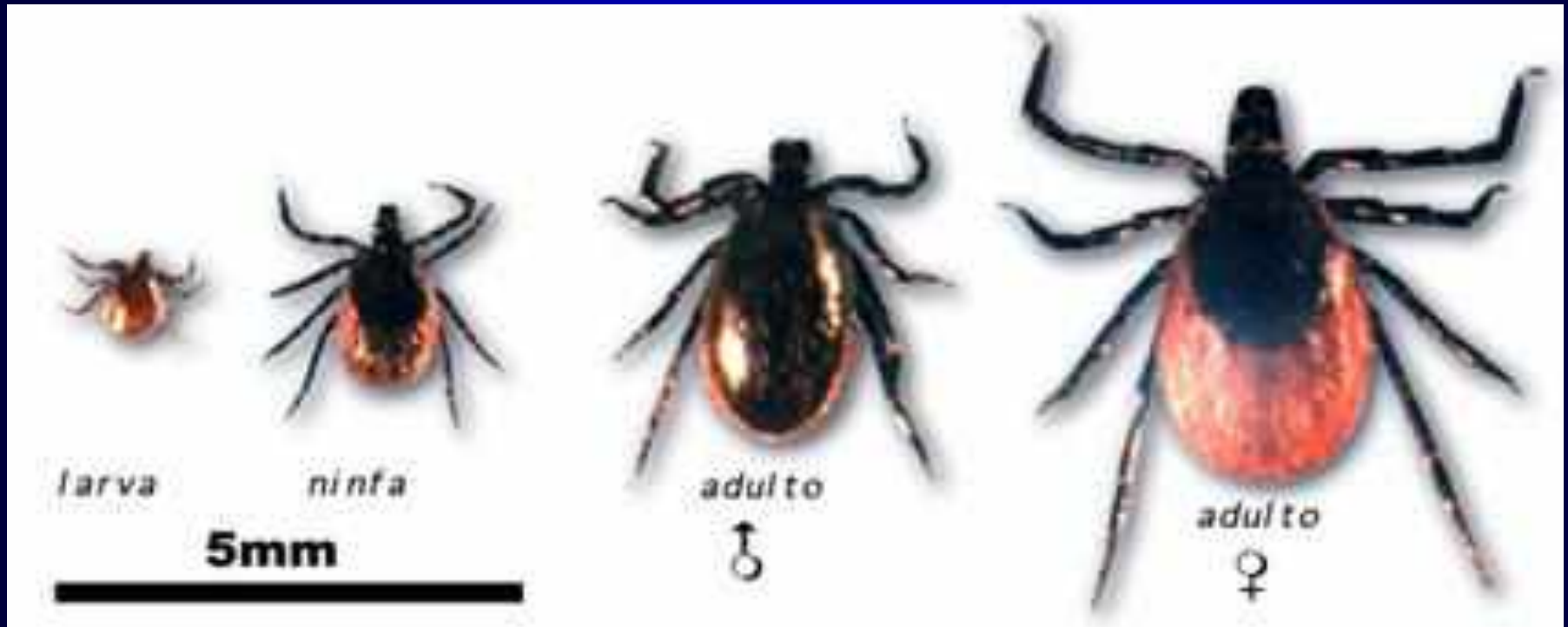
Morfologie di *Borrelia*

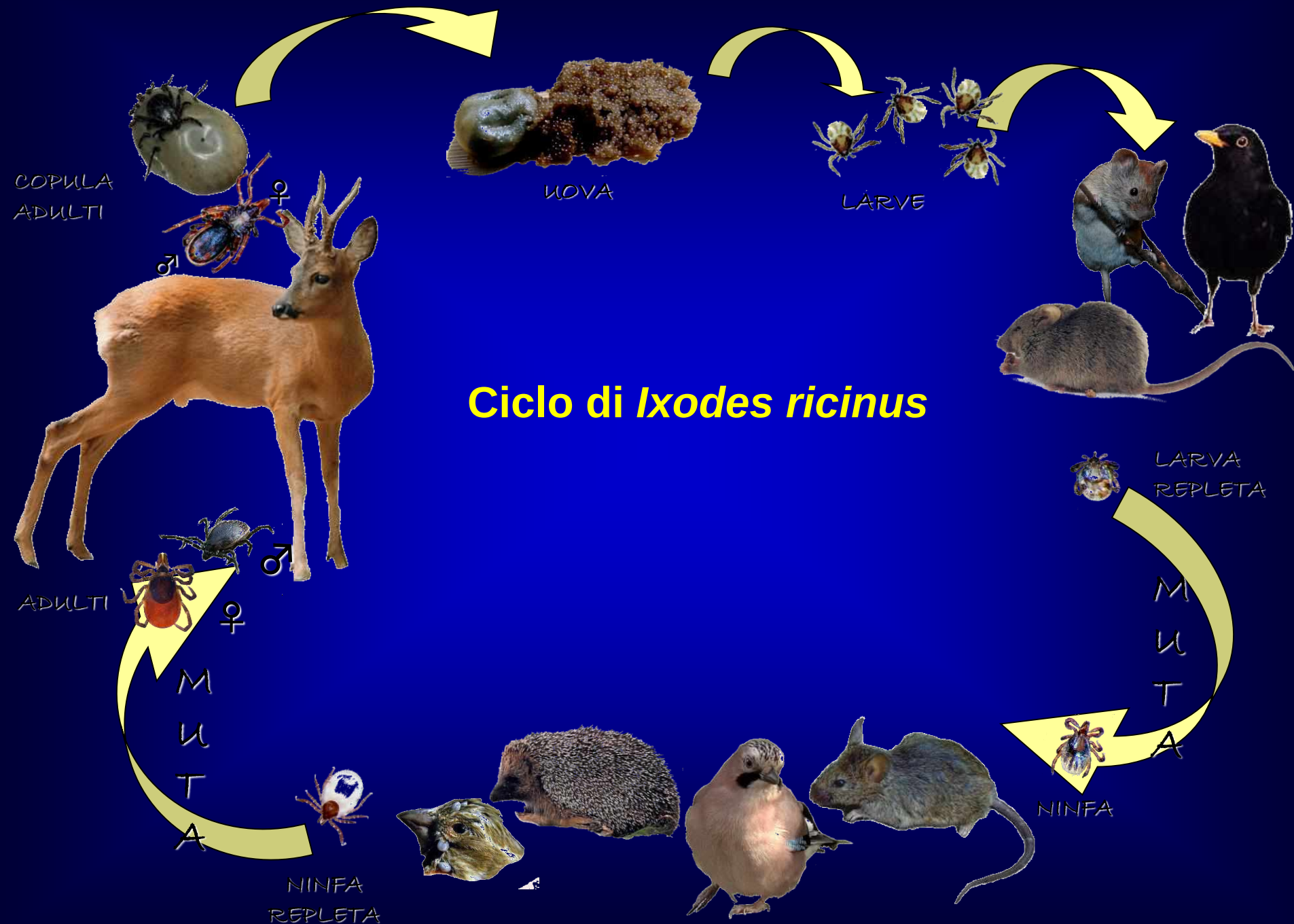




B.burgdorferi, strain BITS
Trieste, 1986

Stadi vitali di *Ixodes ricinus*





Ospiti serbatoio di *Borrelia burgdorferi* su cui *I. ricinus* si nutre

Micromammiferi :

Apodemus selvaticus (topo
selvatico)

Apodemus flavicollis

Ap. Agrarius

Clethrionomys glareolus

Glis glis (ghiro)

Microtus agrestis

Sorex minutus

Etc.

Macromammiferi:

Erinaceus europeus (riccio)

Lepus europeus (lepre)

Sciurus vulgaris e *Sc. carolinensis*
(scoiattolo rosso e grigio)

Uccelli:

Alca torda

Turdus merula (merlo)

Turdus philopmelos (tordo)

Phasianus colchicus (fagiano)

Fringilla coelebs (fringuello)

VIREMIA!!!

La zecca: gli ospiti serbatoio



la zecca: gli ospiti





Ospiti occasionali di *B. burgdorferi* su cui *I. ricinus* si nutre

Uomo

Ungulati : caprioli, cervi, etc.

Ovini

Bovini

Cavalli

Cani

Gatti



Raccolta di *Ixodes ricinus*

**Il metodo di raccolta utilizzato è il
*dragging sample***

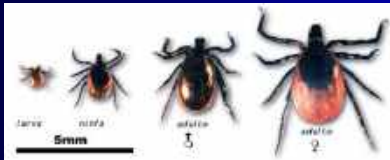


**trascinare sulla vegetazione un panno
bianco di flanella delle dimensioni di 1mq
su una superficie di circa 100mq**



Isolamento Borrelie

Da Ixodes.



Omogenato pull di zecche



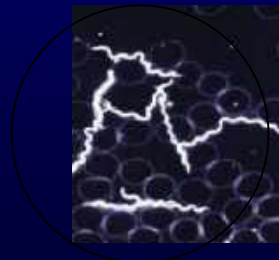
diluizioni scalari con terreno



semina in terreno BSK+ antibiotici



incubazione a 30-34C° ed osservazione



Da Campioni umani :

Biopsia cutanea

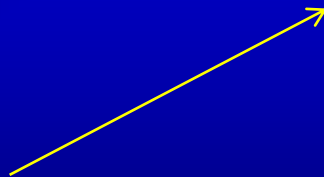
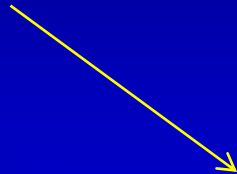
Biopsia miocardio

Sangue

diluizioni seriali e semina in BSK

Liquido sinoviale

Liquor



Classificazione *B.burgdorferi*

- Inizialmente riconoscimento fenotipico:
- Profilo proteico in SDS-Page electrophoresis
- con Ac monoclonali → Serotipi
- Successivamente *in genospecie*, mediante *tecniche molecolari* :

Analisi comparata del DNA : profili di restrizione

DNA/DNA ibridazione

Analisi RFLP dopo PCR spazio rrfA-rrlB

Nested PCR OspA

PFGE DNA

etc

Ricerca sequenze DNA di Borrelia



***Laboratorio Spirochete
Dipartimento di Scienze della vita
Università di Trieste***

Analisi PFGE DNA di 14 isolati da ECM digestione con *Mlu*I

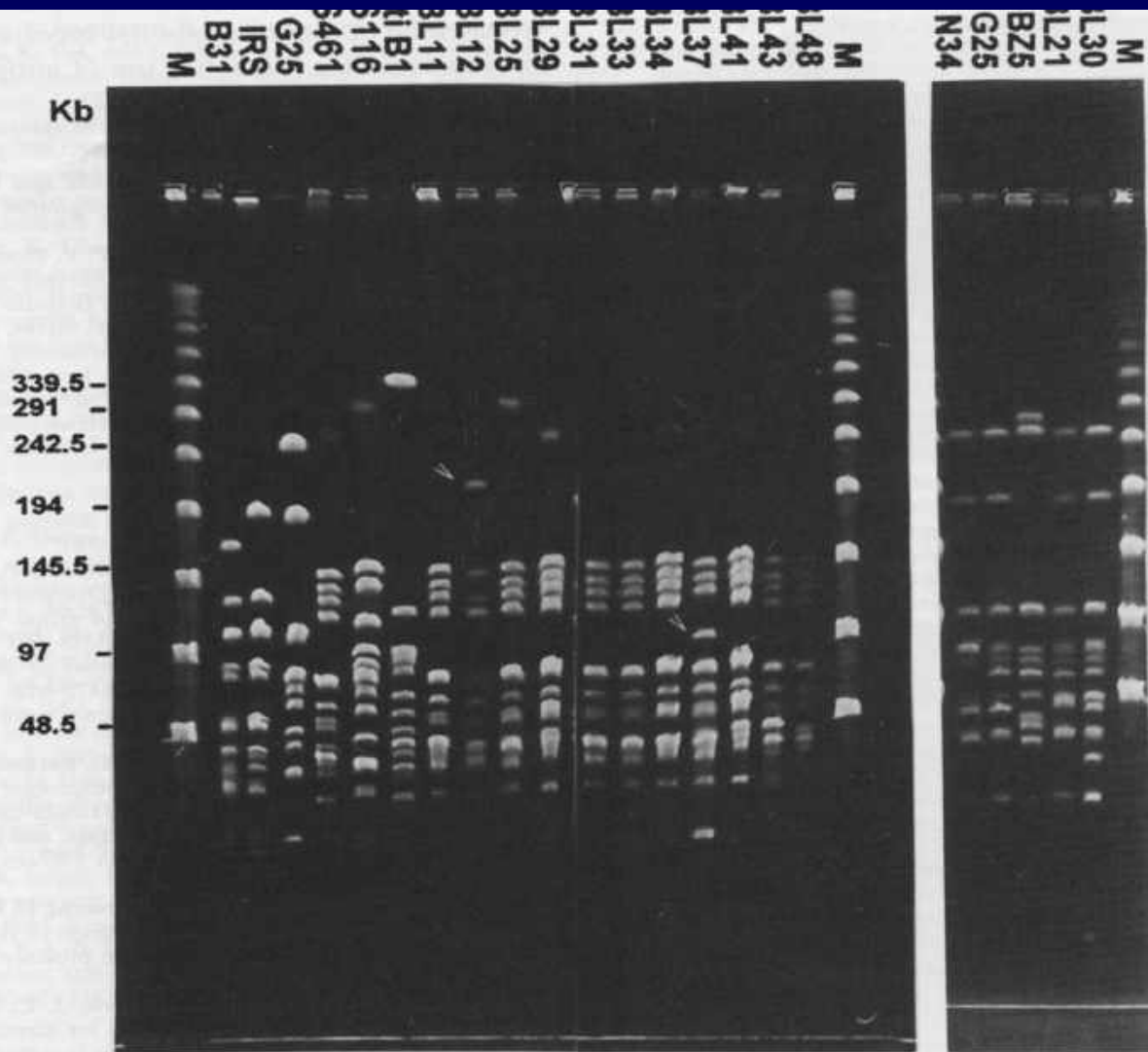


FIG. 3. PFGE of *Sma*I-cleaved genomic DNAs from *B. burgdorferi* sensu lato strains. The digestion products were separated at 200 V for 24 h at 0.25% TBE with two increasing pulse times as follows: from 5 to 15 s (15 h) and from 20 to 25 s (9 h). The names of strains are

Principali specie di *B.burgdorferi*, distribuzione e vettore

<i>B.b. sensu stricto</i>	<i>I.scapularis</i>	USA
	<i>I.ricinus</i>	EURASIA
<i>B. andersoni</i>	<i>I.dentatus</i>	USA
<i>B.lonestari</i>	<i>Amblyoma americanum</i>	USA
<i>B.garinii</i>	<i>I.ricinus</i>	EURASIA
	<i>I.persulcatus</i>	ASIA
<i>B.afzelii</i>	<i>I.ricinus</i>	EURASIA
	<i>I.persulcatus</i>	
<i>B bissetii</i>	<i>I.ricinus</i>	EUROPA
<i>B.lusitanae</i>	<i>I. ricinus</i>	EUROPA
<i>B.valaisiana</i>	<i>I.ricinus</i>	EUROPA
<i>B.Japonica</i>	<i>I.ovatus</i>	Giappone

Genospecie di *B.b.* ed associazione clinica

genospecie

sintomatologia prevalente

B.b. sensu stricto

Forme disseminate (artrite, neuroborreliosi)

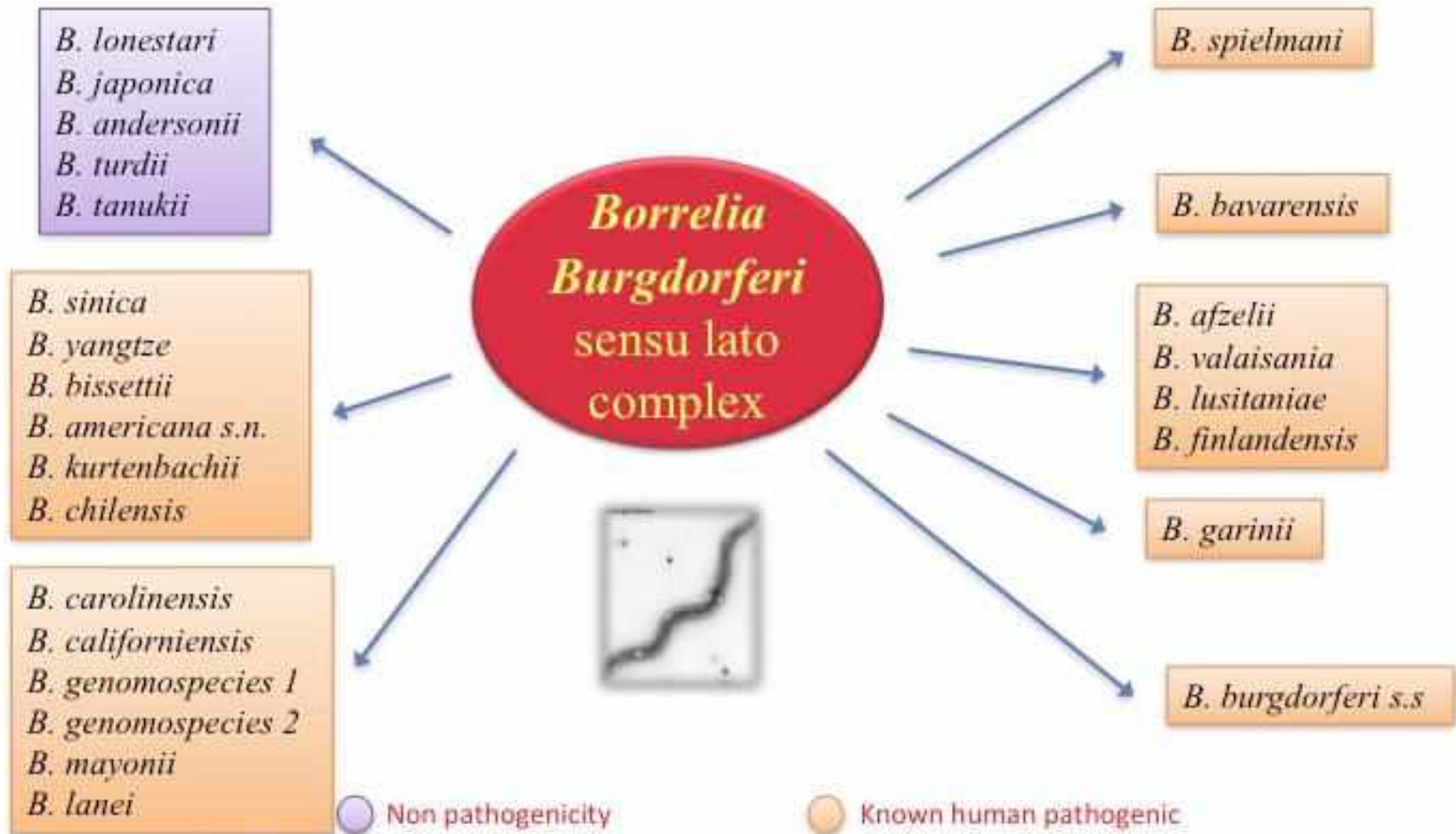
B. garinii

Neuroborreliosi

B. afzelii

Forme cutanee (ACA)
(neuroborreliosi)

Eterogeneità di *Borrelia burgdorferi* s.l.



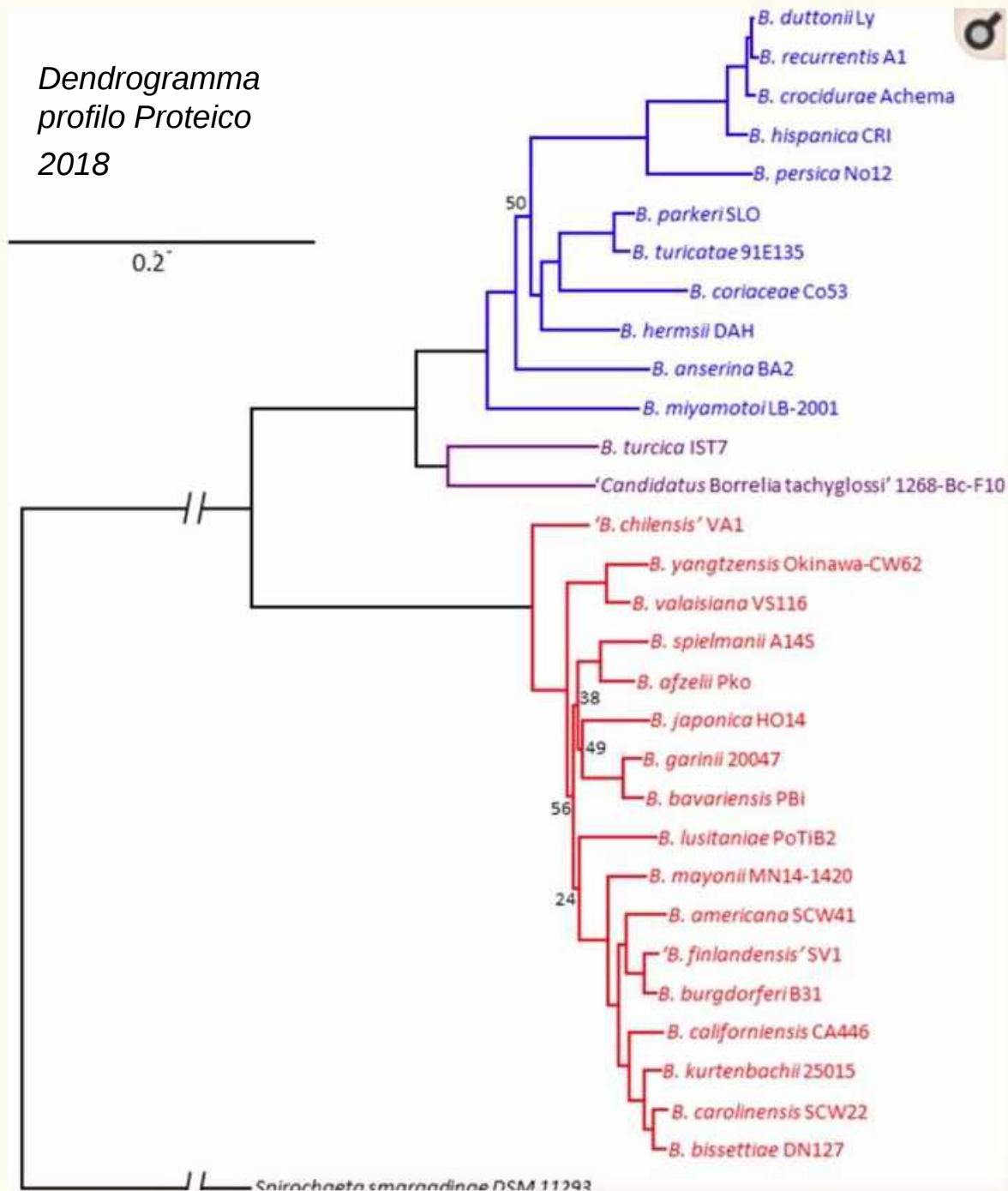
Evoluzione della sistematica:

Due Generi: **Borrelia** comprendente le Borrelie
febbre ricorrente

Borrelia: gruppo Borrelie del Lyme

Esistenza di “specie” intermedie: **Borrelia turcica** (2004)
e **Borrelia taylorii** (2017)

Dendrogramma
profilo Proteico
2018



Margos et al.
Plos One 2018

Successivi isolamenti da uomo a Trieste

<i>Ceppi</i>	<i>distretto</i>	<i>ref.</i>	<i>Genospecies</i>
BITS	<i>I.ricinus</i>	Cinco, et al. APMIS,1989,97,381.382.	<i>B.garinii</i>
D.A.	ECM	Cinco et al. FEMS Microbiol Immunol 1991	<i>B.garinii</i>
Nancy	ECM**	Cinco & Trevisan, Microbiologica, 1992,95.	<u><i>B.afzelii</i></u>
Myo 1	Miocardio***	Cinco, Lardieri, Trevisan et al. Lancet, 1993	<i>Bb sensu stricto</i>
Myo2	Miocardio***	Cinco, Lardieri, Trevisan et al. Lancet, 1993	<i>B.b. sensu stricto</i>
Gualtieri	ECM	n.p.	<i>B.afzelii</i>
BTS3	sangue	np	<i>B.b.sensu stricto</i>

** Paziente seronegativo

*** Cardite, biopsia miocardio

Letters to the Editor

Isolation of *Borrelia burgdorferi* from myocardium

SIR—Lyme borreliosis can vary considerably in its clinical manifestations. Carditis¹ is less frequent than cutaneous, neurological, and joint involvement. Transient atrioventricular block is the most frequent marker of Lyme carditis; less frequent are rhythm disturbances, myopericarditis, and heart failure.² An aetiological role of *Borrelia burgdorferi* in a case of chronic heart disease, characterised by high-grade ventricular arrhythmias and heart failure, was suggested by the isolation of a borrelia strain from a biopsy of the myocardium of a patient with dilated cardiomyopathy.³ Borrelias had not previously been isolated from heart biopsy material, although spirochaetes have been seen in heart tissues at necropsy.⁴

A 20-year-old woman presented with severe heart failure, cardiomegaly, and a mildly hypokinetic left ventricle. She had no history of tick bites, and showed no dermatological changes. She was treated with digitalis, diuretics, and angiotensin-converting enzyme (ACE) inhibitors and progressively improved. 13 days after presentation a haemodynamic study showed normal pressures and normal cardiac output, but the left ventricle was moderately hypokinetic (ejection fraction 44%). At endomyocardial biopsy (five samples from the left ventricle), histological signs of myocarditis were absent, but culture was positive for *B. burgdorferi*. She was treated with penicillin 20 MU a day for 15 days. After 7 months the patient is symptom-free and the heart is normal.

A 47-year-old woman presented in May, 1992, with heart failure and severe left ventricular dysfunction (ejection fraction 30%). After 8 days of treatment with ACE inhibitors, digitalis, and verapamil, haemodynamics were normal and ejection fraction was 47%. Endomyocardial biopsy (seven samples from the left ventricle) showed a diffuse infiltration of lymphocytes with slight interstitial fibrosis. Culture was positive for *B. burgdorferi*. She was treated with penicillin 20 MU a day for 15 days. After 3 months the patient was symptom-free and the heart normal. An endomyocardial biopsy was compatible with the "healing" myocarditis; culture was negative.

Immunofluorescence (Virion) was negative in both patients; the same serum samples were analysed by western blot with, as antigens, borrelia strains B31 and the local isolate BITS.⁵ Only a faint 41 kDa band was visible in the first patient. The first cultured strain, named myo 1, was examined by sodium-dodecyl-sulphate gel electrophoresis.⁶ Protein bands were visible at 22, 23.5, 26, 30, 33, 34, 36, 37, 41, 44, 48, 68, and 74 kDa. Another gel was blotted on nitrocellulose for western blot. Monoclonal antibodies H9724, H5332, H3T, H6831, FR8, F314 (provided by A. Barbour, University of San Antonio, Texas) all recognised the myocardial isolate, confirming it to have the main protein antigens of *B. burgdorferi*. The characteristics of the second isolate are under study.

The diagnosis of carditis, an infrequent clinical manifest-

B. burgdorferi carditis unambiguously affirmed the diagnosis, allowing eradication of the infection. The persistence of *B. burgdorferi* in myocardial tissue in the absence of histological signs of myocarditis may be the cause of a chronic myocardial disease.

G. Lardieri, A. Salvi, F. Camerini

Department of Cardiology, Ospedale Maggiore, 34100 Trieste, Italy

M. Cirico

Institute of Microbiology, Leptospira Reference Laboratory, University of Trieste

G. Trevisan

Department of Dermatology, University of Trieste

- 1 Steere AC, Baisford WP, Weinberg M, et al. Lyme carditis: cardiac abnormalities of Lyme disease. *Ann Intern Med* 1986; 93: 8-16.
- 2 Van der Linde MR. Lyme carditis: clinical characteristics of 105 cases. *Scand J Infect Dis* 1991; 75: 81-84.
- 3 Stanek G, Klein J, Bittner R, Glogar D. Isolation of *Borrelia burgdorferi* from the myocardium of a patient with longstanding cardiomyopathy. *N Engl J Med* 1990; 322: 249-52.
- 4 Marcus LC, Steere AC, Duray PH, Anderson AE, Mahoney EB. Fatal pancarditis in a patient with coexistent Lyme disease and babesiosis: demonstration of spirochetes in the myocardium. *Ann Intern Med* 1985; 103: 374-76.
- 5 Cinco M, Bandi E, Trevisan G, Stanek G. Isolation and characterization of the first tick isolate of *Borrelia burgdorferi* in Italy. *APMIS* 1989; 97: 381-82.

Evidence for an animal origin of vancomycin-resistant enterococci

SIR—Frieden and colleagues' report (July 10, p 76) draws attention to the emerging worldwide problem of vancomycin-resistant enterococci (VRE) in hospital patients. Their finding of two community-acquired infections leads them to conclude that VRE are spreading into the community from hospital origins. We believe the reverse to be true: studies prompted by an outbreak of vancomycin-resistant *Enterococcus faecium* at our hospital indicate a community reservoir of VRE, suggesting that VRE become clinically apparent in debilitated hospital patients whose bowel floras are under the selective influence of antibiotics. Reports so far fail to recognise that the *Enterococcus* spp. are part of the normal bowel flora of many animals and in particular poultry,^{1,2} which may lead to a potential source of VRE.

During eight weeks in 1992, 8 clinical isolates of vancomycin-resistant *E. faecium* were detected in our laboratory; 4 of them from renal patients. Clonal relation between the 8 isolates was shown by direct restriction enzyme analysis with *Sal*I and ribosomal RNA gene restriction patterns with *Eco*R1 (ribotyping). A total of 354 stool specimens, mostly from symptom-free patients, was subsequently screened with highly selective enrichment broth and agar.³ Vancomycin-resistant *E. faecium* was detected in 14% of renal patients, 6% of other

Table 1: nested-PCR on *ospA* gene and RFLP molecular typing results in clinical samples from 29 patients with different manifestations of Lyme borreliosis.



Patient no.	Tick bite	genotype	diagnosis	Serology		N-PCR				
				ELISA + WB		N-PCR blood	N-PCR urine	N-PCR biopsy	N-PCR CSF	N-PCR SF
				IgM	IgG					
1	/	<i>B. garinii</i>	EM	-	+	+	+	-	/	/
2	/	<i>B. afzelii</i>	EM	-	-	-	+	-	/	/
3	+	<i>B. afzelii</i>	EM	+	-	+	+	+	/	/
4	/	<i>B. afzelii</i>	EM	-	-	+	-	-	/	/
5	+	<i>B. garinii</i>	EM	-	-	+	+	-	/	/
6	+	<i>B. afzelii</i>	EM	-	+	+	+	-	/	/
7	+	<i>B. afzelii</i> + <i>B. b. sensu stricto</i>	EM	-	-	+	+	-	/	/
8	+	<i>B. afzelii</i>	EM	-	-	+	-	-	/	/
9	+	<i>B. afzelii</i>	EM	-	-	+	+	+	/	/
10	+	<i>B. afzelii</i> (biopsy) + <i>B. b. sensu stricto</i> (blood, urine)	EM	(+)	+	+	+	+	/	/
11	/	<i>B. garinii</i> + <i>B. afzelii</i>	ACA	-	-	-	+	-	/	/
12	+	<i>B. b. sensu stricto</i>	ACA	-	+	+	+	-	/	/
13	+	<i>B. garinii</i>	ACA	-	-	+	+	-	/	/
14	/	<i>B. garinii</i>	LSA	-	-	+	+	-	/	/
15	++	<i>B. garinii</i> + <i>B. afzelii</i> + <i>B. b. sensu stricto</i>	ACA	-	(+)	-	-	+	/	/
16	/	<i>B. afzelii</i>	Anet.	+	+	+	-	+	/	/
17	/	<i>B. garinii</i> + <i>B. afzelii</i> + <i>B. b. sensu stricto</i>	LSA	(+)	(+)	+	-	+	/	/
18	/	<i>B. garinii</i> + <i>B. afzelii</i> + <i>B. b. sensu stricto</i>	Atx. prof. P-P	+	-	+	-	+	/	/
19	/	<i>B. garinii</i> + <i>B. afzelii</i> + <i>B. b. sensu stricto</i>	LSA	-	-	+	-	-	/	/
20	/	<i>B. afzelii</i>	Anet. + ACA	-	-	+	-	-	/	/
21	/	<i>B. b. sensu stricto</i>	Arthritis	+	+	+	+	/	/	+
22	++	<i>B. garinii</i> + <i>B. afzelii</i> + <i>B. b. sensu stricto</i>	Arthritis	-	+	+	-	/	/	+
23	+	<i>B. garinii</i> + <i>B. afzelii</i> + <i>B. b. sensu stricto</i>	Arthritis	+	+	+	-	/	/	+

24	/	<i>B. garinii</i>	NB	+	-	-	+	/	-	/
25	+	<i>B. garinii</i> + <i>B. afzelii</i> + <i>B. b. sensu stricto</i>	NB	+	+	+	-	/	+	/
26	/	<i>B. garinii</i> + <i>B. afzelii</i> + <i>B. b. sensu stricto</i>	NB	-	-	+	-	/	+	/
27	/	<i>B. garinii</i> + <i>B. afzelii</i> + <i>B. b. sensu stricto</i>	NB	-	+	+	-	/	+	/
28	/	<i>B. garinii</i>	NB	+	+	+	-	/	+	/
29	/	/	NB	+	-	-	/	/	-	/

~~Anet.~~: anetoderma; ~~Att. prof. P-P:~~ ~~atropoderma profundum~~ Pasini-Pierini; NA: non available;

~~±~~: positive result; (+) : borderline-positive reaction; - : negative result; * -: no tick bite; + : one tick bite; ++ : two or more tick bite.

Floris R., G. Menardi, R. Bressan, G. Trevisan, S. Ortenzio, E:Rorai, Marina Cinco. Microbiologica, 30, 2007..

Trieste, Barcolana 2019

