



UNIVERSITÀ DEGLI STUDI DI MILANO

Dipartimento di Patologia Animale, Igiene
e Sanità Pubblica Veterinaria
Sezione di Patologia Generale e Parassitologia

Dottorato di Ricerca in Biologia Animale, XXIII Ciclo
Scuola di Dottorato Terra Ambiente e Biodiversità

Microorganisms associated with hard ticks (Ixodidae): from symbionts to tick-borne pathogens

Tesi di dottorato di: Dario Pistone

Docente guida

Prof. Claudio Bandi

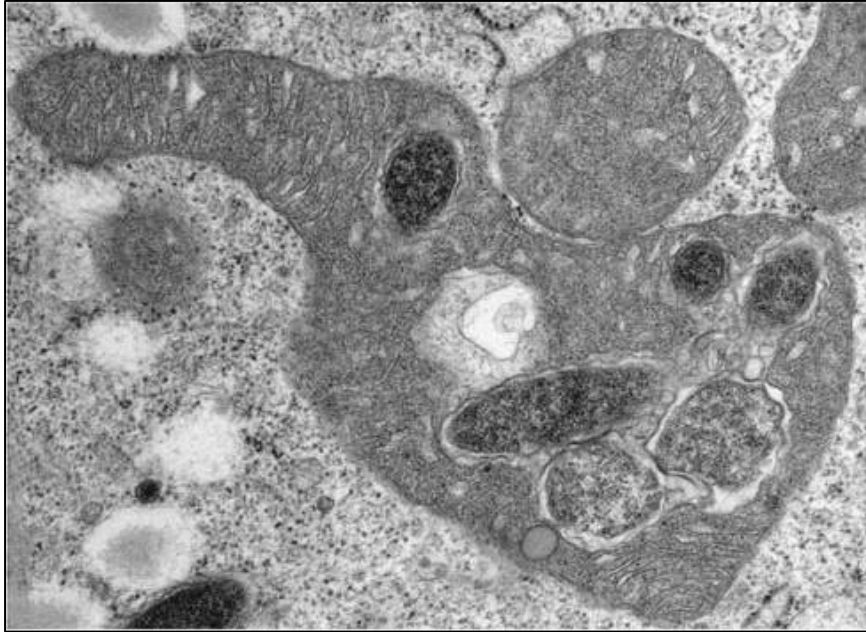
Coordinatore del Dottorato

Prof. Giulio Melone

Supervisor esterno

Dott. Massimo Fabbi

ANNO ACCADEMICO 2010/2011



Cover page:

Image in transmission electron microscopy (TEM) of a mitochondrion of an oocyte of the tick *Ixodes ricinus*.

Midichloria mitochondrii bacteria are located inside the mitochondrion (Prof. Luciano Sacchi – Pavia).

Table of contents

Summary	I
Overview of my PhD experience	V
Preface	VII

PART A: introduction

Chapter 1

Ticks and microorganisms associated with ticks

1.1 Biology of ticks	p. 1
1.2 Ticks as vectors of diseases	p. 6
1.3 <i>Ixodes ricinus</i>	p. 7
1.4 Endosymbionts of ticks and other arthropods	p. 9
1.5 An extraordinary endosymbiont of ticks: <i>Candidatus</i> Midichloria mitochondrii	p. 11

Chapter 2

***Candidatus* Midichloria mitochondrii in ticks and other Metazoa**

2.1 Mito-bacteria in the mitochondria of <i>Ixodes ricinus</i>	p. 13
2.2 <i>Midichloria</i> -like organisms inside eukaryotic cells	p. 15
2.3 Origin of symbiosis: an ancient scenario of endless struggle and cooperation	p. 18
2.4 Living inside an eukaryotic cell	p. 20
2.5 Is <i>Midichloria mitochondrii</i> a very efficient energy parasite?	p. 21
2.6 Mitochondrial pathogens	p. 23

PART B: experimental work

Chapter 3

Genetic variability of *Ixodes ricinus* and diffusion of *Borrelia burgdorferi* sensu latu in Europe and North Africa

3.1 Introduction	p. 25
3.1.1 Genetic variability of <i>Ixodes ricinus</i>	p. 25
3.1.2 <i>Borrelia burgdorferi</i> sensu latu	p. 27
3.2 Materials and methods	p. 30
3.2.1 Tick sampling	p. 30
3.2.2 Laboratory procedures	p. 32
3.2.3 Amplification of nuclear loci, molecular cloning and sequencing	p. 32

3.2.4 Amplification of mitochondrial DNA and sequencing	p. 35
3.2.5 PCR detection and identification of <i>Borrelia</i> species	p. 35
3.2.6 Data analysis	p. 36
3.3 Results	p. 39
3.3.1 Results of the analysis on mitochondrial and nuclear loci	p. 39
3.3.2 Analysis of demographic changes in the populations	p. 47
3.3.3 Genospecies of <i>Borrelia burgdorferi</i> s.l. complex in Europe and North Africa	p. 49
3.4 Discussion	p. 50
3.4.1 Genetic analysis on <i>Ixodes ricinus</i> population	p. 50
3.4.2 Pattern of genetic variation inside the European continent	p. 51
3.4.3 Considerations on Lyme borreliosis	p. 52

Chapter 4

Screening for *Midichloria mitochondrii* in blood and tissues of vertebrate hosts

4.1 Introduction	p. 53
4.2 Materials and methods	p. 55
4.3 Results	p. 57
4.4 Discussion	p. 58

Chapter 5

Bacterial pathogens transmitted by ticks in diverse Italian areas

5.1 Introduction	p. 59
5.2 Materials and methods	p. 63
5.3 Results	p. 65
5.3.1 Lyme borreliosis in the Pianura Padana, Lombardia (Study n.1)	p. 66
5.3.2 Parco del Ticino: <i>I. ricinus</i> population density and bacterial threats (Study n.2)	p. 69
5.3.3 Pathogens transmitted by tick in a North-western Alpine area in Piemonte (Study n.3)	p. 72
5.3.4 SFG Rickettsiae and <i>Anaplasma phagocytophilum</i> in Emilia-Romagna (Study n.4)	p. 75
5.3.5 Screening for pathogenic bacteria in ticks collected on migratory birds (Study n.5)	p. 80
5.3.6 Tularemia outbreak in Toscana (Study n.6)	p. 82

Chapter 6

Conclusive remarks

6.1 <i>Midichloria mitochondrii</i> and <i>Ixodes ricinus</i>	p. 85
6.2 Tick-borne pathogens and diseases in Italy	p. 88