

Overview of my PhD experience

In 2007 I enrolled in the PhD in Animal Biology, and my work since then was supervised by Prof. Claudio Bandi, Faculty of Veterinary Medicine, University of Milan. My research activity was not funded by an academic grant.

The majority of the experimental work was done in the laboratory headed by Prof. Bandi at the 'Dipartimento di Patologia Generale, Igiene e Sanità Pubblica Veterinaria (DIPAV)' – Faculty of Veterinary Medicine – University of Milan. During my PhD all research projects I developed, even collaborating with other Institutions (IRCCS Policlinico San Matteo and Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna – Sezione di Pavia - IZSLER) were coordinated by both my PhD supervisor (Prof. Claudio Bandi), and by the Director of the sections of Milano, Pavia and Varese of IZSLER (Dr. Massimo Fabbi). The activity of research developed in these years were mainly concentrated in the areas of animal biology that are at the boundary with parasitology. In details, my research activities focused on ticks and microorganisms associated with ticks comprehending both endosymbionts and pathogenic bacteria, that are often associated with (or vectored by) hard ticks (Ixodidae).

My PhD research project was initially focused on the intramitochondrial bacterium *Midichloria mitochondrii* and its main host *Ixodes ricinus*. Further development of this project brought me to investigate the genetic variability and population structure of *I. ricinus* in Europe and North Africa; concurrently I performed a screening for the presence of spirochetes belonging to *Borrelia burgdorferi* sensu lato complex. This project was developed in collaboration with Prof. Sandra Urbanelli – University of Roma 'La Sapienza'.

As a secondary line of research I investigated the capacity of the intramitochondrial endosymbiont *Midichloria mitochondrii* to infect vertebrates. At the beginning of my experience the molecular detection of *M. mitochondrii* in blood of mammals and human beings was one of my primary task. Several direct and indirect evidence suggest that *M. mitochondrii* might have an infective potential for vertebrates, possibly without triggering a pathological response.

Another part of my work on *M. mitochondrii* consisted in numerous attempts to culture and growth this intramitochondrial endosymbiont in diverse tick cell lines. Although some promising results were obtained, I decided to not include this part in the final PhD dissertation.

I also developed a research project for the Hospital 'San Matteo' – Fondazione IRCCS of Pavia, in the context of a collaboration with Dott. Massimo Fabbi (IZSLER) and with my supervisor, Prof. Claudio Bandi. The possibility to work in the context of a collaboration among different Institutions (DIPAV – IZSLER – Policlinico San Matteo, Pavia) allowed me to flank the work of people with different scientific backgrounds and to work in different Institutions, respectively oriented to animal research, veterinary diagnosis and human diagnosis/human health. In this stimulating and prolific environment I had the possibility to develop my personal biological and zoological knowledge with applicative perspective. In particular, during my PhD experience I learned to collect ticks by dragging method and directly on the vertebrate hosts. In my work, ticks were recognized by using standard taxonomical key and also by using molecular tool, sequencing specific target genes with a DNA barcoding approach. I also dissected ticks for extraction of organs, DNA extraction and molecular analysis and reconstruction of phylogenetic relationship between different tick species and associated microorganisms. At present, I am also investigating the presence and association

between tick species and different habitats in combination with variation of environmental parameters. Finally, I followed several projects on pathogenic microorganisms, causing vector/tick-borne diseases, under different perspectives. In particular, I designed diagnostic protocols for molecular detection of bacteria belonging to the genera *Rickettsia*, *Ehrlichia* and *Anaplasma* and the pathogenic bacteria *Francisella tularensis* and *Coxiella burnetii* in hard ticks Ixodidae and in other biological matrix. Indeed, the PCR detection of bacterial pathogens in ticks represented a first step that later allow to apply these diagnostic molecular methods in other context. For example the protocols optimized on ticks were also used to detect pathogenic bacteria directly in human matrices (e.g. *B. burgdorferii* s.l. spirochetes in skin biopsies and cerebrum-spinal fluid), animals (e.g. *Rickettsia* sp. in blood of dogs) and environmental source (e.g. *Francisella tularensis* and MAP in water samples) for the interest respectively of IRCCS (humans) and IZSLER. In the framework of the investigation on tick-borne pathogens, I reported the presence of spirochetes of the species *Borrelia afzelii* and *B. lusitaniae*, both implicated in development of Lyme disease in humans, in an highly populated area close to the industrial district of Milan. This investigation allowed to describe human cases of borreliosis in a zone, previously not consider at risk of tick infestation or endemic for Lyme disease.

In 2010 I was selected to undertake a 8-month mobility period, within the framework of my doctorate studies, at the Federal University of Alagoas, UFAL - Brazil. The mobility grant (Erasmus Mundus) allowed me to develop independent research in the laboratory of Chemical Entomology (Laboratório de Química Entomológica, Departamento de Química) at the University of Alagoas and also to spend a short period of time in the laboratory of Prof. Marcelo Labruna at University of Sao Paulo (USP); Prof. Labruna is one of the major expert in ticks and tick-borne diseases of South America.

All research projects developed during my PhD work, including collaboration with other Institutions (IRCCS and IZSLER) were coordinated by my supervisor Prof. Claudio Bandi.