

Preface

In the framework of a project to investigate bacterial pathogens transmitted by ticks, developed in 2003 at the faculty of Veterinary Medicine at the University of Milan, the use of universal bacterial primer pairs (targeting 16S rDNA gene), in a screening of hard ticks, allowed to obtain bacterial sequences lacking significant match in the sequence databases. These sequences were obtained by an engorged female of the tick *Ixodes ricinus*. The great majority of sequenced clones resulted to belong to an unknown bacterium, indicating that this particular microorganism represented a preponderant presence in the bacterial community of the tick. This massive number of bacteria, suggested the possibility to have encountered a symbiont. The research on the microorganism was implemented following various approaches. A specific PCR was developed based on the known new bacterial 16S rDNA sequence. A screening on specimens of *I. ricinus* collected in several localities covering the complete geographical area of distribution of the species, from Scandinavia to North Africa and from Portugal to Russia, consented to confirm the presence of the bacteria in the great majority of females and in males, with lower prevalence. Preliminary phylogenetic analysis based on 16S rDNA sequences clustered this new bacterium as a member of the order Rickettsiales (phylum: Alpha-proteobacteria), comprehending animal and human bacterial pathogens of the genera *Rickettsia*, *Ehrlichia*, *Anaplasma*, and *Wolbachia* that is a parasites or a symbionts of several orders of insects, also present in nematodes. All members of the order Rickettsiales are obliged intracellular bacteria. Specific fluorescent probes targeting ribosomal 16S rRNA were designed and FISH technique (Fluorescent In Situ Hybridization) was applied to obtain information on the location of the bacteria inside the body of the ticks. High bacterial population was detected in the ovaries, as frequently reported for other symbionts. PCR on laid eggs showed 100% positivity confirming the capacity of these bacteria to be passed at the progeny through vertical transmission.

When researches decided to investigate the ultra-structural details on the localization of the microorganism in the ovaries through TEM microscopy (Transmission Electron Microscopy) a great surprise was ready to come. The TEM images showed bacteria in the cytoplasm of the ovarian cells, but some bacteria were also, surprisingly, harbored inside mitochondria of the same cells. No other bacteria had been described to occupy this location, and this micro-organism, with some characteristic of symbiont was temporarily named IricES1 (*Ixodes ricinus* endo-symbiont 1).

One of the researchers (N.L.) working on the microorganism proposed the name *Midichloria mitochondrii* for this bacterium, to acknowledge George Lucas, that introduced the concept of symbiotic organisms in the Star War movie series, to explain the ability of Jedi masters to enter in contact with the Force. I started to work on *M. mitochondrii* in 2008 when several questions were still waiting for an answer. My colleagues were organizing the work of full genome sequencing of this organism and we were also planning experimental work to investigate the infectious potential of this bacterium for vertebrates and humans. The capacity of *M. mitochondrii* to survive in different cell lines, outside of mitochondria, and the interaction with other bacteria in the complex microbiota of ticks were also objects of interests. In 2010 the genome of *M. mitochondrii* was completed. Finally, the possibility to rear and maintain ticks in laboratory conditions associated to the culture of *M. mitochondrii* in tick cells lines, demonstrated in 2011, open new research opportunity above all in association with the knowledge derived from the genomic studies.