Dear Dr. Žákovská,

Thank you very much for the review on my bachelor thesis and your valuable comments.

I would like to apologize for not precise description of the Figures. Next time I will try to make it clearer, thank you for that comment.

About the pictures of *B. miyamotoi* under the microscope - I had the plan to add them in my presentation. Thank you also for this remark.

Figure 1 below shows a draft-map of sites of tick collections that was produced within the frames of the research project QK 1920258 "Spread of ticks and tick-borne diseases: novel and neglected risks to domestic and farm animals and humans" supported by the Ministry of Agriculture, ZEMĚ. Withing the grant period (3 years) thousands of ticks were collected at the localities marked on the map. The classical way of tick collection based on flagging of vegetation (using a piece of tissue of size cca.1m x 1m) was used. Ticks of all developmental stages, mostly nymphs and adults, were collected separately into 50 ml plastic tubes and were kept alive at 4^{o}C until analysis. One half of tick was used for total DNA purification with further PCR analyses of the presence of spirochete species in them, while the second part was used to initiate the culture in liquid MKP medium. I worked with small group of samples from ticks collection that led to selection of 10 isolates that were of interest for us. All cultures that I worked with were obtained from nymphs of *Ixodes ricinus*.

All 10 isolates, that I worked with in frame of my bachelor project were co-infected with different *Borrelia* species, including *B. miyamotoi*. Neither one of tick originated spirochete cultures, that were analyzed previously by other lab members and later on by me, in frame of project QK2019258, was confirmed to have only *Borrelia miyamotoi*. That is why we decided to use the separation of spirochete species by cultivation on solid media as the 'simplest" way for obtaining of monoclonal population of this pathogen.

I hope my answers addressed your comments appropriately. I will add them in my presentation. I want to say once again thank you for your questions, remarks and evaluation of my thesis.

Yours sincerely,

Angela Kienberger

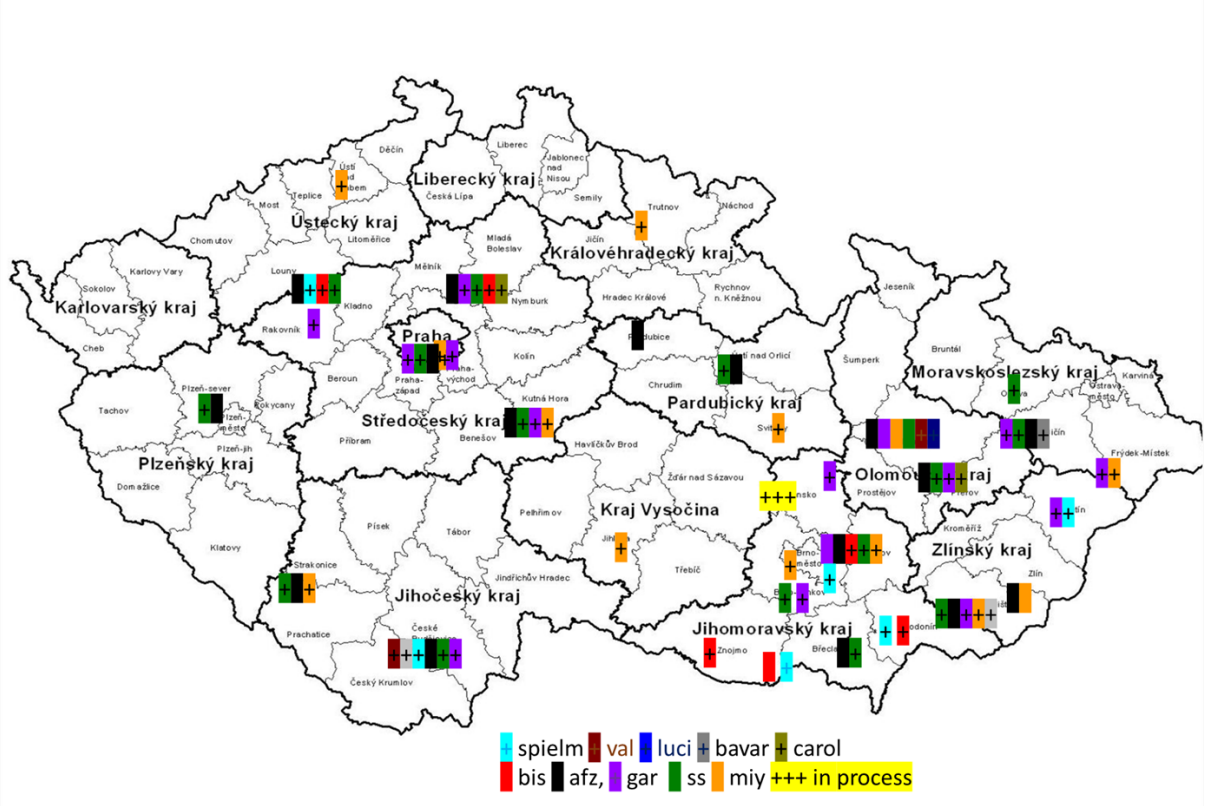

Figure 1: Draft map of sites of tick collections, project QK2019258. Final certified map is available on web pages of the project.

Table 1: Tick isolates selected for my Bc. project (*B. miyamotoi* co-infected with different species of *B. burgdorferi* s.l.)

Area of tick collection / Isolate number	*Borrelia* strains detected by PCR
Branišov 2 (B2)	*B. valaisiana* + *B. miyamotoi*
Branišov 6 (B6)	*B. afzelii* + *B. miyamotoi*
Divči Hrady 3 (DH3)	*B. afzelii* + *B. miyamotoi*
Heroltice 5 (H5)	*B. afzelii* + *B. miyamotoi*
Heroltice 6 (H6)	*B. afzelii* + *B. miyamotoi*
Milovice 2 (M2)	*B. afzelii* + *B. miyamotoi*
Milovice 7 (M7)	*B. burgdorferi* s.s. + *B. garinii* + *B. miyamotoi*
Milovice 8 (M8)	*B. garinii* + *B. miyamotoi*
Milovice 9 (M9)	*B. afzelii* + *B. miyamotoi*
Netolice 3 (N3)	*B. burgdorferi* s.s. + *B. miyamotoi*

Figure 2 shows a map of localities of the isolates selected for this project with the *Borrelia* strains detected by PCR in my isolates.