SCIENTIFIC REPORT of Short-Term Scientific Mission (STSM)

STSM Reference Number: 33760
Cost Action: FA1404
Period of stay: From 06 April 2016 to 16 April 2016
Host: Professor Dr. Antonio CAMARDA, University of Bari "Aldo Moro", Bari, Italy
STSM applicant: Alexandra GRUIANU, Ph.D. Doctoral School in Veterinary Medicine of Bucharest

STSM Project Title: *Epidemiological study of Dermanyssus gallinae in laying hens from Romania*

The purpose of the STSM

The aim of this study was the molecular identification and characterization of *Dermanyssus gallinae* collected during an epidemiological survey performed in South Romania, targeted to gather data about the diffusion of poultry red mites in laying hens farms.

Molecular epidemiologic characterization of *D. gallinae* is based on the sequencing of genes such as the mitochondrial cytochrome oxidase subunit I (COI) gene, and the nuclear internal transcribed spacers (ITS) region of the ribosomal which separates the rRNA genes (Roy et al., 2010).

Also, a morphological identification of *D. galline* is necessary because this species may be confused with *Orinthonyssus sylviarum* (northern fowl mite) which has the same host and environment (Di Palma et al., 2012).

Description of the work carried out during the STSM

During the STSM stage the DNA extraction was performed from specimens of *D. gallinae* using PureLink® Genomic DNA Kits (Invitrogen).

The extracted DNA was used as a template for the PCR targeting the COI and ITS. The PCRs were carried out by using primers previously designed in the host section, and the used thermal cycle was previously determined according to the primers and the expected amplification product.

The PCR products were visualized by agarose gel electrophoresis, following ethidium bromide staining.

In order to prepare the DNA for the sequencing reactions, the PCR products were eluted from the agarose gel, ligated in a specific vector for cloning, and the recombinant plasmids were used to transform *E. coli* Mach1 cells. After selection of positive clones, recombinant plasmids were extract by using PureLink™ Plasmid Miniprep Kit (Thermo Scientific, Milan, Italy) for sequencing.

Also, the morphological identification of *D. gallinae* mites has been performed under the supervision of Professor Dr. Riccardo Lia from the Unit of Parasitology and Parasitic Diseases of the Veterinary Medicine Department.

The main results achieved

I. The PCR amplicons of *D. gallinae* were subjected for sequence analysis of cytochrome oxidase subunit I and internal transcribed spacer. The PCR products were visualized by ethidium bromide staining. The electrophoresis reveled a single band for each sample (A, B, C) and the length of the ITS band was 750 bp (bands 4-A, 5-B, 6-C) and 550 bp for COI band (bands 8-A, 9-B, 10-C). Bands 3 and 11 are for positive control (CP), and bands 2 and 12 are negative control (CN). Band 7 is the size marker (Figure 1).

The electrophoresis from the DNA samples (1 A, 3 B, 5 C) has indicate a length of 500 bp and this was performed for evaluating the quantity of DNA that we added in ligation

mix. Bands 2M, 4M and 6M are size markers (Figure 2).

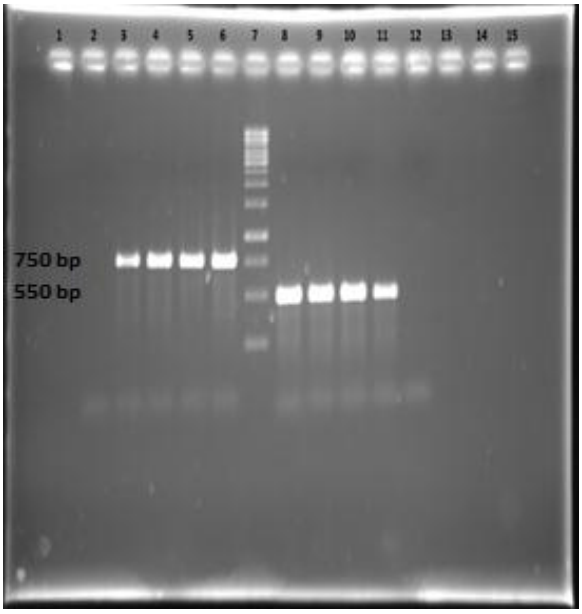


Fig. 1. Agarose gel electrophoresis of PCR (ITS and COI gens) from samples of *Dermanyssus gallinae*

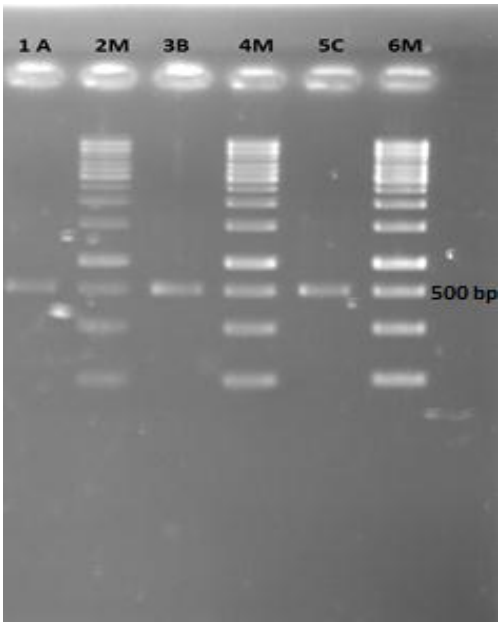


Fig. 2. Agarose gel with the bands for ligation

After transformation, six growth colonies from each sample were selected and PCR was performed to verify the presence of recombinant plasmids. The PCR products were loaded in agarose. The electrophoresis performed showed that the samples B and C were positive for recombinant plasmids. The A sample was negative, probably due to low concentration of PCR product (Figure 3).

Plasmids purification was performed for obtaining high purity plasmid DNA. The electrophoresis was to dose the quantity of plasmid for sequencing. The agarose gel was stained with ethidium bromide showed that the length of the plasmids band (3B and 5C) was 3.5 kb (Figure 4). After sequencing the plasmids, a phylogenetic analysis will be performed and a comparison between the sequence founded in Romania and the sequence detected in other European countries will be done.

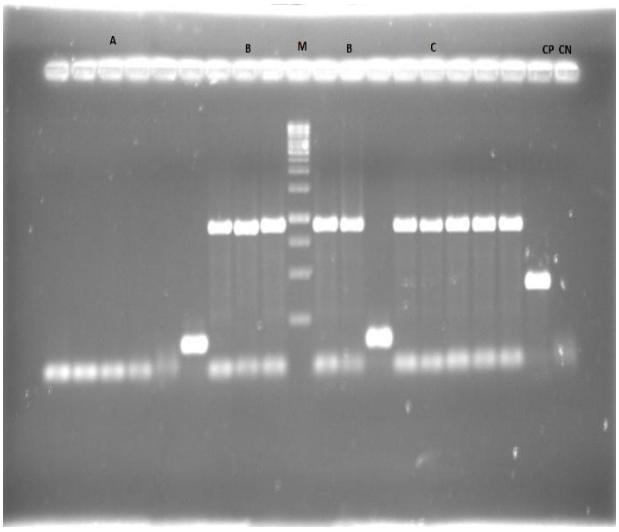


Fig. 3. Electrophoresis gel - A negative, B and C positive for recombinant

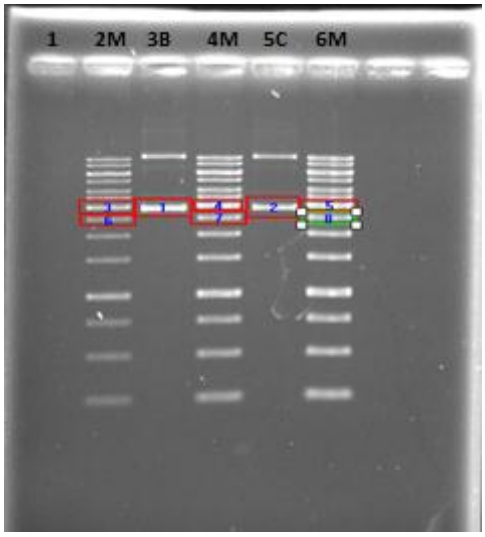


Fig.4. Agarose gel analysis of purified plasmid

II. The morphological identification of *D. gallinae* on stereomicroscope revealed the presence of second cheliceral article (Figure 5). Also, the identification of the parasite showed that genitoventral shield is posteriorly rounded with one pair of seta (Figure 6) and the sternal shield is wider than longer (Figure 7). The anal shield has three setae (Figure 8).

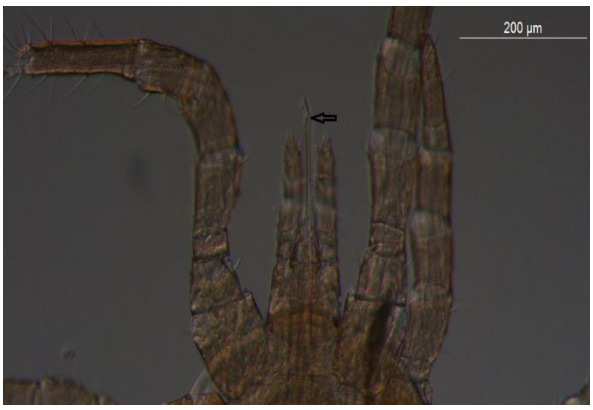


Fig. 5. The cheliceral article (on the arrow) and the palps of *D. gallinae*

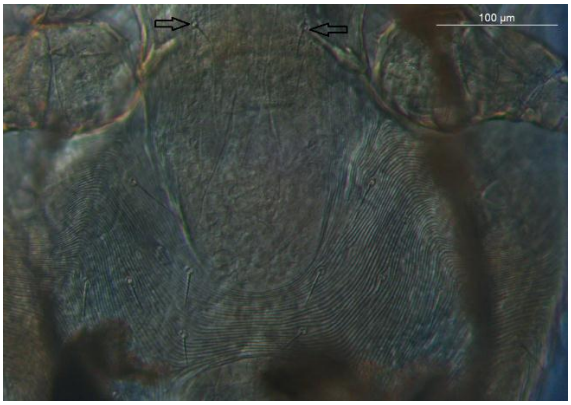


Fig.6. The genitoventral shield of *D. gallinae* with one pair of setae

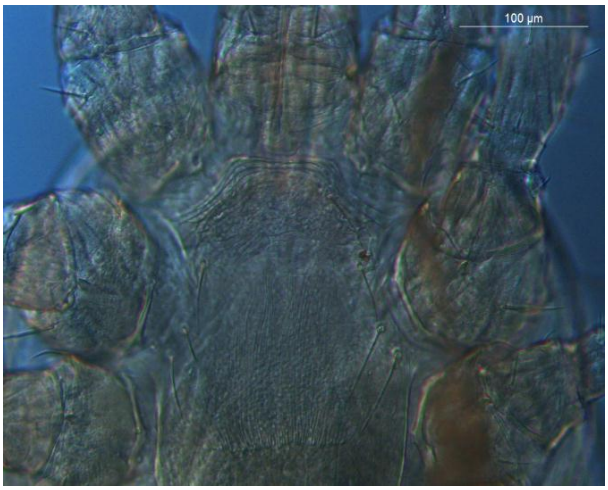


Fig. 7. Wider sternal shield of *D. gallinae*

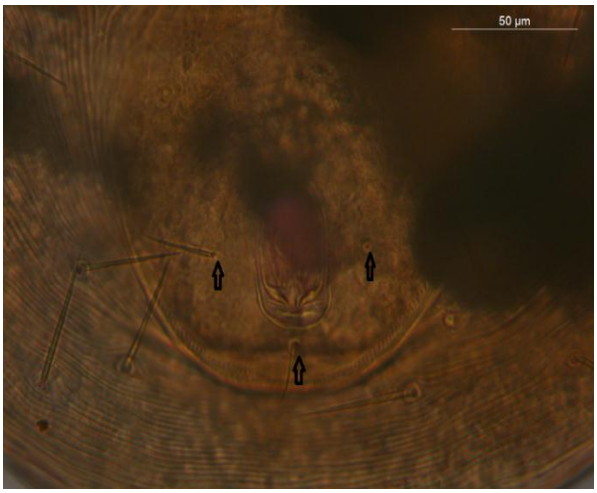


Fig. 8. Anal shield of *D. gallinae* with three setae

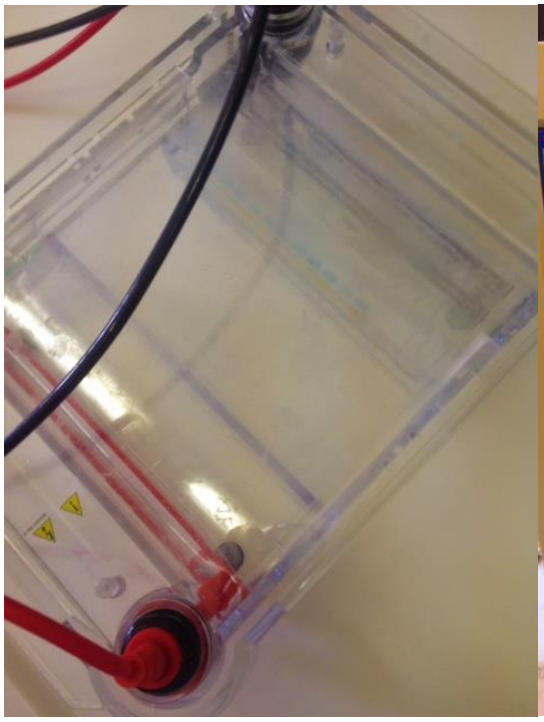
The results obtained highlight that the identification of the mite species is necessary for a better understanding of mites epidemiology and transmission between farms.

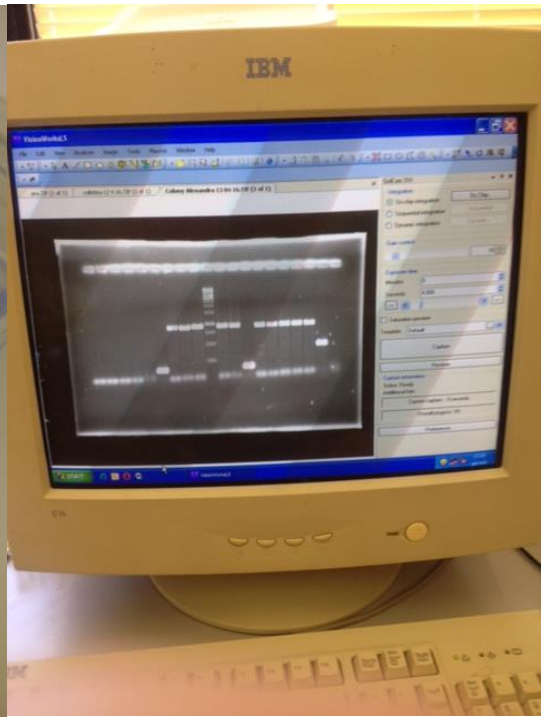
Future collaboration with the host institution

The epidemiological study will be extended in other areas of Romania and in farms with different production systems and the molecular identification will be performed in close collaboration with University of Bari ”Aldo Moro”, Italy. Also, the knowledge and the results obtained from this research stage will be included in upcoming results in co-authoring publications.

References

1. **Di Palma A., Giangaspero A., Cafiero M. A., Germinara G. S., 2012.** A gallery of the key characters to ease identification of *Dermanyssus gallinae* (Acari: Gamasida: Dermanyssidae) and allow differentiation from *Ornithonyssus sylviarum* (Acari: Gamasida: Macronyssidae). *Parasites and Vectors* 5:104
2. **Roy L., Doweling A. P., Chauve C. M., Buronfosse T., 2010.** Diversity of phylogenetic information according to the locus and the taxonomic level: an example from a parasitic mesostigmatid mite genus. *International Journal of Molecular Science* 11(4): 1704-1734

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