Summary of Ph. D. thesis

Bioinformatics analysis of the waterfowl pathogen *Mycoplasma* species

Kovács Áron Botond

Supervisor: Dr. Miklós Gyuranecz

UNIVERSITY OF VETERINARY MEDICINE

Doctoral School of Veterinary Science

Budapest, 2025

Supervisor:

....

Miklós Gyuranecz, Ph.D.

HUN-REN Veterinary Medical Research Institute

supervisor

....

Áron Botond Kovács

1. Introduction

Mycoplasma anserisalpingitidis and *Mycoplasma anatis* are particularly important due to their economic and ecological impacts on waterfowl hosts. *Mycoplasma anserisalpingitidis* was first identified in a goose in 1983 in Hungary, while *M. anatis,* first isolated in the United Kingdom in 1964 during a sinusitis outbreak. High isolation rates from both healthy and diseased birds, along with experimental studies, suggest an opportunistic nature of *M. ansersalpingitidis* and *M. anatis*, the former most commonly isolated from domestic geese, while the latter primarily colonizes both wild and domestic ducks. Mycoplasma infections in waterfowl can spread horizontally, often through migratory waterfowl and vertically. Infections caused by *M. anserisalpingitidis* often result in phallus inflammation, that can progress to necrosis and impaired function, while *M. anatis* infection can cause air sacs and the pericardium to be covered with fibrin, peritonitis, the lungs of the animals to be hyperaemic and slightly edematous. Mycoplasmas typically possess compact genomes-This limited genomic size, combined with the lack of certain DNA repair mechanisms can lead to a comparatively higher mutation rate. Next-generation sequencing can be a way of better understanding the genomic characteristics of these bacteria, leading to more reliable diagnostic methods. There are multiple methods that are based on whole genomes, like the Average nucleotide identity (ANI), average amino acid identity (AAI), the core genome multi-locus sequence typing (cgMLST and Genome wide association study (GWAS). The ANI analysis involves calculating the average percentage of identical nucleotides across the genomes, while AAI is similar, but based on amino acids. GWAS employs statistical methods to correlate genotypes with phenotypes, utilizing whole genomes. There are multiple ways of searching for prophages most prominently using database dependent or independent *in silico* tools.

2. Aims of the study

The aims of the study were:

Ad 1. to set up a core genome multi-locus sequence typing (cgMLST) scheme for *M. anserisalpingitidis*

Ad 2. to evaluate the extent of the genetic and phenotypic variability among *M. anserisalpingitidis* strains and map the variety of the species

Ad 3. to conduct a genome wide association study to evaluate the genetic background of *M. anserisalpingitidis* strains for commonly used antibiotics

Ad 4. to investigate potential past horizontal gene transfer events between *M. anserisalpingitidis* and M. anatis, focusing on prophage like sequences

3. Materials and methods

Sequencing and *de novo* assembly

The DNA of the strains were extracted from 10 ml of logarithmic-phase broth cultures of the strains using the QIAamp DNA Mini kit (Qiagen Inc., Hilden, Germany) following the manufacturer's instructions. Nucleotide sequences were determined using next-generation sequencing on an Illumina® NextSeq 500 sequencer (Illumina, San Diego, CA, USA) following the manufacturer’s protocol. The quality checks of the short reads were performed using FastQC software. A draft genome was assembled for all of the strains with SPAdes software using the default parameters. The contigs of the draft genomes were ordered using ATCC BAA-2147 with the MAUVE algorithm) and annotated with the NCBI Prokaryote Genome Annotation Pipeline.

The core-genome multi locus sequence typing

To establish a cgMLST scheme, a wgMLST scheme was created, which contained all of the CDSs with the chewBBACA software.Then the paralogous alleles were filtered to create the cgMLST scheme, which was set up using the standard 95% presence threshold.

The wgMLST scheme was set up with the “CreateScheme” operation, creating wgMLST scheme. This scheme was used in the “AlleleCall” operation, creating the cgMLST scheme only containing alleles that are present in the majority of the strains. Finally the cgMLST scheme was populated using the “ExtractCgMLST” operation using the standard 95% presence threshold.

Prophage-like sequences in *M. anserisalpingitidis* and *M. anatis* strains

A pilot study was done in two steps, an initial search with PHASTER, PhiSpy and Prophage Hunter and a secondary search on the length filtered PhisPy results. The secondary search was done with PHASTER and Prophage Hunter.

The VIBRANT software was used as a novel approach. The tool analyzes consecutive annotations and compares it with the Kyoto Encyclopedia of Genes and Genomes (KEGG), the Pfam and the Virus Orthologous Groups (VOG) databases. In this step, *M. anatis* strains have been included to determine if the putative prophages have infected this closely related species as well. The prophage-like sequences found by the VIBRANT software were mapped to their respective draft genomes and later to the genomes of other strains, with the Geneious Prime software. The BLAST Ring Image Generator (BRIG) was used to search analyze all of the putative prophages, with lengths exceeding 10,000 bp in the previously assembled scaffold (with MeDuSa) of the *M. anserisalpingitidis* strain MYCAV270.

The genomic and phenotypic description of atypical *M. anserisalpingitidis* strains

Phenotypical characterization

Five *M. anserisalpingitidis* strains (MYCAV61, MYCAV93, MYCAV783, MYCAV785 and MYCAV903) showed significant genomic divergence from the rest of the strain collection. Five additional strains (ATCC BAA-2147, MYCAV34, MYCAV47, MYCAV512 and MYCAV667) were selected as the control group. The concentration of viable mycoplasma cells was determined and diluted to a uniform concentration of 10^4 color-changing units per ml (CCU/ml). For the characterization of the atypical strains, the following phenotypic characteristics were selected: temperature preference, utilization of carbohydrates (glucose and fructose); arginine, urea and pyruvate utilization. In addition to the conventional broth (V1) seven other broths were created based on it (V2-V8), for a total of eight broths. The broths contained either glucose (V1 to V4) or fructose (V5 to V8), arginine (V1, V2, V5 and V6), or urea (V3, V4, V7 and V8) and pyruvate (V1, V3, V5, V7). The test was conducted at two temperatures (33 and 37 °C). These results were then compared using the Kolmogorov-Smirnov statistical test in using the R software.

Growth inhibition test

The five atypical strains, the five control strains and the *M. anatis* type strain (NCTC 10156) were subjected to growth inhibition test by anti-*M. anserisalpingitidis* hyperimmune rabbit serum based on the method described by Clyde (1964). Growth inhibition test was conducted, using 20 µl 'running drops' of *M. anserisalpingitidis* broth cultures were applied at a dilution of 10^4 CCU/ml on Petri dishes containing 7 ml Mycoplasma solid medium. After absorption into the agar, one blank antimicrobial susceptibility disc impregnated with 20 µl *M. anserisalpingitidis* hyperimmune serum was placed on each running drop. This set-up was kept maintained at a CO2 level of 5% at 33 °C for one day, and then at 37 °C for an additional two days. Growth-inhibition zones around the discs were checked daily for 10 days under a stereo microscope.

In silico comparative analysis of the *M. anserisalpingitidis* strains

The comparative analysis of 16S, ITS and 23S rRNA and of the housekeeping genes of the strains

The 16S rRNA, ITS region and 23S rRNA sequences off the strains were extracted. The genes were concatenated and aligned with the MAFFT algorithm. A phylogenetic tree was generated from the alignment with the MEGA-X software using the Maximum Likelihood method with the General Time

Reversible model with Gamma distribution (GTR+G) with bootstrap set to 1000 iterations.

The following housekeeping genes were included in the study: *dnaE*, *fusA*, *parC*, *pyk*, *rpoB*, *rpoC*, *rpoD*, *topA* and *uvrA,* based on Volokhov and co-workers' description, while randomly selected genes were also added: *atpB, ileS, secY, tig* and *uvrD*. The gene sequences were concatenated and aligned with MAFFT algorithm, then a Maximum Likelihood phylogenetic tree was created using the alignment in MEGA-X with General Time Reversible model was chosen with Gamma distribution and invariant sites (GTR+G+I) with 1000 bootstraps.

Average nucleotide identity (ANI) and average amino acid identity (AAI) analysis of the strains

The FastANI software was used to analyze the average nucleotide identity of the genomes. The ANI analysis of the *M. anserisalpingitidis* strains, was conducted both intra- and interspecies; for interspecies analysis *M. anatis* strains were also used. To calculate the AAI values, the CDSs were translated of the strains into protein sequences and the analysis was done with Environmental Microbial Genomics Laboratory's AAI tool, limited to 50 strains, using the five atypical, the reference and 44 randomly selected strains.

Horizontal gene transfer analysis between M*.* *anatis* and *M. anserisalpingitidis* strains

The genomes of both *M. anserisalpingitidis* and *M. anatis* strains were examined for potential horizontal gene transfer (HGT) events using the IslandCafe software. The tool searches for genomic islands within genomes, are good indicators for past HGT events and can be compared with the result of the search for prophage-like sequences and can further elaborate those results.

The genome wide association study

Antimicrobial agents

The antimicrobial susceptibility testing of the strain collection was performed against nine antimicrobial agents: tiamulin, oxytetracycline, doxycycline, tylosin, tylvalosin, tilmicosin, lincomycin, spectinomycin and enrofloxacin. The dilution and storage of the antibiotics, broth microdilution examinations, and determination of the MIC values were performed according to the recommendations of Hannan (2000).

Broth microdilution method

The number of viable mycoplasma cells was determined and diluted to a uniform concentration of 10^4 CCU/ml. Mycoplasma broth medium was used in the assay, each 96-well microtitre plate contained growth controls (broth medium and mycoplasma inoculum, without antibiotic agents), sterility controls (broth medium only, without antibiotic and mycoplasma inoculum) and pH controls (broth medium adjusted to pH 6.8). The minimum inhibitory concentration (MIC) values were determined from the lowest concentration of the antimicrobial agent where no pH and color change could be observed. The antibiotics were applied within the following MIC ranges: 0.25–64 μg/mL (tylosin, tylvalosin, tilmicosin, lincomycin, oxytetracycline and spectinomycin) and 0.039-10 μg/mL (doxycycline, enrofloxacin and tiamulin).

Genotype – phenotype association test and promoter mapping

The genotype and phenotype association was done using the pyseer tool. The genetic distance between the strains was calculated using the cgMLST scheme. The k-mers were counted using fsm-lite software. The genome wide association study was done following the best practices suggestions on the pyseer software's documentation, using the phenotype file containing the MIC values, the distance matrix based on the cgMLST scheme and the k-mers from the fsm-lite count. Linear mixed model was used to better account for the genetic relatedness and similarity of the strains. The association study was repeated nine times in total, once for each antimicrobial agent. Multiple statistical testing can result in p-value inflation; thus, a more stringent criterion is used in genome wide studies $p < 5 \times 10^{-8}$ in our case.

4. Results

Sequencing and *de novo* assembly

We used 110 *M. anserisalpingitidis* strains in this study, resulting in 110 so called draft genome files. On average, the longest contig among the assemblies were 96619 bp (average Phred score was 35.4; average number of short reads: 4753178).

The core-genome multilocus sequence typing of *M. anserisalpingitidis* strains

The cgMLST scheme extracted from the complete CDS set using chewBBACA, included 540 CDSs at 95% gene presence threshold. This represented approximately 68.87% of the CDS set of the ATCC BAA-2147 strain. Strains from the same integration clustered together in the phylogenetic tree; however, cluster I included three strains (MYCAV47, MYCAV49 and MYCAV69) which were not part of the integration. In contrast cluster II included strains with a much more diverse background both geographically and temporally. A correlation was observed between the cgMLST profiles of the strains and geographical origin, as well as collection dates within both clusters. Strains isolated from different tissues within the same animal also showed significant similarity in their cgMLST profiles.

The genomic and phenotypic description of atypical *M. anserisalpingitidis* strains

Phenotypical characterization

Statistically significant differences have been observed between the atypical strains and the control group when weighed by growth speed (p-value = 0.0086, D = 0.25). The cumulative growth over five days showed control strains achieved higher values compared to atypical strains. All of the strains showed a temperature preference for 37 °C. The fructose content was a limiting factor for the growth of the strains, as all reached acidic color change later, with the exception of ATCC BAA-2147 and

MYCAV512 at 33 °C. None of the atypical strains grew in fructose- containing broths at 33 °C or grew very slowly, except for MYCAV61.

Growth inhibition test

The following average distances were measured in the control group: 5.669 mm for MYCAV34, 6.437 mm for MYCAV47, 5.157 mm for MYCAV512, 6.138 mm for MYCAV667 and 4.669 mm for ATCC BAA- 2147. The average distance of the control group was 5.850 mm. The measurement of the average distances of the atypical strains gave the following results: 5.401 mm for MYCAV61, 2.728 mm for MYCAV93, 2.564 mm for MYCAV783, 4.912 mm for MYCAV785 and 3.172 mm for MYCAV903. The average distance of the atypical strains was

3.364 mm. The average distance of the *M. anatis* type strain was 1.408 mm.

The comparative analysis of 16S, ITS and 23S rRNA and of the housekeeping genes of the strains

All of the *M. anserisalpingitidis* strains clustered together, the atypical strains were clearly separated within the *M. anserisalpingitidis* branch. While the *M. anatis* type strain formed a separate branch, the atypical strains were still clearly part of an isolated cluster, distinct from the rest of the *M. anserisalpingitidis* strains.

Average nucleotide identity (ANI) and average amino acid identity (AAI) analysis

A clear gradient is evident across the strain collection, with a notable distinction not only between the five atypical strains and the remaining strains but also among the strains within the main collection itself. The atypical *M. anserisalpingitidis* strains fall short of the usual threshold at which samples can be considered members of the same species (94–95% identity). The *M. anatis* strains only reach approximately 85% identity with the *M. anserisalpingitidis* samples, clearly demonstrating the differences between these two species. The AAI analysis further supported the result of the ANI analysis; however, the differences among the strains were much smaller, due to the presence of silent mutations within coding sequences.

Horizontal gene transfer analysis between *M. anatis* and *M. anserisalpingitidis* strains

The IslandCafe tool found genetic islands in all of the strains, with a minimum of 3 and a maximum of 16 islands detected. However, none of the genomic islands were able to account for the observed differences in the genomes.

The core genome multilocus sequence typing of all the strains

This scheme contained 105 alleles, all of which were found in 95% of both in *M. anatis* and *M. anserisalpingitidis* strains' genomes. The two species were on separate branches and the atypical strains clustered with the rest of the *M. anserisalpingitidis* strains. The phylogenetic tree derived from the joined cgMLST analysis corroborated the results from the ANI, AAI and housekeeping gene analyses.

The genome wide association study of M. anserisalpingitidis strains

Among the three macrolides investigated (tylosin, tilmicosin, and tylvalosin), tylvalosin showed the lowest MIC_{50} value (0.5 µg/ml) and MIC_{90} value (2 µg/ml) against the strains. The MIC values for tilmicosin, tylosin (≤0.25 to >64 µg/ml respectively, with a 16 µg/ml MIC_{50} and a ≥64 µg/ml MIC_{90}), and tylvalosin (≤0.25 to 16 µg/ml) exhibited a wide range. Notably high MIC_{50} and MIC_{90} values were recorded for tilmicosin (≥64 µg/ml for both MIC_{50} and MIC_{90}). The MIC values for lincomycin were grouped around the MIC_{50} value (2 µg/ml), although in the case of one isolate elevated MIC values (>64 µg/ml) could be observed, within a range of 0.25 to >64 µg/ml and with a 4 µg/ml MIC_{90}. Tetracyclines displayed significantly broad ranges of the MIC values (≤0.25

to 64 µg/ml in the case oxytetracycline and ≤0.039 to >10 µg/ml for doxycycline). Their corresponding MIC_{50} value was particularly high (16 and 5 µg/ml respectively), similarly with the MIC_{90} values (64 and 10 µg/ml, respectively). Spectinomycin displayed a MIC_{50} of 8 µg/ml, with a significant number of the strains displaying values over the MIC_{50} value, within a range of 4 to >64 µg/ml and with a 16 µg/ml MIC_{90}. The MIC values for enrofloxacin also exhibited a wide range (0.312 to 10 µg/ml), with a 5 µg/ml MIC_{50} and a 10 µg/ml MIC_{90} values. The tiamulin also showed a broad range of MIC values between 0.156 and 2.5 µg/ml. The MIC_{50} value of tiamulin was 0.625 µg/ml, while the MIC_{90} was 2.5 µg/ml.

The pyseer analysis found various numbers of significant k-mers (p-value ≤ 5x10-8) for five out of the nine antimicrobial agents. In the case of the macrolides for tilmicosin and tylvalosin, the software found 92 and 727 k-mers, respectively, however no significant k-mers could be found in the genomes for tylosin. For lincomycin, the tool managed to identify 1 173 significant k-mers. No significant k-mers were found for either oxytetracycline or doxycycline. The software found 109 significant k-mers for spectinomycin and 436 significant k-mers were found for enrofloxacin. There was no significant k-mer associated with tiamulin resistance. Promotech found 3 437 promoters in the genome of BAA-2147 type strain.

Prophage-like sequences in *M. anserisalpingitidis* and *M. anatis* strains

The initial screening using PHASTER screening found sequences with prophage-like characteristics in all of the strains, however, these hits had exhibited a low score (ranging from 10 and to 40). The PhiSpy software also detected prophage-like sequences in all of the annotated draft genomes with the exception of MYCAV39, MYCAV94 and MYCAV218. The Prophage Hunter software gave positive hits in all of the sequences, however, only MYCAV34, MYCAV77, MYCAV202 and MYCAV670 contained prophage-like sequences that were identified as mycoplasma prophages. All of the methods pointed toward the possibility that our strains contained prophages, but none of the results showed significant overlap with each other. Due to the lack of a consensus in the results from the previous analyses, a new approach was used. The VIBRANT software was chosen as a novel method to further investigate potential prophages in the strains for potential novel prophages. The tool found prophage sequences in 37 out of the 77 strains analyzed. The length of the results sequences varied between 1870 and 59948 bp. As the sequences that were shorter than 10 000 bp are unlikely to be intact prophages, only the prophage-like sequences that were larger than that, were further analyzed. The mapping of these sequences (n = 31) showed that these were intact prophage-like regions and unlikely to be part of a fragmented prophage in the genomes of the strains. Out of these 31 putative prophages 22 showed significant similarity with *M. anatis* NCTC 10156 strain, based on nucleotide BLAST searches of their CDSs (coverage between 57.00–100.00% and identity between 90.98–93.52%). Ten *M. anatis* strains were also checked with VIBRANT software to validate whether these prophage-like sequences were present in other waterfowl mycoplasmas. The VIBRANT software found putative prophages in four *M. anatis* strains, and three of these overlapped with the putative prophages found in strain MYCAV270.

5. Discussion

The core-genome multi locus sequence typing

The developed cgMLST scheme constituted of 540 CDSs, about 68.87% of the CDS set of *M. anserisalpingitidis* type strain ATCC BAA-2147. This scheme resulted in a sequence type assay that not only differentiated between all examined strains in question but also clustered them into sub-clades that showed a great deal of correlation with the metadata of the samples. Both the wgMLST and cgMLST-based phylogenetic trees were significantly similar, which indicates that our core genome scheme is robust enough to be used as a basis of further studies. The observation that strains from the same integration (cluster II) exhibited greater variability and an absence of correlation between genotype and geographical origin may suggest frequent movement among host flocks or potential inter-host contact. The substantial number of Hungarian samples suggests that the infection may be prevalent in the Carpathian Basin; however, the presence of samples from Poland, Sweden, and China indicates that the microorganism is likely present world-wide. China is the leading global producer of geese, making it essential to investigate the prevalence of *M. anserisalpingitidis* infection in the region.

The genomic and phenotypic description of atypical *M. anserisalpingitidis* strains

Evidence indicates distinct genetic boundaries among prokaryotic species nevertheless, while bacterial species can be categorized into taxonomic ranks, these boundaries are likely to differ from species to species. The findings from both the ANI and AAI analysis exhibited a gradient in both the nucleic and amino acid identity, showing that the atypical strains from various locations demonstrated greater similarity to one another than to the other strains. The atypical *M. anserisalpingitidis* strains exhibited a slight variation from this value, nevertheless, this variation was negligible, at just a few percentage points (0.6% at most), and it occurred in only among limited number of strain combinations. The five atypical strains described in our study were isolated from diverse geographical locations including China, Vietnam, and Hungary, in addition to different hosts as swan goose and domestic goose, and from various organs including the cloaca, phallus, and phallus lymph.

The genome wide association study of *M. anserisalpingitidis* strains

There are several ways that bacteria might react with and protect themselves from antibiotics through various mechanisms. Antimicrobial resistance can arise through several mechanisms, including elevated efflux pump activity, alteration of the targets of antimicrobial agents (e.g., macrolides like tilmicosin, tylosin, and tylvalosin), or decreased uptake of antimicrobial agents (e.g., against aminoglycosides like spectinomycin). we identified that various transferases — particularly methyltransferases — frequently exhibit a significant association with decreased susceptibility to several antimicrobial agents, as well as DNA gyrase subunit B, DNA topoisomerase IV subunit A, and efflux pump subunits. This is consistent with the fact that different transferases might play a crucial and important role in antibiotic resistance. The second most common type of CDSs with significant hits were either members of efflux pumps or exhibited efflux pump activity. Bacteria can effectively protect themselves against a wide range of antimicrobial agents by enhancing their efflux activity; combined with decreased influx, these two processes collectively reduce the concentration of antimicrobial agents within cells.

Prophage-like sequences in *M. anserisalpingitidis* and *M. anatis* strains

Out of the 31 putative prophages identified in the *M. anserisalpingitidis* strains, 22 exhibited significant similarity to the *M. anatis* NCTC 10156 strain. Only five putative prophages exhibited a higher level of similarity to any *M. anserisalpingitidis* strains compared to the *M. anatis* NCTC 10156 strain. The cross-examination of the core genome of *M. anserisalpingitidis* indicated that only five putative prophage CDSs were present in the core genome, all identified in MYCAV669, demonstrating that the remaining sequences belong to the accessory genome. Three *M. anatis* strains (MYCAV786, MYCAV787, MYCAV788), showing ~90% similarity in the overlapping regions to one in *M. anserisalpingitidis* strain MYCAV270. There can be multiple reasons behind these inactive, putative prophages, e.g. horizontal gene transfer or phage infection in the past when *M. anatis* and *M. anserisalpingitidis* differentiated from their common ancestor. The fact that the prophage-like sequences were present in some, but not all of the waterfowl mycoplasma strains further strengthen the possibility that these sequences are prophages that infected these strains in the past.

6. Overview of new scientific results

Ad 1. We established the first core genome multilocus sequence typing (cgMLST) scheme for *M. anserisalpingitidis*, along with a dual *M. anserisalpingitidis* – *M. anatis* cgMLST scheme. The precise and robust nature of the cgMLST scheme is established as the gold standard for phylogenetic typing. In addition to phylogenetic analysis, the new scheme successfully clustered the strains according to their metadata, enhancing its utility in epidemiological surveillance.

Ad 2. Characterization of the five atypical strains revealed significant differences from other *M*. *anserisalpingitidis* strains considering both phenotypic and genotypic features. The atypical strains are on the verge of what could be considered as members of the same species and are potentially on the early steps of speciation.

Ad 3. This is the first genome-wide association study on *M. anserisalpingitidis*, representing only the third GWAS performed on mycoplasmas to date. This study revealed multiple antibiotic resistance mechanisms within *M. anserisalpingitidis* strains, identifying significant correlations between five out of nine antimicrobial agents and k-mers within their genomes

Ad 4. Our study successfully identified prophage-like sequences in multiple strains of *M*. *anserisalpingitidis* and *M. anatis*, marking the first time for these avian mycoplasmas.

7. Scientific publications

In peer-reviewed journals

Kovács, ÁB., Kreizinger, Z., Forró, B., Grózner, D., Mitter, A., Marton, S., Bali, K., Sawicka, A., Tomczyk, G., Bányai, K., Gyuranecz, M.: **The core genome multi-locus sequence typing of *Mycoplasma anserisalpingitidis*.** BMC Genomics, 21(1), 403., 2020

Kovács, ÁB., Kreizinger, Z., Forró, B., Grózner, D., Mitter, A., Marton, S., Bali, K., Sawicka, A., Tomczyk, G., Bányai, K., Gyuranecz, M.: ***Mycoplasma anserisalpingitidis* törzsek vizsgálata a maggenomot célzó multilókusz szekvenciatipizáló módszerrel.** Magyar Állatorvosok Lapja, 145, 527–534., 2023

Kovács, ÁB., Wehmann, E., Grózner, D., Bali, K., Nemeshházi, E., Hrivnák, V., Morrow, C. J., Bányai, K., Kreizinger, Z., Gyuranecz, M.: **Characterization of atypical *Mycoplasma anserisalpingitidis* strains.** Veterinary Microbiology, 280., 2023

Kovács, ÁB., Wehmann, E., Sváb, D., Bekő, K., Grózner, D., Mitter, A., Bali, K., Morrow, C. J., Bányai, K., & Gyuranecz, M.: **Novel prophage-like sequences in *Mycoplasma anserisalpingitidis*.** Infection, Genetics and Evolution, 92., 2021

8. Acknowledgements

I would like to thank my supervisor, Miklós Gyuranecz, for the many opportunities, support, inspiration and help through the years. Special thanks go to Enikő Wehmann who not only helped with her insights, but for her professional guidance and help in all of the studies.