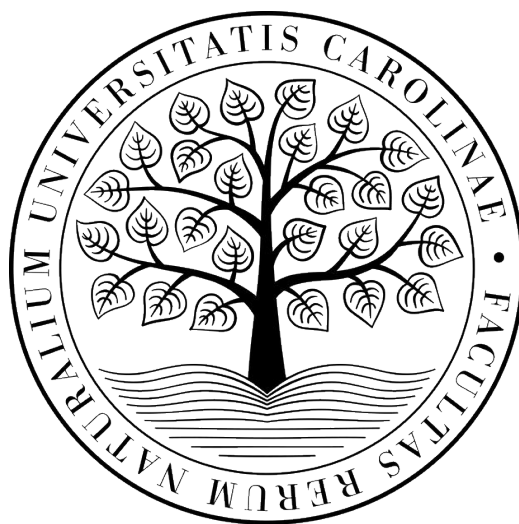


**Univerzita Karlova**  
**Přírodovědecká fakulta**

Studijní program: Zoologie

Studijní obor: Zoologie



**Mgr. Tereza Matějková**

**Integrated multi-omics analysis of chemical signaling in wild  
rodents**

Dizertační práce

školitel: prof. Mgr. Pavel Stopka, Ph. D.

konzultanti: Mgr. Romana Stopková, Ph. D., RNDr. Vladimír Beneš, CSc.

Praha, 2022

## SELECTED PUBLICATIONS

### PUBLICATION I

Moudra, A., Niederlova, V., Novotny, J., Schmiedova, L., Kubovciak, J., **Matejkova, T.**, Drobek, A., Pribikova, M., Stopkova, R., Cizkova, D., Neuwirth, A., Michalik, J., Krizova, K., Hudcovic, T., Kolar, M., Kozakova, H., Kreisinger J., Stopka, P., & Stepanek, O. (2021). **Phenotypic and clonal stability of antigen-inexperienced memory-like T cells across the genetic background, hygienic status, and aging.** The Journal of Immunology, 206(9), 2109-2121.

IF (2022) - 5.422

### PUBLICATION II

**Matějková, T.**, Hájková, P., Stopková, R., Stanko, M., Martin, J. F., Kreisinger, J., & Stopka, P. (2020). **Oral and vaginal microbiota in selected field mice of the genus Apodemus: a wild population study.** Scientific reports, 10(1), 1-11.

IF (2022) - 4.996

### PUBLICATION III

Stopkova, R., Matejkova, T., Dodokova, A., Talacko, P., Zacek, P., Sedlacek, R., Pialek, J., Stopka, P. (2023). **Variation in mouse chemical signals is genetically controlled and environmentally modulated.** Scientific reports, SUBMITTED

### PUBLICATION IV

Stopková, R., Otčenášková, T., **Matějková, T.**, Kuntová, B., & Stopka, P. (2021). **Biological roles of lipocalins in chemical communication, reproduction, and regulation of microbiota.** Frontiers in Physiology, 12.

IF (2022) - 4.566

### PUBLICATION V

**Matejkova, T.**, Dodokova, A., Kresinger, J., Stopka, P., Stopkova, R. (2023). **Microbiome, proteomic and metabolomic profiling of the estrous cycle in wild house mice.** MANUSCRIPT

## ABSTRACT

Symbiotic bacteria living with the host in so-called microbiomes have been one of the significant pillars of all aspects of animal evolution, chemical communication included. However, the phenotype, genotype, and microbiome of laboratory animals kept for generations in sterile conditions changed from their wild ancestors leading to profound differences in the laboratory results and the reality of wild animals. To describe the chemical communication in neglected wild rodents, this thesis focuses on the body parts involved in chemical communication (i.e. mouth, vagina, and intestines) and are also inhabited by microbiomes that produce metabolites with the capability of transmitting chemical signals. Using next-generation sequencing and state-of-the-art proteome and metabolome chromatography-mass spectrometry, this thesis covers the analysis of changes in the microbiome, proteome, and metabolome of wild mice in the context of transferring the wild individuals into the captivity, cohousing wild, and laboratory animals and hormonal changes during the estrous cycles. Moreover, this thesis describes and discusses the differences and similarities in the microbiome, proteome, and metabolome on the level of different species (*Apodemus sp.*), subspecies (*Mus musculus domesticus* vs. *musculus*), and environment (wild vs. laboratory origin). Results show that the core microbiome stays almost intact during the captivity in the case of oral microbiota while vaginal microbiota is less stable. For the first time, we showed that proteome and metabolome of urine transfer information about sex, the environment of origin, and the genetic background of mice. Finally, the results highlighted the high probability of functional interconnection among the vaginal proteome, metabolome, and microbiome. Our research brings comprehensive data that integrates proteome, metabolome, and microbiome results into the multi-omics picture of chemical communication among wild rodents.

Keywords: microbiome, metabolome, proteome, wild rodents, chemical communication, olfaction, mouse

## ABSTRAKT

Bakterie žijící v symbióze se svým hostitelem v takzvaných mikrobiomech jsou jedním z hlavních pilířů evoluce živočichů, včetně evoluce jejich chemické komunikace. Fenotyp, genotyp a mikrobiom laboratorních živočichů chovaných po generace ve sterilních podmínkách se však od jejich divokých předků změnil, což vede k výrazným rozdílům mezi výsledky získanými z laboratorních organismů a z jejich divokých protějšků. Tato práce se zaměřuje na chemickou komunikaci u volně žijících hlodavců. Konkrétně zkoumá části těla, které se přímo podílí na chemické komunikaci (tj. ústa, pochva a střeva) a které jsou zároveň obývány mikrobiomy produkující chemické signály. Změny mikrobiomu, proteomu a metabolomu divokých myší jsou v této práci studovány pomocí sekvenování nové generace a nejmodernější proteomové a metabolomové chromatografie - hmotnostní spektrometrie. Práce analyzuje změny mikrobiomu v rámci přesunu divokých jedinců do zajetí, společného soužití divokých a laboratorních zvířat a také v kontextu hormonálních změn během estrálních cyklů. Dále tato práce diskutuje rozdíly a podobnosti v mikrobiomu, proteomu a metabolomu na úrovni různých druhů (*Apodemus sp.*), poddruhů (*Mus musculus domesticus* vs. *musculus*) a prostředí (přirozený vs. laboratorní původu). Výsledky ukazují, že mikrobiom zůstává během zajetí téměř neporušený v případě orální mikrobioty, zatímco vaginální mikrobiota je méně stabilní. Poprvé jsme ukázali, že proteom a metabolom moči obsahuje informace o pohlaví, původu a genetickém pozadí myší. Nakonec výsledky podtrhly vysokou pravděpodobnost funkčního propojení mezi vaginálním proteomem, metabolomem a mikrobiomem. Náš výzkum přináší komplexní data, která integrují výsledky z proteomu, metabolomu a mikrobiomu do multiomics obrazu chemické komunikace mezi divokými hlodavci.

Klíčová slova: mikrobiom, metabolom, proteom, divocí hlodavci, chemická komunikace, myš

## INTRODUCTION

Experimental animal models have been a basis for biological, research among other factors also for their genetic homogeneity and easy reproduction. Specifically, laboratory mice (*Mus musculus*) are a good example of inbred, laboratory-adapted house mice that were genetically isolated from their wild ancestors more than 80 years ago with their genomes being a mosaic of *M. musculus musculus*, *M. musculus domesticus*, and *M. musculus castaneus* (Yang et al. 2007, Wade et al. 2002). During roughly 1 000 generations of inbreeding, laboratory mice became genetically, physiologically, phenotypically, and behaviorally different from their wild ancestors (Potts et al. 1991, Abolins et al. 2017, Beans 2018). While easier handling, bigger size, and different coat color are easily noticeable differences between laboratory and wild mice, a scientific community has recently become strongly interested in less apparent differences such as differences in genetics, immune systems (Abolins et al. 2017, Viney and Riley 2017), microbiome (Kwon and Seong 2021, Hamilton and Griffith 2019), chemical signaling (Cheetham et al. 2009, Bansal et al. 2021) and others.

In the field of animal communication, bacteria were hypothesized to be the source of olfactory cues already 30 years ago (Albone et al. 1974). Since the different body sites host different microbiomes, each body site has a pool of symbionts that can produce a much more diverse group of odorants than can be found in the genome of the host. Therefore, through symbiosis with bacteria, the host can extend the variety of its olfactory signals with a large number of bacterial odorants without the need to develop new biochemical pathways (Carthey, Gillings and Blumstein 2018). Many of the bacterial products also function as olfactory cues and may change the host's behavior (Ezenwa and Williams 2014).

Considering the fact that olfactory signals influence behavior and such signals are products of bacterial metabolism, there are no doubts that microbiomes are directly involved in behavior. Moreover, since odorants are perceived by the brain in a combinatorial way, i.e. a mixture of molecules creates a sense of specific smell, the odorants might consist of products synthesized by multiple bacterial species. Thus, the link between microbiomes, chemical communication, and behavior opens new possibilities in the field of behavioral genetics.

## AIMS OF THE THESIS

The main aim of my thesis was to contribute to the knowledge of the role of microbiomes in chemical communication in wild-living rodents. To reach this goal, we decided first to describe the microbiome in wild-living rodents under different aspects such as horizontal transfer between wild-living and laboratory mice, changes in microbiota composition after transport from the wild to captivity, or physiological dynamics in the microbiomes over time. Along with that, we focus preferentially on those microbiomes that are in direct contact with the environment, and thus they can be directly involved in chemical communication between the hosts. To cover the topic also from the perspective of the compounds that might serve as chemical signals, we explored the variables that shape the metabolome and proteome in mouse urine. Finally, we linked the methodology and knowledge of our previous work and we studied the potential role of oral and vaginal microbiomes in chemical communication.

Thus, the minor aims of my thesis are:

- 1) To test the degree of horizontal transfer of the microbiome between laboratory and wild mice.
- 2) To demonstrate differences and similarities between microbiomes directly connected to the environment (oral and vaginal) in wild living rodents.
- 3) To assess changes in microbiome composition caused by introduction to captivity.
- 4) To identify variables that cause variation in odorants, i.e., proteome and metabolome.
- 5) To investigate the temporal dynamics of microbiomes that are in direct contact with the environment.
- 6) To analyze the relationship between microbiome, proteome, and metabolome from the point of view of chemical communication.

## MATERIAL AND METHODS

Microbiome, proteome, and metabolome were done on samples from wild rodents, that is, house mice (*Mus musculus musculus*) and field mice (*Apodemus sp.*) or from laboratory mice (e.g. *Mus musculus musculus*, *M. m. domesticus*, C57BL/6J, BALB). Wild animals were captured either in Czechia or Slovakia) and immediately sampled and released back or transferred to the Animal Facility of Charles University, Prague. Microbiome analyzes were performed on the samples from oral microbiomes, gut, and vaginal. Bacterial genomic DNA was extracted from the samples using specialized kits (QIAGEN, Zymo Research) according to the manufacturer's protocols. We PCR amplified specific regions of the 16S rRNA gene using universal primers. For bioinformatics analysis, software such as skewer (Jiang et al. 2014) for demultiplexing samples, dada2 (Callahan et al. 2016) for quality checking, vsearch (Rognes et al. 2016) for clustering sequences into OTUs, the SILVA database version 132 (Quast et al. 2013) for taxonomy classification of sequences, UCHIME (Edgar et al. 2011) with gold.fasta (available at: <https://drive5.com/uchime/gold.fa>) for detecting chimeric sequences, *Decontam* (Davis et al. 2018) to remove contaminants, and the package phyloseq (McMurdie and Holmes 2013) for handling data in R (R Core Team 2015).

All protein samples were preprocessed and the peptide cations were ionized by electrospraying and analyzed on a Thermo Orbitrap Fusion instrument (Q-OT501 qIT, Thermo). MaxQuant software (version 1.6.34) was used to preprocess LC-MS data. MaxQuant's 'match between runs' feature of MaxQuant was used to transfer identifications to other LC-MS/MS runs based on their masses and retention time (maximum deviation 0.7 min). Quantifications were performed using label-free algorithms with a combination of unique and razor peptides. All subsequent analyses were performed in R software.

The volatiles was analyzed using two-dimensional comprehensive gas chromatography with mass detection (GCxGC-MS; Pegasus 4D, Leco Corporation, USA). The mass detector was equipped with an electron ionization source and time-of-flight analyzer, which enables unit resolution. Selected compounds were identified by automated comparison of the ion features in the experimental samples to a library of mass spectra (NIST MS 2.2, USA).

## RESULTS AND DISCUSSION

In this thesis, I followed a strategy of broadening our knowledge of the rarely studied microbiomes in wild living rodents with a focus on the role of bacteria in chemical communication. To cover this topic, we first scrutinized several aspects that influence the microbiome composition such as the immune system (PUBLICATION I), the origin of the animals, i.e. if the rodents are caught in nature or born in breeding facilities (PUBLICATION I, PUBLICATION II, PUBLICATION V), changes in the environment, i.e. transfer from nature to captivity (PUBLICATION II), species specificity (PUBLICATION II), or physiological changes such as estrous cycle (PUBLICATION V). Second, we addressed signaling molecules that might be of bacterial origin and contribute to the chemical communication among the host and its interaction partners (PUBLICATION III, PUBLICATION V). Not only we focused on the volatiles but also examined the liquid phase of the odorants that includes protein carriers for volatiles, i.e., lipocalins whose function as chemical signals was repeatedly demonstrated (Sharrow et al. 2002). Finally, we review all functions of mammalian lipocalins with a special focus on their role in chemical communication and their close relationship with microbiomes (PUBLICATION IV).

Our results demonstrate that the oral and vaginal microbiome is site-specific and is differently susceptible to environmental changes (PUBLICATION II). Surprisingly, the vaginal microbiota is less stable despite its well-known protective function. Similarly, the composition of the oral microbiota also changes significantly during the cohousing of wild and laboratory animals (PUBLICATION I). However, such changes in the composition of the microbiota were not accompanied by similarly profound changes in the immune system (PUBLICATION I). From the odorant point of view, mixtures of proteomes and metabolomes transfer information about the sex, subspecies, and origin of an individual (i.e., if the individual came from the laboratory or was wild-caught) (PUBLICATION III). This information further strengthens the hypothesis that the odorants consist of mixtures of molecules, rather than that one molecule would be a carrier of specific information. Since our proteomic analysis of chemical cues repeatedly described lipocalins as highly abundant particles in orofacial and urogenital secretions (Cerna et al. 2017, Kuntova, Stopkova and Stopka 2018, Stopka et al. 2016, Stopkova et al. 2009, Stopkova et al. 2017), we placed our data in the broader frame of the evolution and neofunctionalization of



lipocalins. That is, we showed for the first time similarities between the roles of lipocalins in chemical communication and microbiome surveillance (PUBLICATION IV).

## CONCLUSIONS

My work showed that the microbiomes of wild living animals are species-specific with distinguishable compositions of the microbiomes even among closely related species such as *Apodemus agrarius*, *A. flavicollis*, *A. sylvaticus*, and *A. uralensis* (PUBLICATION II) or *Mus musculus musculus* and *M. m. domesticus* (PUBLICATION III). Additionally, my results demonstrate that the composition of microbiomes is also tissue-specific with clear differences between different parts of the gastrointestinal tract, namely the duodenum, ileum, colon, and caecum (PUBLICATION I) and also between the mouth and reproductive organs (PUBLICATIONS II and V). Although we showed that there is a transfer of bacteria between wild and laboratory animals, this transfer occurred only when the animals were allowed to be in direct contact with the urine and feces of the other group (PUBLICATION I). However, the transfer was limited and each animal kept its conservative core microbiome after cohabitation with other animals (PUBLICATION I) and also after an introduction from nature to captivity (PUBLICATION II). For the first time, we describe the dynamics of the vaginal microbiome that changes over the estrous cycle, while the oral microbiome did not show significant changes in composition with respect to hormonal changes (PUBLICATION V, Figure 13).

Because of the tight connection between the microbiome and chemical communication, we chose an untargeted approach to analyze the proteome and metabolome of fluids that are rich with chemical compounds (i.e., urine, saliva, and vaginal flushes). Our approach discovered a high amount of proteins (especially lipocalins) and volatile organic compounds that have not been previously described in relation to microbiomes or chemical communication. (PUBLICATION III and V). Furthermore, we tested the relationships among the vaginal microbiome, the proteome, and the metabolome and found a high correlation among these three systems.

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# CURRICULUM VITAE

## Personal data:

MSc. Tereza Matějková

email: [tereza.matejkova@natur.cuni.cz](mailto:tereza.matejkova@natur.cuni.cz)

## Education:

2021 – present: Učitel Naživo

- Additional pedagogical study (not-finished yet)

2019 – 2022: Faculty of Science, Charles University, Prague

- Additional pedagogical study in biology

2018 – present: Faculty of Science, Charles University, Prague

- Ph. D. program, Zoology (not-finished yet)

2016 – 2018: Faculty of Science, Charles University, Prague

- MSc. program, Zoology, (**red diploma**)

2013 – 2016: Faculty of Science, Charles University, Prague

- BSc. program, Biology (**red diploma**)

## Experience in work:

2021 – present: Dep. of Biology Education, Faculty of Science, Charles University, Prague

- Scientific work focusing on didactics and pedagogy in biology

2019 – present: Dep. of Zoology, Faculty of Science, Charles University, Prague

- Laboratory and scientific work related to Ph. D. studium

2017 – 2018: Dep. of Zoology, Faculty of Science, Charles University, Prague

- Laboratory and scientific work related to MSc. studium

## Teaching/Organization/Working with children:

2020 – present: Organization of a biology educational events for basic school students (“Jarní/Podzimní Běstvinka”)

2019 – present: Member of the Biology olympiad working group, category A, B

2014 – present: Faculty of Science, Charles University, Prague

- Organization of a summer camp for successful participants of the Biology olympiad, categories C, D
- Organization of an introductory course for Bc. students of the Biology program at the Faculty of Science, Charles University, Prague

2015 – 2016: Omniveda, Věda nás baví

- The teaching of free-time activities with a focus on scientific experiments for 6-10 years old children

Giving specialized biology lectures for basic and high schools students with a deeper interest in biology

## Received Grants:

2019: GAUK no. 1191419: *Structure, function, and diversity of vaginal microbiome of highly promiscuous rodent Apodemus sylvaticus*, My role: Principal researcher (successfully finished)

## Scientific outcomes:

Stopková, R., Otčenášková, T., **Matějková, T.**, Kuntová, B., & Stopka, P. (2021). *Biological Roles of Lipocalins in Chemical Communication, Reproduction, and Regulation of Microbiota*. *Frontiers in Physiology*, 12.

**Matejkova, T.**, Hajkova, P., Stopkova, R., Stanko, M., Martin, J.F., Kreisinger, J., and Stopka, P., *Oral and vaginal microbiota in selected field mice of the genus Apodemus: a wild population study* (Scientific Reports, 2020)

Moudra, A., Niederlova, V., Novotny, J., Schmiedova, L., Kubovciak, J., **Matejkova, T.**, ... & Stepanek, O. (2021). *Phenotypic and clonal stability of antigen-inexperienced memory-like T cells across the genetic background, hygienic status, and aging*. *The Journal of Immunology*, 206(9), 2109-2121.

Stundl, J., Pospisilova, A., **Matejkova, T.**, Psenicka, M., Bronner, M. E, and Cerny, R., *Migratory patterns and evolutionary plasticity of cranial neural crest cells in ray-finned fishes* (Developmental Biology, 2020)

**Matejkova T.** & Kreisinger J., Hájková P. Stopková R., Stanko M., Stopka P. : *Promiscuity and microbiota in different Apodemus species*, Workshop on Omics Data Integration (2019), Prague, poster

**Matějková T.**, Štundl J., Černý R.: *From folding to neural keel: An evolutionary change in the neurulation within the biggest vertebrate group*, Zoological days Conference (2018), Prague, poster