Description of the main research directions investigated by the institute

At present IAPG comprises of 14 research groups/laboratories, the research of which belongs to three main areas (represented by the three teams taking part in the current evaluation): i) research in the area of biochemical and molecular biology mechanisms involved in the regulation of early development of vertebrates, ii) study of the molecular mechanisms in the area of evolutionary biology and genetics (predominantly of vertebrates) and its influence on biodiversity, genesis, evolution and extinction of species, and stability of ecosystems during climate changes, and iii) study of the basic physiological, molecular genetic, and cellular mechanisms with the aim to use them for biomedical applications. During the period from last evaluation number of changes in IAPG structure has occurred – 2 laboratories were dissolved, other 4 new laboratories were established, and out of 14 now existing laboratories 8 have new group leaders.

i) The **Team of Developmental Biology** includes 6 laboratories: Laboratory of Biochemistry and Molecular Biology of Germ Cells (LBMBGC, head: Andrej Susor), Laboratory of Cell Division Control (LCDC, head: Martin Anger), Laboratory of Developmental Biology (LDB, head: Radek Prochazka), Laboratory of Molecular Morphogenesis (LMM, head: Marcela Buchtova), Laboratory of Cell Signaling (LCB, head: Pavel Krejci), Laboratory of Odontogenesis and Osteogenesis (LOO, head: Eva Matalova).

The research team of the **Laboratory of Biochemistry and Molecular Biology of Germ Cells** (LBMBGC) is focused predominantly on the study of regulation of physiology of the mammalian germ cells with the aim to uncover the role of different protein kinases involved in translation during oogenesis and early embryogenesis. This includes the kinases needed for the regulation of the Cytoplasmic polyadenylation factors which play pivotal roles in the control of specific mRNAs through 3' UTR. The understanding of mechanisms, which regulate oocyte and early embryo development, would have a high impact on the field of cellular biology and mammalian reproductive biotechnology including human (improvement of the conditions for in vitro culture, improving assisted reproductive techniques and understanding of their physiology and pathology) in further perspectives for obtaining in vitro produced preimplantation embryos).

The main research focus of team the **Laboratory of Cell Division Control** (LCDC) is the influence of chromosome segregation in mammalian oocytes and embryos. These cells are larger than average somatic cells and they also show remarkably high frequency of chromosome segregation errors in comparison to the somatic cells. Aneuploidy resulting from the chromosome segregation errors is the single most frequent cause of termination of development in mammals. The intention of the team is to understand the differences between chromosome segregation control mechanisms in oocytes and embryonic blastomeres and somatic cells, which should elucidate the aetiology of aneuploidy during early development.

The **Laboratory of Developmental Biology** (LDB) focuses on the molecular mechanisms regulating oocyte meiotic maturation and early embryonic development in mammals. Pig and bovine oocytes and embryos cultured *in vitro* are being used as experimental models. The research interests are primarily concentrated on following three areas: 1) Identification of genes and signaling

pathways regulating oocyte maturation and developmental competence. 2) Mechanisms of degradation of maternal proteins and embryonic genome activation during the preimplantation development. 3) Molecular studies on the role of oocyte nucleolus in regulation of early embryo development.

The **Laboratory of Molecular Morphogenesis** (LMM) focuses on fundamental morphogenetic processes in organogenesis with special interest in formation of craniofacial structures. Novel molecules and unique roles for known molecules are investigated during cell proliferation, adhesion, migration, differentiation and cell death. Particular attention is paid to hard tissues development, including teeth. Physiological aspects at molecular and cellular levels are investigated and causes of developmental disorders with focus on ciliopathies are examined. The effort of LMM is also to contribute to recent knowledge in basic and biomedical research with links to practical applications in tooth replacement, dental tissue repair after injury and associated oral tumor induction.

The **Laboratory of Cell Signaling** (LCB) focuses on cell communication systems, which use polypeptide ligands and the cell surface receptors. These receptors bind growth factors, cytokines, morphogens, hormones, extracellular matrix components, and other ligands. The main aim is to answer questions related to many aspects of receptor signaling, ranging from molecular and cellular biology of receptor function to development and application of receptor inhibitors. The team also explores the biology of primary cilia, and aims to determine, how defects in ciliary function lead to skeletal abnormalities in humans. Finding cure for achondroplasia is also one of major goals of LCB research.

The major interests of the **Laboratory of Odontogenesis and Osteogenesis** (LOO team) are molecular and cellular aspects of the tooth and bone development, homeostasis and possible reparation/regeneration with focus on *in vivo* context. The research in odontogenesis deals with tooth formation, cell differentiation and extracellular matrix production, innervation and vascularization, and also periodontal apparatus with special attention being paid to health/disease conditions and stem cells. The team specifically focuses also on tooth/bone interface and related structures in craniofacial region. The research in osteogenesis covers both, intramembranous as well as endochondral bones where the growth plate is of particular interest.

ii) The **Team of Biodiversity and Evolution** includes 4 laboratories: Laboratory of Molecular Ecology (LME, head: Petr Kotlik), Laboratory of Mammalian Evolutionary Genetics (LMEG, head: Milos Macholan), Laboratory of Fish Genetics (LFG, head: Petr Rab), Laboratory of Anaerobic Microbiology (LAM, head: Jakub Mrazek).

The major interests of the **Laboratory of Molecular Ecology** are following: i) Evolutionary response of various organisms to global climate and environmental change, including small mammals, fishes, amphibians and crustaceans to show that some temperate species survived in “cryptic” glacial refugia that were located distinctly north of the traditionally recognized southern ones. **ii)** The importance and genetic basis of evolutionary adaptations that species have acquired to survive in such cryptic northern refugia using state-of-the-art molecular techniques including whole-genome sequencing, primarily using the Eurasian bank vole (*Clethrionomys glareolus*) as the model. **iii)** The genomics tools to test population historical colonization scenarios suggested by more traditional phylogeography. **iv)** Identification of key genes underlying adaptations that allowed some bank vole

populations to be more successful during post-glacial colonization, leading to population replacements by studying genes with known link to climate adaptation in other species (haemoglobin) as well as the genome scan approach.

The **Laboratory of Mammalian Evolutionary Genetics** focuses on the following areas of research: i) Phylogeography, phylogeny, species delimitation, systematics, and species distribution modelling in small mammals focusing on various African taxa and clades of rodents and shrews. ii) Multiple evolutionary questions using the house mouse model, revolving around processes during divergence of nascent species, the dynamics of secondary contact using two subspecies, *Mus musculus musculus* and *M. m. domesticus*, forming a natural hybrid zone in Europe detecting as many genetic/genomic, physiological etc. differences between them as possible - behavioural traits potentially reinforcing incomplete reproductive barriers. iii) Among genetic factors contributing to their isolation, attention to two ampliconic gene regions involved in olfactory communication and potentially contributing to the barrier: major urinary proteins and androgen-binding proteins. iv**)** Intragenomic conflict resulting in phenomenal introgression of *musculus* Y-chromosome across the zone deep into *domesticus* territory and hence contradicting Haldane´s rule (i.e. phenomenon of “antispeciation”), including multiple genomic and phenotypic correlates.

The **Laboratory of Fish Genetics** performs research in numerous aspects of fish, as well as some other vertebrate’s, genetics, evolution and biodiversity: i) The origin and consequences of asexual reproduction and polyploidization in evolution using several animal models (*Cobitis, Pelophylax*, etc.) using genomic, cytogenetic, ecological and physiological data with mathematical modelling to reveal molecular and mechanistic triggers of departures from sexual to asexual reproduction and how such change in reproductive mode coincides with speciation. ii) Phylogenomics and conservation genetics in vertebrate speciation and reticulate evolution and speciation. iii) The origin and reproductive mechanisms in hybrid animals that dropped out of the sexual meiotic cycle using genomics and cytogenomics to study mechanisms and molecular bases changing highly conservative machinery in meiosis turning Mendelian segregation to clonal reproduction. iv) The programmed DNA elimination, which eliminates whole parental genome during gametogenesis to understand principles regulating such genome rebuilding. v) The diversity/evolutionary history of loaches of the suborder Cobitoidei, the group to which the major model of the whole LGR, the genus *Cobitis*, belongs, using molecular phylogenetic reconstructions with morphological, geographic or geological data. vi) Identification of the major splits in the evolution of their major clades, the geological periods and geographic regions where major events occurred identifying the driving force of these events. What once started with the phylogenetic relationships of Central European *Cobitis,* now analyses the evolutionary history of freshwater models across all of the Eurasia. vii) Karyotype and sex chromosome differentiation in diverse fish clades, patterns of repetitive DNA accumulation, chromosome rearrangements, genome stability in fish evolution, cytogenomic aspects of the evolutionary dynamics of modified reproductive modes in fishes of genera *Cobitis* and *Hypseleotris*. viii) Cytogenomics of non-teleostean clades, basal teleostean Osteoglossiformes, natural and induced polyploidy in sturgeons and in diverse cypriniform clades. ix) Evolution of sex determination mechanisms in vertebrates, identification and study of sex chromosomes in reptiles, gene content of sex chromosomes, gene dosage compensation, genome

organisation and karyotype evolution, ecological and evolutionary consequences of sex determination transitions

To the subjects elucidated by the **Laboratory of Anaerobic Microbiology** belong: i) Comprehensive elucidation of the ecological, functional, and medical features of the microbial communities with special attention to gut environment where animal and human microbiomes, consisting of bacteria, archaea, fungi, protozoa, and viruses, represent important pseudo-organ playing a fundamental role in health/disease. ii) Using modern sequencing technologies, coupled with bioinformatics, a link between the microbiome and the various diseases such as Inflammatory Bowel Disease (IBD), diabetes, skin cancer, *Clostridium difficile* infection (bacteriotherapy), and celiac disease is being studied. iii) Focus on a link between ruminant and/or hindgut microbiome structure and diet, host genetics and phenotype - methane emissions, rumen and blood metabolites, and milk production efficiency. iv) Cultivation, isolation, identification and characterization of bacteria and fungi with special attention to bacteria with probiotic potential (*Bifidobacteria, Lactobacilli*) and anaerobic fungi with outstanding ability to decompose plant biomass. v) Characterization of microbial communities in other environments, such as fermented milk products and manure digesters.

iii) **The Biomedical Research Team** (BRT) is composed of four laboratories focusing on research relevant to human health: e.g. neurological disease, cancer, and infertility. Three of the four laboratories forming BRT, Laboratory of Cell Regeneration and Plasticity (LCRP, head: Zdena Ellederova), Laboratory of Applied Proteome Analysis (LAPA, head: Petr Vodicka), and Laboratory of DNA integrity (LDI, head: Petr Solc), are in UZFG Libechov campus and work in close cooperation within the PIGMOD (Pig models of disease) Centre. PIGMOD was established in 2013 with support of Research and Development for Innovations Operational Programme. The fourth laboratory, Laboratory of Neurobiology and Pathological Physiology (LNPP, head: Omar Sery), is in Brno and works in close cooperation with Masaryk University.

The **Laboratory of Cell Regeneration and Plasticity** is the largest of the BRT laboratories and has long tradition in development of large animal disease models and their use in preclinical research. The main focus of the laboratory team is on Huntington's disease (HD), Amyotrophic lateral sclerosis (ALS), spinal cord injury, retinopathies and other disorders and injuries of central nervous system (CNS). However, there is also an extensive collaboration with clinicians on development of new surgical approaches, evaluation of their therapeutic effectiveness and introduction in clinical practice in the fields of gastrointestinal tract diseases, wound healing and bone transplantations.

The **Laboratory of Applied Proteome Analysis** (LAPA) uses system biology approaches and both *in vitro* cellular models and *in vivo* animal models to search for disease biomarkers and to elucidate basic disease pathogenesis mechanisms, mainly in neurodegenerative diseases and cancer. LAPA tightly cooperates with other PIGMOD centre laboratories, using the same models and participating in above-mentioned projects related to HD research and ALS/spinal cord injury. The main focus of LAPA is on development of new proteomic analytic approaches for characterization of neural stem cells as both *in vitro* disease models and cell therapy material, on novel ultra-sensitive methods of mutant huntingtin protein quantification; and on characterization of immune system environment both

during cancer progression and as it relates to immune responses to gene therapy application via virus mediated delivery. Independent research projects of LAPA aim to complement use of large animal models with models in a dish, e.g. using human iPS cells derived from HD patients to model disease phenotypes.

The **Laboratory of DNA integrity** focuses on the study of chromosome dynamics and integrity during oocyte maturation and preimplantation development, and their role in developmental disorders and infertility. This includes the elucidation of the roles of several regulatory proteins including Plk1 protein kinase, Aurora kinases, MRE11 nuclease, as well as of the RanGTP–importin-β regulatory pathway in these processes.

The **Laboratory of Neurobiology and Pathological Physiology** is located in Brno and its main focus is on contribution of genetic variability to pathogenesis of multifactorial diseases, with the aim to clarify causes of Alzheimer's disease, schizophrenia, alcohol dependence, age-related macular degeneration and dental agenesis. In addition, LNPP performs basic experimental research aimed at advancing knowledge in fields of neurobiology and body response to potentially toxic nanoparticles.

Research activity and characterisation of the main scientific results

Laboratory of Cell Regeneration and Plasticity

The main focus of LCRP is a biomedical research using minipig as a large animal model suitable for pre-clinical studies. Nowadays, we work on Huntington's disease (HD), spinal cord injury, Amyotrophic lateral sclerosis (ALS), retinopathies, and gastrointestinal tract diseases.

Huntington's disease

In 2015-2019 scientific team of LCRP continued with characterization of the disease progression in transgenic minipigs for Huntington's disease (TgHD) carrying the N-terminal part of human mutated huntingtin with 124 CAG/CAA glutamines, that we previously generated. The breeding of TgHD minipig model and production of animals for experimental use has been supported by Service Contract from the Cure Huntington's Disease Initiative (CHDI) Foundation. CHDI also provided support in the form of Research Agreement focusing on the characterization of the disease progression in these animals. We reported the reproductive phenotype (Macakova et al. 2016), development of neurodegeneration (Vidinská et al. 2018; Ardan et al. 2019), and behavioural abnormalities (Baxa et al. 2019) in this animal model. All studies were performed by LCRP. We also described adipogenic differentiation of bone marrow-derived mesenchymal stem cells derived from TgHD minipigs (Smatlikova et al. 2019), major contribution by LCRP. Together with the other PIGMOD laboratories (LAPA) we found promising biomarkers reflecting immuno-pathological mechanisms (Valekova et al. 2016). Together with Laboratory for Study of Mitochondrial Disorders in Charles University in Prague (Mitolab) we further described the sperm defects, related to above mentioned reproductive phenotype, with focus on mitochondrial metabolism (Krizova et al. 2017) (50% of our contribution, sperm collection, spermiogram, reproductive parameters, immunofluorescence, immunohistochemistry, WB, and manuscript preparation). Within the international collaboration funded by Czech – Norwegien funds, we published together with Mitolab and University of Oslo three papers on deterioration of mitochondrial bioenergetics and ultrastructure impairment in skeletal muscle (Rodinova et al. 2019) (around 25% of our contribution), oxidative stress in primary TgHD fibroblasts (Smatlikova et al. 2019) (80% of our contribution, first and shared corresponding author both from our group) and early signs of behavioural and molecular pathologies (Askeland et al. 2018) (50% of our contribution, shared corresponding author from our group). In collaboration with the Slovak University of Technology in Bratislava we also described the neurological phenotype of TgHD minipigs by magnetic resonance spectroscopy (Jozefovicova et al. 2016) (40% of our contribution). In 2018, we received together with Prof. Knut Stieger from University of Giessen in Germany the Seed Funds form European Huntington's Disease Network for project "Nanoparticle Based CRSIPR/Cas Gene Editing System to treat Huntington's disease". The results from this collaboration were submitted to Scientific Reports in fall 2019 and accepted for publication in January 2020 (Rohiwal et al. 2020) (both first and shared corresponding author were from our team).

CHDI has also supported generation of novel knock-in minipig model (85Q KI-HD), which was generated in Exemplar Genetics (USA) via somatic cell nuclear transfer using Libechov minipig's fibroblasts. Two 27 months old boars were transferred to Libechov in May 2018 in order to establish 85Q KI-HD minipig colony to be used for further model characterization and potential preclinical therapeutic testing. Since May 2019 we received another two-year Research Agreement from CHDI to characterize F1 generation of these 85Q KI-HD minipigs. Additional humanized HD minipig model is in early stage development in cooperation with the Gene Centre of Munich.

One of the top achievements in our HD focused research was the collaboration with Dutch pharmaceutical company uniQure. Together we demonstrated broad brain distribution of AAV5-miHTT gene therapy vector developed by uniQure and strong lowering of human mutant huntingtin in our TgHD minipig model (Evers et al. 2018). Based on these results and partial results from still ongoing longitudinal safety study, uniQure received FDA and EMA approval for first in human clinical testing in January 2019. Furthermore, results of our longitudinal study were recently submitted to Science Translational Medicine. Based on this successful collaboration we raised an interest and started a new collaboration with another pharmaceutical company, Takeda. In 2020 we started the study on allele specific silencing of mHTT in 85Q KI-HD minipig model.

Spinal cord injury and Amyotrophic lateral sclerosis

In 2015-2019, one of the key topics in LCRP as well as Center PIGMOD were the study of potential use of cell and gene therapies in minipig models of spinal cord injury and Amyotrofic lateral sclerosis (ALS). We developed adjustable computer-controlled compression model of spinal cord injury in minipig. This model clinically as well as histopathologically faithfully simulates spinal cord injury in men. We implement preclinical experiments with newly established neural stem cell lines and gene therapy to introduce potential cure. In collaboration with Prof. Martin Marsala (https://profiles.ucsd.edu/martin.marsala) from University of California in San Diego (UCSD) we focused on stem cell therapy for treatment of spinal cord injury and other neurodegenerative diseases affecting spinal cord. In 2015 we published study that characterized the expression of several key proteins related to spinal cord injury (e.g. doublecortin (DCX) and glial fibrillary acidic protein (GFAP)) in pre- and postnatal rat and porcine spinal cord. It was also combined with characterization of DCX expression in spinally grafted porcine-induced pluripotent stem cells (iPS)-derived neural stem cells (NSCs) and human embryonic stem cell (ES)-derived NSCs. Both NSCs sources showed clear DCX expression at 3-4 weeks post-grafting (Juhasova et al. 2015). Our team performed DCX and GFAP detection in pre and postnatal porcine spinal cord, preparation of cells before transplantation, injection of cells into spine, neurological assessment after transplantation, immunosuppression, necropsy, immunohistochemistry and wrote the manuscript, the first author was from our team. We also published a scalable solution for isolating human multipotent clinical-grade neural stem cells from ES precursors (Bohaciakova et al. 2019), where our team performed *in vivo* experiments in minipigs, including spinal cord injury creation, neural precursors preparation before administration and delivery assistance, neurological assessment after NPCs delivery,

immunosuppression, necropsy, and co-wrote the manuscript. Our main success with cell therapy was the publication in Science Translational Medicine where we showed survival of syngeneic and allogeneic iPSC-derived neural precursors after spinal grafting in minipigs (Strnadel et al. 2018) (Our team performed all *in vivo* experiments in minipigs, neural precursors preparation before administration, cell delivery assistance, neurological assessment after NPCs delivery, immunosuppression, necropsy, and co-wrote manuscript). In the field of gene therapy, we again together with prof. Martin Marsala established a new subpial delivery method that enables an effective delivery of adeno-associated virus 9 (AAV9) throughout the cervical, thoracic and lumbar spinal cord, as well as brain motor centers. This novel gene therapy delivery method is promising for treatment of different spinal cord pathologies – ALS, spinal cord injury etc. We published three papers on this topic (Miyanohara et al. 2016), (Tadokoro et al. 2017) and (Bravo-Hernandez et al. 2020). The most recent one, published in Nature Medicine, confirmed widespread gene silencing and blocking of motor neuron degeneration in ALS after spinal subpial delivery of AAV9 (Bravo-Hernandez et al. 2020). In all three papers, we performed *in vivo* experiments in minipigs, subpial delivery assistance, neurological assessment after subpial delivery, immunosuppression, necropsy and contributed tomanuscript preparation.

Optimal route of administration and biodistribution of AAV constructs in our minipigs was also studied in another collaboration with uniQure company. Obtained data were included in publication on Machado-Joseph Disease in Molecular Therapy Methods and Clinical Development (Martier et al. 2019) (all experimental work on the minipigs was done at the PIGMOD Center in Libechov).

Retinopathies

In recent years, we supplemented our long term focus on CNS diseases with research of several diseases of the eye, namely age related macular degeneration (AMD), Stargardt's disease and Usher Syndrome. To this end, we obtained funds for new fully equipped experimental eye surgery unit, and instrumentation for non-invasive follow-up examinations, including the ophthalmic surgery microscope (Hi-R NEO 900A, Haag-Streit), phacoemulsifier/vitrectome (R-Evolution CR, Optikon), ophthalmic green laser (Merilas 532α, Meridian), and optical coherent tomography (OCT) (Optovue, iVue).

In AMD, we investigate the possible replacement of damaged pigmented retinal epithelium (RPE) with transplantation of primary porcine, human and human iPSC derived RPE cells on advanced nanofibrous scaffolds made by Institute of Macromolecular Chemistry in Prague. So far we published three common papers based on this collaboration (Artero-Castro et al. 2019; Ardan et al. 2016; Popelka et al. 2015)(25% of our contribution, 70% of our contribution with first, last and corresponding author from LCRP, and 30% of our contribution respectively).

Statgard's disease is the most common heritable retinal disorder with single causative gene, but no animal model fully replicating disease phenotype is currently available. Together with Prof. Knut Stieger from the University of Giessen we received a three-year bilateral grant (2019-2021) from Czech Grant Agency with a goal to create a minipig model for Stargardt's disease

using CRISPR/Cas9 technology and direct injection of the constructs into a zygote. Both LCRP and LDI laboratories of PIGMOD participate in this project. The role of the laboratory of Prof. Stieger is to develop a new gene therapy that will be tested on the newly generated minipig model.

Since May 2019 we breed Usher 1C pig model that was generated by Nick Klymiuk from Gene Center of Munich and Prof. Uwe Wolfrum from University of Mainz, Germany. Based on our preliminary data of AAV biodistribution in wild type minipig eyes, we were invited to apply for a grant from Usher 2020 foundation to perform preclinical study of gene therapy using these Usher pigs. Together with both laboratories that created the model we plan to perform the gene therapy vitreoretinal surgeries at Pigmod Centre at the end of 2020 and two following years.

Development of new surgical approaches, evaluation of their therapeutic effectiveness and introduction in clinical practice in the fields of gastrointestinal tract diseases, wound healing, and bone transplantations.

LCRP collaborated on research of gastrointestinal tract diseases with physicians from Military University Hospital Prague, General University Hospital in Prague and Institute for Clinical and Experimental Medicine Prague. The aim of this collaboration was training of new and classical surgical techniques, the comparison of their therapeutic effectiveness and introducing of new surgical approaches into clinical practice (NOTES – *Natural Orifice Transluminal Endoscopic Surgery*, POEM – *Peroral Endoscopic Myotomy, ESD-Endoscopic submucosal dissection)*. These collaborations were supported by two projects funded by Czech Health Research Council (AZV ČR) and resulted in several publications: (Dolezel et al. 2018; Martínek et al. 2018; Juhas et al. 2019; Juhásová et al. 2019; Ryska et al. 2017; Kalvach et al. 2018). LCRP team performed minipig anaesthesia, sample collection, laparoscopy, surgery, necropsy, partial immunohistochemistry, and cooperated in manuscript preparation in all of these studies. We also collaborated with physicians from Military University Hospital Prague on minipig preclinical testing of nanobandages in wound dressing, resulting a common publication (https://starfos.tacr.cz/cs/project/OWUVN20170001, LCRP – anesthesia, sample collection, surgery, manuscript preparation). In 2015-2018 LCRP participated in another project supported by Czech Health Research Council (AZV ČR) and focused on bone implants. This collaboration resulted into two publications (Kubíková et al. 2018; Sauerova et al. 2019) (LCRP – *in vivo* experiments, isolation of bone blood for minipig MSCs isolation, propagation and cryopreservation of minipig MSCs, FACS characterization of MSCs *in vitro*, manuscript preparation).

All these studies also represent example of collaboration with local private sector, namely companies http://www.beznoska.cz/; https://www.lasak.cz/; https://www.nanopharma.cz/en/; https://www.studentscience.cz/; https://www.promed.cz/; https://neurgaintech.com/about/.

Laboratory of Applied Proteome Analyses

Research of LAPA is currently focused on two main topics, neuroscience, namely proteomics of neurodegeneration, and cancer research. LAPA tightly cooperates with other PIGMOD centre laboratories, using the same models and participating in above mentioned projects related to HD research and

ALS/spinal cord injury. The main focus of LAPA was on development of new proteomic analytic approaches for characterization of neural stem cells as both *in vitro* disease models and cell therapy material, on novel ultra-sensitive methods of mutant huntingtin protein quantification; and on characterization of immune system environment both during cancer progression and as it relates to immune responses to gene therapy application via virus mediated delivery. Independent research projects of LAPA aim to complement use of large animal models with models in a dish, e.g. using human iPS cells derived from HD patients to model disease phenotypes.

In the field of neuroscience, we characterized surface proteome of H9 NSCs, which could be used for selection of markers of relevant populations for cell based therapies (Tyleckova et al. 2016), and extensively reviewed proteomic approaches to characterization of NSCs in (Zizkova et al. 2015), (90% LAPA contribution, including first and corresponding authors in both papers). In collaboration with Marian DiFiglia's group at Massachusetts General Hospital we developed method for mutant huntingtin protein quantification based on MesoScaleDiscovery platform assay. This assay was used to confirm huntingtin lowering in preclinical study of AAV-miRNA mediated gene therapy in sheep transgenic model of HD (Pfister et al. 2017), LAPA author – measuring of mHTT levels by in house developed MSD assay. The MSD huntingtin assay was also adapted to assess somatic instability of CAG tract at the protein level (Aviolat et al. 2019), LAPA author -development and validation of MSD assay.

Our experience in using NSCs based models allowed us to participate in COST Action "In vitro 3-D total cell guidance and fitness", (CellFit) CA16119 and to obtain funding through InterCost programme of Czech Ministry of Education, Youth and Sport (MEYS) for project "Proteomic characterization of cell membrane surface proteins, secretome and exosomes in human cell based Huntington's disease model. and network" (2018 - 2021). This project aims to analyse the changes in composition of cell surface and secreted proteins of neural stem cells (NSCs) in the presence of mutant huntingtin (mHTT) protein and also changes in proteome and miRNA composition of exosomes secreted by these cells.

Techniques for isolation and characterization of extracellular vesicles (EV) from body fluids as well as cell culture supernatants recently implemented in our laboratory provide another opportunity to study biomolecules present in these EVs as possible disease biomarkers. Implementation of these methods for isolation of EVs from minipig biofluids is described in paper by (Kupcova Skalnikova et al. 2019), 90% LAPA contribution, remaining authors are from LCRP and led to funding of project Proteomic analysis of extracellular vesicles in Huntington's disease (Czech Science Foundation, 2019 – 2021).

Our interest in analysis of immune mediators, e.g. cytokines contributed to characterization of TgHD minipig model, as mentioned in LCRP section (Valekova et al. 2016)(equal contribution of both laboratories). Together with LCRP we also co-operate in development of experimental cell and gene therapies. In collaborative uniQure study (Evers et al. 2018) we have monitored the immune response to the AAV5-based gene therapy of Huntington's disease by studying cytokine levels in cerebrospinal fluid of the TgHD porcine model after intra-striatal application of the viral vectors.

In collaboration with Institute of Experimental Medicine of the Czech Academy of Science, we have analysed factors secreted *in vitro* by mesenchymal stem cells into the conditioned medium, which might have neuroprotective effects in rat model of amyotrophic lateral sclerosis (Řehořová et al. 2019). For another ALS study, led by team of Prof. M. Marsala and colleagues from LCRP, we have optimised a custom made ELISA and analysed levels of anti-AAV9 neutralising antibodies (nAb) in blood serum of pigs with the aim to select animals with low levels of nAb for AAV9 virus vector application (Bravo-Hernandez et al. 2020).

In 2017 was previously independent PIGMOD Centre member Laboratory of Tumour Biology (LTB) merged with LAPA, based on our previous experience in cancer proteomics. Our main area of interest in cancer research is to improve understanding of cancer microenvironment, including secreted factors and immune mediators, on cancer progression and therapeutic approaches.

We breed a porcine model of hereditary melanoma (MeLiM) that was developed at our institute approx. 20 years ago. This model is particularly suitable to study melanoma regression (spontaneous disappearance of the tumours without any treatment). In the last 5 years, we have studied on the MeLiM model the extracellular matrix remodelling during melanoma regression (Planska et al. 2015; 2018), and changes in haematological parameters during regression (Čížková et al. 2019) (all studies primarily by LTB/LAPA members). We have identified a population of recirculating effector/memory αβ T-lymphocytes that play an important role in regression process (Cizkova et al. 2019). We have also provided the tumour cryo-sections for detection of metals in the tissue by MALDI-MS and compared them with the histological findings (Anyz et al. 2017). We have, together with Laboratory of Anaerobic Microbiology at IAPG, mapped the microbiome on the melanoma surface as well as adjacent healthy skin to assess its involvement in melanoma development (Mrázek et al. 2019). We participated also in a study of blood serum cytokines in melanoma patients, where we have analysed the cytokine levels with the aim to map the inflammatory milieu in patients that can reflect the tumour growth or represent potentially relevant targets for therapy improvement (Kučera et al. 2019).

We have published two comprehensive reviews of the MeLiM model (Horak et al. 2019) and on the advances in cytokine detection techniques in cancer research (Kupcova Skalnikova et al. 2017) and reviewed melanoma spontaneous regression in human (Cervinkova, Kucerova, and Cizkova 2017) and possible involvement of microbiome in tumour regression (Kucerova and Cervinkova 2016). Together with colleagues from Masaryk Memorial Cancer Institute and Masaryk University in Brno and from ETH Zurich, we have compared targeted LC-MS proteomic approaches for quantification of proteins in cancer tissue (Faktor et al. 2017) (1/3 contribution of LAPA author).

Laboratory of DNA Integrity

Because of interest in mammalian oocytes that are available only in minimal amounts, we decided to focus our methodology interest in advanced confocal live imaging and computer image analysis because this approach can provide maximum information from few cells. We deeply acknowledge collaboration and support from Jan Ellenberg (EMBL, Heidelberg, Germany), who provided us essential introduction to live imaging and computer image analysis. In this

collaboration, we were interested in Polo-like kinase I (PLK1), which is known as a critical mitotic kinase regulating almost all mitotic events. Because oocytes divide by meiosis and they have acentriolar microtubule-organizing centers (MTOCs) that support spindle formation, we were interested in which PLK1 functions are conserved also in oocytes and which ones are unique for meiotic oocytes. E.g., we have discovered that in contrast to mitosis, PLK1 is not essential for spindle bipolarization, but PLK1 is critical to anaphase I entry independently spindle assembly checkpoint (SAC) satisfaction. Moreover, PLK1 is necessary for the correct microtubule-kinetochore attachment. Although our attempt to publish this exciting story in Development was after about one-year review and revision ping-pong unsuccessful, and we published it finally in PlosOne (Solc et al. 2015) to obtain publication date priority, this work has an excellent response in the scientific community. This paper belongs to the 25% of the most cited article in PlosOne published in 2015.

Our interest in spindle formation and chromosome segregation and acquired expertise in advanced live imaging lead to the establishment of highly productive collaboration with Karen Schilder (Rutgers University, NJ, USA). In this collaborative research, we are interested in how three Aurora kinases (AURK A, B, C) control spindle formation and chromosome segregation. Oocytes lack a classical centriole-containing centrosome. MTOCs work as a functional replacement of centrosomes. Because dozens of MTOCs are formed during meiotic oocyte maturation, mechanisms for sorting and final clustering of MTOCs must operate to create spindle, where two clusters of MTOCs form spindle poles. We discovered that a meiotically specific AURKC is important for MTOC clustering. When this clustering mechanism failed, oocytes are more prone to chromosome missegregation (Balboula et al. 2016). According to our best knowledge, this is the first report showing the importance of MTOCs clustering for correct chromosome segregation.

In mitosis, AURKB has essential roles as the catalytic subunit of the chromosomal passenger complex (CPC). In oocyte, AURKC takes over as the predominant CPC kinase. Because when meiotic AURKC is genetically depleted, its mitotic homolog AURKB probably takes over its function, we were highly interested in the phenotype of mouse females with disruption of both *aurkb* and *aurkc* genes in oocytes (aurka b/c double knock out – DKO). Surprisingly, DKO females are only subfertile, and the majority of oocyte mature normally because remaining AURKA associates with CPC and takes over the function of AURKC(B). On the other hand, part of the DKO oocytes arrests in meiosis I with small spindle because of a low level of AURKA on spindle poles as AURKA moves from spindle poles to CPC to compensate AURKC loss. We published this unexpected story in Current Biology, having David Drutovic as a second author(Nguyen et al. 2018).

To protect genome integrity, cells must not only correctly segregate chromosomes, but they must respond on DSBs by a delay in cell cycle progression and induction of DSBs repair. When we started to study DSBs response in mouse oocytes, little was known about it in mammalian oocytes. We have found that mouse prophase I oocytes respond on increased DSBs by phosphorylation of histone H2AX (γH2AX). Surprisingly, they do not delay meiotic maturation (resumption of meiosis and anaphase I entry) under conditions when increased DSBs induce chromosome fragmentation in about 30% oocytes. We discovered that nuclease MRE11 is essential for H2AX

phosphorylation in meiosis I but not in meiosis II and that MRE11 protects chromosome integrity during meiotic maturation. Our data suggested that DSBs repair operates during meiotic maturation what is in sharp contrast to somatic cells, where DSBs repair is attenuated specifically during mitosis (Mayer et al. 2016).

Because of our interest in DSBs repair in oocytes and embryos we started project on using Cas9 nuclease for precise editing of pig zygote genome to produce genetically modified pigs carrying human V1973X mutation in abca4 gene, which is associated with human Stargardt's disease. The goal of this project is to develop large animal model for preclinical testing of gene therapy. This project is a collaborative effort between LDI and LCRP, as mentioned above in LCRP section.

Our interest in spindle formation and chromosome segregation in oocytes pushes us to try to answer the big question in the field - why do human oocytes require Ran GTPase for the spindle formation, but mouse oocytes do not? We found that the previously described and widely used RanT24N mutant is not a suitable RanGTP inhibitor. As expected, RanT24N disrupts chromosomal cargo gradients (gradient of the spindle assembly factors, SAFs). However, it does not reduce an overall concentration of importin-β cargos (active SAFs) across oocytes. We used double mutant RanT24N, T42A, which inhibits active SAFs more effectively, and small molecule Importazole, to show a requirement of RanGTP – importin-β pathway for spindle formation and correct chromosome segregation also in mouse oocytes. Our work thus indicates that the dependence of spindle formation on RanGTP is common to both human and mouse, supporting the relevance of the mouse model to the mechanisms of human oocyte meiosis. We published this work in the EMBO Journal (Drutovic et al. 2020), having the first and last authors from our lab.

Laboratory of Neurobiology and Pathological Physiology

Since the last evaluation period, LNPP was established as independent laboratory within IAPG. Located in IAPG's section in Brno, LNPP focuses on genetic studies of multifactorial disease pathogenesis.

Research on Alzheimer's disease

Nowadays, Alzheimer's disease (AD) belongs to the main societal problems. Increase in mean population survival leads to the ageing population, with an increasing number of Alzheimer's disease patients. Age is currently considered the main causal factor of this most prevalent neurodegenerative disease, however, genetics and lifestyle are of great importance. The aim of our laboratory is to discover molecular basis of the Alzheimer's disease pathogenesis. The scavenger receptor CD36 is involved in mechanisms of vascular growth, internalization of pathogens (bacteria, fungi), internalization of amyloid beta protein, and gustatory perception of fatty acids. Disturbances of CD36-related processes could also contribute to the development of Alzheimer's disease. Our lab was the first to show a relationship between AD and the gene for CD36 receptor (Šerý et al. 2017). We also discovered relationship between AD and ALOX5AP gene (Šerý et al. 2016, 5). In addition, we collaborate with a wide range of institutions (psychiatric and neurologic) from all over the Czech Republic, using the NGS (next generation sequencing) technology to obtain genetic data on Czech AD patient population.

Research on Schizophrenia

Schizophrenia is a disorder related to dissociation between a perception of reality and experience, affecting about one in every hundred people. It is considered a neurodevelopmental disorder with strong genetic contribution. Our laboratory is engaged in a quest for molecular causes contributing to the occurrence of schizophrenia. We were first to report the relationship between schizophrenia and the gene for the Cortexin 3 (Šerý, Lochman, et al. 2015). Retina as a part of the central nervous system presents unique structure and location making it accessible to direct examination of cytoarchitecture and microvasculature in vivo. We studied novel structural correlates of schizophrenia in the retinal microvasculature using retinal image analysis and optical coherence tomography measurements to assess retinal nerve fiber layer thickness in patients with schizophrenia, their healthy first-degree relatives and gender- and age-matched controls not related to the other two groups. We found significant differences between the diameters of arterioles of patients with schizophrenia and their healthy relatives, between the group of patients and the unrelated controls as well as between the healthy relatives and the unrelated controls. The venules of both the patients and their healthy relatives were wider than those of the unrelated controls. This is the first study finding wider retinal arterioles in patients with schizophrenia (wider retinal venules in schizophrenia have been reported previously). Few, if any, consistent differences in the thickness of either the nerve fiber layer or the ganglion cell layer between the patients and controls were detected. Our manuscript was accepted by Psychiatry and Clinical Neurosciences and should be published in 2020.

Research on alcohol use disorder

Alcoholism arises as a gradual adaptation of the human brain to a regular intake of alcohol. Nevertheless, alcohol-related dispositions have been shown to be approximately fifty percent hereditary. We are focused on genetic, metabolomic, proteomic and neurochemical studies that could improve prediction, mechanistic understanding and possibly extend to diagnostics and treatment of alcoholism and alcohol addiction. Currently, we are focused on consequences of alcohol intake, particularly in relation to a glutamatergic system (Šerý, Sultana, et al. 2015; Kashem et al. 2016; 2017; 2020). For the purpose of this research, we utilise RealTime PCR, Western blot, fluorescence, confocal and electron microscopy.

Research on fatty acid taste perception

In addition to five basic taste modalities (sweet, sour, salty, bitter and umami taste), a growing number of evidence pointed out a possibility of taste perception of fatty acids. This taste modality is important for the final taste of fatty food. Nevertheless, each individual perceives the taste of fatty acids differently and as our laboratory (in cooperation with foreign institution) showed, the perception of fatty acid taste is a genetically influenced trait (Sayed et al., 2015, Mrizak et al., 2015, Daoudi et al., 2015, Karmous et al., 2018, Plesnik et al., 2018). Moreover, it was found out that detection threshold of fatty acids affects not only our food preferences but also could be a risk factor for obesity. Our laboratory deals with the genetic analysis of the gene for CD36 receptor in relation to tests of the fatty acids taste in volunteers.

Research on tooth agenesis

Our laboratory has continued the projects of prof. Míšek a prof. Vaněk. In collaboration with dental clinics, we focus on mutations that cause dental agenesis (oligodontia, hypodontia). Dental agenesis is manifested as an absence of one or more teeth that do not develop at all. It is caused by a mutation in genes that are important for teeth development. Our lab revealed a previously unknown mutation in the gene for PAX9 (Šerý et al., 2015) and in the gene for MSX1 (Bonczek et al., 2018) in families that have oligodontia in anamnesis. Again, NGS (next generation sequencing) technology is utilized in this research.

Research on age-related macular degeneration

According to the World Health Organization, age-related macular degeneration is currently one of the most common causes of blindness. AMD is a multifactorial disease, with risk factors including higher age, inappropriate diet, smoking, cardiovascular disease, etc. In addition, a genetic component is of great importance. Thus, our laboratory conducts research on the genetic influence of this disease. We revealed and described the relationship between the gene for CD36 and an increase of intraocular pressure after administration of Avastin into an eye (Matušková et al., 2018) and between AMD and 4 genes (Matušková et al., 2020).

Research on the presence of the genus Borrelia in the blood-sucking arthropods

Lyme borreliosis is a disease that occurs as a consequence of infection by bacteria of *Borrelia* genus. The most common infection vector are blood-sucking arthropods, especially the *Ixodes ricinus* tick. Our laboratory conducts systematic investigation of ticks from the entire Czech Republic by employing the RealTime PCR method and other analyses. Positive samples are further analysed by utilising DNA sequencing to determine individual *Borrelia* species. Our laboratory revealed the presence of *Borrelia spielmanii* in the territory of the Czech Republic (Bonczek et al., 2015).

Research activity and characterisation of the main scientific results

Research of LBMBGC was in past period focused on the molecular mechanisms which regulate the physiology and pathology of mammalian oocytes and embryos. Gametogenesis and embryogenesis are the key events in sexual reproduction. In the female, meiosis results in a large cell that is competent for fertilization and fundamental for supporting early embryonic development. One of the major problems occurring during mammalian meiosis is the high incidence of errors (*Koncicka et al. 2018, Int J Mol Sci*). These can lead to chromosomal aberrations, which are more prevalent in female gametes and seem to increase with maternal age. Oocyte aneuploidy has severe consequences including pregnancy loss and birth defects. To address these issues, research was concentrating on two major areas of research: intracellular Venues of mRNA translation and regulation of the activities of the most important M-phase protein kinases.

An important characteristic of oocyte and embryo development in mammals is the dependence on the translation and utilization of stored RNAs and proteins rather than de novo transcription of genes in order to sustain rapid development. In the absence of transcription (*Hanna et al. 2019, Genome Biol; Hanna et al. 2018, Nat Struct Mol Biol; Stewart et al. 2016, Epigenomics; Stewart et al. 2015, Genes Dev; Veselovska et al. 2015, Genome Biol*), the completion of meiosis and early embryo development in mammals relies significantly on maternally synthesized RNAs (*Kalous et al. 2018, Int J Mol Sci*). Therefore, the regulation of gene expression is controlled almost exclusively at the level of mRNA stabilization and translation.

We discovered that the RNA distribution in this large cell type indicates the presence of a novel set of regulatory mechanisms needed to ensure that specific gene expression occurs at the right time and in the correct place (*Susor et al. 2015, Nat Commun; Susor et al 2016, CTR; Susor & Kubelka 2017, Results Probl Cell Differ. Book chapter; Jansova et al. 2018, Plos One; Tetkova et al. 2019, Sci Rep*). This contributes to spindle and chromosome organization and thus also plays an important role in the maintenance of genomic stability. Our studies suggest spatio-temporally regulated translational control by the mTOR/eI4F pathway during oocyte and early embryo development. Our projects investigate the translational components that are potentially clinically relevant targets for the development of a healthy oocyte able to sustain embryo development (*Bora et al. 2019, Front Cell Dev Biol*). Moreover, beside of coding transcriptome, we analyze role of non-coding RNAs on the physiology of female germ cells and early embryos (*Ganesh et al. 2020, Nucleic Acids Res*).

Progression through both mitotic and meiotic cycles is controlled by the sequential activation and inactivation of a set of different protein kinases and phosphatases, which play a critical role in the regulation of a large number of important events occurring in the cell during division. Our research team aims to uncover the changes in timing and the degree of activation of the M-phase protein kinases, which are essential for meiotic cell division in the mammalian female germ cell (oocyte) and embryo.

Currently our lab is intensively focused on the study of protein kinases which are involved in translation during oogenesis and embryogenesis (*Virnicchi et al. 2019, bioRxiv; Ellederova et al. 2019, Mol Cell Biol; Jansova et al. 2017, Cell Cycle)*. This includes the kinases needed for the regulation of the

Cytoplasmic Polyadenylation factors, which play pivotal roles in the control of specific mRNAs through 3' UTR. Recently, we revealed that Aurora A kinase activity is not important for this process in mammalian oocytes, although it may be more influential in lower organisms. This suggests that some other kinases must be involved, and the nature of these kinases is central topic to our studies.

The Laboratory of Cell Division Control (LCDC), currently uncovered that the Cyclin A1 distinguishes between male and female meiosis. This molecule belongs to the core cell cycle machinery and therefore it is important to note that it is essential only for male meiosis. Our team discovered that it is not only nonessential for oocyte meiosis, in fact the presence of this molecule in female meiosis will inhibit separase and thus prevent chromosome segregation (*Radonova et al. 2020, Sci Rep*). Our team also studied the regulation of the spindle length in mammalian early embryos. This period is characterized by rapid changes of the spindle length, which is not scaling with the size of the cell. We discovered that the size of the spindle in mouse early embryos has no upper limit and scales with the size of the cell. Our results also revealed that the nuclear to cytoplasmic ratio controls the spindle length in early embryos (*Novakova et al. 2016, PLoS One*). With team of Andrej Susor, LCDC lab collaborated on description of localized translation in mouse oocytes. Our participation was limited to performing live cell imaging experiments with microinjected oocytes (*Susor et al. 2015, Nat Commun*).

The team of LDB focused on three main topics: i) identification of genes and signaling pathways regulating oocyte maturation and developmental competence; ii) mechanisms of maternal protein degradation and embryonic genome activation during the preimplantation development and iii) molecular studies on the role of oocyte nucleolus in regulation of early embryo development.

In the first analyzed topic, the team have further extended our previous data on the role of epidermal growth factor receptor - (EGFR) and mitogen-activated protein kinase 3/1- (MAPK3/1) signaling pathways in regulation of meiosis resumption in pig oocytes. Activation of EGFR network in cumulus cells is essential for maturation of oocytes and cumulus expansion both *in vivo* and *in vitro*. It was shown previously that the pig cumulus-oocyte complexes (COCs) isolated from two different follicle categories (<4 mm and >6 mm) differ in the extent of EGFR phosphorylation upon EGF stimulation. The different extent of the EGFR phosphorylation was associated with lower ability of the small follicle oocytes to resume meiosis and with the inability of cumulus cells to undergo expansion after EGF stimulation. These results indicated that development of full responsiveness of the cumulus cell EGFR to their ligands is developmentally regulated and that it can play an important role in acquisition of oocyte competence on the way to ovulation. We have also shown that in pig COCs, FSH induces expression and the synthesis of amphiregulin (AREG) that binds to EGFR and activates the MAPK3/1 signaling pathway. However, in the recent study (*Prochazka and Nemcova, 2019, Int J Mol Sci*) we found that FSH also caused a rapid minute activation of MAPK3/1 in the cumulus cells, which cannot be explained by the *de novo* synthesis of AREG. The rapid MAPK3/1 activation required EGFR tyrosine kinase (TK) activity, was sensitive to SRC-family kinases and protein kinase C (PKC) inhibitors, and it was resistant to inhibitors

of protein kinase A (PKA) and metalloproteinases. We concluded that in cumulus cells, FSH induces a rapid activation of MAPK3/1 by the ligand-independent transactivation of EGFR, requiring SRC and PKC activities. This rapid activation of MAPK3/1 precedes the second mechanism participating in the generation and maintenance of active MAPK3/1 – the ligand-dependent activation of EGFR depending on the synthesis of EGF-like peptides. The role of MAPK3/1/EGFR signaling pathway in regulation of oocyte meiotic maturation was also summarized in two reviews (*Prochazka et al. 2017, Biol Reprod; Prochazka and Blaha 2015, J Reprod Dev*).

Currently, research of LDB pertains degradation of maternal proteins in early bovine embryos by ubiquitination and its relevance to regulation of major embryonic genome activation. We have concentrated on Skp1-Cullin1-Fbox (SCF) complex, the expression of its invariant components (Cullin 1, Skp1, Rbx1) and its contribution to degradation of maternal proteins. Our up to now results have shown that embryonic expression of all these genes starts in initial stages of development. Especially cullin 1 is activated very early, already at 4-cell stage. Genes participating in ubiquitination are usually activated at 8-cell stage, and the early activation of cullin 1 suggests its necessity for embryonic genome activation. Protein localization analysis showed interesting results especially at the blastocyst stage. There was clear concentration of protein expression and SCF complex activation to trophectoderm (*Benesova et al. 2016, PLoS One*). In the next study, we inhibited the SCF complex by a specific inhibitor MLN4924, and showed that SCF ligases are necessary for the correct maturation of oocytes, cumulus cell expansion, fertilization, and early preimplantation development of cattle (*Kinterova et al. 2019, Biol Reprod*). The systematic long term study of this topic was summarized in a recent review (*Toralova et al. 2020, Cell Mol Life Sci*).

Detailed studies in pig and mouse demonstrated that the oocyte nucleolus (nucleolar sphere) is essential both for completion of the oocyte meiosis and for further embryonic development. In our studies, we aim to characterize the content of the nucleolar sphere by proteomic and genomic approaches as well as the developmental competence conveyed by this structure. First, we have concentrated our attention on genomic and intranuclear characteristics and nucleologenesis in pig oocytes with different developmental competence selected by brilliant cresyl blue (BCB) staining or donor follicle size. The objective of this study was to identify and analyze the transcriptomic profiles of porcine oocytes derived from large or small follicles using RNA high-throughput sequencing technology. RNA libraries were constructed from oocytes of large (LO; 3-6 mm) or small (SO; 1.5-1.9 mm) ovarian follicles and then sequenced in an Illumina HiSeq4000. Differential expression analysis revealed 60 up- and 262 down-regulated genes in the LO compared to the SO group. *BRCA2, GPLD1, ZP3, ND3,* and *ND4L* were among the highly abundant and highly significant differentially expressed genes (DEGs). The ontological classification of DEGs indicated that protein processing in endoplasmic reticulum was the top enriched pathway. In addition, biological processes related to cell growth and signaling, gene expression regulations, cytoskeleton, and extracellular matrix organization were highly enriched processes. In conclusion, this study provides new insights into the global transcriptome changes and the abundance of specific transcripts in porcine oocytes in correlation with their developmental competence (*Gad et al. 1919, Mol Reprod*

Dev). Next, we have confirmed that BCB staining well correlates with the oocyte quality and characterized nucleologenesis in high quality BCB+ oocytes (*Murin et al. 2019, Zygote*).

The Laboratory of Molecular Morphogenesis focused on the molecular signaling affecting formation of craniofacial structures and developmental processes contributing to the limb patterning during evaluated period. Here, we summarize main findings in individual streams of our research interests.

One of our main research topics is focused on the craniofacial patterning. Our study as the first directly compared gene expression profiles in different regions of the face and it has not only recognized general trends in the expression profiles but also identified specific sets of genes that are differentially expressed in the individual prominences (*Nimmagadda et al. 2015, Dev Biol*). We described several candidate genes that can be used to identify the normal frontonasal mass, mandibular as well as maxillary prominences. Next, we examined a role of several key genes in loss-of-function and gain-of-function experiments (*Cela et al. 2016, Dev Dyn; Cela et al. 2016, Front Physiol*). To evaluate cellular and molecular mechanisms contributing to developmental defects, we further analyzed them in several mice models deficient in *Tmem107* or *Cdk13* genes (*Cela et al. 2018, J Dent Res; Novakova et 2019, Front Cell Dev Biol*).

The other main area of LMM interest is the morphogenesis of individual dental structures. Differences in tooth morphology and age related changes during odontogenesis were analyzed in several different species of vertebrates, including mammals (*Dosedelova et al. 2016, J Anat; Landova Sulcova et al 2019, Dev Dyn; Fons et al. 2019, Dev Dyn*). We focused on the variations in the morphology and developmental processes in monophyodont, diphyodont and polyphyodont species that relate to their ability to form replacement teeth and the fate of the dental lamina (*Dosedelova et al. 2015, Plos One; Popa et al., 2019, Development*). Following our work on species-specific differenced in odontogenesis underlying the initiation of variable number of teeth generations, we focused on the signaling processes between the neural crest-derived mesenchyme and the ectoderm of the oral cavity that control successional dentition development and establishment of the structures asymmetry (*Putnova et al. 2017, Front Physiol)* and primary cilia related signaling or defects in odontogenesis (*Hampl et al 2017, J Dent Res*). Our aim was to uncover developmental processes contributing to the replacement tooth formation and their comparison in species with two or multiple tooth generations with intention to reveal species-specific differences, which determine early regression of dental lamina in animals with limited number of successional generations.

Furthermore, members of LMM and LCB explored the relationship between intrinsic stability of FGF proteins and their biological activity for all 18 members of the FGF family (*Buchtova et al. 2015, Cell Mol Life Sci*). Numerous proteins were found to be unstable and their biological activity is limited by their instability. Stabilization via exogenous heparin binding, introduction of stabilizing mutations or lowering the cell cultivation temperature rescues signaling of unstable FGFs. Thus, the intrinsic ligand instability is an important elementary level of regulation in the FGF signaling system. Next, the effect of new synthetic FGFR inhibitors on limb development was evaluated with the aim to find the best component for further *in vivo* studies (*Gudernova et al. 2016, Hum Mol*

Gen; Fafilek et al. 2018, Osteoarthritis and Cartilage). As individual inhibitors exhibit different activity profiles towards FGFRs, the comparison of their actions allowed the examination of limb development under quantitatively and qualitatively different FGFR mediated stimuli. We demonstrated that a complete inhibition of FGFR signaling suppresses limb bud outgrowth leading to absent limbs. More importantly, a partial inhibition of FGFR signaling revealed a previously unknown role of FGFR signaling in skeletogenesis. In future, we plan to follow these findings and expand study on determination of FGF role in anterior-posterior patterning during limb development with focus on zeugopod area.

LCB team further analyzed the effects of several inhibitors on aberrant fibroblast growth factor receptor (FGFR) signalling in models of skeletal dyplasias and cancer cell lines (*Balek et al. 2017, Bone; Gregor et al. 2019, Cell Mol Life Sci*). The composition of signalling complexes associated with activated FGFRs was revealed at the cell membrane, which lead to discovery of novel mediator of FGFR-ERK MAP kinase signalling, the inositol phosphatase SHIP2 (*Fafilek et al 2018, Science Signaling*). We characterized the interaction of FGFR signalling with canonical WNT/b-catenin signalling, in regulation of chondrocyte differentiation (*Buchtova et al 2015, Biochimica et Biophysica Acta, 2015*). We described the interaction of FGFR signalling with the primary cilia (*Kunova Bosakova et al. 2018, Human Molecular Genetics*), and revealed the molecular mechanism of this interaction (*Kunova Bosakova et al. PNAS*, 2019). We developed novel transcriptional reporter system capable of in-cell activity profiling of majority of known oncogenes and discovered a new class of inhibitors of FGFR signalling (*Balek et al. 2018, Biomaterials*). We also contributed to the research of others, our collaboration with the team of prof. Deborah Krakow at University of California Los Angeles lead to article (*Csukasi et al. 2018, Science Translational Medicine*), describing a novel mechanism of hormonal regulation of bone growth. Our collaboration with the team of prof. Jiri Damborsky at Masaryk University Brno lead to development of thermally stable FGFR ligand FGF2, which is now marketed worldwide by ThermoFisher.

Specific domains of LOO research are cystein proteases (caspases) and their novel functions in odontogenic and osteogenic processes. The research includes elucidation of mechanisms mediating their effect, particularly related to diagnostic and therapeutic aspects (as reviewed in *Kudelova et al*. 2015, *J Physiol Pharmacol*). In the period 2015-2019, original data were obtained on the activation pattern of pro-apoptotic caspases in non-apoptotic cells during odontogenesis and related osteogenesis including their novel roles, such as impact on expression of osteocalcin, one of the major osteogenic marker (*Svandova et al. 2018, Front Physiol)*. The osteogenic potential of caspases was investigated also in endochondral bones, particularly the growth plate (*Janeckova et al. 2018, J Histochem Cytochem*). Since caspases are activated in posttranslational manner, exact single cell based techniques were developed and applied for examination and quantification of the active proteins in different cell types (*Ledvina et al. 2017, Anal Bioanal Chem* - in cooperation with Kleparnik et al./IACH CAS/Brno who provided the instrumentation). The effect of caspase inhibition on expression of specific markers was additionally followed in chondrocytes and provided a screening of potential networks and candidate

molecules affected by caspases (*Adamova et al. 2016, In Vitro Cell Dev Biol Anim*).

Simultaneous research in the period 2015-2019 was dealing with Fas/FasL system known as an extracellular switch for caspase cascade but emerging also in important non-apoptotic functions. The findings pointed to a new non-apoptotic function of FasL in bone development associated with Mmp2 expression (*Svandova et al. 2018b, Front Physiol* – in cooperation with prof. Poliard/UniParisDescartes/France who provided the deficient mice). Additionally, a growth dependent phenotype of the mandible was revealed in FasL deficient mice (*Svandova et al. 2019, J Anat* – in cooperation with prof. Poliard from Univ. Paris Descartes/France who provided the deficient mice and performed microCT measurements). The Fas/FasL system was investigated also in forming limbs/growth plate chondrocytes (*Svandova et al. 2017, Histochem Cell Biol*) and in the Meckel´s cartilage where activation of caspase-8 and caspase-2 was shown and considered in coupling apoptosis and autophagy (*Bilikova et al. 2019, Physiol Res*).

The attention was paid also to investigation of periodontal structures and tooth eruption related to formation of functional dentition anchored in the jaw. The results (*Zvackova et al. 2017, Front Physiol*) illustrated developmentally regulated and tissue-specific changes in the balance of two FACITs and three SLRPs, and provided a complex localization of the antigens in soft tissues, the dental pulp, and periodontal ligaments, in the mineralized tissues, predentin/dentin and alveolar bone, and junction between soft and hard tissues. Particular attention was paid to investigation of the alveolar/mandibular bone in order to obtain knowledge welcome also for replacement strategies (such as dental implantology). Osteogenic and angiogenic analysis provided a molecular survey of the early post-condensation stage of mandibular/alveolar bone development which has not yet been investigated *in vivo* (*Vesela et al. 2019, Front Physiol*). Related modulations were investigated also with respect to hypoxia and novel factors (*Bobek et al. 2019, Histochem Cell Biol*).

In cooperation with the King´s College London (during a scientific stay of the LOO member, V. Oralova, at KCL), c-Fos deficient mice, lacking osteoclasts, were investigated. The failure in root development and the loss of the tooth-bone interface was rescued in the presence of donor osteoclasts, thus it was shown that signals from the tooth recruit osteoclasts to clear the bone from around the tooth, allowing the tooth to grow, form roots, and later erupt (*Alfaqeek et al. 2015, J Dent Res*). In cooperation with prof. Radlanski (Univ. Berlin/BRD), morphogenesis of the compartmentalizing bone around the molar tooth up to the eruption stage was analysed and published (*Radlanski et al. 2015, Ann Anat*). Recent knowledge on development of tooth and associated structures was reviewed in the book Stem Cell Biology and Tissue Engineering in Dental Sciences (chapter by *Matalova et al. 2015*).

Also, other earlier established cooperations and joint topics were developed in the period 2015-2019 such as with prof. Smarda on novel functions of the transcription of Myb in osteogenesis and chondrogenesis (*Oralova et al. 2015, Bone; Oralova et al. 2017, Calcif Tissue Int*). In cooperation with the Ghent University, where a LOO member (V. Oralova) spent 2 years of her post-doc period (2017-2018), finalization of results related to specificities of tooth/bone development in zebrafish models was achieved (*Oralova et al. 2019, Biol Open*).

Research activity and characterisation of the main scientific results

LME

The group conducted and published a series of studies using the bank vole as a model system and aimed at elucidating the response of temperate species to the global warming that followed the Last Glacial Maximum.

- They have performed a comparison of genomic SNP variation *via* genotyping-by-sequencing and revealed a complete replacement due to climate change of one population of the bank vole with another population of the same species, but which was probably better adapted to the new climatic conditions. This is a unique example of such response of organisms to climate change (**Kotlík, P., S. Marková, M**. Konczal, W. Babik, J. B. Searle. 2018. Genomics of end-Pleistocene population replacement in a small mammal. Proceedings of the Royal Society B-Biological Sciences 285: 20172624). P. Kotlík (the corresponding author) obtained funding, designed and supervised the work, performed the majority of the analyses and wrote the paper. S. Marková performed the experimental work and revised the text. The co-authors performed part of the data analyses, provided advice and helped revise the text.

- In a study using more traditional as well as next-generation sequencing to revisit the phylogeny of the bank vole, a key study species for understanding the response of European fauna to the climate change following the Last Glacial Maximum, they found significant signatures of selection in the bank vole mitochondrial genome, but little evidence of pervasive effects of the deviation from neutrality on the bank vole phylogeography (**Filipi, K., S. Markova**, J. B. Searle, **P. Kotlík**. 2015. Mitogenomic phylogenetics of the bank vole Clethrionomys glareolus, a model system for studying end-glacial colonization of Europe. Molecular Phylogenetics and Evolution 82:245-257). K. Filipi (at that time a student of P. Kotlík) performed the experimental work, analysed the data and drafted a part of the text. S. Marková supervised the experimental work and data analysis and edited the text. P. Kotlík (the corresponding author) obtained funding, designed the study, supervised the work of K. Filipi and wrote most of the text. The co-author helped revise the text.

- In a work using bank vole RNA-Seq data they demonstrated how useful such data can be to recover the mtDNA transcriptome in a non-model rodent and shed light on mammalian mtDNA transcriptome and post-transcriptional modification. It demonstrated that even though gene content and organisation of mtDNA are strongly conserved among mammals, annotations that neglect the transcriptome are prone to errors (**Marková, S., K. Filipi**, J. B. Searle, **P. Kotlík**. 2015. Mapping 3 ' transcript ends in the bank vole (*Clethrionomys glareolus*) mitochondrial genome with RNA-Seq. BMC Genomics 16). S. Marková performed most of the work and drafted the text. P. Kotlík (the corresponding author) obtained funding, designed the study, performed some analyses and revised the text. K. Filipi (at that time a student of P. Kotlík) performed part of the work and helped write the text. The co-author provided advice and helped revise the text.

- Another their study supported the model where gene conversion reshuffling non-synonymous genotypes between high- and low- expressed globin paralogs in the bank vole enables tuning of erythrocyte thiol levels, thus helping maintain intracellular redox balance under fluctuating environmental conditions. Thus, the study suggests a possible role for gene conversion between differentially

expressed gene duplicates as a mechanism of physiological adaptation of populations to new or changing environments (**Strážnická, M., S. Marková**, J. B. Searle, **P. Kotlík**. 2018. Playing hide-and-seek in beta-globin genes: Gene conversion transferring a beneficial mutation between differentially expressed gene duplicates. Genes 9:492). M. Strážnická (a PhD student of P. Kotlík) performed the work and drafted the text. S. Marková supervised the experimental work and edited the text. P. Kotlík obtained funding, supervised the work of M. Strážnická, and helped write the text. The co-author provided advice and helped revise the text.

- They have also discovered a new species of fish in rivers of Europe. In a collaborative study of genetic variation of the endangered Carpathian barbel in the Danube basin P. Kotlík and his Hungarian colleagues of the University of Debrecen discovered that barbels from the river Crisul Repede system are so different from all other species of barbels as to be considered a separate species. The new species was named Biharian barbel *(Barbus biharicus* Antal, László & Kotlík, 2016) for the Bihár (Bihor) county where it lives (Antal, L., B. Laszlo, **P. Kotlík**, A. Mozsar, I. Czegledi, M. Oldal, G. Kemenesi, F. Jakab, S. A. Nagy. 2016. Phylogenetic evidence for a new species of *Barbus* in the Danube River basin. Molecular Phylogenetics and Evolution 96:187-194). P. Kotlík was invited by the Hungarian colleagues to provide expert advice based on his previous experience with the Carpathian barbels (he discovered and described two new species in 2002). He supervised part of the work, provided advice and helped write the text.

- As another groups of vertebrates studied by the team, frogs are well-known amphibians threatened by disease, pollution, habitat loss and climate change, but some aspects of their natural history are surprisingly poorly known, including the species limits and mechanisms of speciation. The results of their study shed light on the species limits in the European short-call tree frogs and show that introgression played an important role in their evolutionary history and occurred even between taxa supported as distinct species (Gvoždík, V., D. Canestrelli, M. Garcia-Paris, J. Moravec, G. Nascetti, E. Recuero, J. Teixeira, **P. Kotlík**. 2015. Speciation history and widespread introgression in the European short-call tree frogs *(Hyla arborea sensu lato, H. intermedia* and *H. sarda*). Molecular Phylogenetics and Evolution 83:143-155). V. Gvoždík (the corresponding author, a former PhD student and postdoc of P. Kotlík) collected samples, performed the experimental work, analysed data and drafted the text. P. Kotlík supervised the work of V. Gvoždík and revised the text. One co-author co-supervised V. Gvoždík and the other co-authors contributed samples.

- A study using planktonic crustaceans addressed biological invasions, a global issue with far-reaching consequences. This study combined historic information with genetic analysis of resting eggs to reconstruct the invasion of a lake by a Daphnia species in the 1970s from the resting egg bank in the sediments. It shows that genetic data covering the entire invasion process from its beginning can accurately reconstruct the invasion history and that propagule banks can preserve such information, enabling the study of successful invasions (Möst, M., Oexle, S., **Marková, S**., Aidukaite, D., Baumgartner, L., Stich, H.B., Wessels, M., Martin-Creuzburg, D., Spaak, P. 2015. Population genetic dynamics of an invasion reconstructed from the sediment egg bank. Molecular Ecology 24:4074-4093). S. Marková was invited by the Swiss colleagues to provide expert advice based on her previous experience with the genetic analysis of

Daphnia. She participated in the study design and data interpretation and helped write the text.

LMEG

The main focus of the LMEG is the 30-year study of the hybrid zone between two house mouse subspecies as a model of the evolution of reproductive barriers. According to Haldane's rule, sex chromosomes should harbour more incompatibilities than autosomes. As a consequence, transmission of sex-linked genes across a genetic barrier is expected to be hampered. Previously, we reported a remarkable example of a contradiction of this assumption: unidirectional east-to-west Y chromosome introgression of the eastern subspecies (*Mus musculus musculus*) into the range of the western subspecies (*M. m. domesticus*) in W Bohemia/NE Bavaria. In our recent study we first analysed the precise course of the house mouse hybrid zone (HMHZ) using a large material consisting of almost 7500 mice collected across a vast area from the Baltic Sea to the Alps covering ~288,000 km^2 and embracing a ~900 km long portion of the HMHZ. The second goal was to map the spatial distribution and extent of the Y chromosome introgression, we showed to be widespread in Central Europe and locally rather extensive. We also showed that although sex ratio perturbations described in our previous study appear also in other introgression areas, they may not be ubiquitous. Finally, we revealed that although not all Y chromosome types are associated with the introgression, it is not restricted to a single 'winning' haplotype. The study was designed by M. Macholán who actively participated in gathering the material, data analysis and writing the paper (bioRxiv, https://doi.org/10.1101/2019.12.23.887471; submitted to Molecular Ecology).

Based on our previous results we hypothesized that the introgression is a consequence of intragenomic conflict between sex chromosomes, probably involving also some autosomal genes. One of likely battlefields is the conflict over the number of gene copies and over expression. Therefore, we focused on two ampliconic genes, *Sly* and *Slx*, present in many copies on Y and X chromosome, respectively, and known to be engaged in an arms race over sex ratio. We analysed their copy number (CN) variation and expression in males across two replicate regions of the HMHZ in Europe, comparing a range of models to explain the data. In both replicates we found more *Sly* copies than *Slx* copies and more amplicons of both genes in *M. m. musculus* than in *M. m. domesticus*, mirrored in 3–4-fold higher eastern total *Sly* expression. In contrast, *Slx* copy number had little impact on total *Slx* expression levels, suggesting trans-subspecies homeostasis of *Slx* expression. Further, we analysed the proportion of *Sly* CN and expression in the context of a single male, i.e. relative expression/CN of both genes within each male. The results showed two multilocus fronts: the consensus (and X chromosome) zone centre versus a Y chromosome invasion front ~13 km to its west. The data were consistent with two phases of westward introgression. First, a wave of disruption of relative *Slx*/*Sly* regulation biasing sex ratio in favour of females. Second, as an adaptive response, introgression of (high *Sly* CN) *musculus* Y chromosomes redressing the sex ratio imbalance. A further currently localized wave is supported with lower N: introgression of high eastern *Slx* CN despite the strong barrier to introgression on the X chromosome. Our study thus provides empirical insight

into consequences of selfish genes sweeping across semipermeable species barriers. The project was designed by M. Macholán who also participated in methodology development and data analysis; the data have been gathered and a manuscript (finished for Nature Ecology and Evolution) written by M. Macholán, K. Daniszová, Z. Hiadlovská.

Complementary to searching for genomic correlates of the Y introgression, we paid attention to phenotypic traits potentially mediating the phenomenon. Given the paternal inheritance pattern, obvious candidates for traits mediating the introgression are characters associated with sperm quantity and quality. We can also expect traits such as size, aggression, or the length of generation cycles to facilitate the spread. We thus used two consomic strains carrying the non-recombining region of the Y chromosome of the opposite subspecies, allowing us to study introgression in both directions, something impossible in nature due to the unidirectionality of introgression. We analysed several traits potentially related to male fitness. Transmission of the *domesticus* Y onto the *musculus* background had negative effects on all studied traits. Likewise, *domesticus* males possessing the *musculus* Y had, on average, smaller body and testes and lower sperm count than the parental strain. However, the same consomic males tended to produce less dissociated sperm heads, to win more dyadic encounters, and to have shorter generation cycles than pure *domesticus* males. These data suggest the *domesticus* Y is disadvantageous on the *musculus* background while introgression in the opposite direction can confer a recognizable, though not always significant, selective advantage. Our results are thus congruent with the unidirectional *musculus* → *domesticus* Y chromosome introgression in Central Europe. Therefore, we used 31 recombinant lines from eight wild-derived strains representing four localities within the two mouse subspecies. These lines were reciprocally crossed and resulting F1 hybrid males scored for five phenotypic traits associated with male fitness. Molecular analyses of 51 Y- linked SNPs attributed ~50% of genetic variation to differences between the subspecies and 8% to differentiation within both taxa. A striking proportion, 21% (frequencies of sperm head abnormalities) and 42% (frequencies of sperm tail dissociations), of phenotypic variation was explained by geographic Y chromosome variants. Our crossing design allowed this explanatory power to be examined across a hierarchical scale from subspecific to local intrastrain effects. We found that divergence and variation were expressed diversely in different phenotypic traits and varied across the whole hierarchical scale. This finding adds another dimension of complexity to studies of Y introgression not only across the house mouse hybrid zone but potentially also in other contact zones. All the above mentioned results were revealed in close collaboration with colleagues at the IVB, Studenec (J. Piálek, Stuart J.E. Baird and others) who created the consomic and recombinant inbred strains and participated in collecting data and some statistical procedures. B. Vošlajerová Bímová conceived and supervised the consomic strains project, carried out the behavioural experiments, and participated in data analysis and was a senior author of the paper (**Vošlajerová Bímová** et al., Heredity 2020: https://doi.org/10.1038/s41437-020-0330-z). M. Macholán was involved in part of statistical analyses and writing the papers (the recombinant strains study was published in **Martincová** et al., Ecology and Evolution: DOI: 10.1002/ece3.5196).

The experiments with consomic strains showed that the Y introgression can have also behavioural correlates. However, these correlates should be placed in a broader context of different exploratory strategies, population structure and the dynamics of its creation. Indeed, we found that *M. m. domesticus* males longer assess potential danger before entering an unknown area but then are more active in its exploration and more eager to overcome a water barrier. By contrast, *M. m. musculus* males were shown to be more secure-seeking during exploration but to perform better under stressful conditions or to react less dramatically to handling. Based on these contrasts, we expected these taxa to differ also in processes behind establishing social hierarchy. We carried out two experiments using fraternal pairs as the simplest social units over an extended period since weaning to about 100 days of age. We showed that *domesticus* males invested more to growth and quickly created a hierarchy which can potentially relax social stress while *musculus* males were faster in reaching maturity yet the social tension persisted further to adulthood. These results were corroborated by the following study suggesting that the prolonged social stress in *M. m. musculus* results in significantly higher emigration rate of subordinate males compared with dominant ones. This is in sharp contrast with *M. m. domesticus* males in which rapid establishment of social hierarchy through overt aggression leads to a decrease of testosterone and 'stress hormone' corticosterone, and to more frequent migrations of dominant males. Finally, we performed two long-term experiments (run in parallel for the two subspecies) in seminatural conditions using radio-frequency identification tags, with the aim of deciphering the dynamics of establishment of a population structure and possible differences between the taxa. Although the applied method has recently been used both for the mouse and other species as well, we are the first who could struggle with enormous amounts of data and treat them in a meaningful way. Consequently, we are also the first who rendered a statistically robust proof of different social structuring in *M. m. musculus* vs. *M. m. domesticus*. These studies resulted in a series of papers co-authored by all LMEG members; all of them were designed and supervised by the first and last authors (B. Vošlajerová Bímová, Z. Hiadlovská, K. Daniszová). O. Mikula designed and performed key statistical analyses while M. Macholán was involved in managing the studies and writing the papers. Since 2015, the results were published in **Hiadlovská** et al., Gen. Comp. Endocrinol. 2015, doi: org/10.1016/j.ygcen.2015.09.033; **Vošljerová Bímová** et al., Ethology 2016, doi: 10.1111/eth.12462; **Daniszová** et al. Gen. Comp. Endocrinol. 2017, doi: org/10.1016/j.ygcen.2017.06.023; article **Hiadlovská** et al.: „Dominant or subordinate? Two European house mouse subspecies differ in the social rank of dispersing males" is currently under minor revision in Behav. Processes; the paper on seminatural experiments is being prepared for Proc. R. Soc. B).

LFG

- We established and developed a new promising animal model for thorough investigation of asexual reproduction and polyploidy - the spined loaches of the genus *Cobitis*. Using integrative approaches, we characterized evolution and speciation history of this taxon and resolved fundamentals of molecular basis of production of clonal gametes. Such a research brought several unique informations for general biology and evolution: 1) asexual reproduction arises as a by-product of species diversification by merging of genomes with more

substantial degree of differentiation, 2) asexuality is a form of Bateson-Dobzhansky-Muller incompatibilities that seems related to disturbed crosstalk between merged regulatory networks in a hybrid, genome, 3) production of clonal gametes represents a remedy to classical hybrid sterility. Therefore, asexual reproduction appears as an inherent stage of the speciation continuum and consequently, its incidence in nature is much more common than previously believed. Aforementioned findings stem from following publications:

¨-Using conventionall as well as advanced (GISH) chromosomal staining techniques, we investigated structural changes of clonally transmitted karyotypes in hybrid di- and polyploids. We found that despite over 300kya of clonal transmission, hybrid's karyotypes remained remarkably conserved, thereby challenging classical theories assuming fast aneuploidisations of asexual genomes. (**Majtánová Z., Choleva L., Symonová R., Ráb P.,** Kotusz L., Pekárik & **Janko K**. 2016. Asexual reproduction does not apparently increase the rate of chromosomal evolution: karyotype stability in diploid and triploid clonal hybrid fish (*Cobitis*, Cypriniformes, Teleostei). PLoS ONE. 11:e0146872. IF 2015: 3.057), Z. Majtanova, R. Symonová, P. Rab performed the cytogenetic work, L. Choleva and K. Janko arranged most samples and performed molecular analyses. All authors contributed to study design and text.

- In the next study, we combined population genetic, genomic and phylogeographic data with results of experimental crossing experiments to demonstrate that asexuality emerges as a by-product of interspecies differentiation during the speciation process and that it assist in itself in the formation of postzygotic reproduction barrier. (**Janko K,** Pačes J, Wilkinson-Herbots H, Costa RJ, **Roslein J,** Drozd P, **Iakovenko N,** Rídl J, Hroudová M, **Kočí J,** Reifová R, **Šlechtová V, Choleva L**. 2018. Hybrid asexuality as a primary postzygotic barrier between nascent species: On the interconnection between asexuality, hybridization and speciation. Molecular Ecology 27: 248-263). K. Janko designed the experiments and analysed much of the NGS data, J. Roslein, J. Koci performed most genotyping, L. Choleva, N. Iakovenko performed reproductive experiments, sampling was performed by K. Janko and L. Choleva.

- In the following study, we participated on analysis of speciation and sex chromosome evolution of European nightingales. The study was among the first one to demonstrate the role of sex chromosomes in the formation of speciation barriers in the ZW sex determined animals. (Mořkovský L; Janousek V; Reif J; Ridl J; Pačes J; **Choleva L; Janko K**; Nachman MW; Reifova, R . 2018. Genomic islands of differentiation in two songbird species reveal candidate genes for hybrid female sterility. Molecular Ecology 27: 949-958), L. Choleva and K. Janko participated on establishment of laboratory protocols and on data interpretation.

- In one the most valued work by us, we used combination of genotyping, RNAseq, morphological and ecological investigations to reveal the complex background of hybrid and polyploid phenotypes in relation to parental species. The study revealed several outstanding features, among other the fact that overall intermediacy of hybrid d phenotypes does not result from additive expression but instead of a mixture of dominantly expressed genes (**Bartoš, O., Röslein, J**., Kotusz, J., Paces, J., Pekárik, L., Petrtýl, M., **Janko, K.** 2019. The Legacy of Sexual Ancestors in Phenotypic Variability, Gene Expression, and Homoeolog Regulation of Asexual Hybrids and Polyploids. Molecular Biology

and Evolution, 36(9), 1902–1920. doi: 10.1093/molbev/msz114). O. Bartos,J. Roslein performed most lab work and data treatment and also participated on advanced statistical analyses. K. Janko designed the study and performed many statistical analyses and drafted the MS.

- Finally, in the theoretical study we used mathematical modelling to demonstrate that asexual hybrids have unexpected impact on coexisting parental species. Namely, we found that presence of asexuals has similar effect as that of shared pathogen and in such a way, asexuals may provide arbitrage for competing sexual species. (**Janko, K.,** Eisner, J., & Mikulíček, P. 2019. Sperm-dependent asexual hybrids determine competition among sexual species. Scientific Reports, 9(1), 722. doi: 10.1038/s41598-018-35167-z). K. Janko conceptualized the central idea and wrote the MS.

- Asexual animals are often called unisexual because they are formed only by females. The even more surprising is the occurrence of hybrid males in water frogs. The team members studied the role the triploid hybrid males they play in populations and found their complex functions. Such males have very contrasting roles in Europe despite the same genotype constitution. In Central Europe, males sexually parasitize diploid hybrids and just perpetuate their genotype, which is the usual pattern in parthenogens. In North-western Europe, the triploid males are gamete donors for diploid hybrids, thereby stabilizing the mixed 2n-3n hybrid populations (Pruvost, N. B. , P. Mikulíček, **L. Choleva**, H.U. Reyer. 2015. Contrasting reproductive strategies of triploid hybrid males in vertebrate mating systems. Journal of Evolutionary Biology 28:189-204). L. Choleva came with the idea of the contrasting roles in males, helped with the field sampling and funding for this part, and manuscript writing.

- In the second study, the authors analyzed population structures in Central Europe to contribute to the pan-European study presenting that different hybrid and parental genotypes are not evenly distributed across Europe. Instead, their genetic diversity is structured by latitude and longitude and the presence/absence of parental species but not of triploids. Moreover, all-hybrid type of water frog populations can be viewed as evolutionary units that may be on their way towards hybrid speciation (Hoffmann, A, J. Plötner, N. Pruvost, D.G. Christiansen, S. Röthlisberger, **L. Choleva**, P. Mikulíček, D. Cogălniceanu, I. Sas-Kovács, D. Shabanov, S. Morozov-Leonov, H.U. Reyer. 2015. Genetic diversity and distribution patterns of diploid and polyploid hybrid water frog populations (*Pelophylax esculentus* complex) across Europe. Molecular Ecology, 24, 4371-4391). L. Choleva designed and organized sampling of the Czech water frog populations, helped with data analyses and interpretation (Liběchov and Zurich), helped with funding for this part, and contributed to the revision of the manuscript.

- In the third study, they applied the comparative genomic hybridization to show that classic hybridogenesis is not the exclusive way operating in hybrid water frogs. These findings are changing some textbook claims because hybrid water frogs are typical for a linkage between the genome elimination and hemiclonal inheritance. Instead, they showed that *P. esculentus* males from mixed populations with parental *P. ridibundus* have no genome elimination from the germline prior meiotic division (**Doležálková, M., A. Sember**, F. Marec, **P. Ráb**, J. Plötner, **L. Choleva**. 2016. Is premeiotic genome elimination an exclusive mechanism for hemiclonal reproduction in hybrid males of the genus *Pelophylax*? BMC Genetics, 17, 100). M. Doležálková (a PhD student of Lukáš

Choleva) performed the work and drafted the text. A. Sember and P. Ráb supervised the experimental work and edited the text. L. Choleva obtained funding, supervised the work of M. Doležálková, and helped write the text. The co-author provided advice and helped revise the text.

- The small study with the Croatian team presented relatively rare data on amphibian age and southern distribution-range limits of hybridogenetic *P. esculentus* in Europe. The results made the region potentially attractive to study gene flow and the impact of *P. esculentus* on *P. ridibundus*, in the Balkan Peninsula (Čavlović K., I. Buj, D. Karaica, D. Jelić, **L. Choleva**. 2018. Composition and age structure of the *Pelophylax esculentus* complex (Anura; Ranidae) population in inland Croatia. Salamandra 54:11-20). L. Choleva was invited by the Croatian colleagues to provide expert advice based on his previous experience with the genetic analyses of water frogs. He supervised part of the K. Čavlović work (during her stay in Liběchov), helped with the funding of the genetic part, provided advice, and helped write the text.

- In the last water frog study, the team addressed the role of diploid hybrid males, because unisexuality is almost exclusively linked to the female sex, and most studies addressed host-parasite dynamics in populations where sperm-dependent females dominate. The results showed that males also can perpetuate over many generations as the unisexual lineage, and successfully compete with sexual males for eggs provided by sexual females. Natural persistence of such sex-specific hybrid populations allows studying the similarities and differences between male and female reproductive parasitism in many biological settings (**Doležálková-Kaštánková M.**, N. Pruvost, J. Plötner, **L. Choleva**. 2018. All-male hybrids of a tetrapod *Pelophylax esculentus* share its origin and genetics of maintenance. Biology of Sex Differences 9:13). M. Doležálková-Kaštánková performed the work and drafted the text. L. Choleva obtained funding, supervised the work of M. Doležálková, and helped write the text. The co-author provided advice and helped revise the text.

- We analyzed old polyploidization event in the evolution of loach family Botiidae. The major result was that the 35 million years old tetraploid lineage within Botiidae, subfamily Botiinae, resulted from an allopolyploidisation event (i. e. *via* interspecific hybridization) followed by subsequent very strong functional diploidization, i.e. silencing of many gene copies and rearrangements chromosomal markers) (**Sember, A., J. Bohlen, V. Šlechtová, M. Altmanová, Š. Pelikánová, P. Ráb** 2018. Dynamics of tandemly repeated DNA sequences during evolution of diploid and tetraploid botiid loaches (Teleostei: Cobitoidea: Botiidae). PLoS ONE 13 (3): e0195054). A. Sember, Š. Pelikánová and V. Šlechtová made all analyses, P. Ráb and J. Bohlen conceptualized study, M. Altmanová visualized and arranged results, all authors co-drafted manuscript.

- A second major finding was that different ploidy levels in botiid loaches act as impassable barrier for hybridization, although morphological data suggested this possibility (**Bohlen, J., V. Šlechtová, V. Šlechta, V. Šlechtová, A. Sember, P. Ráb** 2016. A ploidy difference represents an impassable barrier for hybridisation in animals. Is there an exception among botiid loaches (Teleostei: Botiidae)? PLoS ONE 11(7): e0159311. doi:10.1371/journal.pone.0159311). A. Sember, V. Bohlen Šlechtová, V. Šlechtová, V. Šlechta made all analyses, J. Bohlen and P. Ráb conceptualized study, all authors co-drafted manuscript.

- We analyzed mechanisms of evolution and species differentiation within other loach family Nemacheilidae where the study revealed a very strong impact of

global sea level fluctuations on the evolution of the freshwater fauna of western Indochina in a series of studies demonstrated in the same region a case where different rates of diversification exist in parallel in closely related taxa, depending on the type of habitat (fast radiation in strongly fragmented habitats, slow in less fragmented habitats). In both groups new species were detected and described (**Bohlen, J., Šlechtová, V.** 2016. *Leptobotia bellacauda*, a new species of loach from the lower Yangtze basin in China (Teleostei: Botiidae). Zootaxa 4205 (1): 65-72. **Bohlen, J.,** M. Petrtýl, P. Chaloupková, C. Borin 2016. *Schistura kampucheensis*, a new species of loach from Cambodia (Teleostei: Nemacheilidae). Ichthyological Exploration of Freshwaters 26: 353-362. Grau, J.H., Hilgers, L., Altmüller, J., **Šlechtová, V., Bohlen**, J. 2017. The complete mitochondrial transcript of the red tail loach *Yasuhikotakia modesta* as assembled from RNAseq (Teleostei: Botiidae). Mitochondrial DNA Part B, Resources, 2: 46-47. **Bohlen, J., Šlechtová, V**. 2017. *Leptobotia micra*, a new species of loach from the Pearl River basin in southern China (Teleostei: Botiidae). Zootaxa 4250 (1): 90-100. IF 2015: 0.994. **Bohlen, J., Šlechtová, V.,** Li, F. 2019. Phylogenetic position of *Bibarba bibarba* Chen & Chen, 2007 (Cobitoidea: Cobitidae) as revealed from molecular genetic data with comments on its sexual dimorphism. Ichthyological Exploration of Freshwaters, 94(4): 297 - 304. **Bohlen, J., Dvořák, T., Šlechta, V., Šlechtová, V.** 2020. Evolutionary units and biogeographical history of the Indochinese freshwater fish *Paracanthocobitis zonalternans* (Teleostei: Nemacheilidae) as reconstructed from phylogenetic and geological data. Molecular Phylogenetics and Evolution (in press). J. Bohlen conceptualized all studies, collected materials, V. Bohlen Šlechtová, T. Dvořák made all analyses all authors co-drafted manuscript

- We introduced and developed comparative genomic hybridization (CGH) method for the purpose of i) sex chromosome characterization, ii) comparisons of interspecific genome divergence and iii) analysis of genome composition in asexual/clonal/unisexual hybrid genomes of diverse taxa studied in LFG (**Symonová, R., Sember, A., Majtánová, R., Ráb, P**.: Characterization of fish genomes by GISH and CGH. Chapter 16, pp: 118 – 131. In: Fish Cytogenetic Techniques (Chondrichthyans and Teleosts), Eds: Catherine Ozouf-Costaz, Eva Pisano, Fausto Foresti, Lurdes Foresti de Almeida Toledo CRC Press, Inc., Enfield, NH 03748, USA). R. Symonová introduced the protocol from her Ph.D. stay in Germany, others adapted the protocol for fish models (e.g. **Majtánová Z, Choleva L, Symonová R, Ráb P,** Kotusz J, Pekárik L, **Janko K.** 2016. Asexual Reproduction Does Not Apparently Increase the Rate of Chromosomal Evolution: Karyotype Stability in Diploid and Triploid Clonal Hybrid Fish (Cobitis, Cypriniformes, Teleostei). PloS One 11 (1)).

- With chromosome painting (WCP) we found shared or independent origin of standard or multiple sex chromosomes in several fish taxa (*Hoplias malabaricus* species complex, genera *Oplegnathus*, *Pyrrhulina*, *Triportheus*), contributing to better understanding sex chromosome evolution among diverse fish clades. (de Moraes, R. L.; **Sember, A**.; Bertollo, L. A.; De Oliveira, E. A.; **Ráb, P**.; Hatanaka, T.; Marinho, M. M.; Liehr, T.; Al-Rikabi, A. B.; Feldberg, E.; Viana, P. F.; Cioffi, M. B. 2019. Comparative cytogenetics and neo-Y formation in small-sized fish species of the genus *Pyrrhulina* (Characiformes, Lebiasinidae). *Front. Genet.* 2019, *10*, 1–13, doi:10.3389/fgene.2019.00678. Xu, D.; **Sember, A**.; Zhu, Q.; Oliveira, E. A. De; Liehr, T.; Al-Rikabi, A. B. H.; Xiao, Z.; Song, H.; Bello, M.B. Deciphering the origin and evolution of the X_1X_2Y system in two

closely-related *Oplegnathus* species (Oplegnathidae and Centrarchiformes) *Int. J. Mol. Sci.* **2019**, *20*, 3571. doi:10.3390/ijms20143571 **Sember A**, Bertollo LAC, Ráb P, Yano CF, Hatanaka T, Oliveira EA, Cioffi MB. 2018. Sex chromosome evolution and genomic divergence in the fish *Hoplias malabaricus* (Characiformes, Erythrinidae). Front Genet. 2018; 9: 71. Oliveira EA a **Sember A** (sdílené prvoautorství), Bertollo LAC, Yano CF, Ezaz T, Moreira-Filho O, Hatanaka T, Trifonov V, Liehr T, Liehr T, Al-Rikabi ABH, **Ráb P,** Pains H, Cioffi MB. 2018. Tracking the evolutionary pathway of sex chromosomes among fishes: characterizing the unique XX/XY_1Y_2 system in *Hoplias malabaricus* (Teleostei, Characiformes). Chromosoma 2018;127: 115–128. Cioffi MB, Yano CF, **Sember A**. Bertollo LAC. 2017. Chromosomal evolution in lower vertebrates: sex chromosomes in Neotropical fishes. Genes 2017;8: 258. Yano CF, Bertollo LAC, Ezaz T, Trifonov V, **Sember A**, Liehr T, Cioffi MB. 217. Highly conserved Z and molecularly diverged W chromosomes in the fish genus *Triportheus* (Characiformes, Triportheidae). Heredity 2017; 118: 276–283.). These studies originated from postdoctoral stay of A. Sember infederal University, Sao Carlos, Brazil. In all studies, A. Sember did CGH experiments and analyzed results, P. Ráb co-drafted manuscripts

- We used repetitive DNA markers to i) reveal karyotype stasis vs. dynamic evolution of the genome, ii) to show distinct rate of karyotype and repetitive DNA differentiation after polyploidy and iii) in species with different ecological characteristics. .(**Sember, A., J. Bohlen, V. Šlechtová, M. Altmanová, R. Symonová, P. Ráb** 2015. Karyotype differentiation in 19 species of river loach fishes (Nemacheilidae, Teleostei): Extensive variability associated with rDNA and heterochromatin distribution and its phylogenetic and ecological interpretation. BMC Evolutionary Biology 15: 251. doi: 10.1186/s12862-015-0532-9).). A. Sember, V. Bohlen Šlechtová, made all analyses, M. Altmanová visualized and arranged results, P. Ráb and J. Bohlen conceptualized study, all authors co-drafted manuscript.

- We also improved chromosome preparation in small-sized fishes (particularly family Lebiasinidae and a killifish genus *Nothobranchius*) and optimized new repetitive DNA probes (U1 and U2 snDNA and repeats generated by RepeatExplorer analysis) (De Oliveira, E. A.; **Ráb, P**.; Hatanaka, T.; Marinho, M. M.; Liehr, T.; Al-Rikabi, A. B.; Feldberg, E.; Viana, P. F.; Cioffi, M. B. 2019. Comparative cytogenetics and neo-Y formation in small-sized fish species of the genus *Pyrrhulina* (Characiformes, Lebiasinidae). *Front. Genet.* 2019, *10*, 1–13, doi:10.3389/fgene.2019.00678 and ongoing project with A. Sember as PI).

- We are developing cytogenomics of African annual killifish species pairs of the genus *Nothobranchius* where we i) examine sex chromosome differentiation and male-specific region (MSY), ii) unravel the origin of X_1X_2Y system in five *Nothobranchius* species and whether they have shared or independent origin and master sex determining gene and iii) decipher karyotype variability and repetitive DNA dynamics and sex turnover (ongoing project with A. Sember as PI).

- Together with colleagues from Faculty of Fishery and Water protection, we discovered and cytogenetically analysed sturgeon individuals with around 420 chromosomes resulting from mating of normal female (around 240 chromosome) with spontaneously arisen triploid male (around 368 chromosomes). In this study, we thus reported the second highest chromosome count among vertebrates in cultured sturgeon (~437 chromosomes) after the

schizothoracine cyprinid *Ptychobarbus dipogon* with ~446 chromosomes. The finding also represents the highest documented chromosome count in Acipenseriformes, this study provided the first clear evidence of a maternal origin for spontaneous polyploidization in cultured Siberian sturgeon (Havelka, M., Bytyutskyy, D., **Symonová, R**., **Ráb, P.,** Flajšhans, M. 2016: The Second Highest Chromosome Count among Vertebrates is Observed in Cultured Sturgeon, and is Associated with Genome Plasticity. Genetics Selection Evolution. *48:12* DOI 10.1186/s12711-016-0194-0). R. Symonová, P. Ráb made chromosome analyses and co-drafted manuscript

- We examined paddlefish chromosomes using all cytogenetic protocols together with physical mapping of ribosomal genes and Hox paralogs combined with microsatellite data to unravel the process of re-diploidization in the paddlefish genome evolution. We showed a partial re-diploidization process of the paddlefish genome represented by a complex mosaic structure (tetraploid, partly tetraploid and already diploid markers) comparable with segmental paleotetraploidy revealed in sturgeons (**R Symonová**, M Havelka, CT Amemiya, WM Howell, T Kořínková, M Flajšhans, D Gela, **P Ráb** 2017: Molecular cytogenetic differentiation of paralogs of Hox paralogs in duplicated and re-diploidized genome of the North American paddlefish (*Polyodon spathula*). BMC Genetics 18:19 DOI 10.1186/s12863-017-0484-8). R. Symonová made all analyses, drafted manuscript, P. Ráb conceptualized and co-drafted study.

- Since the role of chromosome changes in speciation remains a debated topic together with colleagues from Université Laval, Canada, we tested relationship between chromosome changes and speciation in two Lake Whitefish (*Coregonus clupeaformis*) lineages recently colonizing postglacial lakes following allopatric phase using a suite of cytogenetic protocols. While chromosome and fundamental numbers were conserved (2n = 80, NF = 98), we detected extensive polymorphism of subtle karyotype traits such as heterochromatin blocks and repetitive DNAs, documenting that differentiation at the level of sub-chromosomal markers mostly appeared during allopatry. The chromosome structures, i.e. repetitive DNAs, detected are still difficult to sequence and assemble, demonstrating thus value of molecular cytogenetics as a complementary approach to understand the genomic bases of speciation (Dion-Cote, A-M., **Symonová, R**., **Ráb, P.,** Bernatchez, L. 2015: Reproductive isolation in a nascent species pair is associated with aneuploidy in hybrid offspring. Proceedings of the Royal Society B. http://dx.doi.org/10.1098/rspb.2014.2862, Volume: 282 Issue: 1802., Dion-Cote, A. - M.; Symonová, R.; Lamaze, F.; **Pelikánová, Š.; Ráb, P**.; Bernatchez, L. 2017: Standing chromosomal variation in Lake Whitefish species pairs: the role of historical contingency and relevance for speciation. Molecular Ecology, 26,1: 178 – 192). R. Symonová, Š. Pelikánová made and evaluated cytogenetic analyses, P. Ráb co-drafted manuscript and co-conceptualized studies.

- With international team and as side activity of our research on high-ploidy sturgeons, we performed cytogenomic analysis in two, non-teleostean, gar genera (*Atractosteus* and *Lepisosteus*) uncovering a GC chromosomal pattern uncharacteristic for any other fish genome. Bio-informatic analysis of the spotted gar (*L. oculatus*) confirmed a GC compartmentalization on GC profiles of linkage groups. This indicated mammalian mode of compositional organization of gar chromosomes. Gars are thus the only analysed extant ray-finned fishes with a GC compartmentalized genome. (**Symonová, R., Majtánová, Z**., Arias-

Rodriguez, L., Mořkovský, L., **Kořínková, T**., Cavin, L., **Johnson Pokorná, M., Doležálková**, Flajšhans, M., Normandeau, E., **Ráb, P**, Meyer, A., Bernatchez, L. 2017: Genome Compositional Organization in Gars Shows More Similarities to Mammals than to Other Ray-Finned Fish. Journal of Experimental Zoology Part B: Molecular and Developmental Evolution DOI: 10.1002/jez.b. 22719, 328 (7): 607– 619). R. Symonová, Z. Majtanová, T. Kořínková made all cytogenetic analyses, R. Symonová made bioinformatics analyses, M. Johnson Pokorná, M., Doležálková contributed by comparative cytogenetic material, P. Ráb conceptualized and co-drafted study.

- We also cytogenetically analyzed a representative of another non-teleostean clade, the bowfin, *Amia calva*, and using a number of markers documented rather teleostean type of genome organization. (**Majtanová, Z., Symonová, R**., Rodrigues, L.A., Sallan, L., **Ráb, P.:** "Holostei versus Halecostomi" problem: insight from cytogenetics of ancient non-teleost actinopterygian fish, bowfin *Amia calva*. Journal of Experimental Zoology Part B: Molecular and Developmental Evolution DOI: 10.1002/jez.b. 22720, 328 (7): 620 – 628). Z. Majtanová made all cytogenetic analyses, R. Symonová contributed to manuscript draft, P. Ráb conceptualized and drafted study.

- Motivated by the discovery of mammalian type of genome organization, P. Ráb initiated, conceptualized and organized international project financed by Brazilian science foundation entitled “An intercontinental approach of the chromosomal evolution in the order Osteoglossiformes (Teleostei: Osteoglossomorpha)”, a group that represents basal teleostean clade. A collection of altogether 8 papers (not itemized here to save space) described karyotype and chromosome evolution of bonytongue representatives from South America, Africa, Asia and Australia. Among them, we discovered mammalian type of genome compartmentalization in two species of Australian arowana species (*Scleropages* spp.) documenting that this phenomenon is not confined to non-teleostean gars.(Cioffi, M. B., **Ráb; P.,** Ezaz; T., Bertollo; L. A. C., Lavoué; S., Oliveira; E. A., **Sember; A**., Molina; W. F., Santos de Souza; F.H., **Majtánova; Z**., Liehr; T., Al-Rikabi; A. B. H., Yano; C. F., Viana; P., Feldberg; E., Unmack; P., Hatanaka; T., Tanomtong; A., Perez, M. F. 2019: Deciphering the evolutionary history of Arowana fishes (Teleostei, Osteoglossiformes, Osteoglossidae): insight from comparative cytogenomics. International Journal of Molecular Sciences 2019, 20, 4296; doi:10.3390/ijms20174296). A. Sember made CGH experiments, Z. Majtanová made cytogenetic analyses of Australian arowana species, P. Ráb conceptualized and co-drafted study.

- In our long-term research focusing on sex determination and genome organization in reptiles, we implemented up-to-date methods in original way and revealed generally important findings. We published the chromosome-anchored genome of Komodo dragon *Varanus komodoensis* serving as one of the best reptile reference genome, also describing the gene content of sex chromosomes, the putative sex-determining gene and the genetic background leading to unique physiological traits of this enigmatic lizard.(**Johnson Pokorná M, Altmanová M, Rovatsos M**, Velenský P, Vodička R, Rehák I, Kratochvíl L ,2016: First description of the karyotype and sex chromosomes in the Komodo dragon (*Varanus komodoensis*). *Cytogenetic and Genome Research* 148: 284–291, Iannucci, A., Altmanová, M., Ciofi, C., Ferguson-Smith, M., Milan, M., Pereira, J. C., Pether, J., Rehák, I., Rovatsos, M., Stanyon, R., Velenský, P., **Ráb, P**., Kratochvíl, L., Johnson Pokorná, M.: 2019 Karyotype evolution and

conserved sex chromosomes in monitor lizards (Varanidae). Heredity https://doi.org/10.1038/s41437-018-0179-6, Lind AL, Lai YYY, Mostovoy Y, Holloway AK, Iannucci A, Mak ACY, Fondi M, Orlandini V, Eckalbar WL, Milan M5, **Rovatsos M**, Kichigin IG, Makunin AI, Johnson Pokorná M, **Altmanová M**, Trifonov VA, Schijlen E9, Kratochvíl L, Fani R, Velenský P, Rehák I, Patarnello T, Jessop TS, Hicks JW, Ryder OA, Mendelson JR, Ciofi C, Kwok PY, Pollard KS, Bruneau BG (2019) Genome of the Komodo dragon reveals adaptations in the cardiovascular and chemosensory systems of monitor lizards. *Nature Ecology & Evolution* 3: 1241–125.M. Johnson Pokorná, M. Altmanová, M. Rovatsos made all cytogenetic analyses, P. Ráb co-drafted 1 manuscript.

- Using the real-time quantitative PCR we developed the method of molecular sexing giving the essential tool for population sex ratio screening, for conservation management and in commercial breeding. Using this approach we also tested the origin of sex chromosomes in amniotic vertebrates, we i) revealed the age of the sex chromosomes, ii) evaluated their evolutionary stability, and iii) discovered that although the sex chromosomes evolved multiple times independently, the same syntenic blocks were co-opted in sex determination. For many species from various reptile lineages we described the karyotype for the first time and studied also other cytogenetic features including the detection of sex chromosomes expanding thus the knowledge of genome organization and sex determination in reptiles. Of the collection of altogether 16 papers we exemplify by **Johnson Pokorná, M**., & Kratochvíl, L. , 2016:What was the ancestral sex-determining mechanism in amniote vertebrates? Biological Reviews, 91(1), 1-12.

- We also discovered that lacertid lizards share the same sex chromosomes (of ZZ/ZW type) as humans (XX/XY), what allows evolutionary comparative studies of the same gene content under different type of heterogamety. (**Rovatsos M,** Vukić J, **Altmanová M**, **Johnson Pokorná M**, Moravec J, Kratochvíl L (2016) Conservation of sex chromosomes in lacertid lizards. *Molecular Ecology* 25: 3120–3126. In all studies, Johnson Pokorná, M. Altmanová, M. Rovatsos made all cytogenetic analyses and co-drafted manuscripts.

- Our another achievement related to the discovery that polar microinvertebrates, such as tardigrades, colonize surfaces of glaciers and form endemic lineages restricted uniquely to this habitat without gene flow with species from surrounding realms. This demonstrates that diversity of Polar Regions is much higher than previously believed and that adaptation to specific habitats is a major driver. From the collection of 8 papers we exemplify Zawierucha, K., Marshall, C. J., Wharton, D., & **Janko, K.** 2019. A nematode in the mist: *Scottnema lindsayae* is the only soil metazoan in remote Antarctic deserts, at greater densities with altitude. Polar Research. doi: 10.33265/polar.v38.3494, where K. Janko made genetic analyses and co-drafted manuscripts

LAM

Following projects and studies were performed by the LAM team in the evaluated period.

- The RuminOmics project

The 4-year project aimed to integrate expertise and technologies to increase rumen efficiency and decrease the environmental footprint of ruminant production, significantly advancing current knowledge in this sector. The project

applied state-of-the-art –omics technologies to understand how rumen microbiome is controlled by the host animal and consumed diet, and to elicidate impact of these factors on greenhouse gas emissions, efficiency and product quality. The research is undertaken by 11 partners across Europe and coordinated by Prof J Wallace of the University of Aberdeen, Scotland. The main research outputs were:
Metamicrobiome of more than 1000 cows and several reindeers were sequenced.
Relation of the animal genome to the microbiome, feed efficiency, and methane emissions was estimated
Interaction of bacteria, methanogens and anaerobic fungi were described.
Host-microbe interactions in genetically identical and genetically diverse animals were determined
Rumen microbiota transplantations from cow to reindeer and vice versa were tested.
Related changes in the nutrient supply of the cow with the composition and function of the ruminal microbiome, as assessed by methane and N emissions
Tools and bioinformatics for rapid analysis of phenotypes, microbiomes were assessed.
A public metagenomics database was created
The results of the project were published as a complex study in a high impact paper (J. Wallace et al. 2019. A heritable subset of the core rumen microbiome dictates dairy cow productivity and emissions. Science Advances 5(7). K. Fliegerová supervised samples preparation and with and H. Sechovcová were in charge of digesta processing and DNA isolation and sequencing of most sequenced samples, J. Mrázek was involved in data processing, J. Kopečný contributed to the text revisions.
- The “Healthy-Dairy” project
Research activity was focused on the analysis of lactococcal bacteriophages in cheese making factory. Bacteriophages causing slow or insufficient milk acidification and reduced product quality were quantified using qPCR approach based on specific primers with the aim to prevent economic losses in dairy industry. Efficient detection method for phages 936 independent on phage cultivation in dairy products was developed and applied to dairy product manufacture. Outputs of this study were presented as two certified methodologies and four papers in peer-reviewed journals. K. Fliegerová designed experiments, J. Killer contributed to manuscripts and certified methodology preparation, L. Štrosová collected samples and prepared them for sequencing, performed the experimental work, J. Mrázek analyzed the data and together with J. Kopečný drafted the text.
- Evaluation of new phylogenetic marker useful in a taxonomy of bacterial order *Lactobacillales* and families *Bifidobacteriaceae* and *Propionibacteriaceae.* The main aim was to suggest candidate genes that could be applicable as new phylogenetic markers, distinguishing between species, subspecies and strains of different bacterial groups: order *Lactobacillales*, families *Bifidobacteriaceae* and *Propionibacteriaceae.* Suitable specific primers flanking the variable regions were designed and optimal PCR conditions for amplification were set up. New phylogenetic trees with a higher resolution and discrimination power were calculated improving the phylogeny of the tested groups. All novel molecular markers may be applied as classification, identification and phylogenetic

markers in specific phylogenetic taxa within the order *Lactobacillales* and families *Bifidobacteriaceae* and *Propionibacteriaceae*. The results were published in 10 different international journals. Some of the proposed gene markers were also used in characterization, classification and phylogenetic of new species of *Bifidobacterium* and *Cutibacterium* (see in cooperation). J. Killer designed experiments and prepared manuscripts, Ch. Mekadim participated in the experimental work (PCR and sequencing) and J. Mrázek contributed by results discussion and data evaluation.

- Characterization and quantification of the cutaneous and intestinal microbiota of human and animals and their changes during the diseases' progress. The LAM team is involved in many projects, that aim to study interactions between different microbiota (cutaneous and intestinal) and hosts (human and animal), such as characterization and quantification of skin and gut microbiome of human/animal samples, comparison of host's microbial composition in health and disease and determination of diseases related changes in different host microbiota. Following projects were solved in the evaluated period, most of them as part of a collaboration with universities and institutes through the world. The role of our team is the microbial diversity description by tools of molecular microbiology, the anaerobic cultivations, fatty acids analyses, and the discussion and interpretation of the results.

Melanoma-related changes in the skin microbiome

Gut microbiome composition and metabolomic profiles of wild western lowland gorillas

Colonization of germ-free piglets with commensal *Lactobacillus amylovorus*, *Lactobacillus mucosae*, and probiotic *E. coli* Nissle 1917 and their interference with *Salmonella Typhimurium*.

Effect of selected stilbenoids on human faecal microbiota.

Association of plumage iridescence with distinct feather microbiota in a tropical passerine.

Variation in honey bee gut microbial diversity as affected by ontogenetic stage, age and geographic location.

PCR and Omics based techniques to study the diversity, ecology and biology of anaerobic fungi. Those projects resulted in an output of 13 publications in peer-reviewed journals, top are listed below. A. Gomez et al. (2015. Gut microbiome composition and metabolomic profiles of wild western lowland gorillas (Gorilla gorilla gorilla) reflect host ecology. Molecular Ecology 24) showed that habituation levels of gorillas group affect their microbiome. **Jakub Mrázek, Ingrid Koppová and Klára Vlčková contributed to this study by analyzing gut metabolites and performing PCR-DGGE and sequencing analyze to reveal microbiome diversities. Igor Šplíchal et al. (2019.** Colonization of Germ-Free Piglets with Commensal Lactobacillus amylovorus, Lactobacillus mucosae, and Probiotic E. coli Nissle 1917 and Their Interference with Salmonella Typhimurium. Microorganisms 7) demonstrated the effects of targeted colonisation of germ-free piglets by probiotic bacterial strains. J. **Killer (LAM) contributed by the classification of the *Lactobacillus* strains using molecular-genetics techniques. V. Gvoždíková-Javůrková et al. (2019.** Plumage iridescence is associated with distinct feather microbiota in a tropical passerine. Scientific Reports 9) showed that distinctive nanostructure properties of iridescent male feathers or different investment in preening influence feather microbiota community composition and load. J. **Mrázek contributed to this**

study by performing PCR-DGGE and qPCR analyses to describe feather microbiota. Z. Hroncová and colleagues (2015. Variation in Honey Bee Gut Microbial Diversity Affected by Ontogenetic Stage, Age and Geographic Location. PLoS ONE 10) presented effects of the ontogenic stage, age and geographic location on honey bee gut microbial diversity. J. Killer, J. Mrázek participated by providing molecular-genetics (DGGE, 16S rRNA sequencing) data revealing the bacterial composition of the intestinal tract of different ontogenetic stages of honeybees. J.E. Edwards et al. (2017. PCR and Omics Based Techniques to Study the Diversity, Ecology and Biology of Anaerobic Fungi: Insights, Challenges and Opportunities. Frontiers in Microbiology 8) described molecular tools and challenges that are used to evaluate phylogeny of anaerobic fungi. K. Olša-Fliegerová of the LAM team contributed to this publication by the consultation and MS preparation.

Another research interest of the LAM team is the description and characterisation of new anaerobic bacteria and fungi. Three novel representatives of the family *Bifidobacteriaceae*; *Bifidobacterium apri* sp. nov., *Galliscardovia ingluviei* gen. nov., sp. nov. and *Alloscardovia venturai* sp. nov. were described during the evaluated period. The first one was isolated from the digestive tract of wild pigs, the second from the crop of a laying hen and the latter from the oral cavity of a guinea-pig. Thus, knowledge of the systematics of the *Bifidobacteriaceae* family was considerably enhanced. *Agathobacter ruminis* sp. nov. was also introduced within the family Lachnospiraceae. We also investigated the enzymes of fibrolytic butyrate producing bacteria by applying whole genome sequencing approach. Total output of those studies was five peer-reviewed papers. In those studies, J. Killer, J. Kopečný, K. Olša Fliegerová designed the experiments, H. Sechovcová, Ch. Mekadim participated in the experimental work (DNA isolation, PCR and sequencing) and J. Mrázek contributed to data evaluation. All members participated in manuscripts preparation.