DEPARTMENT OF MOLECULAR AND BIOMEDICAL SCIENCES

The research program of the Department of Molecular and Biomedical Sciences is focused mainly on basic research in protein biochemistry, molecular and cellular biology, and genetics. The primary goal of our investigations is the acquisition of new understanding of mammalian pathophysiology with the aim of improving human and animal health.

Toxinology

One of our traditional research topics in the field of toxinology is the study of molecular mechanisms of toxic action of secreted phospholipases A_2 (sPLA$_2$s) from animal venoms. In particular, we are focused on those endowed with presynaptic neurotoxicity (β-neurotoxins). The knowledge that we are gaining by studying toxic sPLA$_2$s is helping us to discover the pathophysiological roles of orthologous mammalian sPLA$_2$s, for example, their role in the development of neurodegenerative diseases such as Alzheimer's disease (AD).

In this year, we published a very important paper on molecular identification of a mitochondrial receptor for ammodytoxin (Atx), a neurotoxic sPLA$_2$ from the venom of the nose-horned viper (*Vipera a. ammodytes*, *Vaa*) (J. Šribar et al., *Sci. Rep.*, 9 (2019), 293). This finding is crucial, not only to deepen our understanding of the motoneuron poisoning by Atx on the molecular level but also to unravel the role of a mammalian sPLA$_2$, an orthologue of Atx, in AD. In the paper, we described the purification of an Atx receptor from neuronal mitochondria and its identification as the subunit II of cytochrome c oxidase (CCOX), an essential constituent of the respiratory chain complex. We demonstrated that Atx inhibits the activity of CCOX that explains the hindering of ATP production by this toxin in a poisoned nerve terminal. Studies are underway to confirm similar activity also in the case of the endogenous sPLA$_2$. Endogenous sPLA$_2$ is present in mitochondria, therefore, we are testing the hypothesis of its involvement in physiological regulation of ATP production by the organelle in normal conditions. In pathological conditions, for example in AD, the activity of the endogenous enzyme is largely increased and damage inflicted to neuronal mitochondria very similar to the one observed in the Atx-poisoned nerve endings. We are trying to confirm a functional link between the endogenous sPLA$_2$, CCOX binding and degeneration of mitochondria, and Atx may serve as an excellent tool to this end.

In the area of sPLA$_2$ research, we concluded the first year of the bilateral project with our Russian partners from the Laboratory of Molecular Toxinology at Shemyakin and Ovchinnikov Institute of Bioorganic Chemistry, Russian Academy of Sciences, Moscow (BI-RU/19-20-029). In this context, we are studying, how the endogenous sPLA$_2$s modulate the functions of nicotinic acetylcholine receptor (nAChR). The new young researcher, involved in these studies, is preparing recombinant mammalian sPLA$_2$s (GII, GV and GX isoforms) and their enzymatically inactive forms to probe their effects on nAChR in the Russian laboratory. In the scope of this collaboration, I. Križaj also presented an invited talk at the II. Life Sciences Forum (joint VI. Congress on Biochemistry and IX. Russian Symposium "Proteins and Peptides") in Sochi, Russia.

In 2019, we have also been intensively studying the *Vaa* venom proteins that affect the blood coagulation process—haemostasis, in particular a serine protease with procoagulant, FVIIa-like activity, named VaaSP-VX, the first procoagulant snake venom serine protease with dual, blood coagulation factor V- and X-activating activity. Experimental part is finished and the paper is drafted (Z. Latinović et al., in preparation).

We also published a review paper entitled "Venomous snakes in Slovenia—composition and action of their venoms" in the Slovenian journal Medicinski razgledi (V. Leban et al., *Med. razgl.*, 58 (2019), 55–74).

A new ambitious international network emerged in 2019, focusing on the improvement of snakebite therapy and development of advanced tools to degrade vessel-occlusive thrombi, aiming to drastically reduce mortality and morbidity due to snake envenoming and thrombosis. Both aims of this consortium of 13 research groups from Europe and Latin America focus on

highly effective snake venom proteinases, metallo- and serine proteinases. Our group has been invited to join and was involved in the preparation of an MCSA-ITN application entitled: “Converting Viper venoms into Public and Private health ESsentials (CoViPPES)” to raise money for recruitment and training of young scientists in the first stage.

In 2019, we concluded a systematic analysis of venoms of two European snakes, medically very relevant nose-horned viper (*Vaa*) and one of the most rare Croatian karst viper (*Vipera ursinii macrops, Vum*).

The local and systemic clinical manifestations of poisoning by the venom of *Vaa* are the result of the pathophysiological effects inflicted by enzymatic and non-enzymatic venom components acting, most prominently, on blood, cardiovascular and nerve systems. To help improve the current antivenom therapy towards higher specificity and efficiency, and to assist drug discovery, we have constructed, by combining transcriptomic and proteomic analyses, the most comprehensive library yet of the *Vaa* venom proteins and peptides (Figure 1). Sequence analysis of the venom gland cDNA library has revealed the presence of messages encoding 12 types of polypeptide precursors. At the protein level, 57 venom proteins belonging to 16 different protein families have been identified, four of which, serine proteases (SVSPs), $sPLA_2$s, snaclecs and metalloproteinases (SVMPs), comprise about 80% of all venom proteins. Peptides detected in the venom include natriuretic peptides, bradykinin-potentiating peptides and inhibitors of SVSPs and SVMPs. Of particular interest, a transcript coding for a protein similar to P-III metalloproteinases but lacking the metalloproteinase domain was also found at the protein level in the venom. The existence of such proteins has been demonstrated for the first time, justifying the proposal of a new P-IIIe subclass of ancestral SVMP precursor-derived proteins (A. Leonardi et al., *J. Proteome Res.*, 18 (2019), 2287–2309).

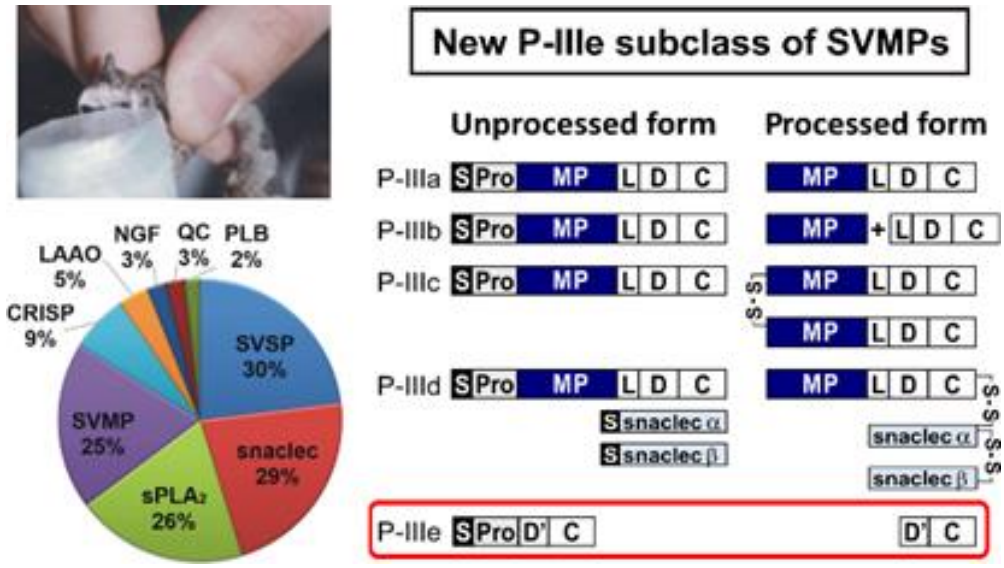

Figure 1. **Comprehensive analysis of the proteome and transcriptome of the nose-horned viper (*Vipera a. ammodytes*, *Vaa*) venom.** The pie diagram is showing relative amounts of the main protein families in the venom: snake C-type lectin-like protein (snaclec), secreted phospholipase A_2 ($sPLA_2$), snake venom serine protease (SVSP), snake venom metalloproteinase (SVMP), Cys-rich secretory protein (CRISP), *L*-amino acid oxidase (LAAO), nerve growth factor (NGF), glutaminyl cyclase (QC) and phospholipase B (PLB). A transcript coding for a protein similar to P-III SVMPs but lacking the MP domain was also found at the protein level in the venom. The existence of such a protein has been demonstrated for the first time. We proposed the introduction of a new P-IIIe subclass of SVMP precursor-derived proteins. The figure is adapted from A. Leonardi et al. (*J. Proteome Res.*, 18 (2019), 2287–2309).

We also studied toxic activities of the *Vum* venom and comprehensively described the proteomic profile of this venom in collaboration with colleagues from the University of Zagreb (UZ). This snake is not medically important, however, its ecology is very special and it is threatened with extinction. Our data opened the way to unravel a unique insecticidal activity of the venom, potentially leading to new pesticides. Comparing pathological properties of the *Vum* venom with those of the *Vaa* venom, and the proteomes of both venoms, we indicated the existence of neurotoxins in viperid venoms structurally unrelated to $sPLA_2$s (M. Lang Balija et al., in preparation).

In collaboration with colleagues from the Centre for Clinical Toxicology and Pharmacology, University Medical Centre Ljubljana (UMCL), we investigated an interesting clinical effect, a profound, transient and reversible thrombocytopenia of functional platelets in patients envenomed by the nose-horned viper venom. In thromboembolic diseases, such as myocardial infarction and ischemic stroke, platelets play a pivotal role. Currently used antiplatelet drugs have one common side effect—a decreased count of platelets with inhibited function. Such condition represents a high risk of life-threatening haemorrhage especially in interventional cardiology and angiology employing antithrombotic approach. Our findings may pave the way to the development of a new group of antiplatelet agents, which will minimize the risk of life-threatening bleeding in antithrombotic approach in interventional cardiology and angiology, and increase the effectiveness of vessel dilatation and emboli aspiration. As we demonstrated, reversible thrombocytopenia in patients poisoned by the *Vaa* venom is caused by snake C-type lectin-like proteins (snaclecs). In 2019, we isolated a pool of these proteins from the venom and purified them to different extents.

The network, including experts from UMCL, immunologists from UZ and our group, continues to collect and analyse samples from patients, who have been envenomed by the nose-horned viper venom and treated with different antivenoms or not, in order to generate new directives for efficient immunotherapy.

2019 was the last year of the Slovenian-Serbian bilateral project (BI-RS/18-19-005). In the scope of this project, our Serbian partners from the Institute of Molecular Genetics and Genetic Engineering, Belgrade, prepared vectors to express snake venom CRISP and new P-IIIe subclass SVMP, and we have started to produce these proteins in bacterial system.

At the end of 2018, we initiated a large research project with the collaboration of two foreign groups, the Department of Biotechnology and Biomedicine from the Technical University of Denmark and the Beijing Genomics Institute from Hong Kong. The major aim of the project is to sequence, assemble *de novo*, annotate and thoroughly analyse the complete *Vaa* genome. From an adult male specimen of this snake, captured in the wild in the north-western part of Slovenia, the liver was dissected and deeply frozen. High-molecular-mass genomic DNA (>100 kilobases) was isolated from this tissue, purified and submitted to combined nucleotide sequencing. The second generation sequencing resulted in 129 gigabases (Gb), and that of the third generation in 161.5 Gb. These data were subjected to a *de novo* assembly process resulting in the final scaffold set with a total length of 1.56 Gb and an N50 contig of 3.38 megabases. In the year 2020, the first draft genome of *Vaa* is expected to be roughly annotated.

Our scientific achievements in the field of toxinology were very well recognised in 2019. We have been invited as lecturers at expert meetings and scientific conferences. Most worth mentioning is the invitation to I. Križaj to organise the section and to deliver a keynote lecture at the 20th World Congress of the International Society on Toxinology, Buenos Aires, Argentina, 8–13 September 2019. Further, I. Križaj was invited to participate in writing a book chapter for an esteemed publishing house (B. Lomonte & I. Križaj (2019): Snake Venom Phospholipase A_2 Toxins. Handbook of Venoms and Toxins of Reptiles, 2nd Edn. (Stephen P. Mackessy, Ed.), CRC Press, Taylor & Francis Group, Boca Raton, Florida, USA, in preparation). And last but not least, a very good work of the Križaj's team was also acknowledged at home, in Slovenia, when the Slovenian Biochemical Society awarded I. Križaj with Lapanje Award, the highest distinction of the society, for the outstanding scientific achievements that made an important contribution to the development of biochemical sciences in Slovenia (Figure 2).

Figure 2. **Professor I. Križaj is receiving the Lapanje award**. At the 13th Meeting of the Slovenian Biochemical Society in Dobrna on September 26th 2019, I. Križaj received the Lapanje award, the highest award of the Slovenian Biochemical Society, for his scientific achievements. The justification of his decoration is available at: http://www.sbd.si/sl/nagrajenci/40/lapanjetova-nagrada/igor-krizaj.

Quick reading highlight: **Animal venoms are rich source of new substances and molecular tools to improve human and animal health.**

Lipid metabolism and signalling

Our work in the field of "Lipid metabolism and signalling" is primarily focused on the identification of cellular pathways of lipid acquisition, trafficking and utilization that may be targeted to reduce the resistance of cancer cells to stress. The survival of cancer cells during severe stress depends on the availability of extracellular lipids and on their capacity to synthesize, mobilise or recycle their own intracellular lipids. By studying the ways in which cancer cells use lipids, we aim to reduce their remarkable ability to adapt to the inhospitable tumour microenvironment and thus reduce tumour growth, metastasis and resistance to therapy.

The paper titled "Lipid Droplets and the Management of Cellular Stress", by E. Jarc Jovičić and T. Petan, was published in the *Yale Journal of Biology and Medicine*, a journal that has been continuously published since 1928 and is edited by Yale medical and graduate students. This invited review paper was prepared for the September 2019 special issue of the journal entitled "Organelles" and was selected as an editor's pick upon publication. In this review, we discuss the emerging roles of lipid droplets as fat storage organelles and major regulators of cellular metabolism. One of the hallmark characteristics of lipid droplets is their capacity to buffer excess lipids and to finely tune their subsequent release based on specific cellular requirements. This simple feature of lipid droplet biology, buffering and delayed release of lipids, forms the basis for their pleiotropic roles in the cellular stress response. In each cell, lipid droplets support the homeostasis of membrane lipid composition and dynamics, take care of damaged proteins and lipids and patrol the cell to form dynamic contacts with other

organelles. They provide protection against excess dietary fat, but also enable optimal energy production in the muscle and heart, complement autophagy during starvation (Figure 3), and regulate inflammatory and immune responses. In each cell, they modulate membrane lipid composition and dynamics, take care of damaged proteins and lipids, patrol the cell to form dynamic contacts with mitochondria and stimulate oxidative metabolism. Lipid droplets are even formed in the nucleus to orchestrate gene expression and nuclear function. These and other ways in which lipid droplets help cells fight against various forms of stress were the focus of this work.

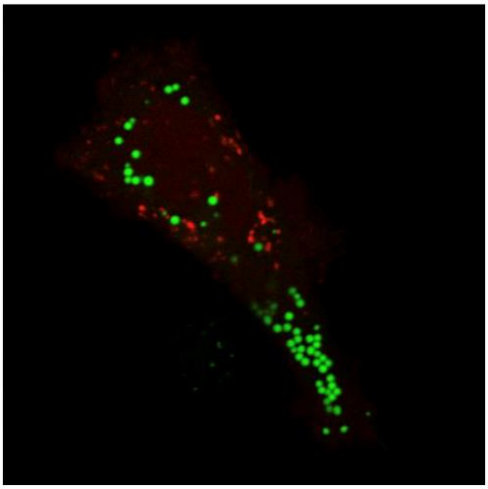

Figure 3. **Lipid droplets accumulate in cancer cells exposed to stress**. HeLa cervical cancer cells were exposed to short-term nutrient starvation. Lipid droplet biogenesis and autophagy are activated under these conditions. Lipid droplets and autophagosomes were visualized with live-cell confocal fluorescent imaging. Lipid droplets were stained with the neutral lipid dye BODIPY 493/503 (green), whereas autophagosomes are coloured red due to the presence of a genetically-inserted autophagosomal marker protein LC3, fused with red fluorescent protein (RFP) (authors: M. Jusović & T. Petan).

The paper “A twist of FATe: lipid droplets and inflammatory lipid mediators”, by E. Jarc Jovičić and T. Petan, was published upon invitation in the special issue of *Biochimie* entitled “Biogenesis and fate of lipid droplets”. *Biochimie* is a journal published by Elsevier on behalf of the Société Française de Biochimie et Biologie Moléculaire and we were delighted to have the opportunity to contribute to this issue. In this review, we discuss the principal ways in which lipid droplets regulate the availability of fatty acids for the production of lipid mediators and activation of inflammatory signalling pathways. On the one hand, lipid droplets sequester polyunsaturated fatty acids (PUFAs) thereby limiting their availability for participation in signalling pathways. On the other hand, lipids derived from the neutral lipid core or the phospholipid monolayer of lipid droplets directly act as signalling mediators or are converted into ones. Most notably, we now have evidence in immune cells and adipocytes that lipid droplet-derived PUFAs may oxidized by cyclooxygenase and lipoxygenase enzyme to produce a plethora of lipid mediators, such as those from the eicosanoid family, that regulate the inflammatory process and also affect tumorigenesis. Lipid droplets thus act as signalling hubs that integrate metabolic and inflammatory processes. Traditionally, the hydrolysis of glycerophospholipids in cellular membranes by various PLA_2 enzymes has been considered the main source and stimulus for lipid mediator synthesis. Here we expand this view by discussing novel evidence that identifies lipid droplets as sources for lipid mediator production, thereby challenging the dogmatic view of phospholipase-driven inflammatory lipid mediator synthesis. Understanding the connections between these emerging topics may in turn lead us to the discovery of important missing links between nutrition, lipid overload and inflammation in some major modern diseases, such as cancer, metabolic diseases and neurodegeneration.

Our recent work in the field of lipid droplets was well accepted in the scientific community as judged by several invited lectures, most notably at the 13th Meeting of the Slovenian Biochemical Society, held in Dobrna, Slovenia, where Dr. Petan was invited to present his work. E. Guštin, our former master student from the Faculty of Chemistry and Chemical Technology at the University of Ljubljana was awarded the Krka Prize for her master thesis titled “Lipid droplets and fatty acid trafficking in breast cancer cells”. M. Jusović received an Ad Futura Postgraduate Scholarship for her PhD studies at the Jožef Stefan International Postgraduate School and joined our group this year. Belen Vilanova Baeza, a master student

from the Edinburgh Napier University, UK, joined our department for a three month research visit via the Erasmus+ mobility programme.

Quick reading highlight: **Targeting the ways in which cancer cells use lipids is a promising strategy to reduce their resilience.**

High-throughput genetics and functional genomics in yeast *Saccharomyces cerevisiae*

Polygenic trait analysis and genome editing methods are among the fastest developing fields in genetics. We developed a method of iterative crossing of yeast strains with diverse genetic backgrounds, with which we were able to prepare a strain with an extremely high level of acidotolerance (D. Slokar & U. Petrovič, unpublished). The method has great potential in the field of biotechnology. We have developed a CRISPR-Cas9 multiplex method, which can simultaneously make up to five specific changes in the yeast genome (G. Žun et al., unpublished). With a combination of these approaches, new industrial yeast strains can be prepared even more effectively.

In collaboration with the group of Prof. Blaž Zupan from the Faculty of Computer and Information Sciences at the University of Ljubljana, we published an article describing a freely available method for the analysis of large numbers of images acquired by microscopic analysis (P. Godec et al., *Nat. Commun*., 10 (2019), 4551). This method enables us to be even more competitive in investigating inter-organelle interactions and other cell-biological phenomena in yeast.

In May 2019, our group organised and hosted the 14th Yeast Lipid Conference, a bi-annual event bringing together researchers from the field of yeast lipid research from the whole world. Uroš Petrovič was also a guest co-editor of the Special Issue on Yeast Lipids of the Yeast journal (Figure 4).

Figure 4. **Cover of the Yeast journal's Special Issue on Yeast Lipids**. The issue's editor was U. Petrovič, principal investigator of the yeast genetics and biotechnology group. He was also the head of the organizing and scientific committees of the YLC 2019 – 14th Yeast Lipid Conference.

Quick reading highlight: **Genetics and genomics of yeasts for the development of biotechnology.**

Evolutionary genomics

The origin and evolution of large multigene families of pore-forming proteins (aerolysins and actinoporins) in lampreys have been investigated (N. Marondini et al., in preparation). The origin and evolution of lysozyme families in eukaryotes were investigated using the phylogenomic analysis. Large amount of novel data has been obtained for the oldest lineages of animals and enabled us to clarify the origin of metazoa-specific lysozyme families (S. Štrukelj et al., in preparation).

In the scope of the bilateral project with Croatia (BI-HR/18-19-030: "Gene-modulatory role of human alpha satellite DNA: physiological and evolutionary implications"), we investigated the association of alpha satellite DNA with transposable elements. Analysis of primate genome data has enabled us to clarify the mechanisms how the alpha satellite DNA inserts into the introns of different genes. The mobility and insertion of alpha satellite DNA into the introns has been enabled by various transposable elements. We have found that alpha satellite DNA in euchromatin genes is associated with various transposable mobile elements, e.g. with different Alu and L1 repeat families, different LTRs from endogenous retroviruses, and with different molecular fossils of DNA transposons. Analysis of the distribution of alpha satellite DNA in the introns of orthologous genes in primates revealed several distribution patterns (absence in the prosimians; presence in Simiiformes, Catarrhini, Hominoidea, Hominidae and Homininae) and different insertion times (10, 18, 20, 30 and 45 million years ago) of this repetitive DNA into the analysed euchromatic genes. Gene ontological analysis of genes associated with alpha satellite DNA revealed that these genes are involved into the specific biological processes and have specific molecular functions (D. Kordiš et al., in preparation).

The diversity and evolution of RNA viruses has been well studied in arthropods and especially in insects. However, the diversity of RNA viruses in the basal hexapods has not been analysed yet. To better understand their diversity, evolutionary histories and genome organizations, we searched for RNA viruses in transcriptome and genome databases of basal hexapods. We discovered 40 novel RNA viruses, some of which are also present as endogenous viral elements derived from RNA viruses (Figure 5). We demonstrated that basal hexapods host 14 RNA viral clades that have been recently identified in invertebrates. The following RNA viral clades are associated with basal hexapods: Reo, Partiti-Picobirna, Toti-Chryso, Mono-Chu, Bunya-Arena, Orthomyxo, Qinvirus, Picorna-Calici, Hepe-Virga, Narna-Levi, Tombus-Noda, Luteo-Sobemo, Permutotetra and Flavi. We have found representatives of the nine RNA viral clades that are present as endogenous genomic copies in the genomes of Machilis (Monocondylia) and Catajapyx (Diplura). Our study provided a first insight into the diversity of RNA viruses in basal hexapods and demonstrated that the basal hexapods possess quite high diversity of RNA viral clades (S. Ott & D. Kordiš, *PeerJ*, (2019), in press).

The diversity and evolution of RNA viruses has also been well studied in vertebrates and invertebrates. However, the diversity of RNA viruses in non-bilaterians and their role in viral origins and evolution is unclear. To understand their diversity better, evolutionary histories and genome organizations, we searched for RNA viruses in numerous transcriptome and genome databases of non-bilaterians. Here, we demonstrate that non-bilaterians possess 18 out of 24 RNA viral clades, which are the following: Birna, Partiti-Picobirna, Toti-Chryso, Reo, Mono-Chu, Bunya-Arena, Orthomyxo, Ophio, Yuevirus, Qinvirus, Hepe-Virga, Luteo-Sobemo, Narna-Levi, Nido, Picorna-Calici, Tombus-Noda, Astro-Poty and Weivirus. Such RNA virome diversity is similar to that of insects, crustaceans and molluscs, but is higher than in platyhelminthes, nematodes, chelicerates or vertebrates. Our study shows that non-bilaterians might be the important reservoir of numerous RNA viruses. The discovery of rich and diverse RNA viromes in the non-bilaterians has important implications for inferring the ancestral metazoan virome, explaining the origin and the timing of appearance of Metazoa-specific RNA viral clades and families, as well as inferring the age and distribution of RNA viral clades and

demonstrating that rich RNA viromes are typical for all invertebrate lineages (S. Ott & D. Kordiš, *PeerJ*, (2019), under revision).

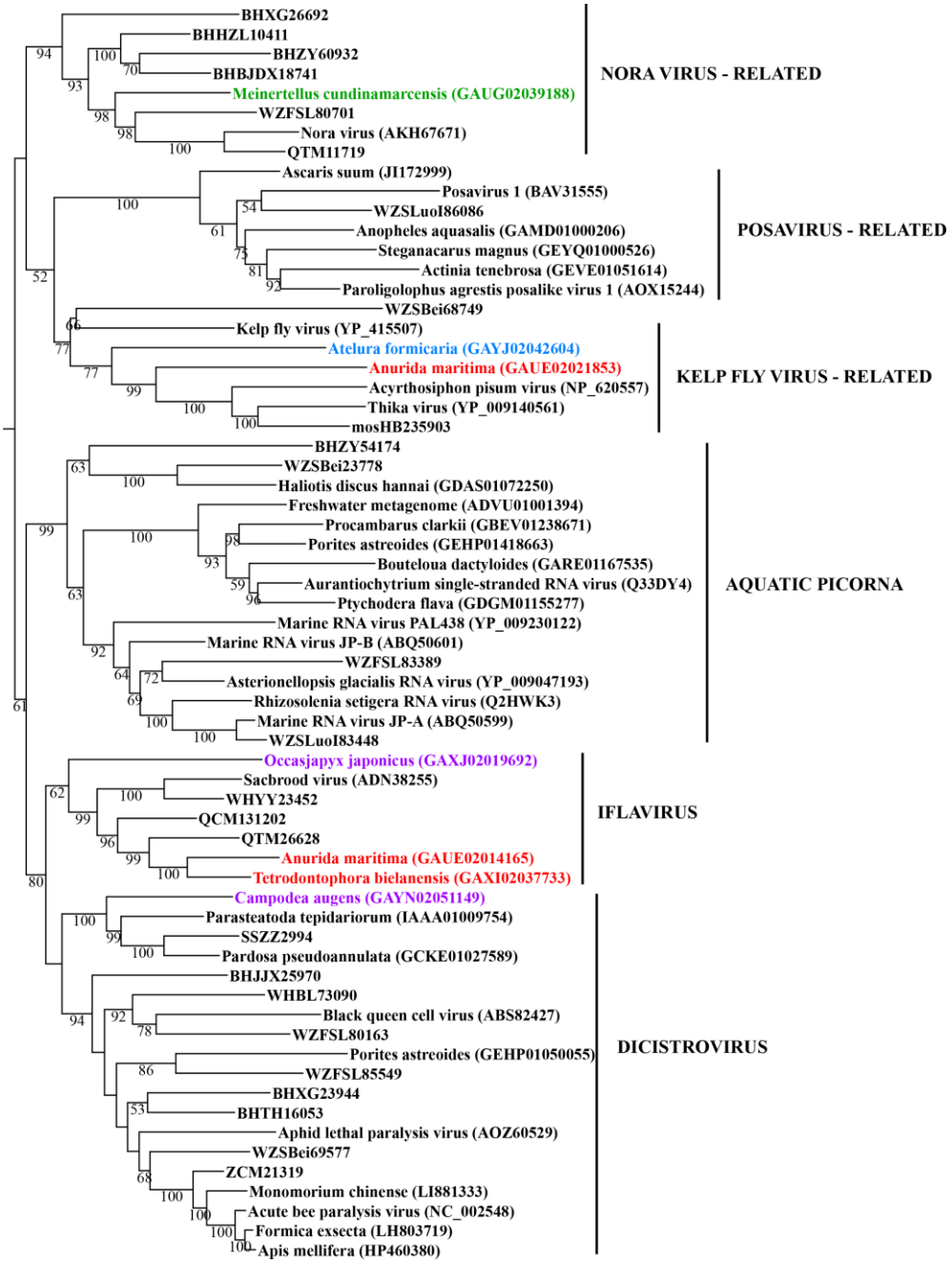

Figure 5. **Maximum likelihood phylogeny of the "Picorna-Calici" clade of RNA viruses in basal hexapods.** Phylogeny is based on the analysis of the viral RdRP domain. The names of the viruses are marked with different colours based on their host taxonomy; springtails (Collembola) are red, Diplura are violet, Monocondylia are green, and Zygentoma are blue. The best fit model of amino acid substitution for this data set was determined as Blosum62+I+G4 according to the Bayesian information criterion. Most sequences were obtained from the GenBank; species names and accession numbers are included. The figure is adapted from S. Ott & D. Kordiš (*PeerJ*, (2019), in press).

Quick reading highlight: **Evolutionary, genomic and structure-function analysis of an unusual fungal lipid droplet-associated protein.**

Other subjects

We also participated in several research projects out of the thematic scope of our department. Two collaborative projects resulted in publications in 2019.

As partners in the Slovenian Research Agency (SRA) project J4-7162, led by colleagues from the Biotechnical Faculty of the University of Ljubljana (BF/UL), we participated in preparing the invited review paper on ceramide phosphoethanolamine (CPE), the major sphingolipid in invertebrates and in some bacterial species (A. Panevska et al., *Biochim. Biophys. Acta – Biomembranes*, 9 (2019), 1284–1292). CPE has been detected in trace amounts also in mammalian cells. Understanding the biophysical and physiological relevance of CPE is still elusive. It is apparent, however, that it differs in the biosynthetic mechanisms of sphingomyelin, due to the specific CPE synthase in invertebrates. In contrast to well-established sphingomyelin/cholesterol interactions that result in the formation of ordered membrane domains, the formation of ordered CPE/cholesterol domains is not favoured. CPE may be crucial for the early development of *Drosophila melanogaster*, and it might be involved in the developmental stages of *Trypanosoma brucei*. As a Bacteroidetes-associated sphingolipid, CPE might also be involved in maintenance of these bacteria in their ecological niches. An efficient detection of CPE in biological systems is needed to better define its distribution and biological role(s).

In collaboration with another research group from the BF/UL we were analysing vitellogenin (Vtg), a female-specific protein and its potential as a molecular marker for sex identification of the European blind cave salamander or proteus (*Proteus anguinus*). In this endangered animal, sexes are namely indistinguishable according to external morphology, which hinders the establishment of efficient captive breeding program. Most importantly, we identified Vtg in the plasma of vitellogenic proteus female with visible oocytes and showed that simultaneously with the degradation of oocytes also the Vtg concentration was decreasing until it dropped under the detection level. Thus, we exposed Vtg as a promising molecular marker for sex identification in proteus, advancing the reproductive programme of this unique species (T. Gredar et al., *Comp. Biochem. Physiol. – Part B: Biochem. & Mol. Biol.*, 235 (2019), 30–37).

As partners in the study of glioblastoma multiforme (GBM), the most common and lethal form of brain tumour, project led by colleagues from the Medical Faculty of the UL, we participated with the confocal microscopy analysis. To improve the therapy of this tumour and patient outcome, sustained drug delivery to glioma cells is needed, while minimising toxicity to adjacent neurons and glia cells. This may be achieved through an anti-proteomic approach based on nanobodies, the single-domain antigen-binding fragments of heavy-chain antibodies of the camelid adaptive immune system. In the work submitted for publication, we report that anti-vimentin, anti-TUFM, anti-NAP1L1 and anti-DPYSL2 nanobodies display cytotoxic effect and reduce glioblastoma cell migration (A. Zottel et al., *Therap. Adv. Med. Oncol.*, submitted).

As partners in the SRA project J7-7424, led by colleagues from the Faculty of Electrical Engineering of the UL, we participated with the analysis of nanoparticles' protein corona composition to explain their cytotoxicity and induction of cytokine secretion in THP-1 macrophages. The paper is in the final stage of preparation (K. Strojan et al., in preparation).

Also in the field of nanoparticles research, this time in collaboration with our partners from the Ruđer Bošković Institute in Zagreb, we participated in establishing the mechanism of formation and morphogenesis of *Arca noae* shell's nanoscale biomineral structures. We accomplished the mass-spectrometric identification of protein components of the shell, potentially involved in the process of biomineralization, i.e. initiation of the extracellular nucleation of aragonite nanocrystals. The experimental part of the work is concluded and the paper is in preparation (V. Čadež et al., in preparation).

Most important publications in 2019

1) Šribar, J., Kovačič, L., Oberčkal, J., Ivanušec, A., Petan, T., Fox, J.W. and Križaj, I.: The neurotoxic secreted phospholipase A_2 from the *Vipera a. ammodytes* venom targets cytochrome c oxidase in neuronal mitochondria. Sci. Rep., 9 (2019), 293

2) Leonardi, A., Sajevic, T., Pungerčar, J. and Križaj, I.: A comprehensive study of the proteome and transcriptome of the venom of the most venomous European viper: Discovery of a new subclass of ancestral snake venom metalloproteinase precursor-derived proteins. J. Proteome Res., 18 (2019), 2287–2309
3) Godec, P., Pančur, M., Ilenič, N., Čopar, A., Stražar, M., Erjavec, A., Pretnar, A., Demšar, J., Starič, A., Toplak, M., Žagar, L., Hartman, J., Wang, H., Bellazzi, R., Petrovič, U., Garagna, S., Zuccotti, M., Park, D., Shaulsky, G. and Zupan, B.: Democratized image analytics by visual programming through integration of deep models and small-scale machine learning. Nat. Commun., 10 (2019), 4551
4) Panevska, A., Skočaj, M., Križaj, I., Maček, P. and Sepčić, K.: Ceramide phosphoethanolamine, an enigmatic cellular membrane sphingolipid. Biochim. Biophys. Acta – Biomembranes, 1861 (2019), 1284–1292
5) Jarc, E. and Petan, T.: Lipid droplets and the management of cellular stress. Yale J. Biol. Med., 92 (2019), 435–452