Sveučilište u Zagrebu

Prirodoslovno-matematički fakultet

Biološki odsjek

Treći simpozij studenata bioloških usmjerenja

Third Symposium of Life Sciences' Students

Knjiga sažetaka

Book of Abstracts

Zagreb,

27. svibnja | May 27th

2017.

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BIUS

UDRUGA STUDENATA BIOLOGIJE

Drage kolegice i dragi kolege, dobrodošli na Treći simpozij studenata bioloških usmjerenja – SiSB3!

Ove godine nastavljamo s tradicijom nakon dva uspješno realizirana simpozija u 2015. i 2016. godini. Priredili smo Simpozij po treći put! Cilj nam je, kao i dosadašnjih godina, na jednom mjestu okupiti i povezati studente koji su zavoljeli znanost o životu, koji žude za istraživanjem svega živoga (a ponekad i granično živoga), čije misli lutaju beskrajno čudesnim poljanama evolucijskih ostvarenja. Svoja iskustva i znanja na ovom Simpoziju međusobno dijele studenti Biološkog odsjeka Prirodoslovno-matematičkog fakulteta Sveučilišta u Zagrebu, kao i studenti ostalih srodnih fakulteta u Hrvatskoj i široj regiji.

Atmosferu ovogodišnjeg studentskog Simpozija zagrijat će počasni gost predavač, profesor William F. Martin sa Sveučilišta „Heinrich Heine” u Düsseldorfu, vodeći znanstvenik u području evolucijske biologije i biokemije. Prisustvovat ćete izlaganjima dvadesetak studenata koji će prezentirati rezultate svog znanstveno-istraživačkog rada. Ove ćete godine po prvi puta imati priliku pregledati postersku sekciju, gdje će prvenstveno studenti koji su radili studentsku praksu, bilo na nekoj od naših institucija ili na studentskoj razmjeni u inozemstvu, ali i drugi imati priliku podijeliti svoja iskustva s Vama.

Pomoću ove knjige moći ćete lakše pratiti sva događanja na Simpoziju. U njoj ćete pronaći raspored svih predavanja, sažetke izlaganja i postera, kao i popis izlagača. Knjiga će biti dostupna i na mrežnim stranicama Simpozija.

Također, srdačno Vas pozivamo da nam se pridružite na zajedničkom ručku u Studentskom prostoru! Za sva pitanja slobodno se obratite članovima Organizacijskog odbora ili pomoćnom osoblju Simpozija.

Zahvaljujemo na neizmjernoj pomoći i podršci Biološkom odsjeku PMF-a te Udruzi studenata biologije BIUS kao službenom organizatoru. Zahvaljujemo i našim sponzorima, Studentskim zborovima PMF-a i Sveučilišta te Plivi Hrvatska d. o. o. na ustupljenim sredstvima i materijalima te na ukazanom povjerenju. Posebno zahvaljujemo profesorima-suradnicima, recenzentima i izlagačima, kao i Vama koji ste svojim dolaskom uveličali ovu manifestaciju.

Organizacijski odbor Trećeg simpozija studenata bioloških usmjerenja

Dear colleagues, welcome to the Third Symposium of Life Sciences' Students – SiSB3!

This year we continue with the tradition after two successful installments of the Symposium in 2015 and 2016. We organized the Symposium for the third time! Our goal is, as in previous years, to rally and connect students who are enthralled by life sciences, who seek to examine every living thing (and sometimes marginally alive), whose thoughts gallop through endlessly wondrous fields of evolutionary achievements. At this Symposium students share their experience and knowledge, be they of the Department of Biology at Faculty of Science, University of Zagreb or of other faculties in Croatia and the wider region.

Professor William F. Martin, the honorary guest lecturer, will kindle the imaginations of this year's Symposium participants. Professor Martin, a leading scientist in the field of evolutionary biology and biochemistry, works at the Heinrich Heine University in Düsseldorf. Afterwards, about twenty students will present to you the results of their scientific endeavours.

This Book will help you to easily navigate all the events at the Symposium. In here you will find all the lectures, presentation summaries, and posters as well as the list of active participants. The book will also be available on the Symposium website.

We cordially invite you to join us at a common lunch in the Student Area! For any questions, please contact the members of the Organizing Committee or Symposium Volunteers.

We would like to thank the immeasurable help and support of the Department of Biology of the Faculty of Science and the Association of the Biology Students BIUS as the official organizer. Also, we would like to thank our sponsors, the Students' Union of the Faculty of Science and the University and to Pliva Hrvatska d. o. o. for the funds and materials provided as well as for their trust in our work. Especially, we would like to thank the professor-associates, reviewers and active participants, as well as to you for augmenting this event with your presence.

Organizing Committee of the Third Symposium of Life Sciences' Students

Raspored

09:30–10:00	Okupljanje i registracija sudionika
10:00–10:50	**Svečano otvaranje Simpozija**
10:50–11:00	**Pauza** za kavu
11:00–12:00	Gost predavač **prof. dr. sc. William F. Martin**: Fiziologija, anaerobni mitohondriji, endosimbioza i kompleksnost
12:00–12:30	**Pauza** za pregledavanje **posterske sekcije**, kava
12:30–13:30	**Predavanja: mikrobiologija, molekulska biologija, fiziologija životinja**
	Predsjedavajući: **Frane Miljković**
12:30–12:45	**Marija Vuk**: Raznolikost i otpornost na antibiotike bakterijskih izolata Dinarskog krškog podzemlja
12:45–13:00	**Hrvoje Mišetić**: Filostratigrafska analiza bakterije *Escherichia coli*
13:00–13:15	**Ivana Milošević** i **Ana Mesić**: Imunohistokemijska izraženost proteina BORIS i STAM-2 u tumorima zametnih stanica testisa
13:15–13:30	**Neven Miculinić**: MinCall – određivač nukleotida temeljen na metodama dubokog učenja za sekvencer DNA MinION
13:30–13:40	**Zajedničko fotografiranje**
13:40–14:40	**Ručak** za sudionike Simpozija
14:40–15:40	**Predavanja: zoologija**
	Predsjedavajuća: **Ana Bekavac**
14:40–14:55	**Igor Pejnović** i **Tihana Pensa**: Promjene sastava zajednica malih bentičkih foraminifera iz odobalja sjeveroistočnog dijela Jadranskog mora u posljednjih 400 godina (Piranski zaljev)
14:55–15:10	**Barbara Čolić**: Genetička varijabilnost nametnika *Pomphorhynchus laevis* (Zoega in Müller, 1776) (Acanthocephala) u hrvatskim rijekama
15:10–15:25	**Ivan Šapina**: Identifikacija smeđe mramorne stjenice *Halyomorpha halys* (Stål, 1855) i njeni mogući putevi ulaza u Hrvatsku
15:25–15:40	**Ivana Grgić** i **Zuzana Redžović**: Procjena opasnosti za Nacionalni park Krka u području antropogenih utjecaja uz grad Knin
15:40–16:00	**Pauza** za pregledavanje **posterske sekcije**, kava
16:00–17:00	**Predavanja: zoologija i botanika**
	Predsjedavajuća: **Zuzana Redžović**
16:00–16:15	**Ivan Šapina**: Pigmejski vražičci s Wallacee (Orthoptera: Tetrigidae): *Hirrius* s Mindanaa i *Zvierckia* gen. nov. sa Sulavezija
16:15–16:30	**Barbara Horvatić**: Problematika izrade ključa za determinaciju božjih ovčica (Coleoptera: Coccinellidae)
16:30–16:45	**Nikola Medić**: Sastav lipida novoizoliranog soja pikoeukariota *Picochlorum* sp. PMFPPE4 u standardnom i fosfor-limitirajućem mediju
16:45–17:00	**Ana Terlević**: Vaskularna flora područja Dotrščina (Zagreb, Hrvatska)
17:00–17:15	**Završna riječ**

Timetable

09:30–10:00	Participants Gathering and Registration
10:00–10:50	**Symposium Opening Ceremony**
10:50–11:00	**Coffee Break**
11:00–12:00	Invited Speaker **Prof. William F. Martin**: Physiology, Anaerobic Mitochondria, Endosymbiosis and Complexity
12:00–12:30	**Poster Section** and Coffee **Break**
12:30–13:30	**Lectures: Microbiology, Molecular Biology, Animal Physiology** Chair: **Frane Miljković**
12:30–12:45	**Marija Vuk**: Biodiversity and Antibiotic Resistance of Bacterial Isolates from the Dinaric Karst Subterranean Habitat
12:45–13:00	**Hrvoje Mišetić**: Phylostratigraphic Analysis of *Escherichia coli*
13:00–13:15	**Ivana Milošević** and **Ana Mesić**: Immunohistochemical Expression of BORIS and STAM-2 in Testicular Germ Cell Tumors
13:15–13:30	**Neven Miculinić**: MinCall – MinION DNA Sequencer end2end Deep Learning Basecaller
13:30–13:40	**Group Photo**
13:40–14:40	**Lunch** for Symposium Participants
14:40–15:40	**Lectures: Zoology** Chair: **Ana Bekavac**
14:40–14:55	**Igor Pejnović** and **Tihana Pensa**: Changes in the Composition of Smaller Benthic Foraminiferal Assemblages in the Offshore in the Northeastern Adriatic Sea Within the Last 400 Years (Piran Bay)
14:55–15:10	**Barbara Čolić**: Genetic Variability of the *Pomphorhynchus laevis* Parasite (Zoega in Müller, 1776) (Acanthocephala) in Croatian Rivers
15:10–15:25	**Ivan Šapina**: First Record of the Invasive Brown Marmorated Stink Bug *Halyomorpha halys* (Stål, 1855) in Croatia
15:25–15:40	**Ivana Grgić** and **Zuzana Redžović**: Risk Evaluation for the Krka National Park in the Anthropogenically Impacted Area Near the Town of Knin
15:40–16:00	**Poster Section** and Coffee **Break**
16:00–17:00	**Lectures: Zoology and Botany** Chair: **Zuzana Redžović**
16:00–16:15	**Ivan Šapina**: Pygmy Blackhoppers from Wallacea (Orthoptera: Tetrigidae): *Hirrius* from Mindanao and *Zvierckia* gen. nov. from Sulawesi
16:15–16:30	**Barbara Horvatić**: Problems That Occur While Creating Determination Key for Ladybirds (Coleoptera: Coccinellidae)
16:30–16:45	**Nikola Medić**: Lipid Classes Composition of the Newly Isolated Picoeukaryote *Picochlorum* sp. PMFPPE4 in the Standard and Phosphorus Limited Medium
16:45–17:00	**Ana Terlević**: Vascular Flora of the Dotrščina Area (Zagreb, Croatia)
17:00–17:15	**Closing Speech**

Gost predavač

Invited Speaker

Fiziologija, anaerobni mitohondriji, endosimbioza i kompleksnost

William F. Martin

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Kloroplasti i mitohondriji zadržali su svoju prokariotsku biokemiju, međutim, njihovi genomi umanjeni su te se većina njihovih proteina kodira jezgrinim genima. Endosimbiotska teorija tvrdi da su bakterijski geni ušli u eukariotske genome preko predaka današnjih organela. Ona također predviđa sporadični unos prokariotskih gena u eukariotsku lozu, dok njihovo stjecanje vremenski odgovara endosimbiotskim događajima. Nasuprot tome, teorije horizontalnog prijenosa gena (LGT) koje se vode pravilom „ono si što jedeš" previđaju konstantan unos prokariotskih gena u eukariotske genome. Koristeći genomske podatke možemo razlikovati endosimbiotsku teoriju od teorije LGT. Grupiranjem i filogenetskom analizom svih eukariotskih genskih porodica koje imaju prokariotske homologe pokazali smo: 1) prijenos gena iz bakterija u eukariote sporadičan je i vremenski odgovara nastanku mitohondrija i kloroplasta 2) nasljeđivanje gena kod eukariota vertikalno je, a razlikovni gubitak uzrokuje prorijeđene genske distribucije, 3) kontinuirani horizontalni prijenos gena specifičan za pojedinu lozu ne doprinosi dugoročnoj evoluciji genskog sadržaja eukariotskih genoma. Nastanak eukariota bio je ujedno i nastanak vertikalnog nasljeđivanja, dok je spolno razmnožavanje bilo nužno za očuvanje vertikalne evolucije loza kako bi se početna loza eukariota sačuvala od Müllerovog sita. Kodirajući nizovi u eukariotskim genomima koji kodiraju za proteine 70 % ili više homologne prokariotskim proteinima najčešće su artefakti. Samo podrijetlo mitohondrija bila je ključna tema koja je pokrenula rasprave o problemima vezanim uz simbiozu u području stanične biologije. Rješenja tih problema istaknute su osobine koje razlikuju eukariote od prokariota: jezgrina ovojnica, energijski povoljna polimerizacija proteina u citosolu te stanični ciklus, uključujući redukcijsku diobu i recipročnu rekombinaciju (spolno razmnožavanje). Čak i sustav unutarnjih membrana u eukariota potječe od mitohondrija; preko vezikula vanjske membrane (OMV) koje su nastale u pretku mitohondrija, unutar citosola arhealnog domaćina. Zarobljeni u citosolu domaćina, OMV-ovi su se spontano nakupljali fuzionirajući međusobno ili s plazmatskom membranom domaćina. Ovim procesima nastaju vezikule mitohondrijskog podrijetla koje se sastoje od bakterijskih lipida. Sustav tih vezikula, koje odlaze prema površini stanice, sparen je s domaćinovim predačkim putem kotranslacijskog umetanja proteina u membranu. Time je stvoren praiskonski, sekretorni endoplazmatski retikul, temelj sustava unutarnjih membrana eukariota. Fiziološki gledano, eukarioti kao grupa manje su raznoliki nego vrste roda Rhodobacter međusobno, a ključni razlog tome je endosimbioza. Gledano sa stajališta stanične biologije, eukarioti su u temelju različiti od prokariota, a osnovni razlog tome su mitohondriji.

Physiology, Anaerobic Mitochondria, Endosymbiosis and Complexity

William F. Martin

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Chloroplasts and mitochondria have conserved their prokaryotic biochemistry, but their genomes are reduced, and most organelle proteins are encoded in the nucleus. Endosymbiotic theory posits that bacterial genes in eukaryotic genomes entered the eukaryotic lineage via organelle ancestors. It predicts episodic influx of prokaryotic genes into the eukaryotic lineage, with acquisition corresponding to endosymbiotic events. Lateral gene transfer theories of the ”you are what you eat” variety predict a constant flux of prokaryotic genes into eukaryotic genomes. Genome data can discriminate between endosymbiotic theory and LGT theory. By clustering and phylogenetic analysis of all eukaryotic gene families having prokaryotic homologues we show (1) that gene transfer from bacteria to eukaryotes is episodic and coincides with the origin of chloroplasts and mitochondria, (2) that gene inheritance in eukaryotes is vertical, sparse gene distributions stemming from differential loss, and (3) that continuous, lineage-specific lateral gene transfer does not contribute to long-term gene content evolution in eukaryotic genomes. The origin of eukaryotes was the origin of vertical lineage inheritance, and sex was required to keep vertically evolving lineages viable by rescuing the incipient eukaryotic lineage from Muller’s ratchet. Coding sequences in eukaryotic genomes that share more than 70% amino acid sequence identity to prokaryotic homologs are typically assembly or annotation artifacts. The origin of mitochondria was the decisive incident that precipitated symbiosis-specific cell biological problems, the solutions to which were the salient features that distinguish eukaryotes from prokaryotes: A nuclear membrane, energetically affordable protein polymerization in the cytosol, and a cell cycle involving reduction division and reciprocal recombination (sex). Even the eukaryotic endomembrane system originated from mitochondria via outer membrane vesicles (OMVs) released by the mitochondrial ancestor within the cytosol of its archaeal host at eukaryote origin. Confined within the host’s cytosol, OMVs accumulated naturally, fusing either with each other or with the host’s plasma membrane. This matched the host’s archaeal secretory pathway for cotranslational protein insertion with outward bound mitochondrial-derived vesicles consisting of bacterial lipids, forging a primordial, secretory endoplasmic reticulum as the cornerstone of the eukaryotic endomembrane system. In terms of physiology, eukaryotes as a group are less diverse than a typical *Rhodobacter* species, and endosymbiosis is why. In terms of cell biology, eukaryotes are fundamentally different from prokaryotes, and mitochondria are why.

Izvori | References:

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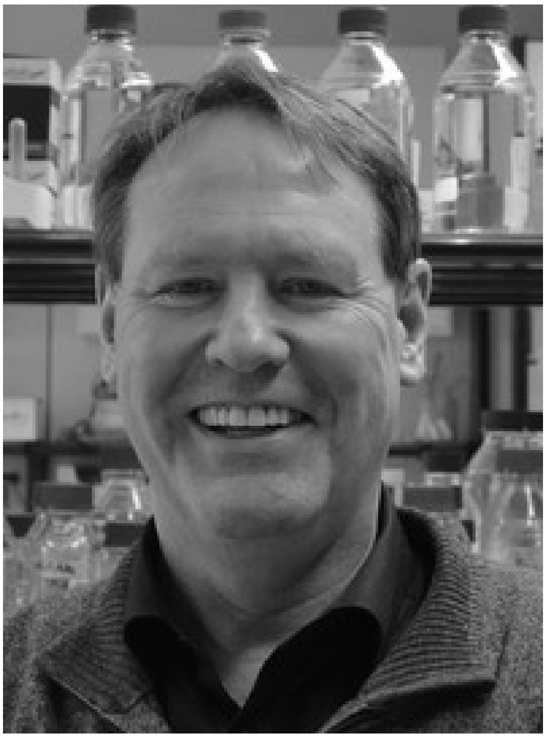

Profesor Martin bavi se istraživanjima u evolucijskoj biologiji i biokemiji. Istražuje evolucijsku prošlost kloroplasta, mitohondrija i hidrogenosoma. U njegovoj grupi raznim laboratorijskim eksperimentima i računalnim metodama pokušavaju spoznati postanak eukariota, ali i otići korak dalje, do samog izvora života. Objavio je preko 250 radova citiranih skoro 25 000 puta.

Professor William F. Martin is involved in the fields of evolutionary biology and biocheimistry. His interests include investigation of plastid, mitochondria and hydrogenosomes evolutionary past. His group uses different laboratory experiments and computational methods to elucidate the origin of eucaryotes and even go a step further, to the very source of life itself. He has published over 250 papers cited nearly 25 000 times.

Više informacija o profesoru Martinu možete pronaći na njegovoj stranici:

Find out more anout professor Martin on his website:

http://www.molevol.hhu.de/prof-dr-w-f-martin.html.

Usmena izlaganja

Talks

Raznolikost i otpornost na antibiotike bakterijskih izolata Dinarskog krškog podzemlja

Marija Vuk[1], Sandra Hudina[1], Snježana Kazazić[2], Ana Šimatović[2], Jasna Lajtner[1], Nikolina Udiković Kolić[2], Ana Bielen[3,*]

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U ovom je radu po prvi puta istražen sastav obraštajnih mikrobnih zajednica krškog podzemlja s minimalnim antropogenim utjecajem, s naglaskom na prisutnost otpornosti na antibiotike u prikupljenim bakterijskim izolatima. Dinarski krški podzemni ekosustavi predstavljaju veliku prirodnu vrijednost za Hrvatsku, dok su njihove mikrobne zajednice, osnova hranidbenih mreža u oligotrofnim podzemnim ekosustavima, većinom neistražene. Stoga je u ovom radu za mjesto uzorkovanja odabran Markov ponor (Lipovo polje, Hrvatska) za koji je u ranijim istraživanjima pokazano da nije zagađen pa može poslužiti za opis antropogeno netaknute mikrobne zajednice iz krškog podzemlja.

Kako bi se sakupio što veći broj različitih bakterijskih izolata, obuhvaćena su tri mikrostaništa koja se razlikuju prema količini vode. Obrisci su uzorkovani s 10 jedinki špiljskih školjkaša *Congeria jalzici* za svako mikrostanište te su nasađeni na neselektivnu krutu hranjivu podlogu TSA (engl. *Tryptic Soy Agar*). Prva banka bakterijskih izolata Markovog ponora sadržava 99 čistih bakterijskih izolata, dobivenih presađivanjem čistih bakterijskih kultura, koji su taksonomski determinirani dvama molekularnim metodama: MALDI-TOF masenom spektrometrijom i određivanje slijeda baza amplikona 16S rDNA filogenetskom analizom. Među izolatima prevladavale su bakterije iz koljena Firmicutes (71,7 %). Osim njih, tu su i koljena: *Proteobacteria* (razred *Gammaproteobacteria*) (15,2 %), *Actinobacteria* (12,1 %) i *Bacteroidetes* (1,0 %). Količina vode u staništu značajno utječe na sastav obraštajnih bakterija: neki su rodovi pronađeni samo na kongerijama izvađenim iz vode (uglavnom predstavnici aktinobakterija), dok su drugi pronađeni na špiljskom higropetriku (*Agromyces*, *Agrobacter*, *Chryseobacterium*) ili na zraku (*Exiguobacterium*). Naposljetku, većina bakterijskih izolata (81,8 %) bila je otporna barem na jedan od osam ispitanih antibiotika, a dva izolata srodna vrstama *Bacillus thuringiensis* i *Pseudomonas helmanticensis* bila su rezistentna na svih osam. Time je pokazano da je rezervoar gena za antibiotičku rezistenciju prisutan i u staništima s minimalnim antropogenim utjecajem.

Zaključno, dobiveni rezultati daju prva saznanja o uzgojivim mikrobnim zajednicama u Markovom ponoru i postavljaju temelje za zaštitu krškog podzemlja od antropogenog utjecaja – ako se u budućnosti utvrdi otpornost bakterijskih vrsta iz Markovog ponora na dodatne klase antibiotika, to će biti indikacija negativnog antropogenog utjecaja.

Biodiversity and Antibiotic Resistance of Bacterial Isolates from the Dinaric Karst Subterranean Habitat

Marija Vuk[1], Sandra Hudina[1], Snježana Kazazić[2], Ana Šimatović[2], Jasna Lajtner[1], Nikolina Udiković Kolić[2], Ana Bielen[3,*]

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This paper exhibits the composition of biofilm microbial communities of the Dinaric karst subterranean habitat with minimal anthropogenic influence, focusing on antibiotic resistance of collected bacterial isolates. The Dinaric karst subterranean ecosystems represent a significant natural treasure for Croatia, and yet their microbial communities, the basis of food chains in oligotrophic subterranean ecosystems, are mostly unexplored. Therefore, Markov ponor (Lipovo polje, Croatia) was chosen as the sampling location. Previous research of this locality showed that it was not polluted and thus can serve to describe anthropogenically-undisturbed microbial community of karst underground.

In order to collect the largest variety of bacterial isolates, swabs were collected from 10 *Congeria jalzici* shells for each of the three microhabitats that vary according to the amount of water. The obtained swabs were streaked on tryptic soy agar (TSA). First bank of bacterial isolates from Markov ponor contains 99 isolates obtained by subculturing single bacterial colonies, which were taxonomically determined by two molecular methods: MALDI-TOF mass spectrometry and sequencing of the 16S rDNA amplicons with phylogenetic analysis. Bacteria from phylum *Firmicutes* are predominant among the isolates (71.7%). Other notable represented phyla are *Proteobacteria* (class *Gammaproteobacteria*) (15.2%), *Actinobacteria* (12.1%) and *Bacteroidetes* (1.0%). The amount of water within the habitat greatly influences the composition of biofilm bacteria: some genuses have been found only on *Congeria jalzici* that has been taken out of the water (mostly representatives of the *Actinobacteria*), while the others were found on hygropetric (*Agromyces*, *Agrobacter*, *Chryseobacterium*) or on air (*Exiguobacterium*). Finally, the majority of bacterial isolates (81.8%) were found to be resistant to at least one out of eight tested antibiotics, while two of the isolates related to *Bacillus thuringiensis* and *Pseudomonas helmanticensis* species were resistant to all of the tested antibiotics. Therefore, it has been shown that the gene pool for antibiotic resistance exists even in habitats with minimal anthropogenic influence.

In conclusion, the obtained results give the first insights into culturable microbial communities in Markov ponor and can serve as a basis for protecting the karst subterranean habitats from anthropogenic influences – if bacterial species from Markov ponor develop resistance to additional classes of antibiotics, it will serve as an indication of negative anthropogenic influence.

Filostratigrafska analiza bakterije *Escherichia coli*

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Genomska filostratigrafija je analitička metoda koja proučava makroevolucijske trendove u evoluciji određene vrste. Filostratigrafske mape su dosada uglavnom rađene samo na eukariotima. U ovom je radu izrađena filostratigrafska mapa bakterije *Escherichia coli* sojeva K-12 i ISC11. Robusnost mape testirana je na dva načina: korištenjem reducirane i nasumične proteinske baze te uvođenjem uvjeta o graničnom broju pogodaka po filostratumu. Prvim se načinom distribucija proteinskih sljedova po filostratumima za oba soja promijenila te su dobiveni jasni signali u pojedinim filostratumima. Varijacijom granične vrijednosti o broju pogodaka po filostratumu za rezultate dobivene na sveobuhvatnoj bazi uočeno je da se porastom granične vrijednosti smanjuje broj proteinskih sljedova u prvom filostratumu, a povećava broj proteina u mlađim filostratumima, posebno u onima koji su imali jasan signal pri testiranju robusnosti na reduciranoj i nasumičnoj proteinskoj bazi. Testiranjem robusnosti filostratigrafske mape oba soja bakterije *E. coli* utvrđena su tri ključna makroevolucijska događaja pri kojima je nastala većina proteinskih sljedova. Nadalje, analizirana je uloga u biološkim procesima, molekularna funkcija i stanična lokalizacija evolucijski starih i evolucijski mladih proteina koji su identificirani u filostratigrafskim mapama bakterije *E. coli* K-12 dobivenim korištenjem sveobuhvatne, reducirane i nasumične baze te najveće granične vrijednosti. Ustanovljeno je da većina starih proteina ima ulogu u staničnim i metaboličkim procesima. s obzirom na funkciju u stanici najviše pokazuju katalitičku i transportnu aktivnost te ulogu u vezanju, a lokalizirani su ponajviše u staničnoj unutrašnjosti i na membrani. Evolucijski su mladi proteini nekarakterizirani i neklasificirani te je njihova uloga u biološkim procesima, funkcija u stanici te stanična lokalizacija nepoznata. Ovo je jedna od prvih filostratigrafskih analiza napravljenih na prokariotskom organizmu i to na onome koji je najkorišteniji prokariotski model u molekularnoj biologiji te prva analiza koja uključuje testiranje uvođenjem uvjeta o grančnom broju pogodaka po filostratumu kao i analizu uloge proteina u biološkim procesima, funkcije u stanici i stanične lokalizacije identificiranih evolucijski starih i mladih proteina iz proteoma bakterije *Escherichia coli* K-12.

Phylostratigraphic Analysis of *Escherichia coli*

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Genomic phylostratigraphy is an analytical method used for discovering macroevolutionary trends in the evolution of species. Thus far, it has mainly been applied to eukaryotes. In this study, the phylostratigraphic map of bacterium *Escherichia coli* has been created for strains K-12 and ISC11. The quality of the maps was tested by using two different approaches. The first approach was to use a reduced and random protein database in order to construct the phylostratigraphic map. This caused a different distribution of proteins among phylostratums for both strains and also showed significant signals in certain phylostratums. The second approach was to introduce a condition according to which there has to be a certain number of hits per phylostratum before a protein can be assigned to it. By applying multiple sets of cut-off values for hits per phylostratum while utilizing the complete protein database, it was detected that an increase of the cut-off value causes a decrease in the number of proteins in the first phylostratum and an increases in the number of proteins in later phylostratums, especially in the ones that contained strong signals while testing the results on the reduced and random protein databases. Based on these tests of the phylostratigraphic maps for both strains, three main macroevolutionary events were identified as points in which the majority of proteins appeared in the proteomes of the tested strains. Furthermore, Gene Ontology analysis was used to analyze the molecular function, biological processes and cellular localization of the proteins that were identified as evolutionarily old or new in the proteome of *E. coli* K-12 strain. This was conducted based on the results obtained using the complete, reduced and random protein databases and also on the results obtained using the highest cut-off score. It was concluded that older proteins mostly have roles in metabolic processes, while their molecular function relates to catalytic and transport activity along with a role in binding partner molecules. They are mostly localized in the cytoplasm and on the plasma membrane. Evolutionarily new proteins are mostly uncharacterized and unclassified so their roles in biological processes, as well as their molecular functions and cellular localization still remain unknown. This is one of the first phylostratigraphic analyses on the prokaryotic organism and the first analysis that includes test based on the cut-off number of hits per phylostratum as well as determination of the molecular function, role in the biological processes and cell localization of the evolutionary old and new proteins from the *Escherichia coli* K-12 proteome.

MinCall – određivač nukleotida temeljen na metodama dubokog učenja za sekvencer DNA MinION

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Uređaj MinION tvrtke Oxford Nanopore Technology prvi je prenosivi sekvencer DNA. Može očitati nizove velike duljine, preko 100 kbp, no još uvijek ima stopu pogrešaka dosta višu od ostalih metoda sekvenciranja.

U ovom istraživanju kreirali smo MinCall, cjelokupni model, od izvornog signala do očitanja nukleotida za MinION. Model se bazira na metodama dubokog učenja i koristi se konvolucijskim neuronskim mrežama u svojoj implementaciji. Kako bi postigao najbolje moguće karakteristike, koristi se najnovijim tehnikama dubokog učenja i arhitekture, specifično rezidualnim neuronskim mrežama, normalizacijom nad grupom i gubitkom vezane vremenske klasifikacije.

Najuspješniji model, u koji je uključeno 270 slojeva neurona, postiže najbolje dosad zabilježene značajke uz preciznost od 90,5 % na podacima dobivenih sekvenciranjem bakterije *E. coli* koristeći kemikalije generacije R9 i 1D-sekvenciranje.

MinCall – MinION DNA Sequencer end2end Deep Learning Basecaller

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The Oxford Nanopore Technologies's MinION is the first portable DNA sequencing device. It was reported that it can produce long reads, over 100 kbp, but its error rate is much higher than in other sequencing technologies.

In this study, we created MinCall, an end2end basecaller model for the MinION generated data. The model is based on deep learning and uses convolutional neural networks (CNN) in its implementation. To achieve the best performances, it uses cutting edge deep learning techniques and architectures, notably gated residual network, batch normalization and Connectionist Temporal Classification (CTC) loss.

The best performing 270 layers deep model shows state-of-the-art 90.5% match rate on *E. coli* dataset gained by using R9 nanopore sequencing chemistry and 1D reads.

Imunohistokemijska izraženost proteina BORIS i STAM-2 u tumorima zametnih stanica testisa

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Tumori zametnih stanica testisa (testicular germ cell tumour, TGCT) najčešći su tumori mladih muškaraca u periodu od puberteta do 40 godina. U Republici Hrvatskoj i Republici Sloveniji bilježi s najveći porast incidencije TGCT-a od svih zemalja Europe. Naglasak ove studije je na skupini seminoma i neseminoma adolescenata i mlađe odrasle dobi. U ovom je istraživanju po prvi put analizirana imunohistokemijska izraženost proteina Brother of the Regulator of Imprinted Sites (BORIS) i Signal Transducing Adaptor Molecule 2 (STAM-2) u TGCT-ima, na uzorku od 61 pacijenta. Ova dva proteina sudjeluju u unutarstaničnoj signalizaciji te se smatra da su povezani s aktivacijom protoonkogena. Utvrđena je pozitivna reakcija na BORIS u 100 % analiziranih uzoraka, neovisno o histološkoj građi, dok je reakcija na STAM-2 bila slaba ili je nije bilo. Uočena je razlika u izraženosti STAM-2 u čistim seminomima i seminomskoj komponenti miješanih tumora zametnih stanica testisa (mixed germ cell tumour, MGCT) u odnosu na ostale komponente MGCT-a. Iako rezultati ovog istraživanja zahtijevaju daljnje potvrde, ukazuju na potencijalno značenje određivanja ekspresije BORIS-a i STAM-2 u TGCT-ima te njihovom eventualnom doprinosu poboljšanju dijagnostike ovih tumora.

Immunohistochemical Expression of BORIS and STAM-2 in Testicular Germ Cell Tumors

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Testicular germ cell tumours (TGCT) are the most common tumours in young males between puberty and the age of 40. Of all European countries, the highest increase of incidence was registered in the Republic of Croatia and the Republic of Slovenia. The aim of this study was to analyse seminomatous and nonseminomatous tumours of adolescents and younger males. This study was the first to analyse the immunohistochemical expression of two proteins, Brother of the Regulator of Imprinted Sites (BORIS) and Signal Transducing Adaptor Molecule 2 (STAM-2) in TGCTs, in 61 samples. These two proteins play a role in intracellular signalisation and are considered to be involved in the protooncogenes activation. All analysed samples were BORIS-positive, regardless of histological type, whereas the reaction to STAM-2 was weak or absent. There was a difference in the expression of STAM-2 in pure seminomatous tumours and seminomatous component of mixed germ cell tumours (MGCT) in comparison with other components of MGCTs. Although this study requires further confirmation, it shows the potential significance of determining BORIS and STAM-2 expression and their possible contribution in the improvement of diagnosing TGCTs.

Promjene sastava zajednica malih bentičkih foraminifera iz odobalja sjeveroistočnog dijela Jadranskog mora u posljednjih 400 godina (Piranski zaljev)

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Piranski zaljev izložen je velikom antropogenom utjecaju (blizina luka Koper i Trst, brodogradilišta Monfalcone, te velika poljoprivredna aktivnost u zaleđu). Šesnaest uzoraka sedimenta iz različitih dijelova jezgre duljine 1,5 m mikropaleontološki je istraživano. U uzorcima frakcije 250 μm određene su vrste i rodovi malih bentičkih foraminifera prema kriterijima za klasifikaciju Loeblich & Tappan (1987). Temeljem dobivenih podataka o sastavu zajednice, izračunat je niz statističkih i ekoloških indeksa. Simpsonov indeks raznolikosti i Berger-Parkerov indeks dominacije ukazuju na veliku raznolikost vrsta u zajednici čitavom duljinom jezgre. Visoke vrijednosti Shannon-Weaverovog indeksa pokazuju dominaciju dvije do tri vrste u svim uzorcima uz odstupanja u uzorcima dubine 90–95 cm i 120–125 cm. Vrijednosti Fisherovog α indeksa također su visoke i njihove oscilacije se podudaraju s onima kod Shannon-Weaverovog indeksa, te oba indeksa ukazuju na okoliš šelfnog mora normalnog saliniteta. Analiza metodom Transfer-funkcije za salinitet pokazala je općeniti trend smanjenja vrijednosti saliniteta od dubljih i starijih prema plićim i mlađim uzorcima unutar jezgre uz mjestimične kraće periode oscilacije vrijednosti, posebice kod uzorka dubine 120–125 cm, čija starost odgovara sredini 19. stoljeća, a siromašan broj jedinki i visoka vrijednost saliniteta od 37,2 ‰ ukazuju na antropogeni događaj tijekom tog perioda. Odnos epifaunalnih i infaunalnih vrsta pokazuje izraženu dominaciju epifaunalnih oblika poput elfidijskih i amonijskih vrsta (zastupljenost u rasponu od 80–90 % unutar zajednice), što upućuje na oligotrofičnost uz morsko dno u posljednjih 400-tinjak godina.

Changes in the Composition of Smaller Benthic Foraminiferal Assemblages in the Offshore in the Northeastern Adriatic Sea Within the Last 400 Years (Piran Bay)

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The Gulf of Piran generally speaking is an area under a significant anthropogenic influence (vicinity of Koper harbor and Trieste city, Monfalcone shipyards, and great agricultural activity in the hinterland). Altogether, 16 sediment samples obtained from different depth intervals of 1.5 m long core were micropaleonthologically studied. Smaller benthic foraminifera from 250 μm fraction have been identified on species and generic level following Loeblich & Tappan (1987) criteria. Based on obtained data, a variety of statistical and ecological indices was calculated. Values of Simpson diversity and Berger-Parker dominance indices imply a great diversity of species along the entire length of the core. High values of the Shannon-Weaver index show that two to three species are dominating in all samples, whereas other species are present in small proportions. The minor deviation of values was recorded in the assemblages 90–95 cm and 120–125 cm deep. Fisher's α index shows high values as well, and although some oscillations of values occur, general trend coincides with those of Shannon-Weaver index. Both indices suggest that sedimentation took place within inner-outer shelf with normal salinity. Applied Transfer function method for salinity show a decreasing tendency of salinity from deeper/older samples towards shallower/younger ones. Short-lasted periods of oscillations of the salinity took place from time to time, especially during the middle of the 19th century, where the corresponding sample from 120-125 cm depth shows a scarcity of individuals as well as a salinity value of 37.2‰ which indicates an anthropogenic influence during the period. Epifaunal to infaunal species ratio shows a great dominance of epifaunal forms such as elphidiids and ammoniids (their presence in the samples varying from 80–90% of the total assemblage), suggesting that oligotrophic conditions were present during the last 400 years.

Genetička varijabilnost nametnika *Pomphorhynchus laevis* (Zoega in Müller, 1776) (Acanthocephala) u hrvatskim rijekama

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Kukaši (Acanthocephala) su koljeno endoparazita na kralješnjacima karakteristični po svom uvlačivom proboscisu (rilu) s kukicom i složenim životnim ciklusom s dva domadara. Za međudomadare najčešće koriste rakove ili kukce, a za domadare sve razrede kralješnjaka. Kukaši su podijeljeni u četiri razreda. Jedan od njih, Palaeacanthocephala, sadržava isključivo vodene nametnike koji kao međudomadare koriste različite vodene račiće (amfipode), a zatim ribe. Premda nisu izravno uključeni u hranidbeni lanac, kao i većina nametnika, njegov su važan dio i po biomasi i po složenom životnom ciklusu. Osim toga, utječu na svog domadara mogućim patogenim djelovanjem ili akumuliranjem teških metala. Zbog potonjeg svojstva i brojnih interakcija dobri su bioindikatorski organizmi. Takve sposobnosti nekada su usko vezane za linije (soj) unutar jedne vrste koje se prepoznaju genetičkom analizom molekularnih markera. Zanimljivom se pokazala široko rasprostranjena vrsta u Europi *Pomphorhynchus laevis* (Zoega in Müller, 1776). U ovom radu istraživali smo njenu genetičku varijabilnost pomoću genetičkog markera mitohondrijskog gena COI. Istraživanje je obuhvaćalo jedinke sakupljene u nekoliko rijeka u Hrvatskoj koje pripadaju crnomorskom slivu (Sutla, Sava, Kupa, Dobra, Mrežnica, Korana, Una, Orljava i Dunav) te u rijeci Lici koja je ponornica. Analizom slijeda nukleotida gena COI u uzorcima uočena je prisutnost kriptične vrste *P. tereticollis* koja je dugo smatrana sinonimom za vrstu *P. laevis*. Promatranjem haplotipova uzoraka samo *P. laevis* nameće se zaključak da su populacije u hrvatskim rijekama evolucijski očuvane i stabilne te vrlo homogene, uz izdvajanje jednog haplotipa iz Dunava. Pretpostavljamo da je to vrsta *P. bosniacus*, no zbog manjka podataka o njoj u GenBanku nemoguće je potvrditi podatak. Zaključno, gen COI pokazao se kao dobar alat u analizi varijabilnosti između i unutar bliskih vrsta.

Genetic Variability of the *Pomphorhynchus laevis* Parasite (Zoega in Müller, 1776) (Acanthocephala) in Croatian Rivers

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Acanthocephala is a phylum of endoparasites on vertebrates, characterized by the presence of an eversible proboscis, armed with spines and complex life cycles with two hosts. First host are usually crustaceans or insects, for the second host they use all vertebrata classes. Acanthocephala are currently divided into four classes; one of them contains mainly aquatic parasites – Palaeacanthocephala. For their host they mostly use aquatic crustacean (e.g. amphipods) and fish. Essentially they are not part of the food chain, like most parasites are not, but they are important part of it due to their large contribution by biomass and complex life cycle. They can affect their host by a possible pathogenic activity or by accumulating heavy metals. Due to their last trait and numerous ecological interactions, they are a good candidate for bioindicator species. These characteristics are sometimes closely correlated to specific lineage of species that can be distinguished by the genetic analysis with molecular markers. *Pomphorhynchus laevis* (Zoega in Müller, 1776) is a widely distributed species in Europe and exploration of its bioindicator potential has had good results. In this study, genetic variability of *P. laevis* with mitohondrial COI gene as a molecular marker was examined. Individual animals were collected from fish caught in rivers in Croatia that are a part of the Black Sea basin (Sutla, Sava, Kupa, Dobra, Mrežnica, Korana, Una, Orljava and Danube) and Lika river, a subterranean river. DNA sequencing analysis of COI gene has shown the presence of a cryptic *P. tereticollis* species which was previously considered a synonym for *P. laevis*. Haplotype-association analysis for *P. laevis* in Croatian rivers has shown that populations are evolutionary stabile and homogeneous, with one haplotype from Danube that stands out. Our assumption is that that specimen was *P. bosniacus*. Conformation for that hypothesis is lacking because it could not be verified due to small number of sequences for that species in GenBank. Nevertheless, COI gene has proven as a good tool for intra and inter species variability.

Pigmejski vražičci s Wallacee (Orthoptera: Tetrigidae): *Hirrius* s Mindanaa i *Zvierckia* gen. nov. sa Sulavezija

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Rod *Hirrius* Bolívar (1887) imao je i ima problematičnu taksonomiju. Rod je osnovao Bolívar za vrstu *H. punctatus* koja je do tada pripadala rodu *Arulenus* Stål 1877. Rod je sličan Discotettiginima Hanckock 1907 zbog manjka frontomedijalne projekcije, posjedovanja glatkog pronotuma, nedostatka trnova i izbočina, ticala postavljena između složenih očiju i jako nakrivljenog lica. Kasnije je Günther dodao još vrsta: *H. montanus*, *H. scrobiculatus*, *H. sarasinorum* 1937 i *H. mindanensis* 1938 što je promijenilo opis roda i otežalo uključivanje u ključeve. Dodane sulavezijske vrste imaju bifurkaciju frontomedijalne koste postavljenu niže, grubi pronotum, čvorasti diskus, nazubljene margine i karine srednjih i stražnjih bedara. Vrste sa Sulavezija – *H. montanus* i *H. sarasinorum* - dijele brojne morfološke odlike i s vrstom *H. scrobiculatus* te su odvojene u novi rod unutar Scelimenina (neobjavljeno ime *Zvierckia* Skejo, Tumbrinck, Šapina i Pushkar 2015 s neobjavljenom vrstom *Z. storozhenkoi* Skejo, Tumbrinck, Šapina i Pushkar 2015). Vrste s Mindanaoa (*H. punctatus* and *H. minanaensis*) ostaju unutar roda koji se trenutno vodi bez potporodičnog smještaja unutar Tetrigida.

Pygmy Blackhoppers from Wallacea (Orthoptera: Tetrigidae): *Hirrius* from Mindanao and *Zvierckia* gen. nov. from Sulawesi

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Genus *Hirrius* had and still has a problematic taxonomy. The genus was established by Bolívar for *H. punctatus*, which was originally placed in the genus *Arulenus* Stål 1877. The genus was characterized among Discotettiginae Hanckock 1907 by the lack of frontomedial projection, smooth pronotum without spines and protuberances, antennal groves positioned between the compound eyes and a strongly oblique face. Later, more species were added: *H. montanus*, *H. scrobiculatus*, and *H. sarasinorum* in 1937, and *H. mindanensis* in 1938 by Günther, who changed the description of the genus and thus made any attempts of including it in an identification key difficult. Sulawesi species have the bifurcation of the frontal costa positioned lower, rugose pronotum with tuberculated discus, armed margins and carinae of mid and fore femora. Species from Sulawesi – *H. montanus* and *H. sarasinorum* - share numerous morphological features and are with Sulawesi *H. scrobiculatus* separated into a new genus within Scelimenini (unpublished name *Zviercikia* Skejo, Tumbrinck, Šapina and Pushkar 2015 with unpublished species *Z. storozhenkoi* Skejo, Tumbrinck, Šapina and Pushkar 2015). Species from Mindanao (*H. punctatus* and *H. minanaensis*) remain within the genus which is currently assessed as without subfamily placement within Tetrigidae.

Procjena opasnosti za Nacionalni park Krka u području antropogenih utjecaja uz grad Knin

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Rijeka Krka sa svojim sedrenim slapištima te brojnim jezerima predstavlja prirodni i krški fenomen te je zahvaljujući ljepoti krajolika, kao i velikoj bioraznolikosti, brojnim endemičnim, rijetkim i ugroženim vrstama 1985. godine proglašena nacionalnim parkom. Cilj ovog rada bio je procijeniti antropogene utjecaje na dio vodotoka rijeke Krke koji je pod neposrednim utjecajem tehnoloških otpadnih voda tvornice vijaka DIV d. o. o. i komunalnog ispusta grada Knina koji su smješteni samo 2 km uzvodno od granice Parka te predstavljaju potencijalnu opasnost za Nacionalni park Krka. Antropogeni utjecaj procijenjen je praćenjem niza fizikalno-kemijskih i mikrobioloških čimbenika, koncentracije otopljenih metala u vodi te citosolskih metala i biomarkera u jetri potočne pastrve (*Salmo trutta* Linnaeus, 1758), bioindikatorskog organizma i tipičnog predstavnika ihtiofaune rijeke Krke. Osim na dijelu vodotoka rijeke Krke uz navedena onečišćenja kod grada Knina, uzorkovanje je provedeno i na izvoru rijeke Krke kao kontrolnom lokalitetu. Fizikalno-kemijski i mikrobiološki čimbenici ukazuju na pogoršanje kakvoće vode u dijelu toka uz grad Knin, gdje je za pojedine čimbenike voda vrlo lošeg ekološkog stanja, dok je na izvoru voda vrlo dobrog ekološkog stanja. Koncentracije gotovo svih metala u vodi i staničnom citosolu jetre pastrva su bile povišene u dijelu toka uz ispuste otpadnih voda u odnosu na izvor, s izuzetkom citosolskih metala Cd, Cs, Pb, Rb i Tl u jetri potočne pastrve, što vjerojatno ukazuje na njihovo prirodno podrijetlo u krškim vodama, posebno Cd. Biomarkeri izloženosti metalima – metalotioneini (MT), potvrdili su povećanu izloženost metalima statistički značajno povišenim koncentracijama u riba s izvora rijeke Krke. S obzirom na to da je Cd jedan od glavnih metala koji pobuđuju sintezu MT, ovaj biomarker vjerojatno odražava povišene razine citosolskog Cd, čije podrijetlo u okolišu treba dodatno istražiti. Prikazani rezultati ukazuju na značajan antropogeni utjecaj na rijeku Krku kod grada Knina, ali pojedini citosolski metali i na povećani unos metala u organizme na izvoru rijeke Krke. S obzirom na to da smo dokazali potencijalnu opasnost za očuvanje vrlo dobrog ekološkog stanja vode, a time i staništa i bioraznolikosti Nacionalnog parka Krka, neophodno je provoditi sustavni monitoring i sustavno praćenje kakvoće vode rijeke Krke.

Risk Evaluation for the Krka National Park in the Anthropogenically Impacted Area Near the Town of Knin

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The Krka River, with its travertine waterfalls and many lakes, represents a natural and karst phenomenon, and was was proclaimed a National Park in 1985 due to the beauty of landscape as well as high biodiversity, many endemic, rare and endangered species. The aim of this study was to assess anthropogenic impact on the Krka River watercourse which is under the direct influence of the industrial municipal wastewater from the DIV d. o. o. screw factory and municipal wastewater from the Town of Knin, which are located only 2 km upstream of the Park borders, therefore presenting the potential threat to the Krka National Park. The anthropogenic impact was estimated by physico-chemical and microbiological water parameters, dissolved metal concentrations in water, as well as cytosolic metal and biomarker concentrations in the liver of brown trout (*Salmo trutta* Linnaeus, 1758), a bioindicator organism and typical representative of the ichtyofauna. In addition to the Krka River sampling site under the pollution impact near the Town of Knin, sampling was also performed at the Krka River source, as the reference site. Physico-chemical and microbiological water parameters showed deterioration of water quality at location near the Town of Knin, for certain parameters even to water of bad quality, while the water from the Krka River source was of high ecological status. Levels of majority of metals in the water and in the trout hepatic cytosol were increased at pollution impacted site of the Krka River compared to its source, with the exception of cytosolic metals Cd, Cs, Pb, Rb and Tl, probably indicating their natural origin in karst waters, especially of Cd. Biomarkers of metal exposure - metallothioneins (MT), confirmed increased metal exposure by significantly increased levels in the fish from the Krka River source. Considering Cd as one of the main MT inducers, this biomarker probably reflected elevated levels of cytosolic Cd, whose origin should be additionally investigated. Presented results indicate significant anthropogenic impact on the Krka River near the Town of Knin, although specific cytosolic metals point to the increased metal exposure in the Krka River source. Since we confirmed potential threat to preservation of a high ecological status of the river water, and thus of the habitat and biodiversity in the Krka National Park, the implementation of comprehensive water quality monitoring strategies of the Krka River is required.

Prvo zamjećivanje invazivne smeđe mramorne stjenice *Halyomorpha halys* (Stål, 1855) u Hrvatskoj

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Smeđa mramorna stjenica *Halyomorpha halys* (Stål, 1855) (Hemiptera: Heteroptera: Pentatomidae), izvorno iz Koreje, Kine, Tajvana i Japana, invazivna je vrsta koja se već proširila Sjevernom Amerikom i širi se Europom. Prvo je zabilježena u Pennsylvaniji, Sjevernoj Americi (2001), a potom je zabilježena u Europi: Austriji (2015), Francuskoj (2013), Njemačkoj (2012), Grčkoj (2014), Mađarskoj (2014), Lihtenštajnu (2009), Rumunjskoj (2015), Srbiji (2015) i Švicarskoj (2008). Pronašli smo dva živa primjerka u gradu Rijeci u siječnju ove godine. Budući da svi ključevi za europske pentatomide određuju *H. halys* kao *Pentatoma rufipes* (Linneaus, 1758), raspravljamo o njenoj morfologiji i razlikama među sličnim europskim pentatomidima. Pošto je *H. halys* invazivna vrsta, raspravljamo i o potencijalnim putevima unosa u Hrvatsku.

First Record of the Invasive Brown Marmorated Stink Bug *Halyomorpha halys* (Stål, 1855) in Croatia

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The brown marmorated stink bug *Halyomorpha halys* (Stål, 1855) (Hemiptera: Heteroptera: Pentatomidae), native to Korea, China, Taiwan and Japan, is an invasive species that has already spread over North America and is spreading over Europe. It was first recorded in Pennsylvania, North America (2001) and later it was recorded in Europe: Austria (2015), France (2013), Germany (2012), Greece (2014), Hungary (2014), Lichtenstein (2009), Romania (2015), Serbia (2015) and Switzerland (2008). We found two live specimens in the city of Rijeka in January of this year. Since all European pentatomid identification keys identify *H. halys* as *Pentatoma rufipes* (Linneaus, 1758), we discuss its morphology and differences between similar-appearing European pentatomids. Since *H. halys* is an invasive species we also discuss potential pathways into Croatia.

Problematika izrade ključa za determinaciju božjih ovčica (Coleoptera: Coccinellidae)

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Božje ovčice ili bubamare (Coccinellidae) porodica su unutar reda kornjaša (Coleoptera). Ekološki i morfološki vrlo su raznolika skupina kukaca. Zbog karnivornog načina prehrane ekološki su veoma značajne te se koriste u biološkoj kontroli štetnika u poljoprivrednim sustavima, gradskim vrtovima te parkovima. Fauna božjih ovčica Hrvatske nedovoljno je istražena. Glavni su razlozi za to nedostatak stručnjaka, kao i sveobuhvatnih ključeva za determinaciju ove porodice. Literaturnih podataka za faunu božjih ovčica Hrvatske je malo te su rijetka ciljana istraživanja samo te porodice. U svrhu izrade determinacijskog ključa pregledana je sva dostupna literatura koja sadržava podatke o božjim ovčicama Hrvatske kao i privatne zbirke koje sadržavaju pripadnike ove porodice kukaca sakupljene u Hrvatskoj. Materijal je pregledan i određen pomoću svih dostupnih ključeva za determinaciju. Kombinacijom postojećih ključeva, kao i novostečenog znanja, izrađen je prvi sveobuhvatni ilustrirani ključ za determinaciju božjih ovčica Hrvatske. Smatra se da će ključ biti koristan ne samo entomolozima iz Hrvatske nego i onima iz regije. Ključ je bogato ilustriran i sadržava 82 crteža čime bi se uporaba, kako profesionalnim entomolozima, tako i amaterima, trebala iznimno olakšati.

Problems That Occur While Creating Determination Key for Ladybirds (Coleoptera: Coccinellidae)

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Ladybirds, or ladybugs (Coccinellidae), are a family within the order beetles (Coleoptera). Ecologically and morphologically they are a very diverse group of beetles. They are very important due to their carnivorous diet, thus being widely and successfully used in biological control of pests in agroecosystems, as well as in urban gardens and parks. Nevertheless, the fauna of Croatian ladybirds is still poorly known. The main reasons for this are the lack of entomological experts, as well as comprehensive identification keys for this family. Literature data and research targeting only Croatian ladybirds family are rare. In order to provide the first identification key for Croatian ladybirds, all available literature and private coleopterological collections in Croatia were reviewed. The material was examined and identified using all available identification keys. Combining existing identification keys and newly acquired knowledge, the first comprehensive illustrated identification key to ladybirds of Croatia was made. It is believed that the key will be beneficial to Croatian entomologists as well as those from the region. The key is richly illustrated and contains 82 drawings which will extremely facilitate its usability by both professional and amateur entomologists.

Sastav lipida novoizoliranog soja pikoeukariota *Picochlorum* sp. PMFPPE4 u standardnom i fosfor-limitirajućem mediju

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Istraživan je sastav lipida novoizoliranog soja pikoeukariota *Picochlorum* sp. PMFPPE4 u standardnom i fosfor-limitirajućem mediju kroz 20 dana uzgoja. Za određivanje krivulje rasta korištene su svjetlosna mikroskopija i protočni citometar. Eksperiment je bio postavljen u triplikatu, te su poduzorci za abundanciju uzimani svaki dan, a za analizu lipida svaki drugi dan. Ovisno o mediju uzgoja, stanice *Picochlorum* sp. dale su drugačiji odgovor koji se manifestirao u različitim krivuljama rasta kao i sastavu lipida. Za razliku od standardnog medija u kojem su stanice prošle kroz eksponencijalnu fazu rasta i ušle u stacionarnu fazu, u fosfor-limitirajućem mediju nije došlo do povećanog razvoja stanica. Koncentracija ukupnih lipida pratila je abundanciju stanica, te je u fosfor-limitirajućem mediju na početku eksperimenta bila znatno viša nego u standardnom mediju. Dobiveni rezultati ukazali su na indukciju mehanizama za preživljavanje u fosfor-limitirajućim uvjetima promjenom sastava lipida i recikliranjem fosfata. Došlo je također i do promjene pojedinih udjela lipida s ulogom energijske rezerve (triglicerida), remodeliranja membranskih lipida (glikolipida i fosfolipida) te porasta indikatora razgradnje. Utvrđena je plastičnost i biotehnološki potencijal novoizoliranog soja *Picochlorum* sp. PMFPPE4. Daljnjom optimizacijom medija za uzgoj bit će moguće dobiti veću koncentraciju lipida koji mogu biti korisni u proizvodnji biodizela i naći primjenu u industriji.

Lipid Classes Composition of the Newly Isolated Picoeukaryote *Picochlorum* sp. PMFPPE4 in the Standard and Phosphorus Limited Medium

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Lipid composition and cell abundance of the newly isolated strain *Picochlorum* sp. PMFPPE4, was determined during 20-day growth rate experiment in the standard and phosphorus limited medium. Growth rate of the culture was determined by cell counts performed by light microscopy and flow cytometry. Experiment was set in triplicates, and subsampled every 24 h for cell counts and every 48 h for determining lipid classes. Depending on the medium, *Picochlorum* sp. showed different response in terms of cell counts and lipid classes. Cells grown in the standard medium showed exponential growth rate and have reached the stationary phase, while the same did not occur in the phosphorus limited medium. Concentration of all lipid classes showed the same trend as the cell abundances during the experiment. Lipid concentrations in the phosphorous limited medium, were much higher than those in the standard medium indicating possible induction of survival mechanisms by changing lipids and recycling phosphate. Also the change in specific lipid classes was recorded as the change in lipid classes with role of energy reserve (triglycerides), remodeling of membrane lipids (glycolipids and phospholipids) and an increase in degradation indicators. The plasticity and biotechnological potential of the newly isolated strain *Picochlorum* sp. PMFPPE4 was recorded. Further optimization of the chemical content of the growth media will possibly provide higher lipid production that could be used in biodiesel industry.

Vaskularna flora područja Dotrščina (Zagreb, Hrvatska)

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Tijekom 2016. godine istraživana je vaskularna flora područja Dotrščine, rekreacijske park-šume smještene na jugoistočnim padinama Medvednice. Istraživana je flora šumskih zajednica koje prevladavaju na ovom području, te otvorenih područja šume kao što su rubovi cesta, oko rasadnika biljaka Šumarskog fakulteta te oko spomenika u sklopu spomen-područja Dotrščina koji obuhvaća južni dio istraživanog područja. Ukupna površina istraživanog područja iznosi 3,3 km^2. Literaturni podaci za područje Dotrščine navode prisutnost četiriju šumskih zajednica čija je rasprostranjenost potvrđena ovim istraživanjem: šuma hrasta lužnjaka i običnog graba, šuma hrasta kitnjaka s crnim grahorom, submontana bukova šuma sa žućkastom grahoricom i šuma hrasta kitnjaka i običnog graba s trepavičastim šašem. Osim navedenih šumskih zajednica, na manjim dijelovima dotrščinske šume nalaze se i šumske kulture bagrema, japanske kriptomerije, pitomog kestena i običnog bora. Šumske zajednice mjestimično su isprekidane drugim tipovima staništa poput ruderalnih, travnjačkih te vlažnih staništa uz potoke. Zabilježene su ukupno 202 svojte, a najbrojnije porodice su *Rosaceae*, *Poaceae* i *Fabaceae*. Najčešći životni oblici su hemikriptofiti (47,4 %), fanerofiti (20,9 %) i geofiti (17,3 %). U flori dominiraju biljke euroazijskog (44,1 %) i srednjoeuropskog (17,8 %) flornog elementa. Zabilježeno je 14,8 % alohtonih biljaka, od čega osam invazivnih, porijeklom iz Sjeverne Amerike i Azije. Najviše invazivnih svojti, njih tri, pripada porodici *Asteraceae*. Pronađene su dvije osjetljive (VU) vrste (*Platanthera bifolia* i *Taxus baccata*) koje su uz još jednu pronađenu vrstu kaćuna (*Neottia nidus-avis*) zakonom strogo zaštićene. U flori dominiraju biljke polusjene, umjereno vlažnih i toplih, uglavnom kiselih tala, obogaćenih dušikom. Usporedbom flore područja Dotrščine sa sličnim (sub)urbanim područjima u Zagrebu utvrđeni su slični udjeli životnih oblika i flornih elemenata, dok je pojavnost invazivnih biljaka bila znatno manja, nego kod ostalih područja. Broj vrsta utvrđenih na području Dotrščine manji je u odnosu na većinu drugih zagrebačkih urbanih područja. Naposljetku možemo zaključiti da je Dotrščina područje relativno niske raznolikosti flore i djelomično je pod utjecajem urbane okoline, što se očituje u povećanom broju otvorenih tipova staništa.

Vascular Flora of the Dotrščina Area (Zagreb, Croatia)

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The vascular flora of the recreation parkforest Dotrščina, situated on the southeastern slopes of the Medvednica Mt, was studied in 2016. This study includes the flora of forest communities which dominate this area, and the open areas such as road edges, the nursery garden of the Faculty of Forestry and surrounding of monuments within the memorial area of Dotrščina, which includes the southern part of the studied area. The total researched area is 3.3 km^2. Literature data for the area of Dotrščina indicate the presence of four forest communities whose presence and distribution were confirmed by this research: pedunculate oak and common hornbeam forest, sessile oak forest with *Lathyrus niger*, submontane beech forests with *Vicia oroboides* and forests of sessile oak and common hornbeam with *Carex pilosa*. In addition to the above listed forest communities, in smaller parts there are forest cultures of black locust, Japanese cedar, sweet chestnut and Scots pine. Forest communities are intermittently interrupted by other habitat types such as ruderal, grassland and humid habitats along streams. In total 202 taxa were recorded, with the *Rosaceae*, *Poaceae* and *Fabaceae* being the most numerous families. Hemicryptophytes (47.4%), phanerophytes (20.9%) and geophytes (17.3%) prevail. The flora is dominated by taxa belonging to Euro-Asian (44.1%) and central-European (17.8%) chorotype. As much as 14.8% of the flora is allochthonous, with eight invasive taxa originating from North America and Asia. The most numerous invasive taxa, three of them, belong to the *Asteraceae* family. Two vulnerable (VU) species (*Platanthera bifolia* and *Taxus baccata*) were found, which are strictly protected by law, as well as another orchid species found (*Neottia nidus-avis*). The dominance of the plants of semi-shade, moderately moist and warm, mostly acidic, nitrogen-enriched soils was observed. The comparison between the flora of the Dotrščina forest and other similar (sub)urban areas of Zagreb showed the similarities in terms of life forms and chorology, while on contrary, Dotrščina exhibits lower number of invasive species. The number of species found in the area of Dotrščina is smaller than in the majority of other urban areas of Zagreb. Finally, we conclude that the flora of the Dotrščina area is relatively low in diversity, and is partly influenced by the urban environment, which is manifested in the increased number of open habitat types.

Posterska izlaganja

Poster presentations

Naseljavanje i kretanje makrozoobentosa u intersticiju sedrenih barijera

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Sedrene barijere pružaju jedinstveni splet mikrostaništa različitih biotičkih i abiotičkih svojstava u kojem obitava posebna zajednica makrozoobentosa. Najveći dio zajednice vezan je uz mahovinske sastojine, no sedrena podloga ispod njih izuzetno je važno refugijalno područje za makrozoobentos, ali i stanište tipičnih intersticijskih životinja. U ovom radu se istražuju migracije životinja u sedrenom intersticiju. Istraživanje je provedeno u Nacionalnom parku Plitvička jezera na barijeri Novakovića Brod uklapanjem pokusnih posuda s blokovima evakuirane prirodne sedre (svi organizmi i organska tvar su uklonjeni) u prirodno stanište intersticija. Uklopljene posude bile su podijeljene u dva sloja: gornji – bliži površinskom staništu mahovinskih sastojina i donji - dublji sloj do dubine od 10 cm. Cilj je istraživanja bio utvrditi obrasce naseljavanja makrozoobentosa, te protok čestica organske tvari kao glavnog energijskog resursa kroz plitki sloj sedrenog intersticija. Koncentracija otopljenog kisika bila je veća u površinskoj vodi nego u sedrenom supstratu i mahovini. Kretanje makrozoobentosa kroz intersticij bilo je raznoliko. Nasumični migranti bili su detritivorni Oligochaeta koji su funkcionalno, morfološki i etološki prilagođeni na život u intersticiju. Usmjereno gibanje bilo je svojstveno jedinkama roda *Hydropsyche*. One su se uglavnom kretale okomito na struju vode čime vjerojatno osiguravaju ostanak u blizini svojih mreža. U donjem dijelu istraživanog sloja, koji je vrlo slabo nastanjen, više je čestica organske tvari jer se resursi sporije troše. U donjem sloju najkrupnije čestice činile su većinu detritusa, a fine čestice bile su najslabije zastupljene budući da su one resurs većine detritivora.

Colonization and Movement of Macrozoobenthos in Tufa Barriers Interstitium

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Special macrozoobenthic community resides at tufa barriers which provide a unique combination of microhabitats of different biotic and abiotic traits. Although this community is mostly tied to the moss mats, tufa underneath them serves both as a habitat and an extremely important refuge area. Here we study macroinvertebrate migrations in the interstitial zone of tufa in Plitvice Lakes National Park at the barrier Novakovića Brod. This survey was conducted by implementing traps with evacuated natural tufa blocks in the natural interstitial habitat (all organisms and organic matter was removed before). Implemented traps were divided into an upper section closer to the moss mats and a lower section which reached the depth of 10 cm. The objectives were: to determine macrozoobenthic migrations through the shallow tufa interstitium and to determine the flow of organic matter as the main energetic resource. The results showed that the concentration of dissolved oxygen was higher in the surface water compared to the water in tufa and moss mats. However, patterns of migration of macrozoobenthic organisms were diverse. Detritivorous Oligochaeta, functionally, morphologically and ethologically adapted to the intestitial life, were random migrators. Characteristic for *Hydropsyche* caddisflies was directed migration, suggesting that movement perpendicular to flow enables animals to stay near their nets. More particulate organic matter was accumulated in the lower and poorly inhabited section of tufa due to slower and lesser animal consumption. Therefore, CPOM was dominant and FPOM were the least represented for being eaten by most detritivore interstitial taxa.

Predstavljanje projekta „Kako će klimatske promjene utjecati na širenje račje kuge?”

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U Hrvatskoj, kao i u ostatku Europe, zabilježen je značajan pad brojnosti zavičajnih populacija deseteronožnih rakova. Među glavnim razlozima ugroženosti su klimatske promjene, regulacija vodotoka te invazivne vrste rakova koje djeluju kao vektor širenja bolesti račje kuge. Uzročnik račje kuge je mikroorganizam *Aphanomyces astaci*, koji se nalazi na popisu sto najgorih invazivnih stranih vrsta. U sklopu projekta u tijeku su istraživanja kako bi se utvrdilo kako klimatske promjene (povišenje temperature vode) utječu na virulentnost račje kuge kod zavičajnih i invazivnih rakova, odnosno predvidjet će se hoće li klimatske promjene značajno povećati opasnost od infekcije zavičajnih rakova račjom kugom. S obzirom na to da su postojeće metode detekcije *A. astaci* uključivale usmrćivanje rakova, kao prvi korak ovog projekta razvijena je neinvazivna metoda detekcije uzročnika račje kuge. Metoda se temelji na uzorkovanju površinske mikrobiote trljanjem kutikule živog raka. Iz tako dobivene mješovite epibiontske mikrobne zajednice izolira se ukupna DNA te se PCR-testom provjerava je li u uzorku prisutna DNA uzročnika račje kuge. Rezultati PCR-testa na DNA izoliranoj iz obrisaka uzetih s površine osam rakova pokazali su potpunu podudarnost s rezultatima dobivenim invazivnom metodom. Rezultati ovog istraživanja dat će nova saznanja o tome što će se dogoditi u međuodnosu zavičajnih i invazivnih rakova (kao prenosioca račje kuge) uslijed klimatskih promjena. Ukoliko se pokaže da će povišenje temperature vode dati dodatnu prednost invazivnim rakovima, tada treba pojačati aktivnosti vezane uz njihovo uklanjanje iz sustava.

Presentation of the Project “How Will Climate Change Affect Expansion of the Crayfish Plague?”

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In Croatia, as well as throughout Europe, a significant decline in numbers of native decapod crayfish populations has been registered. Some of the main reasons of population decline are climate change, regulation of waterways, and invasive crayfish species which act as vectors for spreading the crayfish plague disease. The microorganism *Aphanomyces astaci* is the causative agent of the crayfish plague and it is listed as one of the hundred worst invasive species. This project will investigate how climate change (increase in water temperatures) affects the crayfish plague virulence in native and invasive crayfish, predicting if climate change will significantly increase the risk of *A. astaci* infection within native crayfish populations. Since existing methods for detection of *A. astaci* involve sacrificing the animals, a non-invasive method for the detection of the crayfish plague was developed as the first step of this project. This method is based on the collection of mixed epibiotic microbial communities by scrubbing the cuticule of a living crayfish. Then, the DNA is isolated from the mixed epibiotic microbial communities and the presence of *A. astaci* DNA in the sample is detected by PCR. The results of the PCR-test done on the DNA isolated from the swabs taken from the cuticle of eight crayfish have shown a complete match with the results obtained by an invasive method. The results of this project will help to predict the changes in relationship between native and invasive crayfish species in the light of climate change. If it shows that the increase of water temperature offers additional advantage to the invasive crayfish, then actions for the removal of those species from the system should be reinforced.

Erasmus+ praksa na institutu European Molecular Biology Laboratory (EMBL)

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Akademske godine 2015./2016. obavljala sam praksu u sklopu programa Erasmus+. U trajanju od sedam mjeseci postala sam dio istraživačkog tima na institutu molekularne biologije European Molecular Biology Laboratory, Heidelberg (Njemačka). Praksu sam obavljala u grupi dr. Martina Becka, koji se bavi strukturnom proteomikom. Dr. Beck je jedan od najvećih stručnjaka u otkrivanju građe proteina koji čine jezgrine pore. Cilj mog projekta bio je izolirati dva nukleoporina eukariotskih stanica kako bi im mogli odrediti strukturu elektronskom mikroskopijom. Proces izolacije dva velika proteinska kompleksa uključivao je kloniranje pomoću sustava MultiBac, stvaranje bakulovirusa, njihovu prekomjernu ekspresiju u stanicama insekta i pročišćavanje izoliranih proteina. Ovim izlaganjem želim detaljnije objasniti proces izolacije željenog proteina, te podijeliti svoja osobna iskustva stečena na institutu.

Erasmus Internship at European Molecular Biology Laboratory (EMBL)

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During the academic year 2015/2016 I conducted an internship as an Erasmus+ student. For seven months, I was a part of the research team at the European Molecular Biology Laboratory, in Heidelberg, Germany. I conducted this internship in the group of Dr. Martin Beck, whose main focus is structural proteomics. Dr. Beck is one of the leaders in discovering the structure of nuclear pore complexes. The aim of my project was to isolate two eukaryotic nucleoporins so their electronic microscopy structure could be obtained. The process of isolation included cloning by MultiBac system, creation of baculovirus, overexpression of those proteins in insect cells, and purification of isolated proteins. With this poster I would like to explain the process in more depth, and share experience gained at the institute.

Laboratorijska praksa u Laboratoriju za biofiziku stanica na Institutu Ruđer Bošković

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U sklopu laboratorijske prakse u grupi prof. dr. sc. Ive Tolić na Institutu Ruđer Bošković sudjelovale smo u istraživanju utjecaja augmina na strukturu diobenog vretena. Augmin je oktamerni proteinski kompleks koji omogućava nukleaciju novih mikrotubula na već postojećim i ima ulogu u uspostavi bipolarnosti i napetosti diobenog vretena. Cilj je bio proučiti njegovu ulogu u stvaranju premošćujućih mikrotubula, nedavno otkrivene nove skupine mikrotubula koji lateralno povezuju vlakna diobenog vretena vezana za kinetohore sestrinskih kromatida. U tu smo svrhu transfecirale dvije različite stanične linije, U2OS i HEK293, plazmidima koji su nosili gene za tubulin-α i CENP-A povezane s fluorescentnim reporterskim genima. Potom smo korištenjem malih interferirajućih RNA (siRNA) utišale gen za HAUS6, koji je jedna od komponenti augmina. Nakon tog tretmana, augmin smo detektirale imunocitokemijskim bojenjem. Stanice s obilježenim mikrotubulima, kinetohorama i augminom promatrale smo pomoću konfokalnog fluorescencijskog mikroskopa za pretraživanje. Utjecaj utišavanja gena analizirale smo određivanjem udjela stanica s multipolarnim diobenim vretenom i mjerenjem kontura bipolarnih diobenih vretena.

Internship at the Laboratory of Cell Biophysics at Ruđer Bošković Institute

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During the internship in the group of Prof. dr. sc. Iva Tolić at the Ruđer Bošković Institute we participated in the research of augmin and the mitotic spindle. Augmin is a multimeric protein complex known to facilitate nucleation of new microtubules from pre-existing ones. It has an important role in tension creation within the spindle and establishment of cell bipolarity. The aim was to assess potential role of augmin in the nucleation of recently discovered new class of microtubules, termed bridging fibres, which laterally connect kinetochore fibres attached to sister kinetochores. We transfected two different cell lines, U2OS and HEK293, with plasmids encoding reporter genes and tubulin alpha or CENP-A. Then we silenced a gene encoding an augmin subunit, HAUS6, by small interfering RNA (siRNA). Following this treatment, we detected augmin complex by immunocytochemistry staining. We examined cells, containing labelled microtubules, kinetochores and augmin, by confocal scanning microscope. The effect of gene silencing was assessed by determining the ratio of multipolar cells, and by measuring contours of bipolar spindles.

Optimizacija izolacije DNA i lančane reakcije polimerazom za umnažanje mikrosatelitnih biljega divlje mačke, *Felis silvestris* Schreber, 1777

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Divlja mačka (*Felis silvestris* Schreber, 1777) najmanje je istražena strogo zaštićena vrsta zvijeri u Hrvatskoj. U Europi je genomska čistoća populacije divlje mačke ugrožena zbog iznimno česte hibridizacije s domaćom mačkom, no nije istraženo kakvo je stanje populacije divljih mačaka u Hrvatskoj. Stoga je za potrebe očuvanja i reintrodukcije vrste nužno provesti analizu genomske različitosti populacije divlje mačke u Hrvatskoj. Kao prvi korak u istraživanju, izolirana je genomska DNA iz 40 uzoraka različitih tkiva divlje i domaće mačke. Najbolji rezultati izolacije dobiveni su korištenjem uzoraka krvi i mišića što je bilo vidljivo analizom na agaroznom gelu gdje su najčešće bile vidljive jasne i uske vrpce veličine oko 48 kb. Osim toga, genomska DNA je uspješno izolirana i iz briseva usne šupljine – dobivene su male količine DNA, ali dovoljne za kasniju provedbu lančane reakcije polimerazom (PCR od eng. *polymerase chain reaction*). Izolacija DNA iz zubne pulpe nije bila uspješna vjerojatno zbog premale količine DNA u početnom uzorku. U sljedećem koraku istraživanja, optimizirano je umnažanje 12 različitih mikrosatelitnih biljega divlje mačke PCR-om, korištenjem uzoraka DNA različitih jedinki divlje i domaće mačke. Istražen je utjecaj različitih temperature i vremena pri kojima se početnice vežu na kalup te vremena trajanja produljivanja lanaca na uspješnost amplifikacije. Osim toga istražen je i utjecaj količine DNA kalupa na uspješnost reakcije PCR-a kako bi se izbjegla moguća inhibicija kalupom. Optimalna količina kalupa po reakciji bila je 20 ng. Istražen je i utjecaj višestrukog odmrzavanja i smrzavanja oligonukleotidnih početnica pri čemu su se efikasnijima pokazale svježe početnice. Na kraju, dobiveni rezultati ovih istraživanja omogućiti će analizu genomske različitosti populacije divlje mačke odnosno utvrđivanje genomske čistoće i valoriziranje genomske raznolikosti njezine populacije u Hvatskoj.

Optimization of DNA Isolation and Polymerase Chain Reaction for Amplification of Microsatellite Markers of Wildcat, *Felis silvestris* Schreber, 1777

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Wildcat (*Felis silvestris* Schreber, 1777) is the least studied strictly protected wild carnivore in Croatia. Genomic purity of European wildcat population is endangered because of the frequent hybridisation with domestic cats, while the situation in Croatia is still unknown. Therefore, with the final aim of conservation and reintroduction of wildcat, it is necessary to carry out genome diversity analysis of the Croatian population. As a first step, genomic DNA was isolated from 40 specimens of wildcats and domestic cats. The best isolation results were obtained with blood and muscle samples, with clear and narrow bands visible on agarose gel, indicating DNA fragment size of around 48 kb. In addition, genomic DNA has been successfully isolated from buccal swabs – small amounts of DNA were obtained, but it was sufficient for subsequent polymerase chain reaction (PCR). Isolation of DNA from dental pulp was not successful, probably because of the small amount of DNA in the initial sample. The next step of the study was the optimization of PCR amplification of 12 different wildcat microsatellites, using isolated DNA from different wild and domestic cats as a template. The influence of different annealing and elongation temperatures, as well as elongation time was studied. Impact of template DNA amount on PCR reaction was also investigated in order to avoid possible template inhibition, and it was established that the optimal quantity of DNA template per reaction was 20 ng. The influence of multiple freezing/thawing of oligonucleotide primers was examined: fresh primers were shown to be more efficient. In conclusion, the results of these studies will enable analysis of genome diversity of the wildcat population, determining its genomic purity and valorising the genomic diversity of its population in Croatia.

Uloga kompleksa Cascade u inhibiciji replikacije DNA u bakteriji *Escherichia coli*

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Sustav CRISPR-Cas (od eng. *Clustered regularly interspaced short palindromic repeats* i *CRISPR associated*) bakterijski je mehanizam obrane od stranih genetičkih elemenata. Sustav se sastoji od lokusa CRISPR u koji se ugrađuju razmaknice stranog podrijetla između ponavljajućih sljedova, te pridruženih proteina Cas. Razmaknice unutar lokusa CRISPR služe za prepoznavanje i cijepanje ciljne DNA prilikom ponovljene infekcije istim stranim elementom. Kompleks Cascade vođen je putem CRISPR-RNA, ili crRNA, koja je komplementarna ciljnoj DNA s kojom će kompleks interferirati. Stoga crRNA ima ključnu ulogu u obrani sustavom CRISPR-Cas jer sudjeluje u prepoznavanju strane DNA i njenom cijepanju dovođenjem proteina Cas3.

U ovom radu je istraživano hoće li se kompleks Cascade vođen dizajniranom crRNA vezati na protorazmaknicu na plazmidu i stvoriti strukturu znanu kao R-omča te time spriječiti replikaciju DNA plazmida. U pokusu je onemogućena razgradnja R-omče i plazmida delecijom ostalih cas gena; kako je karakteristika sustava CRISPR-Cas modularnost, za uklanjanje strane DNA potrebno je nekoliko proteinskih kompleksa. U ovom redu obrađivala se faza interferencije i kompleks Cascade koji je njezin izvršitelj, a faza adaptacije i razgradnje onemogućene su delecijom gena cas koji bi prepisivali izvršitelje ovih faza. Ako bi zaista kompleks Cascade s navođenom crRNA zaustavila replikaciju DNA, to bi se moglo uočiti kao manja efikasnost transformacije na selektivnim podlogama. Dobiveni rezultati su pokazali da stanice s kompleksom Cascade imaju smanjen broj transformanata i smanjenu vijabilnost, ali da je efikasnost transformacije slična kao u stanicama bez kompleksa Cascade. Drugim riječima, u slučaju da je kompleks Cascade eksprimiran u soju, pokazano je da bez obzira na postavljena uvjetna ograničenja pri dizajnu razmaknica vijabilnost sojeva se održavala na podjednakoj razini. To upućuje na mogućnost da u živim stanicama dolazi do zaustavljanja replikacije i plazmida i kromosomske DNA pomoću različitih molekula RNA, ne samo crRNA, jer je moguće da kompleks Cascade kao navigacijsku RNA osim crRNA pogreškom može ugraditi neku od normalno zastupljenih mRNA u bakterijskoj stanici.

The Role of Cascade Complex in Inhibition of DNA Replication in *Escherichia coli* Bacteria

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The CRISPR-Cas (clustered regularly interspaced short palindromic repeats and CRISPR associated) system is a bacterial defence mechanism against foreign genetic elements. The system is comprised of CRISPR locus, which incorporates spacers from foreign genetic sequences, and of Cas associated proteins. The role of spacers contained within CRISPR locus is recognition and cleavage of target DNA after repeated infection with the same foreign genetic sequence. Cascade complex is guided by CRISPR RNA, or crRNA, which complements target DNA and induces interference. Therefore, crRNA has a key role in CRISPR-Cas defence mechanism since it recognizes foreign DNA sequences and cleaves them by associating Cas3 protein.

In this research we investigated the role that Cascade complex, guided by a designed crRNA, plays in the inhibition of DNA plasmid replication in the event that it binds to the protospacer on the plasmid and subsequently forms a structure known as R-loop that prevents plasmid DNA replication. Cleavage of R-loops was disabled in this experiment by using strains with deleted cas genes. Characteristic to CRISPR-Cas systems is modularity and several protein complexes are required to remove foreign DNA. In this experiment, the only observed phase was interference, executed by Cascade, and remaining phases were disabled by deleting the cas genes responsible for producing protein executors. If the Cascade complex guided by crRNA actually inhibits the replication of DNA, the effect could be observed as decreased transformation efficiency on selective media. Our results showed that cells with functional Cascade complex have a reduced number of transformants as well as decreased viability, but the transformation efficiency remains similar to cells without the functioning Cascade complex. In other words, if Cascade is expressed in the strain it was shown that regardless of restrictive conditions to some designed spacers, strain viabilities were shown to differentiate in negligible percentages. This could possibly indicate that different RNA molecules, and not just crRNAs, participate in the inhibition of replication of both plasmid and chromosomal DNA, since it could be possible that during inclusion of guiding RNA in Cascade mistakes can be made likely by overabundance of basal transcribed mRNA in bacterial cell.

Razvoj testova za analizu metilacije DNA regulatornih regija gena *GMNN* i *EZH2* i njihova primjena u istraživanju podtipova difuznog B-velikostaničnoga limfoma

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Istraživanja malignih tumora ukazala su na važnost gena uključenih u replikaciju i ponovnu uspostavu kromatina u onkogenezi. Njihova poremećena funkcija može biti povezana s epigenetičkim promjenama. Cilj ovog istraživanja bio je analizirati stupanj metilacije DNA regulatornih regija gena *GMNN* i *EZH2* kod podtipova difuznog B-velikostaničnog limfoma. U tu su svrhu razvijeni testovi za procjenu stupnja metilacije pojedinih CpG mjesta unutar regulatornih regija tih gena. Odabrani su parovi početnica koji lančanom reakcijom polimerazom umnažaju fragmente bisulfitno konvertirane DNA unutar regulatornih regija gena od interesa. Razvijena su tri testa za regulatorne regije gena *GMNN* i četiri testa za regulatorne regije gena *EZH2*. Nakon toga određeni su optimalni uvjeti reakcija pirosekvenciranja. Završno je razvijen po jedan test za svaki gen. Ti su testovi primijenjeni u analizi podtipova difuznog B-velikostaničnog limfoma. Analizirani dijelovi regulatornih regija gena *GMNN* i *EZH2* nisu pokazali značajne razlike između podtipova. Budući da su ovim istraživanjem obuhvaćeni relativno mali dijelovi regulatornih regija oba gena, analizu bi trebalo proširiti na dodatna CpG mjesta te uključiti veći broj uzoraka.

Assay Development for DNA Methylation Analysis of Regulatory Regions of Genes *GMNN* and *EZH2* and Their Application in Evaluation of Diffuse Large B-cell Lymphoma Subtypes

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Cancer research pointed to the importance of genes involved in replication and reestablishment of chromatin in oncogenesis. Gene dysfunction in tumours is often associated with epigenetic changes. The aim of this study was to analyse DNA methylation of regulatory regions of genes *GMNN* and *EZH2* in diffuse large B-cell lymphoma subtypes. Methylation assays were developed for the analysis of those regions at single CpG site resolution. Primers for PCR-amplification of bisulfite treated DNA fragments were designed. PCR conditions were optimized for the amplification of three and four fragments of regulatory region of *GMNN* and *EZH2*, respectively. Subsequently, optimal conditions for pyrosequencing were determined. Finally, one assay per gene was created. Newly developed assays were used in the analysis of diffuse large B-cell lymphoma subtypes. The results showed no statistically significant difference between the subtypes. Since the analysis covered only a small part of the regulatory regions of *GMNN* and *EZH2*, the study should be expanded to additional CpG sites and repeated on a larger group of patient samples.

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