BOOK OF ABSTRACTS

22nd International Symposium on Chironomidae

June 17-19 Niš Serbia

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June 17-19, 2024 in **Niš, Serbia**

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This Symposium is organized with the financial support of the Ministry of Education, Science and Technological Development of Republic of Serbia

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Book of Abstracts

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WELCOME NOTES

Dear Colleagues and Friends,

It is with great pleasure and enthusiasm that we welcome you to the 22nd International Symposium on Chironomidae, hosted by the Faculty of Sciences and Mathematics, University of Nis, starting on June 17th, 2024. This year's symposium holds special significance as we celebrate the 60th anniversary of this distinguished gathering. The city of Nis, with its rich cultural and historical heritage spanning over a thousand years, provides the perfect venue for such a traditional and significant event.

In a time when biodiversity loss among insects is accelerating and experts in taxonomy are scarce, we face the crucial challenge of preserving and advancing our knowledge of Chironomidae. This symposium plays a pivotal role in enhancing our understanding of the applications and approaches involving Chironomidae in aquatic ecology, taxonomy, ecotoxicology, and molecular biology, while also promoting scientific collaboration across the globe.

We are excited to present a diverse program that includes keynote lectures, roundtable discussions, oral and poster presentations, and workshops. These will provide a comprehensive platform for knowledge exchange, professional development, and —most importantly— the transfer of knowledge to the next generation of researchers. In addition to the scientific program, we aim to foster networking through various social events listed in Symposium's program. The historic and vibrant city of Nis, with its blend of cultural heritage and modern amenities, will ensure the fulfilment of our goals and hopefully leave a lasting impression on all of you.

On behalf of the Organizational Board, we wish to express our gratitude to the scientific committee, sponsors, participants, and volunteers for their contributions in making this symposium a success. Welcome to Niš, and here's to at least another 60 years of scientific excellence and collaboration!

Sincerely,

Đurađ Milošević


60 YEARS OF INTERNATIONAL SYMPOSIUM ON CHIRONOMIDAE
2024 NIŠ SERBIA

PROGRAM

PROGRAM – PRESYMPOSIUM ACTIVTIES JUNE 14 – JUNE 16, 2024

@Faculty of Sciences and Mathematics, University of Niš, Serbia			
DAY 1, Friday, June 14			
9:30	Welcome coffee		*amphitheater*
10:00	Morpho & molecular ID	Dr. Dubravka Čerba, Dr. Saša Stanković, Dr. Đurađ Milošević	*BIOEKOCEN lab. Room 120*
11:00	Morpho & molecular ID		
12:00	Lunch break		
13:00	Chironomids in ecotoxicology	Dr. Dimitrija Savić-Zdravković Jelena Stojanović	*Room 120*
14:00	Chironomids in ecotoxicology		
15:00	Coffee break		*amphitheater*
15:30	Midnight school of numerical ecology in R	Dr. Đurađ Milošević	*Room 118*
16:30	Midnight school of numerical ecology in R		
17:30	End of the day 1		
DAY 2, Saturday, June 15			
9:30	Welcome coffee		*amphitheater*
10:00	Morpho & molecular ID	Dr. Dubravka Čerba, Dr. Saša Stanković, Dr. Đurađ Milošević	*BIOEKOCEN lab. Room 120*
11:00	Morpho & molecular ID		
12:00	Lunch break		
13:00	Chironomids in ecotoxicology	Dr. Dimitrija Savić-Zdravković Jelena Stojanović	*Room 120*
14:00	Chironomids in ecotoxicology		
15:00	Coffee break		*amphitheater*
15:30	Midnight school of numerical ecology in R	Dr. Đurađ Milošević	*Room 118*
16:30	Midnight school of numerical ecology in R		
17:30	End of the day 2		
DAY 3, Sunday, June 16			
9:30	Welcome coffee		*Room 120*
10:00	Morpho & molecular ID	Dr. Dubravka Čerba, Dr. Saša Stanković, Dr. Đurađ Milošević	*BIOEKOCEN lab. Room 120*
11:00	Morpho & molecular ID		
12:00	Lunch break		
13:00	Chironomids in ecotoxicology	Dr. Dimitrija Savić-Zdravković Jelena Stojanović	*Room 120*
14:00	Chironomids in ecotoxicology		
15:00	Coffee break		*amphitheater*
15:30	Midnight school of numerical ecology in R	Dr. Đurađ Milošević	*Room 118*
16:30	Midnight school of numerical ecology in R		
17:30	**End of the workshop**		

@Science and Technology Park Niš, Aleksandra Medvedeva 2a, 18104 Niš, *entrance hall*	
18:00 – 21:00h	**Registration and Welcome Cocktail**

PROGRAM ISC22 – MONDAY, JUNE 17, 2024

@ **University of Niš, Univerzitetski trg 2, Niš 18000,** *amphitheater*	
09:00 - 09:20	***Opening ceremony*** **Prof. Dr. Đurađ Milošević**, general chairmain of ISC22 **Prof. Dr. Niko Radulović**, dean of Faculty of Sciences and Mathematics, University of Niš, Serbia
09:20 – 09:40	**Introducing our sponsors**
09:40 - 10:50	**Session 1: Paleoecology I** **Chairperson: Dr. Andrew Medeiros**, School for Resource and Environmental Studies, College of Sustainability, Dalhousie University, Halifax, Nova Scotia, Canada
09:40 - 10:20	**Honorary Thienemann Lecture,** Dr. Oliver Heiri: From the past into the future: A long-term, chironomid perspective on changing environments, ecosystem dynamics and species distributions
10:20 - 10:35	Kathleen Hipwell, Allison Covert, and Andrew S. Medeiros: **Informing freshwater management with the use of paleolimnology; Halifax Regional Municipality, Nova Scotia, Canada**
10:35 - 10:50	Aneta Formáčková, Ladislav Hamerlík, Laurențiu Țuțuianu, Diana Hanganu, Alfred Vespremeanu-Stroe, Enikő Magyari: **Holocene chironomid-inferred mean July temperature reconstruction from Lake Bâlea (2034 m), S. Carpathians**
10:50 - 11:20	**Coffee break**
11:20 - 12:20	**Session 2: Paleoecology II** **Chairperson: Prof. Dr. Ladislav Hamerlik**, Matej Bel University, Faculty of Natural Sciences, Banska Bystrica, Slovakia
11:20 - 11:35	André P. Amaral, Fabio L. da Silva, Natascha Turetzek, Joachim T. Haug, Viktor A. Baranov: **Chironomids beyond "non-biting"**
11:35 - 11:50	Olga Antczak-Orlewska, Gabriela Trębska, Joanna Święta-Musznicka, Daniel Okupny, Ewa Janik, Mateusz Płóciennik, Bartosz Kotrys, Piotr Kittel:

	Chironomidae history in the Siberian alas - the late Holocene environmental changes in the Yana-Indigirka Lowland
11:50 - 12:05	Pierre Lapellegerie, Simona Breu, Fabian Rey, Lucia Wick, Oliver Heiri: **Fossil chironomids indicate a dynamic history of deepwater oxygen in a Swiss lowland lake during the past 13,500 years**
12:05: 12:20	Varvara Bakumenko, Anneli Poska, Simon Belle, Siim Veski: **Chironomidae (Climate) Continentality Conundrum**
12:20 – 14:00	**Lunch break**
14:00- 15:15	**Session 3: Taxonomy & Phylogeny** **Chairperson: Prof. Dr. Torbjørn Ekrem**, Norwegian University of Science and Technology, NTNU University, Norway
14:00 - 14:15	Peter Langton: **The enigma of a multiplicity of keys and a paucity of locks in Chironomidae**
14:15 - 14:30	Piotr Gadawski, Grzegorz Tończyk, Kacper Świerk, Wojciech Giłka, Michał Grabowski: **'Gotta Catch 'Em All' – A DNA barcode library of Polish Chironomidae**
14:30 - 14:45	R. William Bouchard, Jr., Alexander T. Egan, Corrie Nyquist, Dean C. Hansen: **The pupae of Diamesa Meigen, 1835 (Diptera: Chironomidae: Diamesinae) from the Nearctic region with 13 new descriptions, 1 redescription, and a key to known species**
14:45- 15:00	Narcís Prat, Raúl Acosta, Carles Ribera, Valeria Leoncini, Núria Bonada: **The genus Diamesa in the Pyrenees: from Morphology to Barcoding**
15:00 - 15:15	Peter Langton: **An update on the availability of chironomid pupal keys**
15:15 - 15:45	**Coffee break**
15:45 - 17:30	**Session 4: Molecular studies on chironomids** **Chairperson: Prof. Dr. Kun-Hsien Tsai**, National Taiwan University, Taipei (NTU)

15:45 - 16:00	Jose-Luis Martinez-Guitarte: **The heat shock response in Chironomus riparius: an update**
16:00 - 16:15	Pratibha Bomble, Bimalendu B. Nath: **Generation of Reactive Oxygen and Nitrogen Species (RONS) During Multiple Stress Exposure in Chironomus ramosus**
16:15 – 16:30	Elisabeth Stur, Yngve Brodin, Piotr Gadawski, Valerie Levesque-Beaudin, Janne Raunio, Lauri Paasivirta, Torbjørn Ekrem: **Towards a curated DNA barcode reference library for Nordic Chironomidae (Diptera)**
16:30 - 16:45	Pavlo. Kovalenko, Paraskeva Michailova, Mila Ihtimanska, Svitlana Serga, Oleksandr Maistrenko, Peter Convey, Iryna Kozeretska: **Cytogenetic features and associated microbiome of Belgica antarctica Jacobs, 1900 (Diptera: Chironomidae)**
16:45 - 17:00	Nikola Stanković, Dimitrija Savić-Zdravković, Milica Stojković-Piperac, Jelena Stojanovic, Ivana Kostić-Kokić, Tamara Petronijević, Monika Dudić, Đurađ Milošević: **Unraveling the chronic impact of a toxic strain of Trichormus variabilis on Chironomus riparius larvae within the complex framework of multistress conditions**
17:05 - 17:20	**Chironomidae community session (Dr. Torbjørn Ekrem)**
17:20 - 17:30	***IN MEMORIAM***
19:00 - 20:00	**Guided city tour (walking)**
@Cafe restaurant Pozornica - Vinarija, Tvrđava Letnja Pozornica bb, Niš 18000	
20:00	**Wine Tasting, Music & More**

PROGRAM ISC22 – TUESDAY, JUNE 18, 2024

@ Science and Technology Park Niš, Aleksandra Medvedeva 2a, 18104 Niš	
8:30	**Registration**
08:45 - 10:45	**Session 5: Ecology & Ecotoxicology I** **Chairperson: Dr. Dubravka Čerba**, Department of Physical Impacts on Aquatic Ecosystems, Water Research Institute, Bratislava, Slovakia
08:45- 09:15	**Plenary speaker,** Dr Viktor Baranov: **Chironomidae palaeobiology: tale of blood, rust and amber**
09:15 - 09:30	Valeria Lencioni, Serena Oss Papot, Federica Camin, Monica Tolotti, Francesca Paoli, Alessandra Franceschini, Elisa Stella, Maria Grazia Zanoni, Andrea Squartini: **Exploring dietary habits of Chironomids in headwaters: insights from carbon and nitrogen isotopes, gut content and DNA metabarcoding analyses**
09:30 - 09:45	Marcela Sedlačková Přidalová, Ladislav Hamerlík, Milan Novikmec, Veronika Slobodníková, Marta Veselská, Peter Bitušík, Marek Svitok: **Diversity and distribution of chironomids in Central European ponds**
09:45 - 10:00	Kyle Weston: **Microplastic pollution in freshwater ecosystems: the role of chironomids as gateways to the food web**
10:00 - 10:15	Eduárd I. Csépes, Arnold Móra, Gábor Várbíró, Tamás Bozóki, Áron Lukács, Viktória B-Béres: **Influences of temperature and hydrological extremes on the structure of chironomid assemblages in an artificially regulated reservoir**
10:15 - 10:30	Agnes-Katharina Kreiling, Krista M. Veijonen, Camille A. Leblanc: **Chironomid-dominated invertebrate communities of three small sub-arctic lakes**
10:30- 10:45	Nataša Popović, Vladimir Jovanović, Jelena Đuknić, Đurađ Milošević, Dubravka Čerba, Maja Raković, Momir Paunović: **Co-occurrence networks of chironomid species as a measure of ecosystem stability**
10:45 - 11:15	**Coffee break and poster session**

11:15 - 12:45	**Session 6: Ecology & Ecotoxicology II**
	Chairperson: Prof. Dr. Zlatko Mihaljević, University of Zagreb, Croatia, Faculty of Science, Department of Biology
11:15 - 11:30	Eszter Tombor, János Korponai, Zoltán Szabó, Zoltán Szalai, István Kóbor, Enikő Katalin Magyari: **Ecosystem reorganizations and their drivers in the sediments of Pátka reservoir, based on compositional changes in the chironomid fauna**
11:30 - 11:45	Jón S. Ólafsson: **Ontogeny in the diet and mouthpart morphology of Chironomidae larvae**
11:45 - 12:00	Susan Gresens, Dennis Genito: **Comparison of macrobenthos vs. pupal exuviae to measure biological response to stream restoration**
12:00- 12:15	Denis Bućan, Valentina Dorić, Vlatka Mičetić Stanković, Marko Miliša: **Unraveling chironomidae recolonization dynamics in intermittent streams**
12:15 - 12:30	Melita Mustafić, Marko Nikolić, Milica Nikolić, Dimitrija Savić-Zdravković, Jelena Stojanović, Dragana Ašćerić, Đurađ Milošević: **Optimized digestion methods for aquatic insects in microplastics detection**
12:30 - 12:45	Bea Bartalovics, Tímea Chamutiová, Ladislav Hamerlík, Arnold Móra, János Korponai, Zoltán Szabó, Enikő Katalin Magyari: **Long-term changes of Chironomidae assemblages of Lake Balaton**
12:45 - 14:15	**Lunch break and poster session**
14:15 - 15:45	**Session 7: Ecology & Ecotoxicology III**
	Chairperson: Dr. Dimitrija Savić Zdravković, Faculty of Sciences and Mathematics, University of Niš, Serbia
14:15 - 14:30	Biljana Rimcheska, Stoe Smiljkov, Dijana Blazhekovikj-Dimovska: **Chironomid larvae (Insecta, Diptera) in Radika River watershed - case study from the proposed "HPS Boshkov Most" (North Macedonia)**
14:30 - 14:45	Khouloud Sebteoui; Jelena Stanković; Zoltán Csabai, Cihelio Alves Amorim; Meryem Beklioğlu; Erik Jeppesen; Djuradj Milošević: **Adapting or evading? Chironomus riparius versus environmental change apocalypse: Microplastics, heating and salinization**

14:45 - 15:00	Karel Brabec: **How hydromorphology affects chironomid response to eutrophication of streams**
15:00 - 15:15	Laurynas Stasiukynas, Sigitas Podėnas, Fabio Laurindo da Silva: **Impact of dams on Chironomidae (Diptera) communities: A study of diversity of diversity in Lithuania's Šventoji and Žeimena sub-basins**
15:15 - 15:30	Nikola Stanković, Aleksandra Trajković, Dragana Ašćerić, Jelena Stojanović, Melita Mustafić, Dimitrija Savić-Zdravković, Đurađ Milošević: **Effects of different dietary regimes on the growth, development and nutritional compositon of *Chironomus riparius* larvae**
15:30 - 15:45	Zlatko Mihaljević, Valentina Dorić, Dubravka Čerba, Miran Koh, Ivana Pozojević: **The shaping of chironomid assemblages in man-made lakes**
16:00- 19:00	**City tour (bus)**
@New City hotel & restaurant, Vožda Karađorđa 12, Niš 18000	
21:00	**Gala dinner**

PROGRAM ISC22–WEDNESDAY, JUNE 19, 2024

@Science and Technology Park Niš, Aleksandra Medvedeva 2a, 18104 Niš	
08:45 - 09:30	**Keynote talk**, Dr. Milan Randjelović: **Introduction to the innovation ecosystem - how to revive doctoral dissertations from a drawer**
09:30 - 10:45	**Session 8: Ecology & Ecotoxicology IV** **Chairperson: Dr. Valeria Lencioni**, Invertebrate Zoology and Hydrobiology Department, MUSE-Museo delle Scienze, Trento, Italy
09:30 - 09:45	Jelena Stojanović, Dimitrija Savić-Zdravković, Jelena Vitorović, Đurađ Milošević: **Histology of chironomids: unlocking the new universe**
09:45 - 10:00	Valentina Dorić, Ivana Pozojević, Natalija Vučković, Marina Šumanović, Mario Rumišek, Zlatko Mihaljević: **Chironomidae of natural lakes in the Dinaric region of Croatia**
10:00 - 10:15	Viktorija Ergović, Miran Koh, Dubravka Čerba, Marija Ivković, Djurađ Milošević, Zlatko Mihaljević**:** **Unexplored Diptera communities in mountain streams: Insights and steps forward**
10:15 - 10:30	Joanna Leszczyńska, Małgorzata Dukowska, Eliza Szczerkowska-Majchrzak, Łukasz Głowacki: **Chironomini versus Orthocladiinae – factors determining their local species richness**
10:30: 10:45	Dimitrija Savić-Zdravkovic, Predrag Simović, Milena Radenković, Bratislav Predić, Aleksandar Milosavljević, Katarina Stojanović, Djuradj Milošević: **Integrating deep learning for enhanced bioassessment of aquatic invertebrates: a case study in chironomid ecology**
10:45 - 11:15	**Closing remarks**
11:15 - 12:00	**Brunch and poster removal**
12:00	**Field trip**

POSTER PRESENTATIONS

1.01	Aspen Watts: **Determining the impact of anthropogenic disturbances on midge diversity in Loe pool, Cornwall**
1.02	Bernadett Boóz, Zsolt Kovács, Tomasz Revicz, Bálint Pernecker, Petr Pařil, Marko Miliša, Zoltán Csabai, Arnold Móra: **Hidden biodiversity of Chironomidae in Central Europe**
1.03	Pratibha Bomble and Bimalendu B. Nath: **Combination of environmental stressors determine extent of clastogenicity and DNA damage in *Chironomus ramosus***
1.04	Dubravka Čerba, Ivana Turković Čakalić, Viktorija Ergović, Miran Koh, Martina Hervat, Mirta Sudarić Bogojević: **Salty or sweet, doesn't matter, Chironomids like everything**
1.05	Daniel Vondrák, Martin Dvorak, Ladislav Hamerlík, Peter Bitušík, Isabel L. Dittmann, Kateřina Dočkalová, Leopold Füreder, Veronika Pedrini-Martha, Marek Svitok, Jan Turek, Evžen Stuchlík, Reinhard Lackner: **Life at the edge: chironomids of the genus Diamesa in European mountain streams**
1.06	Eliza Szczerkowska-Majchrzak, Małgorzata Dukowska, Joanna Leszczyńska: **Environmental predictors of Chironomidae assemblage abundance after dam removal**
1.07	Cinara Wanderléa Felix Bezerra, Mario Antônio Navarro da Silva, Fabio Laurindo da Silva**,** Luiz Carlos Pinho: **Taxonomy of Brazilian *Coelotanypus* Kieffer, 1913 (Chironomidae: Tanypodinae)**
1.08	Fabio Laurindo da Silva, Chao Song, Elisabeth Stur, Silvio S. Nihei, Luiz C. Pinho, Torbjørn Ekrem, Nicholas J. Matzke : **Tracing ancestral footprints: *Polypedilum* evolution and biogeography in South America**
1.09	Ivna Azinović, Marko Miliša: **Humans vs chironomids: Anthropogenic impact on chironomid populations in tufa depositing environment**
1.10	Ting-Yu Tsai, Pei-Ling Wen, Tsai-Yin Yen, Ruei-Ying Tsai, Wan-Ting Lin, Kun-Hsien Tsai: **Chironomus striatippenis' strategies of trapping mosquito larvae and survival against drought and high temperature**
1.11	Simona Breu, Ieva Grudzinska-Elsberga, Petra Boltshauser-Kaltenrieder, Willy Tinner, Oliver Heiri: **Reconstructing 13,000 years of environmental and water depth changes from chironomid assemblages in Lake Lucerne, Switzerland**
2.01	Martina Jambrović, Ladislav Hamerlík, Radovan Pipík, Rastislav Milovský, Dušan Starek, Daniela Dobríková, Peter Bitušík: **Reconstructing Holocene environmental changes in an alpine lake (Tatra Mountains, Slovakia) using subfossil chironomid remains**
2.02	Nataša Popović, Jelena Đuknić, Jelena Stanković, Bojana Tubić, Nikola Marinković, Maja Raković, Stefan Anđus: **Chironomids diversity in the streams around the Bor mining area**

2.03	Mila Ihtimanska, Julia Ilkova, Monika Subeva, Emilia Varadinova: **Do long-term changes in stream hydrochemistry affect taxa richness and abundance of family Chironomidae (Diptera): Case study of the long-term ecological research of Mesta River (Bulgaria)**
2.04	Soňa Ščerbáková, Dubravka Čerba: **Chironomids in Slovakian reservoirs – Developing the classification scheme for the ecological potential assessment based on exuviae**
2.05	Tamara Petronijević, Jelena Stojanović, Jelena Vitorović, Dimitrija Savić Zdravković, Margareta Kračun Kolarević, Đurađ Milošević, Nikola Stanković: **Can cyanotoxins inhibit the development of benthic organisms? Influence of Microcystin-LR (MC-LR) on Chironomus riparius larvae**
2.06	Tímea Chamutiová, Ladislav Hamerlík, Radovan Kyška-Pipík, Peter Bitušík: **Late-Glacial to Holocene transition in the Western Carpathians (central Europe) evidenced in the subfossil chironomid record from an alpine lake**
2.07	Veronika Slobodníková, Ladislav Hamerlík, Marta Wojewódka- Prybyl, Tímea Chamutiová, Katarzyna Szarlowicz, Peter Bitušík: **Filling the gap in the Carpathian paleolimnology: Subfossil chironomids and cladocerans from sediments of alpine lakes in the Eastern Carpathians (Ukraine) as indicators of environmental change over the past 200 years**
2.08	Małgorzata Dukowska, Joanna Leszczyńska, Eliza Szczerkowska-Majchrzak, Łukasz Głowacki: **Co-occurrence among Chironomini and Orthocladiinae species – a case from temperate zone rivers**
2.09	Predrag Simović, Marija Jakovljević, Vladica Simić, Djuradj Milošević, Ana Petrović: **A preliminary assessment of Chironomidae diversity in tufa-depositing environments in Serbia, Central Balkans**
2.10	Melita Mustafić, Maša Stojanović, Petar Ristić, Bogdan Jovanović, Aleksandar Tomić, Voislav Petrović: **Microplastics altering behavioral traces of aquatic insects: a study on *Chironomus riparius***
2.11	Dimitrija Savić Zdravković, Jelena Stojanović, Maša Stojanović, Petar Ristić, Bogdan Jovanović, Aleksandar Tomić, Aleksandra Trajković, Melita Mustafić, Dragana Ašćerić, Đurađ Milošević: **Optimizing laboratory substrates for ecotoxicological assessments of chironomidae: a focus on substrate composition and larval morphology**
2.12	Piotr Gadawski, Robert Sobczyk, Tomasz Mamos, Tomasz Rewicz, Paul Hebert, Michał Grabowski: **Diversity of Chironomidae fauna in a unique hot-spot – the case of Skadar Lake basin springs**

Honorary Thienemann Lecture

From the past into the future: A long-term, chironomid perspective on changing environments, ecosystem dynamics and species distributions

Oliver Heiri

Department of Environmental Sciences, University of Basel, Switzerland

Chironomid remains from lake sediments have been described in the scientific literature at least since the early 20th century. However, only in recent decades has chironomid palaeoecology developed to the extent that chironomids are now, together with pollen, diatoms and cladocerans, among the most widely used biological indicators in lake sediment studies. In my presentation, I will present a short outline of the history of chironomid-based environmental reconstruction, and, based on some examples from our own research, discuss how different developments in taxonomic, ecological and numerical analysis have led to the broad application of this indicator group in palaeoenvironmental research. Studies on fossil chironomid taxonomy and taphonomy were essential for providing the basis for reliably interpreting fossil chironomid records. In the late 20th century, the development of modern calibration datasets based on surface sediment samples provided a further essential step for unlocking the potential of chironomids for environmental reconstruction, since modern distributions of chironomids could now be examined at the same taxonomic resolution as used for downcore records. Many of the most successful and promising approaches for interpreting fossil chironomid data are still based on relating fossil chironomid records to such modern distributional datasets using multivariate numerical methods, and these have enabled both semi-quantitative and quantitative reconstructions of past environmental variables such as summer temperature, oxygen availability, nutrient concentrations or salinity based on chironomid remains. Chironomid records from lake sediments can also provide a long-term perspective on the stability, sensitivity and resilience of chironomid assemblages, and indirectly lake ecosystems. For example, records are now available that describe the response and recovery of lakes to eutrophication in the 20th century, but also document lacustrine ecosystem dynamics during, or even preceding periods of early human influences thousands of years ago. Finally, chironomid remains can provide information on past distribution changes of individual taxa over geological time scales, although this approach is still underused and encompasses the labor-intensive re-interpretation of the many individual chironomid records presently available. Future developments are expected to, e.g., include the amalgamation and joint interpretation of the many available chironomid datasets to continental scale reconstructions, the continued application of ecological methods developed in modern hydrobiology to fossil remains, but also the incorporation of emerging technologies in the life sciences such as aDNA analysis or proteomics into

chironomid palaeoecology. Combined approaches based on morphological analyses of remains and such novel methodologies would lead to new and innovative applications in chironomid palaeoecology. Importantly, they would also contribute towards further linking contemporary and fossil chironomid taxonomy, a development which would significantly facilitate interactions and joint projects between the fossil and modern chironomid research communities in the future.

Chironomidae palaeobiology: tale of blood, rust and amber

Viktor Baranov

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Non-biting midges are among the most species rich abundant and ecologically diverse – this is a common refrain, that have been done to death by the generations of the Chironomid workers. When discussing how impressive and successful Chironomids are, as a group of Insects, dimensions of spatial distribution, ecological diversity, ecosystem functions performed and amazingly high abundance, but the dimension which is often completely overlooked in the Chironomidae evolutionary success – is a temporal one. Evolutionary history of Chironomids is spanning at least 250 Million Years, originating (based on the dated Molecular phylogeny by Cranston and colleagues from 2011). Over the las 100 years, we have collectively managed to describe over 350 species of fossil Chironomids, revealing many surprising facts about the group. For one thing, “non-biting” midges in the past, quite often had functional mouthparts, and bitten quite a range of different animals, including, likely, non-avian dinosaurs. The other surprising thing we learned about chironomids, is that they tend to demonstrate a surprising degree of morpholocicas stability, with many modern genera, such as Nadeva, Paraphaenocladius, Heterotrissocladius, Smittia and Pseudosmittia have been around for at least 50 million years, with very little changing in their outward appearance. In this talk I will try to bring together last 15 years of our group’s work on Chironomid palaeobiology, to synthesize our most up to date views on evolution of these fascinating insects.

Oral Presentations Abstracts

Informing freshwater management with the use of paleolimnology; Halifax Regional Municipality, Nova Scotia, Canada

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Watershed-scale stress from urbanization can negatively impact freshwater ecosystems and the services they provide, but our knowledge of how to manage these systems is limited by a lack of baseline knowledge. Halifax Regional Municipality (HRM), Nova Scotia, Canada, has the 8th fastest growing metropolitan area in Canada; hundreds of lakes are increasingly influenced by residential development, and most lakes in the urban-suburban core are developed to some degree. Lake monitoring in HRM has shown increased productivity in developed watersheds; however, a lack of historical context impedes lake management. Here, we establish a historical timeline through the analysis of biological indicators (subfossil chironomids) in a paleolimnological approach applied to three HRM lakes. We found that two lakes in residentially developed watersheds experienced a large shift in chironomid taxa towards those indicative of a rise in nutrients (Chironomus, Cladotanytarsus mancus) at specific points of known human impact. Assemblage shifts occurred ~1885 during land clearance, and again at ~1980 during a residential development phase in catchments. This contrasts with a lake far outside of the development zone, which shows opposite trends in many of the same taxa following the designation of the area as a protected watershed (within the municipal drinking water supply catchment). Our analysis shows that a shift from mesotrophic to oligotrophic conditions likely occurred at the time of land clearance in the catchments, and summer eutrophication occurring today has likely been occurring for over a century before the catchments were urbanized in the 1980s. Municipal water quality monitoring programs only contain records for the last two decades, and conclusions about development resulting in eutrophication contrast with the findings of this study, where lakes became meso-eutrophic in the late 1800s and have not recovered since land clearance. This highlights the limitations of poor resolution “snapshot” surface water quality monitoring compared to paleolimnological methods, which can capture high resolution conditions within a lake. As such, this study emphasizes the need for long-term information on a lake’s trophic status to establish patterns and causes of change.

Holocene chironomid-inferred mean July temperature reconstruction from Lake Bâlea (2034 m), S. Carpathians

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Our study is focusing on the chironomid assemblage changes of an 8.44 m long sediment sequence taken from a north facing alpine lake Bâlea in the Făgăraș Mountains (South Romania). Bayesian age-depth modelling using 16 AMS 14C ages suggests that the sediment extends back to ~10 000 cal yr BP. The diverse subfossil chironomid assemblages include more than 40 taxa, most of which belong to predominantly cold stenothermal types with a preference for oligotrophic conditions. Chironomid-based mean summer and mean July temperature reconstructions were done using the WAPLS transfer function and the Swiss-Norwegian and East-Central European (EcE) training sets. Each of them has their advantages and disadvantages, which will be discussed in the presentation. After consideration, it was decided that the use of the EcE dataset is more appropriate to S. Carpathian sites. According to this data set, the record starts with relatively cooler temperatures (~8-9 °C) that are indicative of the post-glacial climate conditions at the time. From around 9 000 cal yr BP, we see a general warming trend with minor fluctuations, potentially representing the Holocene Climatic Optimum (~11-12 °C). Post this thermal maximum, there is a slight decrease around 3 700 cal yr BP, with temperatures oscillating and then increasing again. Gradual changes are also observed in the composition of the chironomid assemblages. The results of the reconstruction of summer air temperatures and stratigraphic changes of chironomids will be presented together with a discussion of ecological changes in the surrounding environment of lake Bâlea during the Holocene.

Chironomids beyond “non-biting”

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The non-biting midges do not always live up to their vernacular name. At least that appears to be the case when we examine the fossil record. Contrary to the rare Podonominae species belonging to the genera Archaeochlus Brundin, Afrochlus Freeman, and Austrochlus Cranston, several fossils from different ages present adult males and females with mandibles, the main biting structures in nematocerous dipterans. Our research unveiled novel mandibulate specimens from the Cretaceous and the Paleogene, revealing an intriguing pattern. The mandibulate fossils seem to be related to at least three distinct lineages of Chironomidae, suggesting a case of repeated evolution. It is still uncertain what the feeding preferences of these species were; however, the morphology of their mouthparts, revealed by multiple microscopy techniques and micro-CT scans, indicates that some species might have been entomophagous while others were blood-feeders. Furthermore, by including fossil species in a family-level phylogeny based on both molecular and morphological data, our study sheds light on the relationships between the major non-biting midge lineages.

Chironomidae history in the Siberian alas - the late Holocene environmental changes in the Yana-Indigirka Lowland

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Alases are shallow thermokarst depressions periodically filled with water from melting ice. They are inhabited by many aquatic organisms, including chironomid larvae. Fluctuations in their thanatocenoses can reflect past environmental changes, evolution of tundra ecosystems and palaeoclimate of Arctic regions. While the vast region of north-eastern Siberia is still underrepresented in palaeoenvironmental studies, we used multi-proxy approach to analyse three sediment profiles from the active layer of the alases in the Belerekh River valley in the Yana-Indigirka Lowland (NE Yakutia).

Two profiles were examined from lower (AL1) and upper (AL2) alas level constrained by Yedoma hills. Radiocarbon dates indicate that their history reaches 2,845±30 years BP and 3,865±30 years BP, respectively. Numerous chironomid larvae head capsules have been found throughout the AL1 profile, indicating continuous aquatic conditions. However, the increased share of semi-terrestrial taxa ca. 2,000 years BP might be associated with alas drying, probably connected with climate change, as previously abundant cold stenotherms (e.g. *Abiskomyia*) disappeared. After this event a shift in the sediment character happened. Among chironomids, taxa tolerating or preferring unstable hydrological conditions started to dominate, indicating ecosystem transformation into a seasonal water body.

The chironomid subfossils serve not only as a bioarchive of the alas evolution, but also past fluctuations in water level, interconnections between the elements of the hydrological system, and climatic changes.

The expedition and the field work were conducted within the INTERACT grant HOLARCLIM (2019), while the palaeoecological analyses were financed by the Polish National Science Centre (2023/07/X/NZ8/00294)

Fossil chironomids indicate a dynamic history of deepwater oxygen in a Swiss lowland lake during the past 13,500 years

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Swiss lowland lakes have been highly impacted by increased nutrient concentrations and oxygen depletion in the recent past. Unfortunately, little is known about their state before the 20th century land use intensification, modification and eutrophication. Chironomid remains preserved in lake sediments can provide long-term information on the development of lake ecosystems and particularly oxygen conditions. Bichelsee is a small lake located on the eastern Swiss Plateau, presently with a strong oxygen depletion during the summer months. Results showed that Bichelsee was originally dominated by Tanytarsus lugens-type, typical for oligotrophic lakes and sensitive to low oxygen, with a relatively high chironomid influx suggesting high oxygen concentration. Around 7,000 years ago, Tanytarsus lugens-type and Chironomus anthracinus-type became co-dominant, the latter being often found in more nutrient-rich lakes with hypoxic deepwater layers. Presence at variable abundances of Chironomus plumosus-type, typical for eutrophic lakes, from 2,000 years ago onwards, indicates further oxygen depletion and possibly eutrophication. Overall, distinct shifts between phases dominated by Tanytarsus lugens-type and Chironomus, together with changes in chironomid influx and percentages of profundal taxa indicate major and abrupt centennial-scale changes in oxygen availability in Bichelsee. Some major changes seem to coincide with shifts in occupation, cultural developments and land use in the lake catchment. Our results indicate that the deepwater environment in Bichelsee seems to have been limited in oxygen since at least 7,000 years and that small lowland lakes, like Bichelsee, may be exceptionally sensitive in their oxygen regime to land use modifications and other natural or human-associated pressures.

Chironomidae (Climate) Continentality Conundrum

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Swiss lowland lakes have been highly impacted by increased nutrient concentrations and oxygen depletion in the recent past. Unfortunately, little is known about their state before the 20th century land use intensification, modification and eutrophication. Chironomid remains preserved in lake sediments can provide long-term information on the development of lake ecosystems and particularly oxygen conditions. Bichelsee is a small lake located on the eastern Swiss Plateau, presently with a strong oxygen depletion during the summer months. Results showed that Bichelsee was originally dominated by Tanytarsus lugens-type, typical for oligotrophic lakes and sensitive to low oxygen, with a relatively high chironomid influx suggesting high oxygen concentration. Around 7,000 years ago, Tanytarsus lugens-type and Chironomus anthracinus-type became co-dominant, the latter being often found in more nutrient-rich lakes with hypoxic deepwater layers. Presence at variable abundances of Chironomus plumosus-type, typical for eutrophic lakes, from 2,000 years ago onwards, indicates further oxygen depletion and possibly eutrophication. Overall, distinct shifts between phases dominated by Tanytarsus lugens-type and Chironomus, together with changes in chironomid influx and percentages of profundal taxa indicate major and abrupt centennial-scale changes in oxygen availability in Bichelsee. Some major changes seem to coincide with shifts in occupation, cultural developments and land use in the lake catchment. Our results indicate that the deepwater environment in Bichelsee seems to have been limited in oxygen since at least 7,000 years and that small lowland lakes, like Bichelsee, may be exceptionally sensitive in their oxygen regime to land use modifications and other natural or human-associated pressures.

The enigma of a multiplicity of keys and a paucity of locks in Chironomidae, the plethora of hypopygial structure and the overall similarity of female genitalia.

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After a consideration of the structures of male hypopygia and female genitalia in Chironomidae, the copulatory structures of the female genitalia of Metriocnemus albolineatus (Meigen), atriclavus Kiefer, beringensis Cranston & Oliver, carmencitabertarum Langton & Cobo, cavicola Kieffer, corticalis Strenzke, ephemerus Langton, eurynotus (Holmgren), fuscipes (Meigen), terrester Pagast, tristellus Edwards and ursinus (Holmgren) are depicted and discussed.

The enigma remains unsolved.

‘Gotta Catch 'Em All’ – A DNA barcode library of Polish Chironomidae

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In all water ecosystems of the Tatra Mountains, the Chironomidae are the most important group, both in terms of biodiversity and abundance. This study focuses on the species diversity of Chironomidae that inhabit Lakes at higher latitudes in the Tatra Mountains. The material was collected using Malaise traps and sweep nets. Analysis of the ~1,200 COI barcodes revealed the presence of 64 BINs which indicates high species diversity. This project is a prelude to develop a reliable reference library of DNA barcodes for Polish Chironomidae using integrative taxonomy approach. Currently, the barcode library for polish Chironomidae is represented by 36 records (26 BINs) and our results provide 61 new BINs for Poland.
Our investigation has proven the strong need to develop a reference library based on reliable taxonomic identifications.

The pupae of *Diamesa* Meigen, 1835 (Diptera: Chironomidae: Diamesinae) from the Nearctic region with 13 new descriptions, 1 redescription, and a key to known species

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Diamesa Meigen, 1835 is a large genus of cold-adapted, non-biting midges that are most commonly encountered in streams at high latitudes, high elevations, or during the winter in temperate habitats. There are 35 species of Diamesa known from the Nearctic and of these, the pupa has been described in detail for 15 species. We provide full descriptions for 13 previously undescribed pupae and a redescription of one species. The pupae remain unknown for 7 Diamesa species known from the Nearctic. Separating the pupae of some Diamesa species is difficult due to their similarity or the variability of some characters. However, there are many useful characters (e.g., arrangement and size of the L-setae, type of respiratory organ, size and arrangement of abdominal shagreen) that can be used to identify most species based on the pupa. Using these characters, we provide a key to the pupae for 28 species of Diamesa known from the Nearctic region.

The genus Diamesa in the Pyrenees: from Morphology to Barcoding

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Diamesa (Chironominae; Diamesinae) is one of the most representative genera from high mountain freshwaters in Europe. In the Pyrenees, Laville (1986) listed 13 species with D. hamaticornis, bertrami, latitarsis and zernyi as the most frequent ones, identified at adult stage. Puntí, Rieradevall & Prat (2009) collected larvae and pupae of 7 *Diamesa* morphotypes in 12 sites in Mediterranean Pyrenean streams, with zernyi and tonsa groups as the most frequent ones. The identification to species of larvae and, for some species groups (i.e. zernyi and cinerella) also as pupae, is challenging and unfeasible based on the morphological characters recognised as valid in Langton (1991) and Rossaro & Lencioni (2015). A recent work (Lencioni et al., 2021) highlighted the potentiality of DNA barcoding in identifying Diamesa larvae to species but, at the same time, a huge incongruence between morphological and genetic species identification mainly for larvae of *D. zernyi* and cinerella groups. In this work we used DNA barcoding to identify larvae and pharate male pupae collected in different localities in the Pyrenees, focusing on the same species groups. The limits and congruence between the two identification methods are discussed, with a focus on pupal material. Specifically, pharate male pupae identified according to Langton (1991) as D. tonsa, cinerella and hamaticornis appeared to be not separable from each other using DNA barcoding. These findings arose a great concern on how many species of *Diamesa* are colonizing Pyrenees freshwaters and, on which, de facto, are at risk of extinction due to climate change in the South European countries.

The heat shock response in *Chironomus riparius*: an update

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The heat shock response was described in the 60s by Ritossa (Ritossa, 1996, 1962), showing that the increase in temperature can produce the activation of genes that are not active in regular conditions or are at a reduced rate. From then on, much research has been done to understand how the heat shock response is activated and the role of the activated genes in the cell. Presently, it is known that they code primarily for a set of proteins known as Heat Shock Proteins (HSPs), which are primarily chaperones (Feder and Hofmann, 1999; King and MacRae, 2015). They are grouped in several families and involved in many cell functions. They have even been studied in Chironomids, mainly in *Chironomus riparius*. However, there is no systematic analysis of the response since the studies have been performed by analyzing some of the members of each family or even a couple of members. Here is presented the first systematic study on *Chironomus riparius*, which involves the different families of HSPs. The different transcriptomes from the databases allow me to search for genes related to the HSPs. In this sense, the transcriptomic analysis allowed me to identify members from the different families: HSP10, small HSPs, HSP40, HSP70, and HSP90. The search has identified almost 40 genes of heat shock proteins. The response was analyzed by real-time PCR in fourth-instar larvae using two different heat shock treatments. First, the standard temperature used in *C. riparius* (35 ºC) to induce the response was selected to perform a long exposure. Second, an experiment exposing the larvae to a sub-lethal temperature (39 ºC) for a short time (five minutes) was used to analyze the recovery from an acute heat shock. A kinetic study analyzed the mRNA levels at several time points to follow the response of the genes analyzed. The results showed a diversity of responses, in some cases previously described. However, this study uses the more extensive number of genes related to heat shock in *Chironomus riparius* and provide a first insight in the global response of the larvae to the temperature stress. Although the heat shock response has been known for a long time, it is still poorly described in many organisms. It requires a systematic analysis to ensure it is appropriately understood, mainly because the HSPs can be involved in other stress responses and regular cell functions.

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Generation of Reactive Oxygen and Nitrogen Species (RONS) During Multiple Stress Exposure in *Chironomus ramosus*

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Reactive oxygen species (ROS) and reactive nitrogen species (RNS) are free radicals, and these highly reactive molecules are produced as metabolic byproducts. Excessive generation of ROS and RNS (RONS) ultimately leads to oxidative stress and subsequent cellular damage through apoptosis. In nature, organisms often encounter multiple environmental stress simultaneously. Most of the studies carried out so far in the field of stress biology involve singular stress. However, organisms quite often face multiple stressors exerting their impact all at the same time. We have addressed this issue selecting environmentally correlated abiotic stressors viz. desiccation (D), heat (H) and starvation (S) on fourth instar larvae of a tropical non-biting midge, *Chironomus ramosus*. Larvae were subjected to singular (D/H/S), combinatorial (D+H,H+S,D+S) and multiple (D+H+S) stresses. The exposure to stressors led to activation of a common oxidative stress mediated response irrespective of mode and type of exposure. Stress induced homeostasis could be ascertained through an increase in antioxidant activities. Interestingly, we also observed a sharp decline in the mitochondrial aconitase enzyme activity. We further observed escalated level of apoptosis and its magnitude was stressors and combination of stressors specific. Present study has indicated how generation of multiple stress induced RONS could be countered by Chironomid larvae which are otherwise known to thrive under adverse environmental conditions.

Towards a curated DNA barcode reference library for Nordic Chironomidae (Diptera)

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The quality of barcode reference libraries is crucial for accurate species identification using DNA barcoding. We performed a global BIN discordance analysis involving more than 1000 Nordic species and 54334 sequences, and found discordance at the family, subfamily, genus and species level in 260 BINs. Some of these were trivial, resulting from gross misidentifications or contaminations, and could easily be resolved through examination of associated images and flagging of erroneous records in BOLD. Others were caused by different taxonomic interpretations by the identifiers. In order to solve conflicting determinations, we gathered identifiers from Finland, Norway, Poland and Sweden for a five day workshop to compare material in our collections. As a result, we were able to clarify close to 150 BINs of 140 species and create a dataset for future stability of Chironomidae reference barcodes. About 100 BIN discordances could not be resolved at present due to uncertainties concerning the nominal species, and several species still retain multiple BINs indicating large intraspecific genetic divergence and potential cryptic diversity at this taxonomic level. We will show examples from our work and argue for thorough quality assurance measures before new Chironomidae reference barcodes are added to BOLD in the future to avoid creating unnecessary conflicts that will prevent the use of chironomid reference barcodes in molecular identifications.

Cytogenetic features and associated microbiome of *Belgica antarctica* Jacobs, 1900 (Diptera: Chironomidae)

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Chironomids are widely used in genetic and ecological studies. However, the Antarctic endemic midge Belgica antarctica Jacobs, 1900 has received more limited attention to date. This species is extremely pertinent given its endemism to a region subject to extreme environmental conditions and also to some of the most rapid climatic changes globally in recent decades. We present the first standard polytene chromosome map of *B. antarctica*. We show that, despite the rapid recent climate warming in the Antarctic Peninsula region, inversions are present in populations of B. antarctica, which were first documented as much as 40 years ago. We also report two new inherited inversions. The data analysis showed the presence of somatic aberrations that have not been investigated before and may provide an informative method for assessing pollution, which has been growing rapidly in parts of the Antarctic in recent decades. Associated microbiota have a key influence on the survival of organisms under harsh and changing climatic conditions. Analyzing 34 whole-genome sequences of B. antarctica available in the NCBI database, we identified 14 bacterial taxa associated with this chironomid or with its substrate. Separately, did not identify endosymbiotic bacteria such as *Wolbachia* or *Spiroplasma* using PCR (44 specimens from seven locations were tested) and metagenomic analysis. Our data suggest low bacterial diversity in the *B. antarctica* microbiome. The data obtained provide a basis for further population studies of *B. antarctica.*

Unraveling the chronic impact of a toxic strain of *Trichormus variabilis* on *Chironomus riparius* larvae within the complex framework of multistress Conditions

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Understanding the complex interactions between cyanobacteria and *Chironomus* larvae is vital to maintaining the health of freshwater ecosystems and reducing potential negative impacts on biodiversity. The study aimed to explore the potential of the toxic cyanobacterial strain *Trichormus variabilis* on the larvae of *Chironomus riparius* in the presence of 4 common stressors NH4+, NO3-, PO43- and Cd2+. Control larvae were treated with TetraMin food in the presence of the mentioned stressors. The experiment followed OECD test protocol 218 for assessing long-term larval exposure to sediment pollutants, and lasted 13 days. Examination of the chronic effect of the toxic strain of *T. variabilis* on *C. riparius* larvae under multistress conditions included analyses of mortality, growth, hemoglobin concentration in hemolymph and DNA damage in cells of larvae that were treated in the experiment. Cadmium treatments resulted in the death of all tested larvae, whether they were treated by TetraMin or the cyanobacterial strain. Other stressors caused a lower percentage of lethality in combination with *T. variabilis* compared to the combination with the control food TetraMin. Larvae treated with *T. variabilis* in multistress conditions had slower development, lower biomass, lower hemoglobin concentration, but higher rate of DNA damage. Considering that benthic cyanobacteria are proven as potent producers of toxins, their presence poses a significant threat to other benthic organisms. This research showed that permanent members and constituent of the benthos, *C. riparius* larvae may be strongly affected by toxic cyanobacteria, whose harmful effects are greater in the presence of stressors.

Exploring dietary habits of Chironomids in headwaters: insights from carbon and nitrogen isotopes, gut content and DNA metabarcoding analyses

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Gut content analysis (GCA) and stable isotope analysis (SIA) were used to characterise the diet and the food web structure of invertebrate communities in streams and ponds differently fed by glaciers in the Rhaetian Alps (Italy). The relative importance of allochthonous and autochthonous food resources was quantified using stable isotopes of carbon (^{13}C) and nitrogen (^{15}N). GCA was performed on a selection of species, including 13 Diptera Chironomidae (non-biting midges) that represent the most frequent and abundant taxon in these habitats: *Diamesa bertrami, Diamesa latitarsis, Diamesa steinboecki, Diamesa zernyi, Pseudokiefferella parva, Eukiefferella minor, Metriocnemus eurynotus* gr.*, Parametriocnemus stylatus, Thienemaniella clavicornis, Tvetenia calvescens, Macropelopia* sp.*, Zavrelimyia* sp.*, Micropsectra atrofasciata* gr. In addition, the gut microbial structure of Chironomid larvae from the most food-poor glacial habitats was evaluated and compared with microbes from abiotic matrices (via metabarcoding 16S rRNA). New insights were provided as to which bacteria ingested from the environment can be configured as 'food' and which, among those present in the gut, can be considered the stable, resident metabolic 'chefs' of the host animal. Overall, the diet and trophic category (functional feeding groups) of the different species were reviewed. A certain trophic flexibility and omnivory was found, which may facilitate the adaptation of Chironomids and other invertebrates to changes in the quality and quantity of available resources due to glacier retreat. This knowledge will therefore improve our ability to predict future trends in alpine biodiversity in the context of ongoing climate change.

Diversity and distribution of chironomids in Central European ponds

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Small and shallow lentic waterbodies – ponds – are often overlooked ecosystems which may substantially contribute to local and regional freshwater biodiversity, and which may serve as refugia for many rare and endangered species. We focused on chironomids, a usually neglected group of dipterans (Diptera: Chironomidae) that paradoxically represents the dominant part of macrozoobenthos in many freshwater ecosystems. Using larval and exuvial material sampled in ponds scattered across the entire area of Slovakia, we summarize the main trends in the diversity and composition of chironomid communities and discuss the most important environmental drivers affecting the distribution of the species. We also estimated habitat conditions that support the highest diversity of local communities. Temperature and the proportion of surrounding forests significantly influenced the alpha diversity of chironomid communities, while urban land cover and pond size had no significant effect. Ponds with a mean annual air temperature of approximately 4.8°C and a low proportion of surrounding forests are expected to harbor the most diverse chironomid communities. The indication potential of the group is discussed, and we argue that chironomids are a sound indicator group for studying changes in the pond environment. The study was supported by the Slovak Research and Development Agency under contracts APVV-16-0236 and APVV-20-0358, and by the scientific grant agency VEGA (project No. 1/0400/21).

Microplastic Pollution in Freshwater Ecosystems: The Role of Chironomids as Gateways to the Food Web

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The pollution of the marine and terrestrial environments by plastic waste is a growing problem worldwide. Slow rates of degradation result in their accumulation across aquatic ecosystems, where they slowly break down into successively smaller pieces – so-called microplastics. In recent years, the presence and threat of microplastics in freshwater ecosystems have been recognized, and preliminary results suggest that benthic fauna, including chironomids, are at a heightened risk of microplastic ingestion. This presentation will include a review of the literature on freshwater microplastic pollution with a focus on Chironomidae, including results from a qualitative meta-analysis of laboratory ingestion studies of chironomids exposed to varying microplastic types and concentrations. The presentation will also include a preliminary PhD proposal for research in the Philippines which will investigate the role of chironomids as gateways for microplastics to enter the freshwater food web, where they may eventually be consumed by humans.

Influences of temperature and hydrological extremes on the structure of chironomid assemblages in an artificially regulated reservoir

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Climate change-related warming and water level fluctuations are among the main threats to freshwater communities. In this study, we examined the changes in the structure and biodiversity of the chironomid assemblages of Lake Tisza reservoir, the largest artificial lake in the Carpathian Basin, in relation to weather and hydrological extremes, represented by air temperature (T) and water discharge of the River Tisza (Q). Samples were taken between 2009 and 2022, once a year (in August, due to the operating characteristics of the reservoir) in the four basins of Lake Tisza. Our results show that both T and Q significantly affected the physical and chemical characteristics of the lake. While extremes in water discharge have significant influence on the composition and diversity of the chironomid assemblages, no temperature-driven alterations were found, although some taxa were associated with high and low temperatures. The chironomid assemblage structure of different combined QT groups (e.g. low Q with high T, etc.) showed clear differences, and our results support the impact of temperature on chironomids through water discharge. However, it is important to stress that these effects were probably not direct, and rather related to changes in other environmental variables during extreme years. As the scenarios predict an increase in the frequency of extreme climate events, significant compositional and diversity changes in chironomid assemblages are also expected. These processes may ultimately have long-term effects on the entire food web, even potentially hindering the lake's ability to fulfill its nature conservation purpose.

Chironomid-dominated invertebrate communities of three small sub-arctic lakes

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Lakes in the Faroe Islands are small and relatively simple ecosystems which lend themselves well to study species interactions and how they are affected by changing environmental conditions. In June 2022, we started a collaborative research project on temporal changes in feeding ecology and life histories of Arctic charr and Brown trout. The project aims to identify effects of climate change on community structure and trophic ecology of fishes in small sub-arctic Faroese lakes. We compare feeding resources, fish diet and morphology across the three lakes Leynavatn, Saksunarvatn and Toftavatn, which represent contrasting combination of fish species. Data from 2022 are compared to a similar study performed in 2000 (NORLAKE project). Here, we focus on the benthic invertebrate communities of the three lakes, which are dominated by Chironomidae. Preliminary results show that the invertebrate communities of lakes Saksunarvatn and Toftavatn are similar, whereas the deepest of the lakes, Leynavatn, is markedly different. Tanytarsini and Orthocladiinae are abundant in all three lakes, but each lake has a distinct composition of Chironomidae. In Leynavatn, the dominant species are *Tanytarsus* spp. (Tanytarsini), *Corynoneura* spp. (Orthocladiinae) and *Micropsectra* spp. (Tanytarsini); in Saksunarvatn *Psectrocladius* spp. (Orthocladiinae) and *Micropsectra* spp. (Tanytarsini); and in Toftavatn *Synorthocladius* sp., *Cricotopus sylvestris* (both Orthocladiinae) and *Arctopelopia* sp. (Tanypodinae). We discuss these findings in connection with fish diet and potential changes in the lake ecosystems over the past 22 years.

Co-occurrence networks of chironomid species as a measure of ecosystem stability

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The stability of an ecosystem depends on the community composition, but also on the connections that may arise between coexisting community members. Various changes such as climate change, pollution, and habitat degradation affect community structure. To test the stability of the chironomid assemblages' structure in the Danube on the time scale, we first constructed the consensus network of chironomid species co-occurrence using the signed weighted topological overlap approach. Consensus chironomid networks for three distinct Danube sectors were compared among the JDS sampling expeditions in 2007 (JDS2), 2013 (JDS3), and 2019 (JDS4). Many links in each sector were characteristic for one expedition (year). In Sector 1 (Upper Danube), the only persistent link was found between *Rheopelopia* sp. and *Rheotanytarsus* spp. In Sector 2 (Middle Danube) links between *Dicrotendipes nervosus*, *Microchironomus tener,* and *Polypedilum nubeculosum* were strong and constant throughout the years. In Sector 3 (Lower Danube) the same interactions between *D. nervosus* and *P. nubelucolum* were observed, accompanied by *Cladotanytarsus* spp. and *D. nervosus*, *M. tener* and *Procladius* sp., and interactions of *Polypedilum scalaenum* with *M. tener*, *D. nervosus* and *Procladius* sp. The positive correlation between abundances of *Cryptochironomus* sp. and *Stictochironomus* sp. in Sector 2 seen during JDS2 and JDS3, changed into a negative correlation during JDS4. The same direction of changes appeared within Sector 3, between *M. tener* with *P. nubeculosum*, *Cladotanytarsus* spp. with *Tanytarsus* spp., and *Polypedilum scalaenum* with *Tanytarsus* spp. abundances. The results showed both stable and differing correlations of chironomid abundance in space (different sectors) and time (sampling years).

Resilience of alpine lake chironomid communities in response to climate change: a view from the South Carpathian Mountains

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Remote mountain lakes experience accelerating ecological change worldwide due to the ongoing warming. With their variably accessible lakes and consequently alternating grazing pressure, alpine lakes in the Carpathians have experienced trophic level increases to varying degrees in the last couple of centuries. In this study, we analyse Lake Ana (1940 m) and Lake Peleaga (2122 m), from the Retezat Mountains. The sediment cores cover ~400 years, and our aim is to reconstruct the rate of ecosystem change and to identify early warning signals of critical transitions. Our working hypothesis is that if these lakes went through substantial ecosystem reorganization, this must be a response to summer warming since 1980 CE. To verify our assumption we use a multi-proxy approach including Pb^{210}/Cs^{137} dating, chironomid, Cladocera analysis, SPDU (chlorophyll derivatives), main and trace element concentration, organic matter content (LOI, loss-on-ignition), TN, C/N ratio and $\delta^{15}N$ measurements and chironomid-based temperature reconstruction. The chironomid assemblages indicate a cold, oligotrophic environment and the communities remain relatively stable through the investigated period, possibly as a result of the conservation actions, probably also supported by the shadedness of Lake Ana. Our chironomid-based temperature reconstructions show warming from the 1990s and 2000s, respectively, but the inferred temperatures of the youngest samples exceed the temperatures of the 17th century just in the case of

Lake Peleaga. The results of the reconstruction were influenced by human activity: in the 1990s, the reconstruction showed a decrease in temperature in both lakes, due to the effect of fish introduction. Since the analyzed macroinvertebrate assemblages showed considerable resilience to the dominant stressors (warming and anthropogenic disturbance), we conclude that the chironomid communities have not yet passed critical transitions. Their resistance to change is rather exceptional in comparison with other alpine lakes in the South Carpathians, and highlight the importance of protection. The research is funded by the Climate Change Multidisciplinary National Laboratory 5B sub-project (RRF-2.3.1.-21-2022-00014) of the National Research, Development and Innovation Office

Ontogeny in the diet and mouthpart morphology of Chironomidae larvae

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Chironomid larvae go through four larval stages, hatching from eggs, molting, and then going through metamorphosis from larvae to pupae. During when the larvae feed and grow is in many respects reflected in the larval mouthpart morphology. In my presentation I will be presenting results on a comparative study on the diet and mouthpart morphology of chironomid larvae with emphasis on the inter larval stage comparison.

Comparison of macrobenthos vs. pupal exuviae to measure biological response to stream restoration

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In the Mid-Atlantic USA, physical restoration of stream channels is intended to reduce the influx of nutrients and sediment to Chesapeake Bay. However, the depauperate macroinvertebrate communities of urban streams have not shown much improvement in biotic integrity following the physical restoration of the stream channel. Can sampling of chironomid emergence, as floating pupal exuviae, provide a complementary measure of biotic response compared to the standard benthic "D-net" sampling protocol? Cloister's Branch of Towson Run (Baltimore County, Maryland) was restored in 2018 to reduce bank erosion, improve riffle-run-pool channel form, and improve groundwater-surface water interaction. Care was taken in the restoration design to preserve the existing forested riparian buffer to the maximum extent possible. Pre-restoration monitoring of water quality was conducted on Cloisters Branch plus three other non-restored streams in 2013. Floating chironomid pupal exuviae were collected on 3 dates pre-restoration for comparison with standard benthic sampling by Baltimore County. Channel restoration was completed in 2018. Post-restoration biological monitoring of the Cloisters Branch (and the three "reference" streams) was renewed in 2023. Sampling methods were compared at 5 stations along Cloister's Branch. Non-midge genus richness increased from 2013 to 2023 at four of 5 stations. Simulium, *Tipula*, Amphipoda, and Turbellaria decreased in abundance post-restoration and were replaced by Trichoptera and Zygoptera genera that were absent/rare pre-restoration. Chironomini became dominant post-restoration. Shifts in chironomid and non-chironomid assemblages were both significant. Lower salinity/conductivity facilitated biotic recovery. We infer that restoration improved food-web complexity through improved in-stream habitat structure and protection of forested riparian buffers.

Ecology & Ecotoxicology

Unraveling Chironomidae colonization dynamics in intermittent stream (Croatia)

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Intermittent streams naturally alternate between wet and dry phases that depend on temperature and precipitation patterns, nowadays altered by climate change, affecting the seasonality and life cycle of aquatic invertebrate communities. Among the others, Chironomidae are valuable bioindicators that respond rapidly to environmental change in terms of abundance and community structure and are common colonizers of intermittent streams. We investigated the seasonal colonization pattern of chironomids in 11 sites (10 lotic and 1 lentic) along an intermittent stream in continental Croatia in relation to the environmental conditions (e.g., water temperature, nutrients, and substrate type, current velocity). In all, 8282 individuals were collected belonging to 23 genera and 13 species. The most common species were *Diplocladius cultriger* and *Mesocricotopus thienemanni*. *D*. *cultriger* is usually found in silty bottoms and intermittent streams, while *M*. *thienemanni* is usually known from lentic habitats, but in this study, it was found in both lentic and lotic sites. Lotic sites were completely dry from June to November, while lentic sites retained water all year round. The lowest species richness was observed two weeks after rewetting (11 taxa) in December, while the highest richness was recorded four months after rewetting (in April). Chironomids started to colonize the stream only five days after rewetting occurred. This study emphasizes the importance of understanding the ability of certain chironomids to thrive under such stressful environmental conditions.

Optimized Digestion Methods for Aquatic Insects in Microplastics Detection

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The detection of microplastics in aquatic ecosystems has become a pressing environmental concern due to their pervasive presence and potential adverse effects on aquatic organisms. Aquatic insects, particularly those in the larval stage, serve as valuable bioindicators for assessing microplastic contamination. However, accurately identifying and quantifying microplastics in these organisms requires optimized digestion methods that efficiently break down biological tissue while preserving microplastic particles for analysis. This study focuses on developing and refining digestion protocols to enhance the accuracy and reliability of microplastics detection in aquatic insects. Various chemical digestion agents, including potassium hydroxide (KOH), hydrogen peroxide (H_2O_2), and enzymatic treatments with proteinase K, were tested for their efficacy in decomposing organic matter without degrading microplastics. The performance of these methods was evaluated based on digestion efficiency. Best results for adults and exuvia are obtained in cases where Chironomidae were exposed to KOH for 7 days first at room temperature and then placed in a water bath for 7 days at 60°C. The optimized digestion protocol allowed for clear visualization of Chironomus riparius under microscopic examination. For larvae, the best results are still achieved with hydrogen peroxide (H_2O_2) for 15 minutes at room temperature followed by examination under UV light.

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Ecology & Ecotoxicology

Long-term changes of Chironomidae assemblages of Lake Balaton

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Lake Balaton represents a special water body type in Hungary and Europe, it is also at the centre of social interest. Knowledge of its macroinvertebrate fauna and its changes during the last 200 years are of prime importance. In this study we use paleolimnological methods to reconstruct ecosystem changes in Balaton through the study of chironomid larval assemblages. We took 28-45 cm long sediment cores. We identified 1857 head capsules from Siófok basin belonging to 12 morphotypes from 2 subfamilies (Chironominae and Tanypodinae), 1569 head capsules from Keszthely basin belonging to 20 morphotypes from 3 subfamilies (Chironominae, Orthocladiinae and Tanypodinae) and 1929 head capsules from Tihany basin belonging to 14 morphotypes from 3 subfamilies (Chironominae, Orthocladiinae and Tanypodinae). The most frequent taxon was Procladius sp. Cluster analysis divided the chironomid assemblages into 2 significant zones in each sample. We conclude that there have been significant changes in the water quality of the lake in the last one and a half century. The number of Stempellina sp. decreased and the number of Chironomus plumosus-type increased at the beginning of the 20th century. This indicates an increase in the trophic level of Balaton. This was a critical time in the life of the lake, with an unprecendented rate of change in its macroinvertebrate fauna. These changes most likely connected to the growth of human population and related activities that strongly affected the habitat quality of the lake.This research is funded by NKFIH National Multidisciplinary Laboratory for Climate Change (RRF-2.3.1.-21-2022-00014).

Chironomid larvae (Insecta, Diptera) in the Radika River watershed – a case study from the proposed "HPS Boshkov Most" (North Macedonia)

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Chironomid larvae were studied at selected river sites in the Radika River catchment to identify the indicator taxa and analyze their contribution to the assessment of the ecological quality of the rivers included in the planned reservoir "HPS Boshkov Most". Quantitative macroinvertebrate samples were collected in six rivers along the Radika River watershed (Mala River, Lazaropolska River, Garska River, Rosocka River, Tresonecka River, and Jadovska River) in two seasons (Summer and Autumn, 2012) using a Surber sampler. Chironomids were identified to the genus or species level. We found a total of 11 taxa of the chironomid larval fauna belonging to 4 subfamilies: Chironominae, Tanypodinae, Orthocladiinae, and Prodiamesinae, in which indicators of high/good quality, e.g. *Prodiamesa olivacea* (Meigen, 1818) were noted. The most abundant subfamily was Orthocladiinae with a total of 5 taxa. Among the recorded taxa the most widespread species was *Polypedilum pedestre* (Meigen 1830) (all 6 sites), followed by *Paratendipes* sp., *Prodiamesa olivacea*, *Eukiefferiella quadridentata* Cernovskij, 1949, *E. longicalcar* Thienemann, 1926 and *Thienemannimya* sp. (at 5 sites); where the least recorded taxa (at 4 sites) were *E. alpestris* Goetghebuer, 1941 and *E. longipes* (Staeger, 1839). Concerning its abundance, the most common chironomid species was *E. longicalcar* together with *Rhithrogena* gr. *semicolorata* (Ephemeroptera) and *Ancylus* sp. (Gastropoda) from Jadovska River. In conclusion, we can emphasize that the overall composition of the recorded indicative chironomid species can serve as good indicators for the future conservation of the pristine environment and can contribute to the further conservation of the Radika River watershed.

Adapting or evading? *Chironomus riparius* versus environmental change apocalypse: Microplastics, heating and salinization

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Climate change, salinization, and microplastic pollution are emerging as increasing threats to aquatic ecosystems, inciting our investigation into their combined effects on non-biting midge larvae of *Chironomus riparius* in mesocosms. The endpoints were the survival, growth, development, and the average number of ingested particles per larvae. The larvae were exposed to a warming scenario (+4.5 °C), along with a gradient salinity level (2/4/6 and 8 g/L), and environmentally relevant concentration (8 g/m^2) of two sizes of polyethylene microspheres (10-45µm and 63-75 µm). Findings indicated that the synergic effect of heating, microplastics and 8g/L salinity had the most significant impact on larval growth rate, with a decrease of 42.9 %. Similarly, the survival rate significantly decreased by 27.8 %, compared to control conditions. The highest average number of ingested particles were recorded in the larvae exposed to heating, microplastics and salinity of 4 g/L, with the value of 31 particles/larvae. In fact, the average number of ingested particles was significantly higher in the treatments subjected to heating scenario in comparison with ambient temperature. This unique experimental approach is a first step toward understanding the complex responses and potential ecological consequences of these multiple stressors on *Chironomus riparius* larvae, providing important information for the assessment and management of aquatic ecosystems in the face of global environmental challenges.

How hydromorphology affects chironomid response to stream eutrophication

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Chironomid larvae represent an important component of the taxonomic and functional diversity within macroinvertebrate communities in streams. Although a considerable amount of information is available regarding the environmental preferences and sensitivity of individual species to anthropogenic stressors, this insect group is rarely utilized for routine assessment based on detailed identification. The primary analysis of this study focused on a dataset representing gradients of organic pollution/eutrophication and hydromorphological degradation in small streams (23 sites). The sites and their environmental conditions were described using a comprehensive set of characteristics, including water chemistry, hydromorphology, and land cover at multiple spatial scales. Chironomid larvae were identified to the lowest taxonomic level based on current knowledge. Strong relationships were observed between chironomids and environmental indicators of stream impairment (TOC, nutrients, River Habitat Survey scores). Chironomid taxa richness increased in response to either eutrophication intensity or degraded hydromorphology. Additionally, indicator taxa for different pressure magnitude classes and combined pressure conditions were identified. Chironomid species traits were considered to interpret their spatial distribution, taxa co-occurrence, and role in macroinvertebrate communities. The results were put into the context of monitoring data. The study will contribute to the selection of new chironomid indicators of eutrophication and hydromorphological conditions.

Impact of Dams on Chironomidae (Diptera) Communities: A Study of Diversity in Lithuania's Šventoji and Žeimena Sub-Basins

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Rivers are important habitats for biodiversity and environmental health. Maintaining their water quality is crucial to prevent biodiversity loss. While dams historically benefited human lives, their increasing construction since the 20th century has raised environmental concerns. Dams alter morphometric and chemical parameters, impacting river ecosystems. The environmental effects vary based on factors like climate and geomorphology and thus at regional level. In this work, we analysed the effects of dams on six streams in Lithuania monitoring the Chironomidae community as bioindicators of water quality and ecological integrity. Streams are located in the sub-basins of Šventoji and Žeimena: Plaštaka, Kiauna, Luknelė not dammed and Skerdyksna, Dubinga and Šešuola dammed. As the six streams were researched and four study sites were selected in each of them, thus collecting material from 24 sites in the whole study. The data collection spanned from spring to autumn every two weeks, covering the period from 2021 to 2022. Between 2021 and 2022, a total of 336 samples were collected from the six studied streams across all 24 collection sites. Using a 1 mm mesh pond net, more than 11,000 larvae from approximately 60 genera of Chironomidae were collected. We will discuss the impact of dams on Chironomidae diversity, biology and ecology, including a list of new species from Lithuania.

Effects of different dietary regimes on the growth, development and nutritional compositon of *Chironomus riparius* larvae

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Phytoplankton are closely associated with eutrophication, a process where excessive nutrient production stimulates the overgrowth of algae which can lead to reduced oxygen levels and changes in water chemistry, impacting chironomid larvae. The aim of this study is to test phytoplankton as a food source for *Chironomus riparius* larvae and determine if their chemical composition affects larval growth and development. The effects of different dietary regimes were investigated following OECD protocol 218. Control groups were fed using TetraMin® food and four treatments were exclusively exposed to *Chlorella sp.* algae suspension and dry matter, dry yeast, and biofilter content from minnow aquariums, over a period of fourteen days. Statistical analysis indicated no significant difference in survival rates between the control group and the experimental groups, however, there were notable differences in total wet weight and morphometric parameters among the treatments. Larvae that consumed *Chlorella sp*. dry matter and dry yeast exhibited higher mass and better morphometric characteristics compared to those fed algal suspension and biofilter content. The larvae showed varying nutritional compositions based on their diet. The ones that were fed control food had the highest carbohydrate percentage (18.1%), whereas lower percentages were observed in larvae fed *Chlorella sp*. suspension, yeast, biofilter, and *Chlorella sp.* dry matter (5.7%, 5.5%, 5.4%, 4.3%, respectively). Proteins were most abundant in larvae fed biofilter (48%), while lower percentages were found in larvae fed control food, *Chlorella sp.* suspension, yeast, and *Chlorella sp.* dry matter (45%, 36%, 22%, 22%). The highest lipid percentage was detected in larvae fed control food (7.1%), while larvae fed biofilter and *Chlorella sp.* cell suspension had undetectable lipid levels. Larvae that were provided with dried algal biomass exhibited the greatest mass, whereas those given fresh algae or biofilter had notably lower masses. This supports the potential use of dried algae as a suitable food source for maintaining *C. riparius* larvae in laboratory conditions.

The shaping of chironomid assemblages in man-made lakes

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Man-made freshwater habitats are widely used to support the environment for human needs, e.g. for flood control, irrigation, water supply, or power generation. In this study, littoral chironomid assemblages from man-made lakes in the ecoregions Dinaric Western Balkans (n=22) and Pannonian Lowlands (n=15) were analyzed. A total of 110 chironomid taxa were recorded in 113 samples collected from 2016 to 2021. The NMDS analysis based on the chironomid assemblages showed a clear separation of the study sites into two distinct cluster groups: The Dinaric group and the Pannonian group. According to the Simper analysis, the average similarity in the Dinaric group is 48.3 %, with the four most contributing taxa being *Stempellina bausei*, *Ablabesmyia monilis*, *Potthastia longimana,* and *Procladius choreus*. In the Pannonian group, the average similarity is 27.2 % and *Tanytarsus* sp., *Cladotanytarsus* sp., and Orthocladiinae gen. sp. contribute the most to the similarity. Based on the interactive forward RDA analysis, five out of sixteen environmental variables were found to be significant in shaping the chironomid community: transparency, COD, temperature, total N, and Chl *a*. Two out of four land use parameters were also important in structuring the chironomid community: catchment area and intensive agriculture. Sites in the Pannonian Lowlands ecoregion are under greater influence of different types of pollution from land use and agriculture. In particular representatives of the Chironomini (e.g. *Glyptotendipes barbipes*, *Parachironomus gracilior*, *Chironomus luridus*, *Einfeldia* sp.) are associated with increased eutrophication parameters. Based on our results, we can conclude that littoral larval chironomids that can be identified at the species/genus level have a great potential for monitoring artificial and heavily modified lentic habitats and that their chironomid assemblage can emulate the composition of the entire macroinvertebrate community.

Histology of chironomids: unlocking the new universe

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Histology is a widely used method for the microscopic study of tissues, providing valuable insights into tissue morphology and cellular organization. Histopathological analyses further enhance this by detecting deviations from normal tissue conditions, enabling effective diagnosis of pathological changes. While these methods are traditionally applied to vertebrate species, their application to invertebrates like chironomids offers significant potential. As chironomids are great bioindicators of aquatic pollution and are often used in ecotoxicological assessment, histological analysis of different life stages of *Chironomus riparius* gave us information about the modification of internal structures as the organism transitions through different developmental stages. Moreover, histopathological examinations of *C. riparius* larvae have identified specific organs that are more susceptible to the presence of micro- and nanopollutants. By extending histological and histopathological techniques to aquatic invertebrates, we can gain a deeper understanding of sub-organismal structures. This knowledge enhances our comprehension of the mechanisms underlying various physiological responses. In the context of aquatic ecotoxicology, these methods open new dimensions in understanding how different pollutants impact model organisms at the tissue level.

Chironomidae of natural lakes in the Dinaric region of Croatia

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Natural lakes are rare in Croatia due to the permeability of the geological deposits of the Dinaric karst. Their ecosystem services or functions differ. Some serve as important drinking water reservoirs, others as recreational areas, but they all harbor different ecosystems. Among the inhabitants of these ecosystems are Chironomidae, an extremely diverse and successful fly family that can be found in practically all habitats. Due to their omnipresence, ecological niches, and species richness, they are valuable indicators for the assessment of ecological water quality. The aim of this study was to assess the diversity of Chironomidae communities on the shores of Croatian natural lakes. Sampling of larvae was carried out in the summer of 2018 according to a modified proportional stratified sampling protocol on seven lakes. It resulted in 210 samples with over 25,000 chironomids belonging to more than 50 taxa. Lake Visovac (a riverine barrage lake) had the highest abundance of chironomids, while Lake Kozjak (also a barrage lake) had the highest species richness. Both abundance and species richness were lowest in Lake Vrana, an isolated cryptodepression on an island. A cluster analysis based on species composition and abundance divided the lakes into three distinct categories: lakes with elevated salinity harboring species such as *Chironomus aprilinus*, barrage lakes displaying high abundance and taxa richness, and oligotrophic lakes with low abundance and taxa richness. These results enrich our understanding of the distribution of Chironomidae and their ecological preferences in the understudied and rare habitats of natural lakes in karst terrain.

Unexplored Diptera communities in mountain streams: Insights and steps forward

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Altitude and topography play a decisive role in freshwater ecosystems, as they determine their hydrological, ecological, and biological aspects. In stream ecology, streambed slope, water temperature, flow velocity, depth, and stream width can significantly influence habitat variability and affect the diversity of local communities. In this study, we collected dipteran species in macrozoobenthos samples (using a benthos hand net, mash size 500µm) from 14 streams, each sampled at two different altitudes, in three seasons: summer 2020, spring, and autumn 2021, on three mountains: Papuk, Psunj and Medvednica (Croatia). In addition to benthic community collection, we measured standard environmental parameters *in situ*. We used HCl to determine water hardness and measured the depth and water velocity as well as the width of the stream bed. In 673 samples we identified 210 taxa with 82 species (69% Chironomidae). Dipteran community formation as a function of different variables was analyzed using redundancy analysis and variational partitioning analysis was used to estimate the influence of spatial drivers. Dispersal is high when sites are closer together and low when sites are further apart. Spatial factors highly influence the dipteran community on Medvednica, while the communities on Papuk and Psunj were primarily influenced by environmental variables. The studies on mountain stream Diptera are still scarce, also in Croatia, which emphasizes the need for more comprehensive research. Frequent sampling and extensive datasets are crucial for understanding the variability of stream communities and predicting their responses to future climate change scenarios.

Chironomini *versus* Orthocladiinae – factors determining their local species richness

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Chironomidae is one of the most common and abundant groups of dipterans inhabiting freshwater ecosystems. In rivers of Central Poland, the tribe Chironomini from subfamily Chironominae and subfamily Orthocladiinae are the dominant taxa within chironomid communities. The main aims of our study were to identify the dependence of Chironomini and Orthocladiinae local species richness (LSR) on habitat species richness (HSR), correlations with environmental factors and seasons, and to assess which of these patterns are expressions of stochastic and which of deterministic processes (a metacommunity perspective). Chironomid samples were collected over an annual cycle, once a month, from seven lowland rivers located in Central Poland. On each sampling occasion basic environmental parameters were measured or estimated in the laboratory from additionally collected inorganic and organic substrates. The influence of HSR on LSR was significant in the Orthocladiinae, and insignificant in the Chironomini, showing a domination of deterministic HSR-related processes in the Orthocladiinae. Multiple regression of all LSR values of each taxon on environmental parameters and seasons explained over 70.0% of LSR variability in the Orthocladiinae and below 40% in the Chironomini, indicating that abiotic deterministic processes were more relevant in the Orthocladiinae. A positive impact on LSR was exerted by depth (both taxa), HSR and inorganic substrate index (Orthocladiinae). In contrast, LSR was negatively affected by seasonality (Orthocladiinae) and velocity (Chironomini). These two chironomid taxa presented different responses to environmental factors, and different dependence on HSR and seasonality, which seems to be strongly associated with their dispersal abilities, phenology, and ecological demands.

Integrating Deep Learning for Enhanced Bioassessment of Aquatic Invertebrates: A Case Study in Chironomid Ecology

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Aquatic insects are essential bioindicators for assessing the health of freshwater ecosystems. Our research emphasizes the ecological importance of Chironomidae and challenges traditional taxonomic approaches by focusing on Functional Feeding Groups (FFG). This shift toward FFG recognition offers a more ecologically relevant and direct method for assessing environmental impacts. In our study, we utilize an integrated approach that moves beyond conventional taxonomic systems. We developed a sophisticated deep learning model using Convolutional Neural Networks (CNNs) via an EfficientNetB1 framework. This model, trained with a dataset comprising 274 images across various classification groups, achieved a remarkable 98.94% accuracy in mandible identification. We also identified essential mandible features critical for accurate classification into FFGs, such as the apical tooth and inner teeth, which are key to distinguishing between groups that were used by the model. Our findings demonstrate the significant advantages of integrating deep learning in the bioassessment of aquatic ecosystems. By automating the identification of specific morphological traits, our approach can finally bring chironomids into routine monitoring programs. This advancement is crucial since chironomids are an important component of macroinvertebrate communities but are often omitted from bioassessment due to the lack of taxonomic expertise. By shifting bioassessment toward trait-based metrics, we can overcome the obstacle of the taxonomist shortage, resulting in more accurate and useful ecological monitoring in the future.

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Poster Presentation Abstracts

Determining the Impact of Anthropogenic Disturbances on Midge Diversity in Loe Pool, Cornwall

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Anthropogenic impacts on the natural environment are leading to a global loss of biodiversity, particularly affecting both freshwater ecosystems and insects. The loss of freshwater insect biodiversity has impacts on all trophic levels as well as on ecosystem services. The identification of the dominant drivers of biodiversity loss is restricted due to the short timescales covered by monitoring datasets, hampering effective ecosystem management. Palaeoecological datasets, in this study of chironomid larval head capsules, provide a long-term perspective on changing insect populations. A series of sediment cores were taken from Loe Pool, SW England, and assessed for chironomid sub-fossils. Loe Pool is a Site of Special Scientific Interest (SSSI) with a known history of varied anthropogenic activity, including mining, water treatment, and agriculture. This centennial-scale record shows the negative impacts of both mining and agriculture-associated nutrient pollution on the assemblages of chironomids present over time. Despite signs of recovered biodiversity, the original community composition and diversity have not been restored. These results will inform restoration and conservation efforts at the site and will be complemented by studies on two other sites across the UK.

Hidden biodiversity of Chironomidae in Central Europe

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Non-biting midges (Insecta: Diptera: Chironomidae) are frequently the most abundant, widespread, and diverse insect group in freshwater ecosystems. Globally, the number of chironomid species is estimated to exceed 20,000, with considerable diversity observed at both local and regional scales. However, many country-specific taxa lists tend to underestimate the real species richness, as indicated by DNA barcode reference data. New species continue to be described, even within Europe. In the framework of the DRYvER project (Horizon2020 #869226), chironomid samples were collected during six sampling campaigns in 2021 and 2022. Samples were obtained from Hungary, Croatia, and Czechia, and chironomid larvae were morphologically identified to the most precise taxonomic level possible, often at the species level. Additionally, pupae and pharate larvae exhibiting pupal characteristics were included to aid in clear identification. In addition to morphological identification, a subset of larvae underwent molecular analysis using traditional DNA barcoding protocol and targeting mitochondrial cytochrome oxidase subunit I marker. In total, 155 taxa were identified from a collection of 117,871 specimens (81 taxa from Hungary, 102 from Croatia, and 111 from Czechia).

We documented the first occurrence of 20 species in Croatia and 3 in Czechia. Notably, eight larval forms defied classification using existing morphological keys. These taxa may represent previously undocumented larval forms of described species or potentially new species awaiting formal description.

Combination of Environmental Stressors Determine Extent of Clastogenicity and DNA damage in *Chironomus ramosus*

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The response of chironomid midges to multiple combinational environmental stressors is a relatively unexplored area of research. In the present study, we estimated threshold levels of tolerance to the individual as well as a combination of stressors like desiccation (D) and/or heat (H) and/or starvation (S) in the fourth instar larvae of the tropical non-biting midge *Chironomus ramosus*. We estimated the lethal time (LT) at specific doses using regression and Probit analyses based on the percentage of pyknotic polytene chromosomes as a measure of cell death. Larvae (n=30) were exposed to single and multiple combinational stressors (D+H, H+S, D+S, D+H+S). In the treated larvae, we examined 1) the status of polytene chromosomes in the salivary glands and 2) the status of DNA damage using γH2AX marker by immunofluorescence technique. The integrity of chromosome morphology was completely lost during simultaneous exposure to multiple stressors, i.e. D+H+S, as compared to any single stressor. LT_{90} values (in hrs.) obtained were: D (0.75±0.09), H (30±3), S (65±5); D+S (0.75±0.09), D+H (0.5±0.02), H+S (19±1.3); D+H+S (0.55±0.02). The data suggested desiccation as the most potent stressor either as an individual factor or in combination with heat stress. When the larvae were exposed to all three stressors the impact was similar to D+H. It was also obvious that starvation had the least impact amongst all three stressors. γH2AX signals indicated DNA double-strand breaks and were seen as bright spots (foci) under a fluorescence microscope. γH2AX immunofluorescence assay corroborated our light microscopic observations of stress-induced pyknosis of polytene nuclei. It was a clear indication that amongst the three stressors, *C. ramosus* larvae were most vulnerable to desiccation followed by heat stress and the quantum of DNA damage was maximum in the desiccated larvae.

The findings may be useful in biomonitoring studies using *Chironomus* as an indicator species of aquatic ecosystems.

Salty or sweet, doesn't matter, Chironomids like everything

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Chironomid larvae inhabit diverse environments, including brackish or marine habitats. Veliki Brijun island in the National Park Brijuni archipelago hosts such unique aquatic systems, comprising of various artificial water bodies with differing salinity levels, influenced by the surrounding sea and precipitation rate. As part of the project survey, samples of macrozoobenthos, including Chironomidae, were taken from three water-bodies in the coastal wetland Saline (abb. MJ, SrJ, VJ) and Brijuni pond (SB) in April and October 2022. Except for salinity, variations were observed in substrate type, macrophyte presence, and analyses indicated elevated levels of ammonium, organic nitrogen, nitrate, and total nitrogen at specific sites. The abundance of chironomid larvae was high, but the diversity was relatively low. The highest number of taxa (13) was recorded in SB – freshwater pond with macrophytes, and in other water-bodies - with increased salinity, diversity was low and halophile species were present. In April, only *Chironomus salinarius* and *Chironomus aprilinus* were present in the biggest water-body (VJ), while in the autumn, there were representatives of *Chironomus* and *Glyptotendipes* genera (Chironominae). In a smaller lake (SrJ), only *C. salinarius* was sampled during spring and in the autumn samples, we recorded the presence of *Kiefferulus* cf. *tendipediformis* and *Glyptotendipes* sp. (Chironominae). *C. salinarius* and two species of *Halocladius* (Orthocladiinae) were recorded in the smallest water-body (MJ). The influence of perceivable water-level and salinity fluctuations in these ecosystems, with cumulative anthropogenic influence (incl. introduced game), are reflected in the Chironomid community structure. Since restoration activities of the area are planned, continued and new monitoring activities are necessary.

Life at the edge: Chironomids of the genus *Diamesa* in European mountain streams

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Chironomids of the genus *Diamesa* Meigen, 1835 prefer cold, oxygen-rich running waters. We investigated *Diamesa*-dominated stream communities in three European mountain regions (the Central Pyrenees, the Ötztal Alps, and the Tatra Mountains) to better understand their ecology, diversity, and physiology. Species identification of *Diamesa* larvae is challenging, therefore, we supported their morphological characterization by DNA barcoding, employing two molecular markers – the mitochondrial cytochrome oxidase subunit I and the internal transcribed spacers region (Dvorak et al., 2024). In samples from the Ötztal Alps, new methods of glycogen and lipid content determination in a single chironomid larva showed very low energy stores in streams both with and without glacier presence in the catchment (glycogen and lipid concentration <0.01% and <5% of dry weight, respectively; Dvorak et al., 2023). Our results demonstrate that individuals living in extreme environments of mountain streams are adapted to survive with reduced energy stores. The presence of *Diamesa steinboecki* Goetghebuer, 1933, a species adapted to the environment of glacier-fed streams, in the Spanish part of the Pyrenees was observed for the first time. Water temperature seems to be the main driver of *Diamesa*-dominated stream communities' distribution. At our study sites (22 streams in the 3 regions), the relative abundance of *Diamesa* species was significantly higher in streams with a mean July water temperature <6.5°C.

Our results show that changes in *Diamesa* populations can be used for tracking degradation of alpine streams affected by climate warming.

Environmental predictors of Chironomidae abundance after dam removal

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The aim of this research was the analysis of an impoundment's impact on a Chironomidae assemblage in the lowland Drzewiczka River (central Poland). To identify the environmental factors that play a key role in shaping the abundance of chironomids, the study was conducted in three annual periods characterized by different discharges connected to the functioning of the reservoir: before dredging of the reservoir (modified flow), during dredging (natural flow) and after dredging (modified flow). In each period, samples of the benthic chironomid larvae were collected from the impoundment's tailwater from five selected habitats (pool, stagnant, overgrown by macrophytes, bank, rifle). Orthocladiinae and Chironomini predominated among the chironomids in each studied period with the highest abundance noted in the period with natural discharge. To model the response variables of benthic Chironomidae abundance from abiotic factors (current velocity, river width, water depth, water temperature, dissolved oxygen, percentage of substrate covered by vascular plants, inorganic bottom substrate, and quantity of benthic coarse (BCPOM), fine (BFPOM) and transported (TPOM) particulate organic matter), GLM were used. The best-fitting model included a negative effect of BCPOM on chironomid abundance.

Taxonomy of Brazilian *Coelotanypus* Kieffer, 1913 (Chironomidae: Tanypodinae)

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Coelotanypus Kieffer, 1913 is a genus of 21 described species distributed in the Afrotropical, Australasian, Nearctic, and Neotropical Regions. Eleven named species are recorded from South America. *Coelotanypus* larvae occur in benthic sediments of lakes, including artificial impoundments and slow-flowing reaches of rivers. The objective of this work is to explore the diversity of *Coleotanypus* species in Brazil both morphologically and based on DNA analysis, providing new records and descriptions of new species. We analysed samples collected in four out of the six biomes of Brazil (Amazon, Atlantic forest, Caatinga, and Pampa) and found eight species. Two new species are described from a male, pupa, and larva collected in Pernambuco (Caatinga, northeastern Brazil) and one from a male collected in Rio Grande do Sul (Pampa, southern Brazil). Five known species (*C. dimorphus*, *C. tibialis*, *C. mendax*, *C. lobensis* an *C. humeralis*) have their distribution extended. *Coelotanypus tibialis* is redescribed and remarks on immature stages of *C. dimorphus* are also provided.

Tracing Ancestral Footprints: *Polypedilum* Evolution and Biogeography in South America

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Polypedilum stands as one of the largest genera within the family Chironomidae, boasting around 440 documented species distributed globally. Its larvae inhabit diverse aquatic environments, predominantly dwelling in sediments but also thriving amidst macrophytes and in phytotelmata. Despite the execution of phylogenetic analyses on the genus in recent decades, none have delved into biological processes such as diversification, dispersal, and vicariance within a biogeographic framework. The primary objective of this investigation was to propose a hypothesis regarding the divergence time of *Polypedilum* lineages, with a particular focus on species native to South America, through phylogenetic reconstructions. To accomplish this goal, we meticulously analyzed concatenated multigenic data encompassing 3159 characters derived from 143 individual specimens, employing both maximum likelihood and Bayesian inference frameworks. Our analysis confirmed the monophyly of the genus *Polypedilum*, as well as the monophyly of its subgenera *Asheum* and *Cerobregma*. The initial split within *Polypedilum* occurred around 75 MYA, marking the end of the Cretaceous period. Subsequently, there was significant diversification during the Paleogene epoch, a trend observed in several other genera within the Chironomidae family. This study offers significant insights into the evolutionary history and biogeography of *Polypedilum* species within South America. The next phase will entail conducting diversification analyses to deepen our understanding of the latitudinal gradient of diversity within this ecologically significant group of aquatic insects.

Humans vs chironomids: Anthropogenic impact on chironomid populations in tufa depositing environment

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Tufa is a calcium carbonate, a biologically mediated deposit in karst streams. Algae, cyanobacteria, plants, mosses, and macroinvertebrates help create it forming the tufa barriers. The moss mats developing at these sites are habitats for a large number of organisms as well as for various types of macroinvertebrates. One of the champions of tufa deposition in Europe is the Mrežnica River (Croatia). Due to the waterfalls formed in tufa barriers, this river is a popular destination for outdoor activities such as kayaking, swimming, fishing, etc. The goal of this study was to ascertain the level of this human impact on the macroinvertebrates. We sampled one pristine location, one heavily impacted by humans, and two with medium and/or no human impact along the reach. Chironomids were our focal group, however, we also studied the response of other macroinvertebrate taxa for comparison of resilience/recovery of populations downstream. The decrease in numbers of Chironomids was vast, however, the Chironomids have been the most resilient taxon in comparison to EPT taxa, both at the impact site and they were also the taxon whose abundances recovered the fastest downstream.

Although human impact is extreme and decreases both the area of moss mats as habitats and consequently all the populations of macroinvertebrates, there are nooks and crannies that may serve as recolonization and revitalization sites.

Chironomus striatippenis' strategies of trapping mosquito larvae and survival against drought and high temperature

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Chironomid larvae inhabit temporary aquatic habitats and overcome obstacles, such as drought, heavy rain, and predators. This study investigated its interaction with *Aedes* mosquitoes as well as its behavior and gene expression under adversity. During the entomological survey, we observed that chironomids often coexisted with other mosquitoes in their habitats. *Chironomus striatipennis* Kieffer was used in our experiments. We found that gravid female mosquitoes preferred to lay eggs in the water from chironomid habitat. Next-generation sequencing (NGS) showed that the dominant gastrointestinal bacterium of these larvae was *Novispirillum* sp. During larval competition, *Aedes albopictus* (Skuse) larvae preferentially preyed on the chironomids. Mosquitoes growing in the water from chironomid habitat delayed pupation, and most of the emerging adults were males. Chironomid larvae build tubular nests to defend themselves. Low water level, mud, and high temperature promoted nesting, while low temperature reduced larval survival. Light stimulated the expression of heat shot protein (*hsp*) in larvae in the saliva nests and the expression of hemoglobin gene in larvae in the mud nests. The increase in temperature also upregulated the expression of hemoglobin gene.

This study revealed the possible survival strategies of chironomid populations under climate change. In the future, the chironomid intestinal bacteria which attract mosquitoes would provide an alternative of eco-friendly management of freshwater habitats and vector biological control.

Reconstructing 13,000 years of environmental and water depth changes from chironomid assemblages in Lake Lucerne, Switzerland

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Lake Lucerne is a deep oligotrophic lake located in central Switzerland. Strong morphodynamical processes led to geologically verifiable changes in the lake level of Lake Lucerne during the mid-to late Holocene. Here we present a first reconstruction of environmental and ecosystem changes from Lake Lucerne based on fossil chironomids that covers the past 13,000 years and focusses on potential changes in relative water depth. Chironomid assemblages were analysed in a sediment core from the littoral zone. Pronounced changes in chironomid concentrations and assemblages indicate strong environmental changes since the Late Glacial period. In order to estimate potential changes in past water level, chironomid assemblages were passively added to a dataset representing modern chironomid assemblages along water depth ranges of 0-25 m in six different Swiss lowland lakes. Ca. 7400-6500 cal. BP, a considerably lower water level was reconstructed than before. In this core section, chironomids were only found in low concentrations. After 6500 cal. BP, water level appeared to be slightly higher, and from 4000 cal. PB onwards, a further increase in the water level was inferred. These findings agree with the geological evidence for the region. However, from 2500 cal. BP onwards, the chironomid assemblage fluctuates strongly, and it is uncertain if the drivers of this were changes in lake level fluctuations.

Our results show the dynamic history of the near-shore ecosystems of Lake Lucerne and confirm the potential of chironomids to reconstruct relative depth changes in large, deep and stratified lakes in Central Europe.

Reconstructing Holocene environmental changes in an alpine lake (Tatra Mountains, Slovakia) using subfossil chironomid remains

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Sediment deposits from Popradské pleso (High Tatra Mountains, Slovakia), a lake of glacial origin, were investigated using subfossil chironomid remains to determine the duration of the glacier influence on the lake, as well as to reconstruct the impact of Holocene climatic oscillations. Chironomid analysis was done on two sediment cores, which were then merged based on loss-on-ignition and total organic carbon results, to span the entire epoch. During Early Holocene the sedimentary deposits consisted of fine laminated mud, and their chironomid community was species-poor and dominated by *Micropsectra radialis*-type and *Pseudodiamesa nivosa*, indicating very cold, ultra-oligotrophic and oxygen-rich conditions. The transition from a glacially influenced lake system occurred at ~9,900 cal years BP, lasting until ~9,700 cal years BP, with sediments consisting of homogenous mud. In the chironomid record, this was marked by a decrease in abundance of previously dominant taxa, and a concurrent increase of abundances of *Tanytarsus lugens*-type and *Psectrocladius psilopterus*-type, suggestive of a shift towards more productive conditions, with higher water temperatures. Post-transition, the chironomid community of the organic-rich gyttja deposits was dominated by thermally plastic *T. lugens*-type, *P. psilopterus*-type and *Heterotrissocladius marcidus*-type, and by an overall increase in taxonomic richness. Though Holocene climatic oscillations did not significantly impact the community, an increase in abundance of *H. marcidus*-type in the youngest parts of the sediment core is attributable to decreased productivity caused by lower temperatures during the Little Ice Age.

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Chironomids diversity in the streams around the Bor mining area

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Copper mining in the Bor region has a long tradition, which helps to improve the living standards of the local population but can also have a negative ecological impact. The biodiversity of this area is generally unknown. Among aquatic macroinvertebrates, chironomids are quite abundant and thus contribute significantly to the diversity of the macroinvertebrate community. To determine the diversity of Chironomidae around the Bor mining area, samples were collected at 18 sites in 2022. A total of 38 taxa were identified. *Brillia bifida* and *Polypedilum convictum* were the most frequent species found at 44% of localities. The highest Shannon diversity was recorded in Mali Pek (2.2) and Brestovačka reka 2 (2.11), while the lowest was in Rukjavica 2 (0.54). The highest Chao 1 index was recorded in Mali Pek (16.88) and the lowest in Cerova reka (2). Equitability was high (0.75-0.95) and uniform between sites, except in Rukjavica 2 (0.49). Species richness varied greatly among sites. At the Rukjavica and Brestovačka reka sites an increase in species richness was observed when moving downstream and away from the city. The alpha diversity of chironomids was generally high, with the exception of the Rukjavica 2. On the other hand, these hilly rivers and streams have numerous microhabitats that provide shelter for various species. Compared to similar ecosystems, the diversity could be even higher. As no previous data is available, the present study is the first to investigate the diversity of chironomids in this area, providing a baseline for further investigation of mining influence on chironomids.

Do long-term changes in stream hydrochemistry affect taxa richness and abundance of the family Chironomidae (Diptera): A case study of the long-term ecological research of the Mesta River (Bulgaria)

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The Mesta River is a transboundary river between Bulgaria and Greece and a representative water site within the Long-Term Ecological Research (LTER) Network. The river was selected as a model watercourse for conducting surveys of ecological long-term studies because it has been surveyed for the last 40 years in terms of hydrobiology and hydrochemistry. We investigated genera and species of the family Chironomidae (Diptera), whose representatives are commonly one of the most abundant and diverse taxa in freshwater ecosystems due to their adaptive capabilities and ecological plasticity. The relationship between the taxonomic richness and abundance of chironomid larvae and the environmental variables - water temperature, dissolved oxygen concentration, and oxygen saturation was discussed. The data were collected and measured at four sampling sites, located in the upper, middle, and lower sections of the river on Bulgarian territory between 1115 m and 392 m a.s.l. The analysis included the list of chironomid taxa and abundance data, as well as characteristics of a water quality measurement from 1979 to 2020. The values of the environmental variables showed a slow but constant increase throughout the investigated period. The relationship between the distribution of the taxa's richness and abundance and environmental variables was statistically significant (p=0.03) as both richness and abundance decreased through the period. Species from the genera *Brillia* Kieffer, 1913, *Cricotopus* van der Wulp, 1874 and *Eukiefferiella* Thienemann, 1926 had higher abundance in the samples from 1986-1991, while genera from the tribes Chironomini and Tanytarsini started to become more abundant after the year 1992.

Chironomids in Slovakian reservoirs – Developing a classification scheme for ecological potential assessment based on exuviae

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The CPET method is convenient and an often-applied method using exuviae. Our aim was to test the applicability in the assessment of reservoirs´ status in Slovakia. We tried to adapt the British evaluation process to conditions in our country. Chironomid exuviae were collected in 23 reservoirs in Slovakia, in the period 2012-2015. Seven reservoirs are used as water supply while others are multi-purpose. Samples were taken from the surface or leeward shore of reservoirs twice a year (except 2012), using a hand net (250 µm mesh size). The collection and processing of samples were carried out according to the procedure STN EN 15196:2007. In total, 183 taxa were recorded belonging to Tanypodinae (16), Buchonomyiinae (1), Diamesinae (2), Prodiamesinae (1), Orthocladiinae (56), and Chironominae (107) subfamilies. During this research, we recorded 9 new species for Slovakia. Data from 150 taxa scores were used to calculate and create a suitable alternative for the Nutrient Sensitivity Index (NSI), which was tested and correlated with nutrients, in order to apply it in the classification scheme for the ecological potential evaluation. Finally, reservoirs were clustered into groups: 1. Deep reservoirs on higher altitudes (7 water-supply, 5 multi-purpose); 2. Shallow reservoirs on lower altitudes (9 multi-purpose); 3. Shallow reservoirs on low altitudes with a high average annual flow rate and very low average water retention time (2 multi-purpose reservoirs). For the first two listed groups, the maximum ecological potential was derived from NSI values of the water-supply reservoirs.

This is the first attempt in Slovakia to utilize chironomid exuviae in the practical application for ecological potential assessment in accordance with EU legislation within the Water Framework Directive.

Can cyanotoxins inhibit the development of benthic organisms? Influence of Microcystin-LR (MC-LR) on *Chironomus riparius* larvae

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Cyanobacteria produce a wide range of secondary metabolites, many of which are toxic, such as microcystin-LR (MC-LR), the most toxic and widespread variant of cyanotoxins. The toxic effect of MC-LR on aquatic species has been documented, but there is insufficient understanding regarding the impact of MC-LR on *Chironomid larvae*, which have an important role in freshwater food chains. This study investigated the acute toxicity of several concentrations of MC-LR (5, 10, 15, 20, 30 µg L^{-1}) on the non-biting midge *Chironomus riparius* larvae (Diptera, Chironomidae). Mortality, hemoglobin concentration, parameters of oxidative stress (advanced oxidation protein products (AOPP), malondialdehyde (MDA), catalase (CAT), and superoxide dismutase (SOD) activity), and DNA damage were investigated. The results showed that no concentration of the toxin caused significant mortality. However, the level of DNA damage was significantly higher in larvae treated with all concentrations of MC-LR compared to control, where the degree of damage increased proportionally with concentration (ANOVA and Post hoc LSD test; $p < 0.05$). Hemoglobin concentration was reduced in larvae of all treatment groups, inversely proportional to the toxin concentration. The highest toxin concentration significantly reduced the CAT activity of the treated larvae, and the levels of AOPP significantly increased when exposed to 10, 15, and 20 µg L^{-1} MC-LR concentrations.

The findings underscore the significant impact of MC-LR on *Chironomid larvae*, as evidenced by DNA damage, reduced hemoglobin concentration, and altered oxidative stress parameters. These results emphasize the need for further research to fully comprehend the ecological implications of cyanobacterial toxins on freshwater ecosystems.

Late-Glacial to Holocene transition in the Western Carpathians (central Europe) evidenced in the subfossil chironomid record from an alpine lake

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We analysed subfossil chironomids from a sedimentary record of an alpine lake in the Tatra Mountains (Nižné Temnosmrečinské pleso, 1674 m a.s.l.) with an aim to reconstruct past environmental changes during the Late-Glacial/Holocene transition. The low organic matter content and low chironomid abundances in the sediments before ~14,950 cal yr BP suggest oligotrophic conditions with limited aquatic productivity due to extreme climatic conditions. The assemblage structure represented by both cold-adapted and rheophilic taxa (*Diamesa, Pseudodiamesa*, *Micropsectra radialis*-type and *Pseudokiefferiella parva*) indicates that the lake ecosystem was strongly affected by an intensive inflow of cold glacial meltwater. The significant change at ~13,700 cal yr BP is indicated by the replacement of cold-adapted taxa by taxa with wider thermal tolerance, such as *Procladius* and *Tanytarsus lugens*-type. This change could be attributed to the transition between the cold Oldest Dryas and warm Bölling period. The short-lasting cooling during the Older Dryas, not always clearly visible in the stratigraphic diagrams, could be indicated by the rapid increase and dominance of the cold stenothermal *Pseudodiamesa* (~13,200 cal yr BP). The chironomid shift after ~12,000 cal yr BP can be associated with climate warming on the onset of the Holocene. The gradual disappearance of cold-adapted taxa is connected to decreasing influence of meltwater from mountain glacier and/or snowfields. The dramatic increase of organic matter content suggests increased lake productivity, reflected by significant structural changes and newly appeared taxa inhabiting a wide range of habitats.

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Filling the gap in the Carpathian paleolimnology: Subfossil chironomids and cladocerans from sediments of alpine lakes in the Eastern Carpathians (Ukraine) as indicators of environmental change over the past 200 years

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One of the most understudied parts of the Carpathians, when it comes to paleolimnological research, is its Ukrainian part. Thus, we analyzed short cores (20–40 cm) from three alpine lakes in the Eastern Carpathians (Ukraine) to reconstruct environmental changes using subfossil chironomids and cladocerans. The studied lakes, Brebenescul, Nesamovyte and Dohyaska are situated above local treeline (1580–1800 m a.s.l.) in the Chornohora and Svydovets massifs, the highest parts of the Ukrainian Carpathians. These mountains are a good example of an area that is currently experiencing intense degradation, mainly uncontrolled tourism. The combination of ^{210}Pb and ^{137}Cs dating approved that we captured an approx. 200-year history, which gives us an opportunity to study human impact on the lake ecosystem. The subfossil assemblages were relatively stable and taxonomic diversity low throughout the observed period. Cluster analysis identified two zones with slightly different assemblage structure. The lower layers show a more oligotrophic lake conditions. The most abundant taxon in Brebenescul and Nesamovyte was the cold-stenotherm *Tanytarsus lugens*-type, and in lake Dohyaska, *Tanytarsus mendax*-type and *Chydorus sphaericus* (Cladocera), which tolerate relatively warmer conditions. The taxonomic changes indicate climate warming and the expansion of aquatic vegetation. Vegetation growth could be promoted by warming, but also by the increased supply of nutrients to the lake due to soil erosion connected to the ever-increasing impact of human activity.

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Co-occurrence among Chironomini and Orthocladiinae species – a case from temperate zone rivers

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Exploring interactions within assemblages of benthic organisms is crucial to maintaining ecosystems, understanding their complexity, and outlining potential directions for their future development. The main aim of our study was to indicate and interpret the co-occurrence patterns within two dominant chironomid taxa, the Chironomini and Orthocladiinae. The co-occurrence of species of each pair was assessed using the pairwise probabilistic model for species co-occurrence, while the null model of co-occurrence was applied to test if a taxon was competitively structured. Benthic samples were collected once a month for over a year from seven lowland rivers of Central Poland. On each sampling occasion, values of several environmental variables were measured in the field, including velocity, river width, river depth, water temperature, dissolved oxygen, bottom substrate covered by submersed aquatic macrophytes, while inorganic substrate index (SI), and the amount of organic and inorganic matter were assessed in the laboratory. The results of our analyses showed that the co-occurrence patterns differed between the Chironomini and Orthocladiinae, despite randomly and non-randomly associated pairs being observed in both taxa. Positively associated pairs were numerous within the Chironomini and Orthocladiinae, but negatively associated pairs appeared only in the Chironomini.

This indicated a competitive structure of the Chironomini, which was confirmed by the null model of co-occurrence, and its considerable control by biotic deterministic processes. In turn, Orthocladiinae seemed to be under a stronger influence of abiotic factors. This coincides with the statement that the Chironomini is usually considered a more eurytopic taxon, while the Orthocladiinae is a more specialized one.

A preliminary assessment of Chironomidae diversity in tufa-depositing environments in Serbia, Central Balkans

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In this work, we examined the Chironomidae community in six tufa barrier habitats in Serbia. The karst regions of Serbia are situated on the periphery of the large calcareous massifs of the Dinaric Alps and Carpathian-Balkan Mountains, covering approximately 10% of the area. Tufa formations often create unique habitats within karst freshwater environments, providing surfaces for colonization by different insect larvae. Chironomidae larvae play a significant role in the ecological processes of the tufa environments as they inhabit crevices, pores, and surfaces covered in algae and macrophytes (Dorić et al., 2024). During the single-time study, 332 individuals and 29 taxa were recorded. Twenty taxa were identified at the species level, while nine were identified at the genus level. Orthocladiinae was the most diverse subfamily, represented by 17 taxa (60 % of all individuals), while the other subgroups were less represented; Diamesinae (four taxa), Tanytarsini and Tanypodinae (both with three taxa each), and Chironomini (two taxa). The most abundant taxa were *Parametriocnemus stylatus*, *Conchapelopia* sp., and *Micropsectra* sp. Moreover, based on their feeding habits, the taxa were dominated by gatherers (47.8 %), followed by grazers (28.6 %) and predators (20.5 %).

This study may be essential to fill the knowledge gap regarding the freshwater insect diversity in the karst Balkans region. Moreover, the diversity of chironomid communities can provide valuable insights into the ecological health and integrity of these distinctive and highly vulnerable habitats.

Optimizing Laboratory Substrates for Ecotoxicological Assessments of Chironomidae: A Focus on Substrate Composition and Larval Morphology

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Non-biting midges (Chironomidae) are vital bioindicators sensitive to environmental changes. This study investigates how substrate composition affects larval performance, adapting OECD-recommended substrates for ecotoxicity studies focused on mentum deformities as biomarkers. To evaluate the impact of substrate composition on larval Chironomidae, our methodology involved controlled laboratory experiments using five different substrate formulations. These included the standard OECD substrate mix of 75% sand, 20% clay, and 5% peat, as well as variations that either incorporated medical clay or omitted one of the standard components such as sand, clay, or peat. Each treatment was replicated across multiple test samples to ensure statistical validity. Survival rates and wet mass were recorded, and larval mentums were examined at the end of the growth period. Geometric morphometrics was employed on photomicrographs of 178 specimens to analyze morphological variations and deformities in the mentums. Results indicated that substrates with sand and peat, and those with coarse sand alone, had the highest survival rates of 85% and 82%, respectively, proving suitable for ecotoxicological testing. Larvae on these substrates showed mentums close to the "average" form, appropriate for detailed analysis. In contrast, substrates like P+T+MG and G+P, which caused the most significant mentum deviations, were unsuitable fortests tracking mentum changes.

In conclusion, selecting substrates close to standard formulations is crucial for maintaining larval health and ensuring reliable morphological assessments in ecotoxicological studies. This approach enhances bioassay accuracy and aids precise environmental impact evaluations, particularly when mentum deformations are used as a biomarker.

Diversity of Chironomidae fauna in a unique hot-spot – the case of Skadar Lake basin springs

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In the present study, using non-biting midges (Diptera: Chironomidae) as flagship taxa of freshwater ecology, we are focussing on the interesting model represented by the area of the Skadar Lake system (Montenegro and Albania), a well-known hotspot of freshwater biodiversity composed by the young Skadar lake (originated 1200 before present) and by its old system of springs (originated during Pliocene). In this study, we present the results of a study aiming to investigate the diversity of Chironomidae fauna of the springs in the Skadar Lake basin. During field campaigns, 1387 specimens representing 171 BINs were collected from 40 springs. 104 of them (61%) are BINs of rare species represented by one, two, or three specimens per BIN. 148 BINs occur on one or two sites. The most frequent recorded BINs (23%) were identified using the BOLD Taxonomy Engine such as *Prodiamesa olivacea* (BOLD: AAD7458) and *Cricotopus sylvestris / Cricotopus glacialis* (BOLD: AWE262336). The development of an accurate reference library of cytochrome oxidase subunit 1 (cox1) gene sequences for midges inhabiting the Skadar Lake system will be of great interest not only for basic science but also for the aim of conservation purposes.
Our investigation has proven the strong need to develop a reference library based on reliable taxonomic identifications.


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