

# 18th International Symposium on Chironomidae

Trondheim Norway 4-6 July 2011



## Scientific Program and Abstracts

 NTNU

Museum of Natural History  
and Archaeology

## Acknowledgements

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## **Index**

<b>Acknowledgements.....</b>	<b>2</b>
<b>Welcome to Trondheim!.....</b>	<b>4</b>
<b>General program.....</b>	<b>5</b>
Monday, July 4.....	5
Tuesday, July 5.....	6
Wednesday, July 6.....	7
<b>Abstracts.....</b>	<b>9</b>
<b>List of participants.....</b>	<b>52</b>

## ***Welcome to Trondheim!***

Dear all participants and accompanying persons

It is a great honour for us to welcome you to Trondheim and to the 18th International Symposium on Chironomidae. As hosts for the meeting, the Symposium Committee and the NTNU Museum of Natural History and Archaeology have done their best to make this meeting a memorable one and we hope that you will have a pleasant stay in Trondheim.

The international symposia on Chironomidae have traditionally brought together scientists from a wide range of research areas. This year's conference is no exception and we have contributions in taxonomy, systematics, ecology, palaeolimnology, cytology, genetics and toxicology. More than 80 participants from 36 countries attend the meeting and we are proud to see that both students and senior scientists are well represented. Hopefully, you will find the presentations and discussions inspiring for future work on Chironomidae!

The Symposium Committee

*Kaare Aagaard*

Kaare Aagaard

*Elisabeth Stur*

Elisabeth Stur

*Torbjørn Ekrem*

Torbjørn Ekrem



## General program

### Monday July 4

- 09:00-10:30 Registration at conference venue
- 10:30-10:45 Welcome
- 10:45-11:00 Official opening of the 18th International Symposium on Chironomidae by Museum Director Prof. Axel Christophersen
- 11:00-12:00 **The Honorary Thienemann-lecture: Oliver Heiri**  
*Traces of past environments in the chemical composition of chironomid remains: stable isotopes in chironomid palaeoecology.*
- 12:00-13:30 Lunch at Kjelhuset
- 13:30-14:15 **Palaeolimnology. Chair: Ian Walker**  
Andrew S. Medeiros: *A Chironomidae-based stratigraphic record of pronounced 20th century warming in a large, deep Canadian Arctic lake.*  
Victor Frossard: *Chironomid-based reconstruction of the trophic history of Lake Annecy during the last 150 years using an intralake multiple core approach.*  
Gürçay Kıvanç Akyıldız: *Preliminary results on subfossil chironomid training set from Turkey.*  
Gaute Velle: *Chironomid as Palaeoenvironmental proxy: where, when, and which environmental variables can be inferred?*
- 14:15-14:45 Coffee Break
- 14:45-15:15 **Palaeolimnology. Chair: Ian Walker**  
Jos Schilder: *Within-lake productivity affects the role of methane as a carbon source for benthic and planktonic food webs in Lake De Waay, The Netherlands.*  
Roberto Quinlan: *The ecology of subfossil Chironomidae and Chaoboridae assemblages from southern Canadian lakes.*
- 15:15-17:00 Poster set-up and session
- 17:15 Group photo in front of university main building
- 19:45 Reception at the Nidarosdomen cathedral

## **Tuesday July 5**

- 09:00-10:15    **Taxonomy and systematics. Chair: Endre Willassen**  
Susan Gresens: *"Where to draw the line?" Phenotypic variation within the Cricotopus sylvestris species group, across a nearctic-palearctic gradient.*  
Andrey B. Krasheninnikov: *New data on chironomids in the Middle Urals.*  
Andrey Przhiboro: *The Chironomidae collection of the Zoological Institute (St. Petersburg): history, current state and role for further research.*  
Masaru Yamamoto: *A review of the genus Tudayusurika from Japan (Chironomidae: Orthoclaadiinae).*  
Viktor A. Baranov: *"Enigmatic" chironomids (Chironomidae; Diptera) of Ukraine.*
- 10:15-10:45    Coffee Break
- 10:45-12:00    **Taxonomy and systematics. Chair: Endre Willassen**  
Peter S. Cranston: *A molecular phylogeny for the Chironomidae.*  
Alyssa M. Anderson: *Strength of combined forces: molecular and morphological methods reveal cryptic diversity and three new species of nearctic Micropsectra.*  
Larissa Gunderina: *DNA markers for the identification of species of the genus Chironomus.*  
Joel Moubayed-Breil: *New species of Paratanytarsus Thienemann & Bause 1913 (Diptera: Chironomidae) from the Mediterranean region (Corsica, southern France and Lebanon).*  
Patrick Ashe: *Additions and corrections to Part 1 of 'A World Catalogue of Chironomidae (Diptera)'.*
- 12:00-13:30    Lunch at Kjelhuset
- 13:30-15:00    **Ecology and biomonitoring. Chair: Susan Gresens**  
Bruno Rossaro: *Benthic quality index in European lakes.*  
Naime Arslan: *Distribution and diversity of littoral Chironomidae fauna of Lake Sapanca (Turkey) in comparison with environmental parameters.*  
Vít Syrovátka: *The response of chironomid assemblages to mineral richness gradient in the Western Carpathian spring fens.*  
Brigitte Lods-Crozet: *Biodiversity of Chironomidae (Diptera) in high alpine ponds in the Swiss National Park (Switzerland).*  
Christopher E. Luszczek: *The Chironomidae of Nunavut; their biogeographical distribution and limnological niches.*  
Les Ruse: *Trait-based surveillance of flood channel effects on the River Thames.*
- 15:00-17:00    Poster session (w/ Coffee)
- 18:30            Bus for Conference Dinner leaves in front of the Cathedral (see map)
- 19:00            Conference dinner at Ringve Botanical Garden

## **Wednesday July 6**

### **09:00-10:15 Ecology and biomonitoring. Chair: Les Ruse**

Valentine Cartier: *How salinity affects development of Chironomus salinarius (KIEFFER): an experimental study.*

Elísabet R. Hannesdóttir: *Temperature related differences in community structure and life-cycles of Chironomidae in streams in geothermal area, Hengill, in Iceland.*

Leonard C. Ferrington Jr.: *Cold tolerance, longevity and oviposition by Diamasa mendotae (Mutkowski).*

Ian Kenneth Grant Boothroyd: *Chironomidae (Diptera: Insecta) and algal diversity and colonisation in a geothermally-influenced stream in New Zealand.*

Pragati Bhosale: *Larval Chironomids (Chironomidae: Diptera) from fresh water ecosystems of Balaghat in Marathwada region, (M.S.) India.*

Barbara Hayford: *Rare Chironomidae in Mongolia.*

### **10:15-10:30 Coffee Break**

### **10:30-12:00 Ecology and biomonitoring. Chair: Les Ruse**

Peter H. Langton: *Chironomidae collected in lakes of Ellesmere Island in 1991 by Dr. Kate Sylvester: a base line data set for the monitoring of global warming in the arctic.*

Malka Halpern: *Endogenic bacteria facilitate chironomids survival in polluted habitats.*

Amit Lerner: *Reflected polarization, egg density, and habitat availability guide chironomid females to oviposition sites.*

Samantha J. Hughes: *Bioassessment of Portuguese reservoirs using chironomid pupal exuviae: preliminary results*

Tae-Soo Chon: *Species abundance distribution of benthic chironomids in streams and lakes.*

Martin Spies: *Chironomidae types at the Linnean Society, London.*

### **12:00-13:30 Lunch at Kjelhuset**

### **13:30-14:30 Toxicology, cytology and genetics. Chair: Paraskeva Michailova**

Tobias S. Kaiser: *Genetic architecture of lunar and circadian emergence times in the marine midge Clunio marinus (Diptera: Chironomidae).*

José-Luis Martínez-Guitarte: *Analysis of stress response and endocrine function in Chironomus riparius following exposure to putative endocrine-disrupting compounds.*

Takashi Okuda: *A new insight on the desiccation tolerance mechanism in the sleeping chironomid, Polypedilum vanderplanki.*

Bimalendu B. Nath: *Experimental insights into the salient features of gamma radiation tolerance of Chironomus ramosus.*

### **14:30-15:00 Coffee Break**

15:00-15:45	<b>Toxicology, cytology and genetics. Chair: Paraskeva Michailova</b> Salman A. Al-Shami: <i>Detection of DNA damage in Chironomus kiiensis Tokunaga after exposure to sediments from different polluted rivers in Malaysia.</i> Veronika V. Golygina: <i>Study of molecular and cytological structure of centromeric regions in Chironomidae.</i> Paraskeva Michailova: <i>Polytene chromosomes of Chironomidae (Diptera) as a bioassay of trace-metal-induced genome instability.</i>
15:45-16:00	Break
16:00-17:00	<b>Chironomidae Symposium Forum. Chair: Patrick Ashe</b> In memoriam Publication of Symposium Proceedings Vote and announcement of host for the 19 <sup>th</sup> International Symposium on Chironomidae
Ca. 17:00	Closure
Ca. 18:00	Social event at Lillegården, Tyholtveien 2 (see map)



## **Abstracts**

All abstracts are listed in alphabetical order on the family name of the first author. For papers that are presented orally, the name of the presenting author is underlined. Papers that are presented as posters are marked with (P)

The abstracts are not to be regarded as published in the definition of the International Code of Zoological Nomenclature.

## Preliminary results on subfossil chironomid training set from Turkey

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Subfossil remains of freshwater midges (Diptera) are being increasingly valued as indicators of limnological and climatic changes. In order to use subfossil chironomids as indicators of ecological changes, preparing a training set of that region is required. However, this required data set is already missing for Turkey. Our goal is to provide multi proxies from approximately 50 shallow lakes, which were chosen from different regions and different altitudes in Turkey. Up to now, six shallow lakes were collected through our project which was started at the end of 2010. Altitude varies between 2037 m and 5 m among the lakes. Gravity corer was used to take surface samples in the deepest location for contemporary training set. Cores of sediment were also extruded and sampled in the field at 0.5 cm slices. 55 subfossil chironomid taxa were determined from within 290 head capsules. *Apedilum* (Townes, 1945) genus was identified as new and the larva of *Tvetenia bavarica* type was identified as first for the Turkish fauna. The most abundant species identified were *Cricotopus sylvestris* type, *Psectrocladius sordidellus* type, *P. vernalis* type, *Tanytarsus mendax* type, *Polypedilum nubifer* type and *Halocladius fucicola* type. Other typical parameters to consider such as water chemistry, water properties; physical and sedimentary properties were also taken for the transfer function and statistical analysis.

## Detection of DNA damage in *Chironomus kiiensis* Tokunaga after exposure to sediments from different polluted rivers in Malaysia

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Rapid industrialization and urbanization has resulted in an increased input of chemical contaminants into aquatic environment in Malaysia, at time when no adequate methods for ecotoxicological monitoring are available. To overcome this impediment, here, we examined lethal and genotoxic effects of sediments from different rivers of the northern Malaysia against *Chironomus kiiensis*. Fourth instar larvae were exposed to sediments from Selama River (SR), Permatang Rawa River (PRR) and Kilang Ubi River (KUR) at various durations (6, 12, 24 and 48 hrs). The DNA damage in larval cells was detected using the alkaline single cell electrophoresis technique. Pollution level indexed by the levels of physico-chemical parameters in the water and sediment showed progressive increase from SR to PRR to KUR. Variations in the pollution levels could be explained by increasing quantities of polluting elements received among the three rivers. DNA analyses revealed greater damages in cells derived from larvae maintained on polluted sediments, in particular those from KUR. The effects on the genomic material of *C. kiiensis* larvae occurred in a time-dependent manner, with damage level increasing as exposure time progressed. Our results highlight the genotoxic properties of polluted sediments, more importantly; this study showed that *C. kiiensis* larvae could respond to different level of pollution with respect to exposure time.

## Efficiency of sorting Chironomidae surface floating pupal exuviae samples from urban trout streams in Northeast Minnesota, USA

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Collections of Chironomidae surface-floating pupal exuviae (SFPE) provide an effective means of assessing water quality in streams. Although not widely used in the United States, the technique is not new and has been shown to be more cost-efficient than traditional dip-net sampling techniques in organically enriched streams. The intent of this research was to document the efficiency of sorting SFPE samples relative to dip-net samples in trout streams with catchments varying in amount of impervious surface. Samples of SFPE were collected from 17 trout streams in Duluth, MN, USA; dip-net samples of the entire macroinvertebrate community were also collected from these streams. We quantified time needed to sort subsamples of 100 macroinvertebrates and SFPE; time to subsample up to 300 SFPE was also recorded. The average time to sort subsamples of 100 specimens was 22.5 minutes for SFPE samples, compared to 32.7 minutes for 100 macroinvertebrates in dip-net samples. Average time to sort up to 300 SFPE was 37.7 minutes. These results indicate that sorting SFPE samples is more time-efficient than traditional dip-net techniques in trout streams with varying catchment characteristics.

## Strength of combined forces: molecular and morphological methods reveal cryptic diversity and three new species of nearctic *Micropsectra*

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*Micropsectra* is a particularly species-rich genus within the Chironomidae, with representatives found in a variety of freshwater habitats throughout the Nearctic and Palearctic regions. Taxonomic research of Nearctic *Micropsectra*, however, is less advanced than that of Palearctic species, often making species-level identification difficult. Furthermore, while some *Micropsectra* species are thought to be Holarctic in distribution, close examination of Palearctic and Nearctic populations can reveal genetic and morphological differences large enough to be regarded as diagnostic on the species-level. Thus, the intent of this study is to use genetic and morphological characters to help resolve these taxonomic issues and provide a framework for better understanding of North American *Micropsectra*. Larval specimens of *Micropsectra* were collected from six streams throughout Minnesota, U.S.A. during late winter/early spring of 2010 and reared to adulthood, resulting in a total of 100 adult specimens and associated pupal exuviae. The head, wings, hypopygium, and exuviae of all specimens were slide-mounted directly, while DNA was extracted from the thorax and abdomen before mounting. Specimens were identified to species group level. A subset of 48 specimens, with several representatives of each morphological species, was selected for DNA extraction, PCR amplification, and sequencing of mitochondrial cytochrome oxidase I (COI) and nuclear CAD genes. Sequences from these specimens were compared to previously published *Micropsectra* sequences. Our results indicate that we have found three previously undescribed species and one additional species new to the north-central United States. Two of the new species found in this research initially appeared morphologically identical to species known from the Palearctic, however our molecular data indicated that they are genetically distinct. Careful reexamination of adult and pupal morphology revealed slight but consistent diagnostic differences. These results emphasize the importance of using molecular tools in conjunction with traditional morphological techniques when studying Chironomidae diversity, especially when relying on diagnoses from other regions.

# Mouthpart deformities in *Chironomus (Camptochironomus) tentans* and their relevance to habitat characteristics

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A shallow, eutroph and metal contaminated lake [one of the Ramsar sites in Turkey: Lake Uluabat] was investigated monthly from August 2004 to July 2005 for deformities (mentum, antenna, mandible, and epipharyngis) of *Chironomus (Camptochironomus) tentans* larvae. 1800 chironomid larvae were examined and Lake Uluabat was found to contain twelve taxa, dominated by *Chironomus (Camptochironomus) tentans* Fabricius 1805. A total of 327 *C. (C.) tentans* were examined, 55,04% of which possessed deformities. All samples of *C. (C.) tentans*, collected from two stations where water circulation is reduced, showed the highest incidence of deformities throughout the study. Deformities were found in all mouthparts in *C. (C.) tentans* but mentum and epipharyngis deformities were the most frequent. At each sample location, in situ measurements of water (depth, pH, dissolved oxygen and temperature) were taken. In addition, concentrations of eight metals (Cd, Cr, Pb, Cu, Ni, Fe, Zn and Ag) were examined monthly in lake water (for all samples at each sampling station, n = 9) and sediment (n = 9). Analyses of sediment and water from Lake Uluabat indicate the presence of metal pollutants such as nickel, zinc, copper and lead. The relationship between deformity and water-sediment toxicity relationship was analyzed by Correspondence Analysis. According to our results, the total deformity rate strongly correlated with high contents of Ni, Zn, Cd and Pb in sediment and pH, PO<sub>4</sub> in water. The mentum deformities positively correlated with Ag and Zn in sediment, SO<sub>4</sub> and COD in water and negatively correlated with Cd in water and Cu in sediment. Antenna deformities positively correlated with Cr in sediment and COD, NO<sub>3</sub>-N, SO<sub>4</sub> in water. Epipharyngis deformities positively correlated with Zn in water and with Ag in sediment.

## Distribution and diversity of littoral Chironomidae fauna of Lake Sapanca (Turkey) in comparison with environmental parameters

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Lake Sapanca is located in the Marmara region at an elevation of 30 m above sea level and is the second largest lake in the region. The surface area is 46.8 km<sup>2</sup> and maximum depth is 55 meters. The numerical and proportional distributions of benthic invertebrates in Lake Sapanca were surveyed at six different stations. Also some physicochemical parameters and some microbiological parameters of the water were analyzed. According to the results benthic invertebrate fauna consisted of Oligochaeta (42,5 %), Chironomidae larvae (30,5 %) and the varia (27 % [Nematoda, Gastropoda, Bivalvia, Amphipoda, Isopoda, Ostracoda, Plecoptera, Ephemeroptera, Trichoptera, Hemiptera, Coleoptera, Ceratopogoniidae, Culicidae, Tabanidae and Chaoboridae]). The littoral chironomid fauna of the lake consists of a total of 20 species, 3 of which belong to Tanypodinae, 1 to Prodiamesinae, 4 to Orthocladinae, 10 to Chironomini and 2 to Tanytarsini. Shannon-Wiener index results showed that Lake Sapanca had an index of 2,32 richness at 2nd station had the widest diversity; while 4th station had the poorest. According to Bray-Curtis similarity index, the 5th and the 6th stations were found to be very similar to each other; while 1st and 2nd stations were observed to be the most different from all of the other stations in terms of the not only dynamics of the benthic fauna but also chironomid fauna. The relationships between the dynamics of organisms and environmental parameters were supported by Pearson Correlation Index and Canonical Correspond Analysis.

# Additions and corrections to Part 1 of 'A World Catalogue of Chironomidae (Diptera)'

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An update is provided of the more significant changes affecting Part 1 of 'A World Catalogue of Chironomidae (Diptera)'. These include new taxa, new combinations and new synonymies that have been proposed since the publication of the Catalogue in December 2009.

## Chironomidae (Diptera) in the fauna of Ukraine

Viktor A. Baranov



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A complete inventory of data on Chironomidae (Diptera) up to 2010 is summarized, with the distribution of the certain species in the Ukraine. The total number of species recorded from Ukraine is 302. They belong to six subfamilies (Chironominae - 132, Tanypodinae - 27, Telmatogetoninae - 1, Prodiamesinae - 4, Diamesinae - 7, Orthocladiinae - 91). Other recorded names are either nomina dubia or Chironomidae incertae sedis. Subfamily Podonominae have not been recorded yet, but is possible for Carpathian Mountains. Most of these records are originated from hydrobiological papers of 1960–1970s rather than taxonomical or faunistic; some of them are probably based on unreliable identifications based on immature stages alone, and the whole list must be considered with precaution. The distribution of this fauna over the different areas of Ukraine is distinct. More recent collections from various rivers of Carpathian Mountains, Bucovina and other parts of the Danube basin in Ukraine have now increased number of Chironomidae species recorded this area up to 206. The majority are Orthocladiinae, Chironominae (Tanytarsini) and Diamesinae. Total number of Chironomidae species in Dnieper basin is 102, and in Severskiy Donets is 64. Chironomid fauna of Crimea Mountains is quite poor: only 26 species have been recorded. 21 species were recorded for the first time in Ukraine, 9 of them in cold springs of wood and steppe. In general, the Ukrainian chironomid fauna can be considered as rich and diversified in relation with a large diversified habitats and biotopes.

## "Enigmatic" chironomids (Chironomidae; Diptera) of Ukraine

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Most of the Chironomidae species described from Ukraine was based on the larval stages. Some species were described without establishing the types and compliance with the ICZN. Particularly interesting are two species of Orthocladiinae described by Dr. V. Polishchuk. The types for them have not been designated at all, but descriptions have some important details that allow identification of these species. The first of them, *Smittia hiberna* Polyszczuk, 1963, possesses the following combination of characters: S1 and S2 both bifid, the absence of the procercus, presence of two pairs of the ventromental plates, resembling species of the genus *Pseudosmittia*. All specimens were found in the Desna River

basin. The second species, *Limnophyes l. hiberna* Polyszczuk, 1972, was described based on the larva, which was found in a pool of melted water in Odessa Region. Description and figure indicate that the specimen belongs either to *Bryophaenocladus* or *Gymnometriocnemus*. However, the absence of the bifurcation at the posterior parapods indicates that it is rather a species of *Bryophaenocladus*. Study of the chironomids fauna in the basin of the Desna River, Seversky Donets River and the Black Sea Region allows to lift the veil of secrecy over these two species. 34 larvae and one male *Pseudosmittia virgo* Strenzke, 1950 was collected in the tract Gorelaya Dolina (Kharkiv Region), and in the mosses from the city park in Sevastopol, we found three larvae of *Bryophaenocladus furcatus* (Brundin, 1947). These species, based on the author's descriptions were identified by us, respectively, as *S. hiberna* and *L. l. hiberna*. However, without detection of these species in the type localities, and the association of larvae with adults (or pupae) we cannot synonymize them definitely. In the latter case, for both of these species we need to designate neotypes.

## Environmental change in Vindelfjällen, northern Sweden, during the last 5000 years

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Despite the numerous findings of pre-historic human occupation of Vindelfjällen, SW Swedish Lapland, no palaeoclimatic studies from the time of human occupation have been conducted in this part of the Scandes Mountains. We use biological and geochemical proxies in lake sediments to reveal past environmental change in this area. Results will elucidate the relationships between climate change, natural resources and human activity. We selected Vuoksjavratje, (850 m a.s.l.), a typical small (area 5.9 ha, maximum depth 11m) Swedish low alpine lake, for this study. The studied sediment sequence encompass the last ~5200 yrs based on a chronology of eleven <sup>14</sup>C AMS radiocarbon dates on terrestrial macrofossils. We also retrieved undisturbed surface sediments and dated these from <sup>210</sup>Pb. Preliminary results show variable chironomid head capsule (hc) concentrations ranging from 3 to 90 hc/cm<sup>3</sup> sediment. Sedimentation rate varies from ~0.33 to 2 mm/yr. Thus the variability in head capsule concentration is partly due to variations in the sedimentation rate, but most likely also to a changing environment. Based on the down core chironomid composition and a Norwegian chironomid-air temperature training set, Holocene July temperatures have been reconstructed. In the uppermost part of the surface core, *Constempellina* – *Thienemanniola*-type is common (up to 10 %). This type is not present in the modern training set. Despite this, the reconstructed July temperature deviates only by 0.26 °C compared to meteorological data for the reference period 1961-1990. The presence of chironomid types typical of running water and terrestrial origin indicate periods of increased runoff and surface erosion in the catchment. Elemental data from X-ray fluorescence core scanning confirm periods with increased catchment erosion through larger input of minerogenic material and an elemental composition indicating increased grain size.

## A new species of *Tanytarsus* v. d. Wulp from geothermal waters of New Zealand

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The range and diversity of geothermal features occurring at the earth's surface are a characteristic of New Zealand. Despite the significance of these geothermal areas, descriptions of the flora and fauna of these geothermal areas has been sporadic and much remains to be learnt. A species of *Tanytarsus*

has been known to occur in geothermal areas of the South Island for some years. Recent collections from geothermal areas of the North Island has revealed the same species occurs at a range of locations, where it is often the dominant benthic dwelling invertebrate, and the only chironomid present. This paper describes the main features of the larva, pupa and adult male of the new species and also describes the characteristics of the geothermal habitats in which it occurs.

## **Chironomidae (Diptera: Insecta) and algal diversity and colonisation in a geothermally-influenced stream in New Zealand**

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New Zealand has a range of geothermally-influenced ecosystems with distinctive ecological features and biotic communities. In geothermally-influenced streams, distinctions in aquatic flora and fauna occur longitudinally downstream from the source of thermal springs. Amongst the more prominent features of aquatic geothermal ecosystems are members of the dipteran family, particularly Ephydriidae and Chironomidae. Despite the significance of geothermal areas within New Zealand, studies of the ecology of these extreme environments have been sporadic. However, recent studies of geothermal ecosystems in New Zealand have determined the diversity and characteristics of aquatic geothermal ecosystems. In this paper the chironomid communities of several geothermal ecosystems and are described, and some of the environmental factors influencing their distribution are examined. A study of the colonisation of a currently undescribed obligate geothermal chironomid '*Tanytarsus* sp. a' in the Hot Stream at Waimangu geothermal system is described. Colonisation was assessed using submerged ceramic tiles left in situ in the Hot Stream for up to 85 days. *Tanytarsus* sp. a occurred in densities up to 91,000 m<sup>2</sup> and colonisation of the tiles was immediate, with densities of up to 26,000 m<sup>2</sup> present after 48 hours. Algal communities were dominated by fine filamentous cyanobacteria, *Phormidium*, the diatom *Achanthes*, and green algae *Stigeoclonium* and *Spirogyra*.

## **How salinity affects development of *Chironomus salinarius* (KIEFFER): an experimental study**

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*Chironomus salinarius* KIEFFER is a common species of brackish environments such as coastal lagoons. Coastal lagoons are characterized by a high temporal variability of environmental factors, particularly temperature and salinity. This indicates that *C. salinarius* can tolerate important variations of these factors. We examined the effect of salinity on survival and time of development of *C. salinarius*, in order to determine its environmental tolerances and preferences. Levels of salinities between 0 and 50 were tested. Too few individuals survive in environments with salinity between 40 and 50. The survival was optimal for low salinities ( $\leq 5$ ) and high salinities ( $\geq 20$ ), and weak at intermediate levels. The time of development increased with the increase of salinity levels. These results suggest the existence of two physiological strategies, depending on the salinity level. The alternation between these two strategies is highlighted at intermediate levels of salinity with a lower survival rate.

# Chironomid larvae assemblages' structure in association with two submerged macrophyte species

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Spatial and temporal trends of a phytophylous chironomid community on the submerged plant species *Myriophyllum spicatum* L. and *Ceratophyllum demersum* L. were studied at three sites in a floodplain lake (Lake Sakadaš, Kopački Rit Nature Park, Croatia) during summer 2004. Analysis of functional feeding groups and the influence of environmental parameters on Chironomidae larvae assemblages were also conducted. Chironomid community was very abundant and the recorded larvae belonged to three subfamilies: Chironominae (Chironomini and Tanytarsini), Orthocladiinae and Tanypodinae. In both species stands larvae of *Cricotopus sylvestris* gr. and *Endochironomus albipennis* type dominated. However, the total abundance of *E. albipennis* type was seven times greater in association with *C. demersum* and *C. sylvestris* gr. had abundance 1.5 times greater in *M. spicatum* stands. Other representatives of Chironominae were more abundant in *C. demersum* stands, such as *Paratanytarsus* sp., *Glyptotendipes pallens* type, *Polypedilum sordens* type and *Parachironomus varus* type. Most larvae in the community on *M. spicatum* belonged to grazers while active filterers and grazers were most abundant in *C. demersum* stands. ANOSIM indicated statistically important differences between July and September based on the chironomid abundance, and there were no differences between the sampling stations. According to BIO-ENV analysis, environmental variables with highest influences on chironomid community structure, for both plant species, were: water temperature, depth and oxygen concentration, with Secchi depth and chlorophyll a concentration in a lesser extent.

## Species abundance distribution of benthic chironomids in streams and lakes

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Benthic macroinvertebrate communities have been considered as an efficient indicator group for presenting ecological integrity in aquatic ecosystems. Especially chironomids are diverse and occupy a unique position regarding maintenance of biodiversity and reflection of environmental impact. Chironomid assemblages were collected from 7 streams and 18 lakes across different levels of disturbance in Korea. Structural properties residing in chironomid communities were analyzed by species abundance distribution. Rank-abundance models were evaluated according to abundance pattern (e.g., geometric series, log-series, lognormal distribution) and niche apportionment (e.g., dominance pre-emption, random assortment, random fraction, dominance decay). The log normal distribution was accepted frequently in chironomid communities for both lakes and streams, while geometric and log series appeared to be dependent upon environmental impact pertaining to the sampling sites. The dominance decay model seemed to be more fitted to chironomid communities in clean conditions, while random assortment and dominance pre-emption models were more suitable in presenting communities at the disturbed sites for both streams and lakes. Characterization of communities in streams and lakes were further discussed in response to disturbance.



# A molecular phylogeny for the Chironomidae

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A first highly sampled phylogeny estimate is provided for Chironomidae using data from ribosomal genes (18S and 28S), a nuclear protein-coding gene (CAD), and a mitochondrial protein-coding gene (COI), analysed using mixed model Bayesian and maximum likelihood inference methods. The elusive, most-recently described subfamilies Chilenomyiinae and Usambaromyiinae, proved are unsampled. Monophyly of all sampled subfamilies is confirmed, excepting Prodiamesinae contains *Prosilocerus* previously Orthocladiinae. Semifamily Chironominae is confirmed only by exclusion of Telmatogetoninae, closer to Brundin's suggestion. Buchonomyiinae is sister to all extant Chironomidae, conforming more to Murray and Ashe's argumentation. Semifamily Tanypodinae is non-monophyletic: austral Aphroteniinae is sister to all Chironomidae (less Buchonomyiinae). Podonominae is next sister or to Tanypodinae alone. Southern African Harrisonini is a diamesine, embedded within austral tribe Heptagiini, in turn sister to Diamesini. Tanypodinae tribe Pentaneurini and 'non-Pentaneurini' taxa are reciprocally monophyletic. Podonominae molecular findings are substantiated - a monophyletic tribe Podonomini, Boreochlina a grade and *Lasiodiamesa* sister to all other Podonominae. In Orthocladiinae, a tribal system of Orthocladiini and Metriocnemini finds support excluding *Corynoneura*- and *Brillia*-groups (latter sister to *Stictocladus*). Marine *Clunio* and *Thalassosmittia* are deep orthoclads as proposed by Strenzke. *Shangomyia* + *Xyiaomyia* is sister to all other Chironominae justifying a high rank proposed by Sæther and Wang. Tanytarsini is monophyletic, Pseudochironomini is poorly sampled but, with a weakly supported inclusion of *Nandeva*, is sister to Tanytarsini. Tribe Chironomini excludes *Shangomyia* + *Xyiaomyia* and monophyletic clade centred on *Microtendipes*, with 6 segmented larval antenna and alternate Lauterborn organs, sister to Pseudochironomini plus Tanytarsini. Diversification, deduced by divergence time analysis (BEAST), shows Permian origination (perhaps sister to all other Culicomorpha), with subfamily stemgroup origination from the mid-late Triassic to early Cretaceous. Crown group origination ranged from Podonominae on short stem from mid-Jurassic to long-stemmed Aphroteniinae in late Cretaceous. Dates allow some vicariance via Gondwanan fragmentation.

## Towards a phylogeny of the genus *Paratanytarsus* (Diptera: Chironomidae)

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The genus *Paratanytarsus* is known from all biogeographical regions except tropical Africa and is widely distributed in the northern hemisphere. About 20 species are known from Europe, half of them are also found in Norway. In this study the phylogeny of a selection of the species within the genus is evaluated using nuclear genes (CAD 1 and CAD 4 at present), and the relationship with the closely related genus *Micropsectra* is investigated. So far 35 individuals from 14 species are included. Present results support the monophyly of the genus although with low branch-support. Several species show notable intraspecific genetic variation. Individuals of *P. dissimilis*, *P. tenuis*, *P. setosimanus* and *P. austriacus* from both Northern Europe and Canada have been included and indicate substantial trans-Atlantic genetic divergence. Individuals identified to *Paratanytarsus austriacus* came out as paraphyletic with *P. hyperboreus*, and great genetic divergence in CAD and COI gene sequences suggest the presence of cryptic species. One of the main goals of this study will be to find out whether *Paratanytarsus* really is a monophyletic group and to reveal some of the biogeographic history of the genus. The subsequent work will include two more nuclear markers and more species to resolve the more basal nodes in the phylogeny.

## Chironomids in the groundwaters of a karstic cave system of the Dolomiti Bellunesi National Park (Northern Italy)

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The results of a survey carried out in 2004-2005 and 2007 on the aquatic fauna of the Piani Eterni Complex, one of the longest and deepest cave system in Italy, located in the Dolomiti Bellunesi National Park (Eastern Italian Alps), are reported. They show a considerable presence of chironomid larvae, with regard to subterranean waters reported up to now only inside interstitial environments. These larvae were almost totally represented by immature stages (maximum a third larval age) of the Orthocladiinae *Eukiefferiella* sp., very probably a single species, whose presence revealed to be rather considerable at all sampled depth levels of the karstic cave system as well as in its outlet, a spring named Fontanon. The presence almost exclusive of this species inside the Piani Eterni karstic Complex and the finding of a specimen of a terrestrial Orthocladiinae larva at a depth of -200 m of the same Complex are discussed.

## Cold tolerance, longevity and oviposition by *Diamesa mendotae* (Mutkowski)

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Adults of *Diamesa mendotae* commonly emerge in winter in Minnesota when air temperatures are well below freezing. Large numbers can often be collected from on snow near trout streams that do not freeze because of groundwater input. Field collected adults have been shown to depress freezing point to less than -20 degrees C. In laboratory experiments conducted at 6 degrees C, field-collected adults have average longevitys of 18.6 days, but maximum longevitys are 48 days for males and 54 days for females. No statistically significant changes in freezing point depression have been observed as adults age up to 18 days. Females readily oviposit in test vials under laboratory conditions over a wide range of days post-collection. Timing of oviposition influences post-oviposition survivorship, average and maximum longevity, and size of egg mass. These results demonstrate that females preferentially reduce their reproductive output rather than metabolize freezing point depressants as a strategy for emergence into winter conditions when air temperatures are likely to be below freezing.

## **A four years Study of the impact of *Bacillus thuringiensis* var. *israelensis* (Bti) on chironomid population of temporary marshes in the Camargue Rhône delta**



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Since August 2006, experimental B.t.i. treatments are realized in order to control nuisances induced by *Ochlerotatus caspius* and *Oc. detritus* in the Camargue Rhône delta. The study presented here takes place in a large program which aims to take into account the impact of B.t.i. treatments on food webs. Direct effects are studied on chironomids and indirect effect on Algae, Odonata and Red Flags. Aerial B.t.i. treatments are realized by the "Entente Interdépartementale pour la Démoustication" with plane. Chironomid populations are studied on three temporary marshes. Comparisons of the densities, before (T0) and after treatments (2 days, 5 days and 11 days) were made through 15 campaigns of treatment. Chironomid assemblages are dominated by *Polypedilum nubifer*, *Chironomus* spp. and *Procladius choreus*. For the dose of three liter/ha of vectobac 12 AS, our results did not highlight catastrophic effect, but some decrease of densities are noted five days after treatment. This decrease is followed by a recovery of the densities, eleven days after treatment. This could be explained by a punctual effect on the first stages larvae, present in the marsh the day of the treatment.

## **$\delta^{13}\text{C}$ signatures in chironomids remains as a proxy for past changes in lake functioning: Lake Annecy as a case study**



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The benthic food web, especially chironomids, is increasingly considered in whole lake functioning as a significant contributor of carbon (energy) to the highest trophic level. We studied high resolution temporal evolutions (150 years) of chitinous remains of chironomids  $\delta^{13}\text{C}$  extracted from two sediment cores (30 and 60m) in Lake Annecy. Due to the high number of remains and recent advances in spectrometry,  $\delta^{13}\text{C}$  temporal evolution was conducted for each of the main taxa of the chironomid communities. The lake has been enriched by nutrients and reached a mesotrophic state at ca 1940. A remediation plan implemented in 1967 decreased nutrients in the pelagic zone from the 80s until the present. The aim of the study was to understand temporal changes in lake carbon flow during its recent history. From ca 1850 to ca 1930s  $\delta^{13}\text{C}$  of *Micropsectra contracta* and *Sergentia coracina* were constant at around -32‰. From ca 1930 until today,  $\delta^{13}\text{C}$  constantly decreases as low as -38‰. These changes in  $\delta^{13}\text{C}$  chironomids remains occurred simultaneously with changes in chironomids communities studied at the same time. The progressive  $^{13}\text{C}$  depletion of *M. contracta* and *S. coracina* resulted in an increasing amount of respired carbon assimilated in the trophic foodweb to which they belong. The implication of an increase of the methanogenic-methanotrophic trophic pathway is discussed. The divergence between  $\delta^{13}\text{C}$  of chironomids remains and the biological and chemical characteristics in the pelagic zone monitored since ca 1980s highlights the importance of considering the benthic food web to reconstruct the whole lake ecological functioning. These results reinforce the existing link between the chironomids assemblage characteristics and functional properties in lakes.

# **Chironomid-based reconstruction of the trophic history of Lake Annecy during the last 150 years using an intralake multiple core approach**

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Lake Annecy (45° 51' N- 6° 10' E, 446 m a.s.l) is the third largest natural lake in France. It is monomictic and deep (max. depth: 65 m). The lake is often considered as the "cleanest lake in Europe". Lake Annecy was oligotrophic until first signs of eutrophication were noticed in the early 1940's. Water quality degraded until the end of the 1960's. A remediation plan implemented in 1967 resulted in the return to an oligotrophic state. Given the lack of detailed instrumental data before the 1970's, we have developed a paleolimnological approach to assess the trophic history of Lake Annecy during the last 150 years. Chironomid subfossil assemblages and organic matter (Corg, Norg) were studied at high resolution in three cores distributed along a bathymetric transect (30 m, 56 m and 65 m). Chironomid fauna and organic matter analysis of the deepest core suggest a major shift in oxygen conditions at ca. 1930. Oxyphilous taxa were replaced by hypoxia-tolerant taxa. The last decade (i.e. since 2000) is characterized by the near-complete disappearance of the profundal fauna likely linked to the prevalence of anoxic conditions. Parallel analysis of the transect cores can pinpoint the timing and the extent of the hypo/anoxia in the water mass: hypoxic levels reached 56 m at ca. 1940, and 30 m at ca. 1950. During the last 3 decades, no sign of recovery in the chironomid communities could be seen at any depth. This study highlights the degraded state of modern chironomid fauna compared to its pre-1930 state despite the reoligotrophication evidenced from the chemical survey of pelagic water. Our results emphasize the relevance of our paleolimnological approach based on intra-lake coring transects and benthic indicator analysis for the reconstruction of the recent trophic history of lakes and assessment of their present state.

## **Larval Chironomids (Chironomidae: Diptera) from fresh water ecosystems of Balaghat in Marathwada region, (M.S.) India**

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The larval Chironomidae is a widely distributed group of insects, often occurring in most of the freshwater ecosystems. The present study deals with occurrence of larval chironomids from fresh water aquatic ecosystems of the Balaghat ranges from the Marathwada region (M.S.) India. Collection was carried out during the period of June 2009 to May 2010. As result in these study three genera belonging to subfamilies Chironominae and two genera from the subfamilies Tanypodinae were encountered. Chironominae showed high species richness in the comparison of Tanypodinae.

## Study of molecular and cytological structure of centromeric regions in Chironomidae

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To study molecular organization and patterns of divergence of DNA sequences in centromeric regions of chironomids we had chosen sibling species from the *plumosus*-group (*thummi*-cytocomplex) that contains several species which differ greatly by the size of centromeric heterochromatin and *C. dorsalis* (*pseudothummi*-cytocomplex) that has rather unusual cytological structure of centromeric regions in chromosomes AE and BF: each chromosome appeared to be dicentric as it contains two C-positive bands in centromeric region instead of just one as is usual for other *Chironomus* species. Microdissection of centromeric region of chromosome EF of *plumosus*-group species and centromeric regions and C-positive bands of chromosomes AE, CD and BF of *C. dorsalis* had been performed and DNA-libraries of dissected regions had been created. DNA-clones were also obtained from available DNA-libraries. FISH were used for the analysis of localization of DNA-libraries and DNA-clones on polytene chromosomes of corresponding species. It was found that studied *plumosus*-group species differ greatly in the level of homology of sequences from centromeric regions of different chromosomes. In most species all four chromosomes of karyotype show strong hybridization signals of DNA probes and DNA-clones from chromosome EF centromere, whereas in two species – *C. entis* and *C. muratensis* – such hybridization could be observed only in three big chromosomes which mean that centromeric DNA of short chromosome G is greatly diverged from other chromosomes of karyotype. This result was further confirmed by interspecific FISH of *C. borokensis* DNA-probe that showed high homology to centromeric region of chromosome G but almost no homology to centromeres of big chromosomes of *C. entis* and *C. muratensis*. FISH of DNA-probe from the centromeric region of chromosome CD of *C. dorsalis* – the only clearly definable centromeric band on the long chromosomes of *C. dorsalis* – had shown that C-positive bands of chromosomes AE and BF contain DNA sequences homologous to sequences from centromeric region, but the intensity of the hybridization was not as strong as in the centromere of chromosome CD. Strong signals were also observed in some bands on chromosomal arms D, F, G, E and C. The study was conducted with financial support of grant RFBR 09-04-01440a and programs of Presidium RAS №25.2 and “Genofonds and genetic divergence”.

## “Where to draw the line?” Phenotypic variation within the *Cricotopus sylvestris* species group, across a nearctic-palearctic gradient

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The genus *Cricotopus* is globally widespread and diverse, with 4 subgenera. The *sylvestris* species group is the largest within the subgenus *Isocladius*, including twelve west-palearctic species, and at least 4 nearctic species. Larvae are typically associated with aquatic plants or macroalgae in lentic habitats. Adults of most species have conspicuous patterns of light and dark bands on the abdomen and legs. Species in the *sylvestris* group exemplify the taxonomic problems within *Cricotopus* as a whole: characters used to distinguish species can be quite variable with overlapping values across similar species. Although pigmentation plays an important role in species identification, it shows so much intraspecific variation that “light” and “dark” forms of several species have been noted. Because pigmentation played a large role in description of some nearctic species, it is not always clear where to draw the line between them, or the degree to which nearctic and palearctic species populations have

diverged. Based on DNA-barcoding data, members of the *sylvestris*-group collected from the United States, Canada and Norway form 6 homogenous clusters which may represent species populations. Analyses of adult morphology and pigmentation patterns were conducted to quantify the degree of variation in features and diagnostic ratios commonly used for taxonomy. Because a significant range in adult size was observed over this latitudinal range, traits were examined for the presence of allometry, i.e., a disproportional change in dimensions with increasing body size, which could further complicate species discrimination. By comparing such patterns of phenotypic variation with the genetic similarities revealed by “barcoding”, we indicate the extent to which variation in phenotype is a response to local environmental conditions, vs. genetic variation among geographically distant populations.

## **Response of Chironomidae (Diptera) to impoundments in small lowland streams**

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River damming is one the most pervasive human disturbances of the world's ecosystems. Dams play a significant role for the modern civilization, providing effective flood control, water supply, irrigation, inexpensive power generation and recreation, but their functioning causes huge damage in the natural environment. Recently, ecologists' opposition against the construction of dams and a new trend toward the removal of old dams aggravate. Respective discussion concerns large dams in different geographical regions, including Poland. However available reference data concern mostly the impact of large-scaled impoundment on river communities. This study evaluated for one year the effects of three small dams located in low order sections of the Bzura, Mrożyca and Mroga Streams (Central Poland) on their benthic communities (Chironomidae). These rivers are a part of the Vistula drainage basin. Six sampling sites were established in these streams (one above and one below each dam reservoir) at a distance of several dozen km from one another. These streams display different seasonal hydrological regimes because of various functions of these reservoirs (except for flood protection) and various forest percentages of their valleys. Composition of benthic fauna was compared using multivariate techniques, both below and above each reservoir. The highest macrobenthic density was reached at one upstream site (over 25 000 ind.m<sup>-2</sup> in the Bzura Stream), but the biodiversity of the respective community was low. Generally, at each site benthic macroinvertebrates were dominated by Oligochaeta and Chironomidae (midges constituted from over 30% to over 90% of the total macrobenthic density). Among these dipterans Chironomini and/or Prodiamesinae were the most abundant taxa.

## **DNA markers for the identification of species of the genus *Chironomus***

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The genus *Chironomus* is rich of species. Chironomids inhabit various freshwater ecosystems. The species composition of chironomid communities is an important indicator of the quality of these ecosystems. However, the accurate detection of *Chironomus* species by morphological traits is difficult. Cytogenetic identification of chironomid species is restricted to the late fourth instar larvae. DNA sequencing of mitochondrial and nuclear genes effectively identifies chironomid species, however it is expensive and time-consuming. The PCR with species-specific primers is a rapid, accurate and inexpensive method for the identification of species. Species-specific primers yield the products of amplification only in the target species. The aim of this work was to develop the species-specific PCR primers to identify species of the genus *Chironomus*. The internal transcribed spacer 1 (ITS1) of the ribosomal DNA locus

was used as the target for forward primers, the 5.8S rDNA – for reverse primers. The ITS1 and 5.8S sequences of thirteen species of genus *Chironomus* were used to design species-specific primers. Eight forward and two reverse primers were designed. The species-specificity of each of the primer pair was tested against eighteen species of genus *Chironomus*. The species-specificity was confirmed for four primer sets. Two primer pairs specifically detect species of two sibling species groups. The obtained results confirm that the PCR with species-specific primers could be useful as a valuable tool for the identification of chironomid species. This work was supported by the grant RFBR 10-04-00899, and by the Programs of the Presidium of the Russian Academy of Sciences (No. B25 and B27). The work was conducted with the use of equipment of the CCU “DNA Sequencing” SB RAS (<http://sequest.niboch.nsc.ru>)

## ***Vibrio cholerae* is a pathogen of chironomids**



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Quorum sensing is the phenomenon whereby bacteria use signal molecules to communicate with each other. For example, to establish a successful infection, pathogenic bacteria become virulent only when they reach a certain local concentration in their host. Recently, Ng and Bassler (2009, Ann. Rev. Genet.) highlighted the dilemma that in contrast to what is observed for other bacterial virulence gene expression, quorum sensing seems to repress *V. cholerae* virulence factors expression (e.g., cholera toxin). Here I present a novel insight that may enlighten the way *V. cholerae* quorum-sensing signals regulate its genes. Chironomids (Diptera, Chironomidae), which are widely distributed and frequently the most abundant insect in fresh water worldwide, are natural reservoirs of *V. cholerae* (Broza & Halpern, 2001, Nature). Quorum-sensing signals in *V. cholerae* upregulate the production of an extracellular enzyme, hemagglutinin protease (HAP), which degrades chironomid egg masses and prevents the eggs from hatching, demonstrating that HAP is a virulence factor against chironomids (Halpern, 2010, Mol Ecol). Indeed, in a yearly survey *V. cholerae* and chironomids showed a pattern of a predator-prey population dynamics. From a global point of view, chironomids are much more abundant in the environment than humans, so quorum-sensing signals of *V. cholerae* and the gene they regulate should be understood with regard to their role in chironomids rather than humans. Further research is needed to understand the role of cholera toxin in the environmental existence of *V. cholerae*.

## **Temperature related differences in community structure and life-cycles of Chironomidae in streams in geothermal area, Hengill, in Iceland**

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Six spring-fed streams in a geothermal area in Hengill, SW Iceland were studied, 3 cold and 3 influenced by geothermal heating, ranging 6.8 -22.8 °C. Ten benthic samples were collected within each stream on nine occasions between September 2006 and August 2007. Invertebrates from the samples were counted and identified under a microscope along with measuring the head width and length of Chironomidae larvae. Water temperature, pH and conductivity were measured. The aim of the

study was to look at invertebrate communities in streams that varied in temperature and relate the community difference to possible changes occurring with global warming. Chironomidae dominated all streams, except in the warmest stream where *Radix peregra* dominated followed by Chironomidae and *Simulium vittatum* (Simuliidae). Average Chironomidae densities differed seasonally, ranging from 10 to 44,939 individuals m<sup>-2</sup> in all streams. The streams were separated into cold and warm streams, based on the PCA analysis, the first axis explained 30.5% of the variance in the Chironomidae species composition. Axis 2 explained a further 22.8% of the variance. Colder streams were dominated by *Eukiefferiella minor*, *E. claripennis*, *Orthocladius frigidus*, *Thienemanniella* sp. and *Diamesa bohemani/zernyi* gr. and warmer streams by *E. minor*, *E. claripennis*, *Orthocladius* cf. *oblidens* and *Micropsectra* sp.. Beta diversity (Sørensen's index) was highest between the cold streams on average ranging between 0.99 and 1.56. A Spearman correlation was performed between Sørensen's index and temperature difference for each month, revealed a significant correlation ( $P < 0.05$ ) in November 2006, and March, May, July and August 2007. The beta diversity declined as the temperature difference increased, that is streams with more similar water temperature had Chironomidae communities that were more alike. *E. claripennis* has possibly more than one generation in some of the warm streams, but is univoltine in the cold streams with summer emergence.

## Rare Chironomidae in Mongolia

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Documentation of rare species of freshwater invertebrates lags behind that of terrestrial and freshwater vertebrates, often due to inadequate distributional and ecological datasets. The Mongolian Aquatic Insect Survey has created a comprehensive database of aquatic insects from freshwater habitat in Mongolia, providing a unique opportunity to analyze the occurrence of rare species of Chironomidae. Data from surface floating pupal exuviae samples were used in the analysis and only sites from one type of habitat, the headwater stream/spring seep habitat were included to reduce variability due to physical and environmental variables. The resulting dataset was composed of sixty-two sites from three mountain regions in Mongolia; the Altai, Khentii, and Sayan Mountains. Classification of the study sites by hierarchical cluster analysis resulted in a class of 33 rare species, defined by limited geographical ranges and low relative abundances at a given site. Rare species varied by subfamily, from 58% in Orthoclaadiinae to 3% in Podonominae and Prodiamesinae. The variation in percent of rare species by subfamily likely reflects the relative richness in each subfamily from freshwater habitat in Mongolia rather than propensity for rarity. A map of rare species of Mongolian Chironomidae is presented as a tool for management of headwater streams and spring seeps in Mongolia's preserves and national parks.



# Traces of past environments in the chemical composition of chironomid remains: stable isotopes in chironomid palaeoecology

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Head capsules of chironomid larvae are abundant in lake sediments and, in suitable environments, can preserve for ten thousands of years without visible signs of degradation. Microscopic identification of these remains is possible, usually to genus or subgeneric morphotype level. As a consequence, past changes in the taxonomic composition of chironomid assemblages can be studied based on lake sediment records and used to reconstruct past variations in climatic or limnological conditions. Recently, it has become apparent that the stable isotopic composition of chironomid head capsules can provide similarly useful information about past environments as past changes in assemblage composition. The chitinous head capsules of chironomid larvae contain high amounts of the light elements C, N, O, and H. Fossil chironomids can therefore potentially be analyzed for the abundance of the stable isotopes C-13, N-15, O-18, and D (or H-2). In my presentation I will present some applications of stable isotopic analyses of fossil chironomid exoskeleton remains, including the analysis of O-18 for the reconstruction of past climatic changes, N-15 for the reconstruction of past nitrogen pollution in lakes and C-13 for the reconstruction of past changes in the carbon sources of lake foodwebs. In order to develop isotopic analyses of fossil chironomids as a palaeoenvironmental proxy-indicator several obstacles need to first be overcome and I will present some case studies indicating how these difficulties can be addressed. For example, chironomid remains are relatively light and therefore isotopic analysis must be optimized for very small sample mass. In addition, chemical pretreatment can change the isotopic composition of the exoskeleton fragments and therefore care must be taken that sample processing does not influence stable isotope measurements. Also, very little information is available about whether the stable isotopic composition of chironomid head capsules is representative for the entire larva and in how far this is influenced by larval diet or the isotopic composition of the ambient water. To further expand these approaches, and improve their applicability, an important aim for future research will have to be to develop high quality datasets documenting the isotopic values of living chironomids and their remains in lakes in a range of environments and in respect to a gradient of isotopic values in the lake water or in the food available for the larvae. It can be expected that with the development of such datasets, and with the expected increased availability of isotopic measurement facilities, stable isotopes in chironomid larvae and in fossil chironomid remains will play an increasingly important role in freshwater ecology and environmental reconstruction based on lake sediments.

## Bathymetric distribution of benthic macroinvertebrates in small and shallow Lake Shoji, Fuji Five Lakes, Japan

Ⓟ

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Lake Shoji located at the north foot of Mt. Fuji, is one of the Fuji Five Lakes, which are especially familiar to the Japanese people for their beautiful landscapes. In this paper, in order to clarify the current status of the benthic community of Lake Shoji and examine the difference with the last set of quantitative data by Kitagawa (1973), the distribution of benthic macroinvertebrates was studied in this lake, and a comparison of the benthic fauna and density was made between the present and previous studies.

On March 1, 2010 sampling surveys were carried out using a standard Ekman - Birge grab (15×15 cm), taking three replicate samples at each of 4 stations in the northeast parts of Lake Shoji. The average density of benthic community was  $4,107 \pm 3,957$  ind./m<sup>2</sup>, comprised of oligochaetes, chironomids and chaoborid larvae. Their densities were  $2,063 \pm 1,270$  (50.2%),  $1,996 \pm 2,945$  (48.6%) and  $48 \pm 52$  (1.2%), respectively. On the other hand, the average biomass (wet weight) of these benthic macroinvertebrates was  $2.72 \pm 1.57$  (62.7%),  $1.45 \pm 1.26$  (33.4%) and  $0.17 \pm 0.28$  (3.9%) g/m<sup>2</sup>, respectively. Oligochaetes and *Prosilocerus akamusi* and *Procladius* sp. of chironomid larvae were the dominant taxa, and make up 49.4%, 4.8% and 8.5%, respectively, of the total benthic macroinvertebrates in density, and 34.8%, 54.5% and 6.0% in biomass. *P. akamusi* was 2.5 times greater than that in 1973. *P. akamusi* density was related to ignition loss of sediment and water temperature. *Procladius* sp. was closely related to water depth. Chaoborid larvae inhabited the deeper regions (8.6-11.3m). Large environmental changes must have affected oligochaetes and chironomids densities, especially in the deeper regions with low dissolved oxygen concentrations. Consequently, the eutrophication of this lake is proceeding.

## Bioassessment of Portuguese reservoirs using chironomid pupal exuviae: preliminary results

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Many of Portugal's reservoirs are characterized by extreme diurnal fluctuations in amplitude, as water is drawn off for hydroelectric production and water supply. This is particularly marked in years with low levels of rainfall. This physical instability severely limits the macroinvertebrate community able to colonize habitats, especially in the littoral zone of these artificial freshwater bodies, hampering the development of ecological assessment methods. In attempt to overcome this constraint, we made a preliminary appraisal of the use of chironomid pupal exuviae for assessing ecological status in 18 reservoirs, covering 3 typologies and a defined quality gradient across the national territory. Exuviae were collected in spring and summer 2006 following the CEN 2006 protocol, sorted in the lab and identified to genus. Preliminary analyses using nMDS ordination and UPGMA classification indicated distinct chironomid communities in reference reservoirs for each typology, an association that was weaker among non-reference reservoirs. ANOSIM R values indicated distinct but slightly overlapping communities for reference/non reference reservoirs in North and South typologies but not for the "run of river" reservoir typology. SIMPER analyses were used to further assess the percentage of similarity between reference and non-reference reservoirs for each typology and identify the principal chironomid genera contributing to these differences. For example, SIMPER results clearly showed that taxa associated with Northern typology reference reservoirs were *Procladius*, *Tanytarsus*, *Harnischia* and *Cladotanytarsus* while *Cladotanytarsus*, *Microchironomus*, *Cryptochironomus* and *Chironomus* occurred in non-reference northern reservoirs. "Run of river" reservoirs communities were less distinct between reference sites and non-reference sites, which shared *Cricotopus*, *Dicrotendipes*, *Polypedilum* but not *Cladopelma* (reference) and *Procladius* (non-reference). Spearman Rank Order Correlation between principal taxa and pressures indicated that most taxa were negatively correlated with changes in land use. However, *Parachironomus* was positively correlated with agricultural land use and organic enrichment and *Microchironomus* with increasing urban area.

# Genetic architecture of lunar and circadian emergence times in the marine midge *Clunio marinus* (Diptera: Chironomidae)

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The marine midge *Clunio marinus* (Diptera: Chironomidae) is found in the intertidal zone of the European Atlantic coast. In adaptation to its habitat, it has timed adult emergence to the tides, displaying circadian and lunar rhythms. As tidal regimes differ along the coast, *Clunio marinus* comprises many timing races which differ in various aspects of their lunar and circadian rhythms. In a crossing experiment with *Clunio marinus* strains that differ in both circadian and lunar emergence time, we found genetic control of the lunar emergence time. The pattern suggests genetic adaptation to the tidal regime of the place of origin. Genetic control and local adaptation of circadian emergence time has been shown previously (Neumann 1967) and was also observed in my crosses. Our experiment revealed that while both lunar and circadian emergence phase are polygenic traits, they are not inherited independently. This suggests either that a set of genes influences both traits or that evolution has shaped the genetic architecture to stabilize adaptive combinations of lunar and circadian emergence phase through genetic linkage. From the crosses we created the first linkage map of the *Clunio marinus* genome, also including a dozen of cloned circadian clock genes and light receptor genes. By Quantitative Trait Locus (QTL) analysis we could identify two loci each controlling circadian and lunar emergence time and within these loci four genes that are potentially involved in the traits. Currently we are testing these candidate genes for sequence and expression differences specific to the timing races. Eventually, this may reveal the first known molecular components of the lunar clock.

## Strong genetic differentiation and postglacial origin of populations in the marine midge *Clunio marinus* (Diptera: Chironomidae) (P)

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The marine midge *Clunio marinus* (Diptera: Chironomidae) is characterised by a one-dimensional distribution along the European Atlantic coast, where its lunar and circadian emergence rhythms are genetically adapted to the local tidal regimes, resulting in a series of "temporal races". *Clunio marinus* is restricted to rocky coasts and thus the temporal races occur in different rocky patches. We studied ten populations of *Clunio marinus* from five different regions, spanning the major rocky mainland coasts from Spain to Norway, using amplified fragment length polymorphisms (AFLP), microsatellites and mitochondrial cytochrome oxidase I (COI) sequences. Star-like patterns of COI haplotypes within regions indicate postglacial colonisation. A high degree of shared polymorphisms in AFLP markers suggests colonisation from a single source, implying postglacial evolution of timing adaptations in relation to the local tidal regime. In contrast, no COI haplotypes are shared among regions. We hypothesize that different levels of differentiation of nuclear vs. mitochondrial markers in the source region were carried forward during postglacial expansion. Despite the recent origin of populations, all markers reveal distinct genetic differentiation between rocky coasts on a scale of 650 to 1150 km. Differentiation between rocky coasts is not correlated to timing adaptations, suggesting that geographic isolation is prevalent between rocky coasts and that this facilitated the evolution of local timing adaptations. At the same time there is little genetic differentiation within rocky coasts on a scale of 2 to 6 km; leaving open the possibility that within rocky coasts with large variation in tidal regimes, temporal adaptations evolved in the face of gene flow.

# **Distribution and diversity of Chironomidae along an altitudinal gradient of a subantarctic fluvial system in the Cape Horn Biosphere Reserve, Chile (55°S)**

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The distribution of Chironomidae in the Róbalo River during the austral summers of 2008, 2009 and 2010 is reported. The Róbalo River, located in the Cape Horn Biosphere Reserve (55 ° S), provides drinking water to the world's southernmost town, Puerto Williams. The altitudinal gradient of the Róbalo watershed is characteristic of other rivers in the Cape Horn region with a representative mosaic of Andean highlands, evergreen, mixed and deciduous forests. The river was divided, from the headwater (577 meters above sea level) to the mouth of the river into five stations each separated by 100 m of altitude. At each station Surber samples and malaise traps were collected each year. Chironomids were the most abundant benthic macroinvertebrate in the watershed, for all three years studied. Longitudinal differences were noted in chironomid abundance with populations at stations with the highest altitudes, 577 and 482 meters above sea level, significantly greater than populations measured in stations at lower altitudes (one-way parametric ANOVA and SNK,  $p=0.013$ ). Chironomids were dominated by the subfamilies of Orthocladiinae and Podonominae. Other subfamilies present included Aphroteniinae, Chironominae, Diamesinae, and Tanypodinae. The results are similar to studies in New Zealand and Andean Patagonian rivers.

## **New data on chironomids in the Middle Urals**

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The aim of my work was to investigate the fauna of chironomids of the subfamilies Diamesinae, Prodiamesinae and Orthocladiinae of the Middle Urals. Much has been done in this field by researches such as V.V. Gromov, A.O. Tauson, M.S. Aleksevina, I.V. Pozdeev and N.N. Pankov. But most of these studies concentrated their attention only on the immature stages. Especially, the work by V.V. Gromov who studied several species in their three stages of life should be mentioned. The studies of adult midges summarized here were carried out by I.V. Pozdeev (2010) and me (Krasheninnikov, Makarchenko, 2009; Krasheninnikov, 2009, 2010). The results of the work show that during the 7 years of studies of the chironomid fauna (from 2004 to 2011), I found 93 species: 29 of them new to the Urals, 11 new to Russia and one new to science. In subfamily Orthocladiinae 85 species are listed, in Diamesinae 6 species, in Prodiamesinae 2 species. Simultaneously I carried out some phenological observations in two places situated on the territory of the Middle Urals, on the river Sylva and a hydrosulphuric spring near the stream Irgina. The results of these studies revealed differences in flying periods for different species: 12 species fly only in spring, 18 species fly from spring to summer, 15 species from spring to autumn, 34 species fly only in summer, 6 species fly from summer to autumn, 4 species fly in spring and in autumn, 3 species fly only in autumn and one species fly from autumn to spring including winter.

## **Chironomidae collected in lakes of Ellesmere Island in 1991 by Dr. Kate Sylvester: a base line data set for the monitoring of global warming in the arctic**

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Chironomidae were collected in four lakes in arctic Canada from ice melt through the period of exposed water, by skimming the water surface with a hand net. These samples for the greater part comprised pupal exuviae, though some pharate adults, drowned adults and larvae were also present. The contents of the guts of arctic charr were also preserved: they were of pharate adults. 43 taxa were identified. The species lists of the four lakes are compared: the larger, less oligotrophic lake H supported more species; the smaller, more oligotrophic lakes, A, B and C contained species in common that were not represented or rare in Lake H and each yielded a species not found in any of the other lakes. The material revealed that a rapid succession of species occurred as the ice melted. Charr gut contents suggest circadian emergence patterns and intermittent feeding by the fish. Two species represented only by females have since been described as new.

## **A reassessment of the Chironomidae of Lough Neagh, Northern Ireland, the largest lake in the British Isles**

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The list of Chironomidae recorded from Lough Neagh is updated. The contribution of smaller neighbouring pools to the lists made by collecting adults on the shore has in the past led to species being erroneously recorded for the lake. The use of exuviae to establish the development of species in a particular habitat is emphasized.

## **Reflected polarization, egg density, and habitat availability guide chironomid females to oviposition sites**

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In the previous symposium on Chironomidae we first informed our early observation that chironomids select their oviposition habitat using polarized light reflected from the water surface. Soon after we conducted an experiment presented here and recently published which aims to quantitatively validate the phenomenon, and investigates the effect of the presence of conspecific eggs and habitat availability on the female preference. Two multiple choice field experiments were conducted, which included egg traps reflecting varied light intensities and percent polarization; under artificially illuminated and

habitat limited environment and under naturally illuminated and habitat unlimited environment. Isodar analysis was used to quantify the egg density dependent and the habitat availability effects on the egg-batch (EB) distribution. At both experiments, traps reflecting high percent polarization were also the most attractive for the ovipositing females regardless of the reflected intensity, including more than 60% of the EB laid. In the habitat-limited environment, oviposition was found to be dependent on EB density as eggs were found in unpolarized traps. However, under habitatunlimited environment, this dependence was weakened or completely gone, as no EB were found in unpolarized traps, as was indicated by the isodars. A positive correlation was found between the Total Organic Carbon (TOC) in the water on which the chironomid larvae feed, and the percent polarization reflected from the water surface. During sunset, the active time of the chironomids, the reflected intensity from their natural pond decreased to 4% while the reflected polarization was found stable at about 70% as it depends on the water turbidity. We therefore conclude that polarization and not the reflected intensity is the visual cue guiding the ovipositing females as it can be used to assess the habitat quality for their offspring. Oviposition is density dependent in habitat limited environment which weakens when habitat availability increase.

## Microhabitat preferences of Chironomids in two lower mountainous streams in Slovakia

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Chironomids are not commonly used for assessing the quality of freshwater systems. Nevertheless, their high species diversity within a wide range of habitats points to their potential high indicator ability. The main objective of this study is to specify the habitat preferences of preimaginal Chironomids from two Slovakian streams. The investigated streams (Udava, Olsava) are typical small mountainous running streams (catchment area: 214 km<sup>2</sup> (Udava), 340 km<sup>2</sup> (Olsava). Samples were collected at eight localities by kick-sampling using a 25x25 cm hand net (mesh size 500µm). All presented substrates (mineral and organic) were collected in three seasons. A Redundancy analysis was performed using hydromorphological and physiochemical variables to obtain the variation in taxonomical composition. Habitat preferences were determined using Spearman Rank Correlation and Indicator Species Analysis. Overall, the chironomids community was divided into four groups, each represented by a unique composition of environmental variables. The variation was mainly explained by depth and flow velocity followed by size of substrate and bank vegetation. Chironomini taxa were dependent on higher depth and slower flow velocity on mesolithal on the open localities. Orthocladiinae taxa predominantly occurred on the localities with more bank vegetation and their collinear variables: shading, submergent roots and fallen trees. Microlithal and organic substrates affected the presence of species *Brillia modesta*, *B. longifurca*, *Paratrissocladius excerptus* and *Parametriocnemus stylatus*. Habitats with more extreme conditions with higher flow velocity and absence of fine material replaced by macrolithal had lowest species diversity. Finally this work aims at using in the future chironomids and their habitat preferences for freshwater quality assessment

# **Biodiversity of Chironomidae (Diptera) in high alpine ponds in the Swiss National Park (Switzerland)**

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A long-term monitoring program was initiated in 2002 on running and standing waters in a high elevation cirque landscape (Macun Lakes) in the Swiss National Park. The region comprises two contrasting basins with different water sources, a glacier-fed basin and a precipitation-fed basin. Sampling of different permanent and temporary ponds was conducted in 2002, 2004, 2005, 2007 and 2009. Macroinvertebrate assemblages were dominated by chironomids with 42 taxa. The Orthoclaadiinae were the dominant subfamily with 22 taxa. In 21 permanent ponds sampled one to four times through the sampling period 37 taxa were found. *Zavrelimyia melanura* (63%) was the most frequent species followed by *Paratanytarsus austriacus* (50%), *Heterotrissocladius marcidus* (47%), *Corynoneura scutellata* gr. (43%), *Pseudodiamesa nivosa* and *Limnophyes* sp. (40% both). Significant and positive correlation were made between chironomid richness and pH, conductivity, aquatic vegetation. The geomorphological characteristics like pond surface area, mean and maximum depth had no pertinent effect on the chironomid richness. Ordination of the chironomid distribution indicated that the presence/absence of a tributary and the location in the south or north basin separated ponds. The results suggested that high Alpine chironomids are sensitive to subtle differences in environmental conditions and may thus make good sentinels of environmental change to alpine waters.

## **Long-term patterns of chironomid assemblages in a high elevation stream/lake network – Implications to global change**

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In a hydrographic system composed of a stream/lake network at high altitude (Cirque of Macun, Swiss National Park), a long-term monitoring was initiated in 2002. The region comprises two contrasting basins with different water sources, a glacier-fed basin and a precipitation-fed basin. Monitoring encompassed annual measures of physico-chemistry along with samples of chironomids in ten sites at five annual periods (2002, 2004, 2006, 2007, 2010). Chironomid assemblages were dominated in term of occurrence by the Diamesinae subfamily (*Pseudodiamesa branickii*, *Diamesa* gr. *zernyi/cinerella*, *Pseudokiefferiella parva*) and the Orthoclaadiinae *Tvetenia calvescens* and *Parametricnemus stylatus*. The long-term trends, the influence of water sources along with changes in air temperature regime will be discussed.

# Overview and update of the Mesozoic Chironomidae

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The published and new original data on the Mesozoic Chironomidae are reviewed. The occurrence of the family in the Cretaceous, Jurassic and Triassic and the taxonomic composition are considered and analyzed, with remarks on the fossils erroneously described as Chironomidae and later transferred to other families. The contribution of N.S. Kalugina to the knowledge of paleodiversity of the Mesozoic chironomids (especially, of preimaginal stages) is mentioned and acknowledged, as well as her analysis of the differences between the Mesozoic and recent chironomid taxocoenoses in the context of changes in inland aquatic ecosystems presumably connected with the radiation and expansion of angiosperms. The Chironomidae with well-developed proboscis are reviewed, including the recent *Archaeochlus* and *Austrochlus* (Podonominae), the previously described Early Cretaceous and the newly described Late Jurassic members of subfamilies Aenneinae(?), Tanypodinae and Podonominae. Our recent Jurassic finds (new species of *Cretaenne* and *Podonomius*) demonstrate that strongly elongate piercing mouthparts occurred in chironomids already in the Jurassic and such type of mouthparts was much more common than now. In spite of intensive investigations of the Triassic beds during the last decades, the only undoubted find of adult Chironomidae is known from the Late Triassic without any finds of immatures (as well as in the Early Jurassic). Recently, among the most ancient Middle Triassic Diptera from Vosges (France) we described a larva (*Anisinodus crinitus*) assigned to the Chironomoidea (the earliest Culicomorpha known up to date).

## The Chironomidae of Nunavut; their biogeographical distribution and limnological niches

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Littoral macroinvertebrate communities are often dominated by chironomid midges. This is especially true in the northern latitudes of Canada where non-biting midges comprise upwards of 80% community composition. Understanding the composition and function of these ecosystems cannot be accomplished without detailed examination of midge diversity. Littoral kick and sweep samples taken from the shorelines of lakes and ponds across the Kivalliq and Baffin regions of Nunavut, Canada during the summers of 2008 - 2010. A suite of limnological variables was taken at each location including; major ions, trace metals, and nutrient content such as Carbon, Nitrogen, and Phosphorus measures. Dominant taxa including chironomids, amphipods, and *Chaoborus* were identified to the species level in order to examine their diversity and distributions. Ordination analysis, including Redundancy Analysis (RDA) was used to determine environmental variables responsible for structuring contemporary midge communities. Results show Chironomini to be associated with warm water temperatures, and high Phosphorus and Dissolved Organic Carbon. Tanytarsini and Tanypodinae also have affinities for warmer conditions but appear to favour shallower water bodies. Orthocladiinae and Chaoboridae, however, correlate with colder lake temperatures. Identifying natural variation in community composition is important to our understanding of biogeographic patterns in Arctic regions. High taxonomic resolution studies are required to identify community differences in depauperate ecosystems. The purpose of this study was also to carry out a species level RDA in order to elucidate finer scale environmental trends. While chironomids are used as indicators in many paleo-environmental studies, the examination of current populations has implications for environmental management and monitoring. Species-environment relationships can be used to track changes in aquatic ecosystems and can also yield clues to the geographical origins of these species.



## **Analysis of stress response and endocrine function in *Chironomus riparius* following exposure to putative endocrine-disrupting compounds**

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Potential adverse effects of endocrine-disrupting chemicals (EDCs) in human health and ecosystems have become a major research issue. EDCs can alter a hormone function affecting its synthesis or secretion. Their mechanisms of action have been extensively studied in mammals and can involve interaction with hormone receptors. Although effects on reproduction and development have been described in invertebrates, data at molecular level are scarce. *Chironomus* is widely used in aquatic toxicology so it is a very appropriate test genus for research about potential EDCs. In an attempt to identify genes as potential biomarkers for environmental monitoring of EDCs, the effects of several compounds (cadmium, BPA, 4-NP, BBP, DHEP, TBTO, PCP, 4-BP, 4-MBC) with known endocrine disruptor activity in vertebrates were studied. Ecotoxicity was assessed analyzing alterations in gene expression profiles of inducible and constitutive genes related to cellular stress response. Ecdysone, a steroid hormone, plays a key role in the induction and modulation of morphogenetic events throughout development; its first target inside the cells is the ecdysone receptor. The potential effect on endocrine system was analysed studying EcR, one of the two genes that form the heterodimeric ecdysone receptor complex. By RT-PCR were analyzed the effects of acute and short time exposures to the compounds on the expression of these genes. The results show significant changes in the expression level of hsp70 and EcR genes with a different susceptibility to each of the EDCs tested. These data provide, for first time, a molecular mechanism for the action of these compounds in *Chironomus* suggesting that EcR could be a potential biomarker of exposure to EDCs. Overall, the study shows a differential gene-toxin interaction and adds novel genomic tools for biomonitoring environmental xenobiotics. Identification and validation of putative marker genes can provide a fast, sensitive and high throughput assay to test compounds improving also our understanding of the molecular mechanism underlying their toxicity and mode of action. Funded by CICYT, CTM2009-07189.

## **Further down the litter processing chain: effects of litter and detritivore diversity on chironomids consuming faecal particles**

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Previous research on the importance of biodiversity for ecosystem functioning in streams has focussed on the decomposition of leaf litter, a pivotal component of stream nutrient and energy cycles. This research has provided evidence for the potential importance of the biodiversity of leaf-eating detritivores (“shredders”) for aquatic decomposition processes. However, shredder feeding constitutes only one linkage in a larger freshwater “litter processing chain” that regulates the transformation of allochthonous resources in headwater streams, and its subsequent export and uptake. We investigated the knock-on effects of shredder and leaf litter diversity on processes of nutrient uptake and transformation further along the processing chain, with a specific focus on growth and ecosystem engineering of a tubicolous chironomid. Eggs of the particle feeding chironomid, *Chironomus riparius* Meigen, were hatched and reared in laboratory microcosms. Twelve faecal particle types, derived from three shredder species feeding on one of four different leaf litter species, were weighed out into

different 12 mg mixtures, and fed in various combinations to the chironomids. Effects of faecal particle combination for the growth, fat content, and tube constructing behaviours of the chironomid larvae were assessed. Outcomes for chironomid growth and tube-building behaviours depended not only on the leaf species the particles were ultimately derived from, but also crucially on differences in the degree of processing entailed in production of the faecal particles by the three shredder species.

## A Chironomidae-based stratigraphic record of pronounced 20th century warming in a large, deep Canadian Arctic lake

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A high-resolution examination of the subfossil remains of the Chironomidae was conducted on a sediment core from Baker Lake, a large, deep Arctic lake in Canada. The core was sectioned at 0.5 cm resolution and  $^{210}\text{Pb}$  dating was used to establish a chronology. A downcore analysis of over 60 taxa indicated a pronounced gradual decline of several cold-water indicator taxa (*Abiskomyia*, *Pseudodiamesa*) beginning at approximately 1940 and reaching 0% relative abundance at approximately 1990. Several taxa indicative of warmer conditions first appear in sediments beginning in the 1940s and increase in abundance in more recent sediments. In addition, the arrival of *Cladotanytarsus mancus* gr., a warm-water adapted taxa indicative of higher nitrogen concentrations, in recent sediments (circa 1985), increased to 12% of the total chironomid community by the surface (0-0.5 cm) sediment interval. As summer surface-water temperature was identified in Redundancy Analysis (RDA) as a primary variable structuring midge communities in 68 lakes across multiple regions of the eastern Canadian Arctic, a paleo-temperature transfer function was generated from subfossil midge assemblages. The biostratigraphic results from Baker Lake were applied to this temperature inference model, and inference results indicated a 3°C increase in mid-summer surface water temperature over the last 60 years. This also corresponded strongly to the instrumental record available since 1950. Thus, the gradual decline of cold-water adapted taxa, and subsequent increase of several taxa indicative of warmer regions, is a strong signal of recent environmental change within the area of Baker Lake, Nunavut.

## New *Polypedilum* Kieffer species inhabiting bromeliads in Honduras and Brazil (Chironomidae: Chironominae)

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The chironomids inhabiting bromeliads have been studied during the past decade in connection with two projects, one in southeastern Brazil and one in Honduras. So far three *Polypedilum* species have been collected from bromeliads in Brazil, namely *Polypedilum* (*Probolum*) *marcondesi* Pinho et Mendes, *Polypedilum* (*Polypedilum*) *solimoes* Bidawid-Kafka and a new species, *Polypedilum* (*Polypedilum*) n. sp. A. Additionally a new species, *Polypedilum* (*Polypedilum*) n. sp. B., was taken in Honduras. The Brazilian bromeliad species investigated were: *Canistrum lindenii* (Regel) Mez, *Neoregelia laevis* (Mez) L.B. Smith, *Nidularium innocentii* Lemaire, *Vriesea philippocoburgii* Wawra, and *Vriesea vagans* (L.B. Smith) while the focus in Honduras was on *Tillandsia guatemalensis* L.B. Smith. Larvae of all species were isolated from the leaf axils of the bromeliads and reared in the laboratory. In Brazil specimens were also

collected in emergence traps placed on top of the bromeliads. *Polypedilum* (*Probolum*) *marcondesi* was recently described and placed in a new subgenus. The remaining three species all belong in *Polypedilum* s. str. The male of *Polypedilum* (*Polypedilum*) *solimoes* Bidawid-Kafka is redescribed and the female and immatures are described and figured for the first time; the two new species are described and figured based on all life stages.

## Polytene chromosomes of Chironomidae (Diptera) as a bioassay of trace-metal-induced genome instability

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Chironomids are a ubiquitous group of aquatic insects that are very sensitive to environmental stress. Due to their excellent salivary gland chromosomes it is possible to define the genome response of several Chironomid species to various stress agents. The aim of this study was to quantify the genotoxicologic changes in populations of widely distributed chironomid species from aquatic basins in Bulgaria, Italy, Russia, U.K. and Poland, which were exposed to high concentrations of trace metals. We analyzed the structural and functional alterations of the larvae genomes belonging to three different cytotaxa of the genus *Chironomus* ("*thummi*", "*lacunarius*", "*pseudothummi*"), and genera *Glyptotendipes* and *Kiefferulus*. On the basis of the observed structural chromosome rearrangements (para- and pericentric heterozygous inversions, deletions, deficiencies and amplifications), we estimated a Somatic index (S) for each population. The highest S indexes were observed in *C. riparius* populations from stations with high concentrations of trace metals in sediments. We observed a broad spectrum of somatic rearrangements that were related to the genotoxic concentrations of polluting agents in sediments. Moreover, each species showed a specific genome response to stress agents. The observed specific responses are discussed in the light of the specific DNA structure of each species as well as the different gene linkage groups created by different chromosome arm combinations. In larvae from polluted sediments two key structures of the salivary gland chromosomes (Balbiani Rings and Nucleolar Organizer) sharply reduced their activity to levels below those under standard conditions. The polytene chromosomes can be used as tools for evaluating the genotoxicity of the aquatic environment. Their changes provide cost-effective early-warning signals of genotoxic concentrations of environmental pollutants. This project was supported by: DO 02 - 259/08, Sofia and Gene Pools and Genetic Diversity, St. Petersburg.

## A comparative analysis of chironomid community by applying the taxonomic distinctness index

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The widespread family of Chironomidae represents the most varied and often the most abundant group in macroinvertebrate fauna. They are an important element in estimating the effects of natural and anthropogenic disturbance in aquatic ecosystems. This family is still relatively unexplored, especially

in lotic systems in Serbia where there has not been any research for a significant number of years (25 years). The aim of this study was to determine the qualitative and quantitative structure of chironomid community in the South Morava river basin, and to compare it with the previous data for the same drainage basin using the taxonomic distinctness index ( $\Delta^+$ ). Likewise, we studied the performance of  $\Delta^+$  on chironomid assemblages. Samples were collected at 10 sites in two seasons during 2010 making this study comparable with the previous data from 1981. A total number of 3567 specimens were collected and 57 taxa from Chironominae (26), Diamesinae (2), Orthocladiinae (21), Prodiamesinae (1) and Tanypodinae (6) subfamilies were identified. The differences between Chironomidae assemblages in 2010 and 1981 were not significant.  $\Delta^+$  was strongly correlated with the traditional species diversity indices (Margalef's index  $r=0.815$ ,  $p=0.004$ ; Simpson's diversity  $r=0.967$ ,  $p<0.001$ ; Shannon's diversity  $r=0.914$ ,  $p<0.001$ ). It was also correlated with individual variables, such as dissolved oxygen ( $r=0.896$ ,  $p<0.001$ ) and ammonia ( $r=-0.988$ ,  $p<0.001$ ). The results require additional studies to confirm that taxonomical distinctness index could be used as a strong diversity index.

### **New species of *Paratanytarsus* Thienemann & Bause 1913 (Diptera: Chironomidae) from the Mediterranean region (Corsica, southern France and Lebanon)**

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New species of *Paratanytarsus* are described, from Corsica, southern France and Lebanon, based on the male imago and associated pupal exuviae. These descriptions increases the number of valid western Palaearctic species in the genus to 23. The pupal exuviae of the new species resemble those of *P. bituberculatus* (Edwards). Characters are given for distinguishing the adult males and the pupal exuviae from related species.

### **A new species of *Heterotrissocladius*, a rheophilic element, from basaltic and karstic helocrenes in France and Algeria (Diptera: Chironomidae)**

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Imagines and pupal exuviae of a new species of *Heterotrissocladius* are described on the basis of male and female pharates collected in temporary karstic and basaltic helocrenes in Algeria and France. Morphological adaptations on the pupae are believed to be related to the hyporheic nature of the interstitial habitat but are not correlated with the type of rock. The presence on the pupal exuviae of toothed thoracic horns, pointed spinulae on the tergites and numerous macrosetae on the anal lobe probably facilitates anchoring within fissures of the helocrenes. The description of the new species increases the number of valid species of *Heterotrissocladius* worldwide to 22 which includes 9 species in the Nearctic, 12 in the Palaearctic and 6 in the Oriental region.

## ***Rheotanytarsus* sp. nov., a low mountain element from streams located in the eastern Pyrenees and Corsica (Diptera: Chironomidae)**

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Adults and pupal exuviae of *Rheotanytarsus* sp. nov. are described on the basis of male pharates and pupal exuviae collected in low mountain streams located in the eastern Pyrenees and Corsica. Male adults are characterized by: the hairy pattern of the eyes; the shape of the anal point in dorsal and ventral view; the presence of an unusually long, finger-like, digitus on the superior volsella. Pupal exuviae can be separated from other related species by the following combination of characters: frontal apotome with minute broad tubercles bearing frontal bristle-like setae; thoracic horn bearing spinulae almost in one row extending from the near the base to half the horn length; laterally elongated point patches on tergites II-III becoming subrectangular to subcircular on tergites IV-VI.

## **Experimental insights into the salient features of gamma radiation tolerance of *Chironomus ramosus***

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Adaptability of natural populations of *Chironomus tentans* and *C. riparius* to ionizing radiation was first observed in the 1960s. Later, reports on natural populations of *C. balatonicus*, *C. thummi* and other midge species came from different laboratories including contaminated areas of Chernobyl nuclear reactor explosion in mid-eighties. These observations implied remarkable physiological sustenance of chironomid midges in high radiation environments. We have been studying the laboratory population of the Indian species of midge, *C. ramosus* for the past several years to explore the various biological consequences of gamma radiation stress and its underlying mechanism of radio-tolerance. Initially all the developmental stages of *C. ramosus* were evaluated for tolerance to various doses of gamma radiation and survival experiments showed greater tolerance of adults (> 3000 Gy). Salivary glands (SG), the most physiologically active organ of chironomid midges, were used to study cellular and molecular effect of gamma radiation stress. Viability of SG cells was assessed using a vital-dye exclusion method developed in the laboratory. Radiation tolerance corroborated with increased activities of antioxidant enzymes (e.g. superoxide dismutase, catalase) and elevated levels of Hsp 70 expression in the irradiated SG cells compared to that of non-irradiated glands. Following exposure to gamma radiation stress, chromosomal sites showing 'puffing' activity were identified in the polytenic nuclei of SG cells and 19-C region of chromosome IV was found to be the most conspicuous locus puffed out during the regime of gamma radiation stress. A review of the ongoing experimental studies and the salient findings on radiation induced proteins, chromosomal puffing response and teratogenic consequences will be presented. Differential tolerance profile observed in various developmental stages of *C. ramosus* leads us to propose this chironomid midge as a candidate 'biological dosimeter' during environmental crisis management of any radiation leakage. Implication of all these issues will be discussed.

# Holocene climate variability in northeastern Siberia inferred from chironomids in lake sediments

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In order to trace ancient climate-driven hydrological changes in a Yakutian permafrost lake environment, a high-resolution lake status record of Lake Satagay was obtained from the Vilyuy-Lena region. The lacustrine record spans the last 7500 years of the mid- to late Holocene. The downcore variability in the composition of organic matter and the assemblages of fossil bioindicators give evidence of climate-driven and interrelated changes in biological productivity, lacustrine trophic states, and lake-level fluctuations. The lake status record reveals a long-term trend towards lake-level lowering in the course of climate deterioration after 4.2 cal. ka BP and reduced evaporation as well as progressive sediment infill. This long-term trend is overprinted by short-term fluctuations at centennial time scales with high lake levels and decreased biological productivity during cool climate spells with reduced evaporation, as also observed in modern thermokarst lakes of Central Yakutia. The short-term climate spells seem to be related to sun-spot variations and are coherent with a 350-yr cycle of solar activity, which represents a multiple of the prominent 88-yr Gleissberg solar cycle. The short-term cycles were most pronounced during the mid-Holocene climate transition between 6.5 and 4.2 cal. ka BP, following the regional climate optimum and heralding late Holocene climate deterioration.

## A new insight on the desiccation tolerance mechanism in the sleeping chironomid, *Polypedilum vanderplanki*

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*Polypedilum vanderplanki* is a temporal rock pool dweller in the semi-arid regions of Africa. The small and shallow pools are easily dried up during the dry season and consequently the *P. vanderplanki* larvae also become completely dehydrated. They are however able to revive within an hour after rehydration upon the next rain as if without ill effects. This developmental arrest with zero-metabolism is called “cryptobiosis” or “anhydrobiosis”. Larvae accumulated a great amount of trehalose (about 20% of larval dry weight) as compatible solute replacing water and LEA (late embryogenesis abundant) proteins (initially isolated from plant seeds which are desiccation tolerance in general) upon dehydration. Physico-chemical analysis revealed that the anhydrobiotic larvae enter a glass state to protect molecules and cell membranes of the larvae from severe desiccation stress. Nevertheless, to our surprise DNA was damaged due to generation of superoxide during anhydrobiosis and followed by the perfect repair. Indeed the expression of genes encoding the DNA repair enzymes occurred upon entering anhydrobiosis, indicating DNA damage including double-strand breaks and their subsequent repair. The mature antioxidant proteins also accumulated in the dry larvae and the total activity of antioxidants increased by a 3–4 fold in association with anhydrobiosis. Here I will review desiccation tolerance mechanism including a new insight how *P. vanderplanki* larvae deal with the oxidative stress to survive complete desiccation.

## ***Riethia manauara*, a new Amazonian chironomid (Diptera: Chironomidae) from Brazil**



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The genus *Riethia* was erected by Kieffer in 1917, and it includes non-biting midges whose larvae inhabit the bottom of lotic and lentic systems, associated with sandy and fine particulate organic matter. The genus comprises six species described, all of which, except *R. truncatocaudata* Edwards were known only from the Australasian region. In the present study, one new species of *Riethia* is described in all stages and generic diagnoses to the immature stages are emended. The larvae were collected using a hand net in aquatic systems in the Amazon rainforest in northern Brazil, and the specimens examined were slide-mounted in Euparal. The type material is deposited in the Reference Collection of Laboratório de Entomologia Aquática (LEA), Universidade Federal de São Carlos (UFSCar), São Carlos, Brazil and in the Invertebrate Collection of Instituto Nacional de Pesquisas da Amazônia (INPA), Manaus, Brazil. The immatures stages diagnosis must be emended as follows: pupa with transverse band of dorsal spines on conjunctives III/IV and IV/V present or absent. Pedes spurii B present or absent. Larva may have antenna with 5 or 6 segments. Lauterborn organs on apex of 2nd or 2nd and 3rd segments. *Riethia manauara* sp. n. can be separated from other species by the combination of the following characters. Male: hypopygium with superior volsella hooklike, basally pubescent; distal margin with about six long simple setae. Median volsella short, with two simple setae. Inferior volsella with flattened scales; numerous apically pectinate and three distally non-pectinate. Pupa: cephalothorax strongly granulose. Hook row extending nearly on 1/3 the width of tergite II. Conjunctives III/IV and IV/V bare. Longitudinal creases extending the full length of tergites II–VI. Larva: antenna with 6 segments. Lauterborn organs on apex of 2nd and 3rd segment.

## **Why bother to identify *Chironomus* species in contaminant monitoring?**

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Chironomid larvae have great potential as contaminant biomonitors because they are present in a wide variety of freshwaters, including those that are highly contaminated. Since chironomid species are notoriously difficult to identify, closely related species are often pooled for contaminant analysis. However, in doing so, one presumes that contaminant concentrations do not differ markedly among the pooled species. We tested this assumption on the widespread midge *Chironomus* by collecting larvae of this genus from lakes located along a metal-contamination gradient and then identifying species using a combination of molecular and morphological techniques. By measuring trace metal concentrations in each *Chironomus* species we discovered that, within a given lake, the concentrations of some metals (Cd and Se) differed among sympatric species. Among lakes, metal concentrations in the various *Chironomus* species differed in a consistent manner such that certain species had consistently higher concentrations of some metals than did others. To determine why species sharing the same habitat differ in their metal concentrations, we measured larval sulfur isotopic ratios. These measurements revealed that *Chironomus* species sharing the same habitat tend to feed at different depths in sediment where metal concentration and/or bioavailability are likely to differ. Overall, our results suggest that a “one-size fits all” approach for *Chironomus* species may not be valid and that behavioral differences among these morphologically-similar larvae influence their exposure to contaminants.

# **The Chironomidae collection of the Zoological Institute (St. Petersburg): history, current state and role for further research**

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The report overviews the Chironomidae collection at the Zoological Institute of Russian Academy of Sciences in St. Petersburg. It is the oldest, one of the largest and most important collections of Chironomidae in Russia. Most of the collection was unknown to specialists for decades. The collection consists of three main parts: dry specimens (about 20000 pinned adults), material in ethanol (over 25000 vials with larvae, pupae and adults) and permanent slides (over 12000 slides with larvae, pupae and adults). Major periods of the collection formation are considered, based on a study of the collection itself, published sources and archival data. An attempt is made to demonstrate the role of different researchers in creation of each part of the collection. Collectors and expeditions, whose contribution was most important, are listed, as well as the regions of collections. The role of chironomid researchers, who made important contributions to the creation, enrichment or study of the collection, is described. Among them, Alexey Chernovskii, Vera Pankratova, Carl Lundström, Maurice Goetghebuer, Alevtina Shilova and Ole Sæther should be mentioned. According to a preliminary estimate, the collection contains type specimens about of 160 species described by 21 authors from the former USSR territory, and thousands of specimens determined by these and other experts. In addition, the collection includes a lot of unprocessed material from the vast territory of the former USSR, including many distant regions, which are still difficult to access. The modern importance of the collection is considered, as well as its value for further research of systematics and biodiversity of the Chironomidae. The Chironomidae collection of the Zoological Institute is particularly important because it will enable one to interpret correctly many species names proposed in 1930-1980ies, mostly based on brief descriptions of larvae and currently often considered as nomina dubia.

## **Chironomidae of semiaquatic lake shore habitats in Karelian Isthmus (NW Russia)**

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Semiaquatic shore substrata are poorly studied habitats of Chironomidae. Lakes Bol'shoe Rakovoe (Eteläjärvi) and Okhotnich'e (Muolaanjärvi) are shallow mesotrophic wetlands in Karelian Isthmus (ca. 80 km NW St. Petersburg), surrounded by thick swing moor hundreds meters wide formed by herbaceous vegetation and Sphagnum. We aimed to identify species structure of immature chironomid assemblages in highly specific habitats and to provide their quantitative assessment. Diptera in water margin zone (20-m part of swing moor adjoining lake littoral) were studied in 2001 at five sites using two techniques. Quantitative samples of substrata (turf and litter) were taken with a grab-net. Rearings of adults from substrata were made in laboratory. Thirty-two samples ( $S=1.27\text{m}^2$ ) were taken in July and October; 2970 emerging chironomid adults were identified. Nineteen Chironomidae species were found, three of Tanypodinae, ten of Orthocladiinae and five of Chironominae. *Tavastia yggdrasil*, *Thienemanniella minuscula* and *Polypedilum trigonus* were first recorded from Russia, and eight more species, from NW Russia. Orthocladiinae accounted for over 99% emerging adults, with three species predominant and numerous on all sites, *Paraphaenocladus impensus*, *Limnophyes minimus* and *L. natalensis*. Species structure is discussed and compared with data on similar habitats. Mean abundance of chironomid immatures varied from 1246 to 32060 ind./m<sup>2</sup>, mean biomass, 0.104 to 2.053 g(wet weight)/m<sup>2</sup>, depending on site and season. At each site, values of abundance and biomass in July were several times higher than in October. In July, Chironomidae comprised 3-15% of Diptera



biomass and 1-5% of macroinvertebrates biomass; in October, 13-49% and 7-20%, respectively. Factors affecting species distribution and abundance are discussed. Comparison of our data with results of the littoral zone study (Skvortsov, Belyakova, 1999) showed that species lists were totally different. Probably, two factors determine specific features of swing moor habitat: stable water regime (neither prolonged flooding nor distinct drought occurs, as swing moor floats at water surface) and incoming coarse organic matter (mostly remains of herbaceous plants without allochthonous components).

## **The ecology of subfossil Chironomidae and Chaoboridae assemblages from southern Canadian lakes**

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Subfossil assemblages of Chironomidae and Chaoboridae were examined in 172 lakes spanning the Great Lakes region of southern Canada. The lakeset spanned a gradient of depth (shallow polymictic to deep stratified), trophic status (oligotrophic to eutrophic), deepwater oxygen (anoxia to saturation). There were several surprises when examining the environmental distribution of various taxa. For example, *Corynoneura/Thienemanniella* was more abundant in deeper stratified lakes, and *Eukiefferiella/Tventia* was found primarily in stratified lake assemblages, despite neither taxa not being considered profundal in traditional chironomid-based lake classification schemes. Comparisons of assemblages from polymictic vs stratified lakes indicate that there may be candidate taxa for inferring polymictic vs stratified conditions, such as *Cladopelma* or *Einfeldia* spp., which may be useful for interpreting paleoenvironmental records where a lake or pond may have experienced shifts in thermal regime. An expanded hypolimnetic oxygen inference model using both subfossil chironomids and chaoborids produced a modest improvement ( $n = 109$ ,  $r^2_{(jack)} = 0.70$ , RMSEP = 1.83 mg L<sup>-1</sup> [O<sub>2</sub>]) compared to the original 2001 VWHO model ( $n = 54$ ,  $r^2_{(jack)} = 0.54$ , RMSEP = 2.15 mg L<sup>-1</sup> [O<sub>2</sub>]).

## ***Chironomus aprilius* Meigen, 1830, production in underground brackish waters of Bafq, Iran**

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Blood worms are known as useful live food for cultured aquatics. *Chironomus aprilius* Meigen, 1830 is an endemic blood worm in sediments of the earthponds of Bafq area in the center of Iran. The mean of chironomid larvae density in sediments of the enriched earthpond was 8800±2690/m<sup>2</sup>, maximally in winter. Collection of egg masses was performed by innovative collectors prepared with low cost materials. May, June and July were the best time for egg collection. Culture of blood worms was successful in glass tanks with 16h. light, at 24-26°C temperature. Blood worms were withdrawn 14 days after egg incubation. Suitable concentration for harvesting was 1250 eggs/m<sup>2</sup> (1-2 egg masses/m<sup>2</sup>) which lead to 89% hatching. Enrichment of earthponds or plastic tanks including sediment is recommended for culture of chironomid larvae. Large blood worms were mainly available in winter. Results of body analysis of the larvae indicated high dry matter with good quality.

# Studies on distribution and diversity of Chironomid larvae (Insect: Diptera), with special emphasis on water quality in Dr. Salim Ali Lake, Aurangabad, M.S. India

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Dr. Salim Ali Lake in Aurangabad (19°46'48"N, 75°17'24"E) was under investigations for the study of influence of some physico-chemical parameters on distribution and diversity of Chironomidae larvae. Samples were collected from seven sites in lake over a period of one year, from Oct. 2009 to Sept. 2010. Total of 9 taxa belonging to 2 subfamilies, Chironominae and Tanypodinae were identified. Chironominae was the richest sub-family in the lake; the frequent and abundant genus was *Chironomus*. The Tanypodinae sub-family showed certain sensitivity to low dissolved oxygen concentration and high nutrient levels, and was not found at points of high pollution levels.

## Additional notes about the revision of the genus *Orthocladius* s. str. Van der Wulp, 1874

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Palaeartic and Nearctic species belonging to the genus *Orthocladius* van der Wulp, 1874 were revised repeatedly (Soptonis, 1977; Rossaro et al., 2003; Sæther, 2003; Spies & Sæther, 2004; Sæther, 2005). Recently the examination of the material from Goetghebuer's collection deposited at the IRSNB and from Brundin's collection deposited at the NHM allowed us to add new knowledge to this intriguing genus. An attempt to summarize the present knowledge resulted in the production of a new key to West Palaeartic species belonging to the subgenus *Orthocladius* s. str. known as adult males and pupal exuviae. Species belonging to the subgenus *Symposiocladius* Cranston and some Nearctic species, which were made available for examination, were also included in the key. The adult males can be separated on the basis of the morphology of the adult male genitalia above all, but there are species which cannot be easily separated on the basis of adult male morphology alone. The pupal exuviae often allow a better separation, but there are species whose adult males are better separated than pupal exuviae. The best situation is when both stages are available, unfortunately some species deposited at IRSNB are known only as adult males, so some questions still remain open.

## Benthic quality index in European lakes

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The development of benthic quality indices for European lakes is hindered by the lack of information concerning some lake types and some anthropogenic pressures. Most information is from northern European lakes stressed by acidification and from deep lakes subjected to eutrophication; for other lake types (Mediterranean for example) and for other pressures (hydromorphological alteration, toxic stress) there is practically no information about the response of benthic macro-invertebrates; this hinders the possibility of an inter-calibration of the indices among the MS in EU. In the present communication some benthic quality indices are proposed considering the littoral, sublittoral and profundal zones in small and large lakes from the Alpine region in response to eutrophication above all. The samples available were collected with grabs or by scuba divers in different years since 1950. The indicator taxa which prevailed in these lakes were chironomids and oligochaetes; so these taxa were used above all to develop the indices. The indices were validated using different databases. At present the analysis of the response of benthic macroinvertebrates to other pressures is still in embryo.

## Trait-based surveillance of flood channel effects on the River Thames

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An artificial anabranch was opened to the main River Thames by the end of 2001. Chironomid pupal exuviae have been regularly sampled from sites along the main river since 1977 including sites above and below the anabranch. Two sites on the anabranch have also been surveyed since 2003. Feeding and habitat preferences were attributed to taxa while additional environmental-tolerance traits were derived from the long-term data of the Thames. Canonical Correspondence Analysis, with spatial variation partialled out, was used to select temporal environmental variables explaining chironomid taxa distribution. Taxa with significant t-values in the regression with selected environmental variables were then included in the subsequent univariate analysis of variance of traits by General Linear Modelling. From 1977 up to 2001 lower Thames sites, above and below the anabranch, showed no significant changes in any of the traits investigated. Four surveys from 2002 to 2009 have revealed some significant changes, either in comparison with before construction or between sites, downstream of the anabranch. These changes suggest that the anabranch is providing a sink for poor-quality sediments. Immediately downstream of the anabranch less sediment is depositing in the Thames, grazers have increased, as have chironomids sensitive to ammonia while chironomids tolerant of low oxygen conditions have decreased.

## Key to the species of the genus *Tanytarsus* van der Wulp from Brazil

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The Chironomidae research in Brazil started nearly 200 years ago, but a significant increase of studies on taxonomy, systematics and ecology has only occurred through the last 50 years, especially in the last 20 years. A total of 379 species are recorded from Brazil and almost half of them were described after the Catalog of Spies & Reiss (1996) of Neotropical and Mexican Chironomidae (Mendes & Pinho 2011). For many insect groups, Brazil (and the whole Neotropical region) is a species-rich area. However, a precise estimation of chironomid species richness is still not possible due to the absence of records for many regions of the country. Faunistic studies are thus necessary, but they must be assisted by regional taxonomic keys. Trivinho-Strixino & Strixino (2003, 2011) have given the most complete contribution for larvae identification of Brazilian chironomids. Recent studies have provided identification keys for adults, pupae and larvae of Brazilian chironomids (Roque & Trivinho-Strixino 2008; Mendes & Andersen 2008, 2009; Pinho et al. 2009, Wiedenbrug & Trivinho-Strixino, 2009). In the tribe Tanytarsini, the genus *Tanytarsus* van der Wulp, 1874 is one of the most species rich, widely distributed and an eurytopic genus. The aim of our contribution is to present a taxonomic key for adult males of 25 *Tanytarsus* species recorded from Brazil. A dichotomous key with figures is given based on external morphology, which is very diverse in *Tanytarsus* and usually allows for differentiation of species and identification of diagnostic characters.

## Discrimination of the Genus *Cricotopus* (Diptera: Chironomidae) from Turkey by Cytochrome c oxidase subunit I – preliminary results

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The family is more than 120 million years old and has undergone extensive adaptive radiation to occupy a wider range of microhabitats at present than any other aquatic insect group. Chironomids (Diptera) are often the most abundant group of insects in freshwater environments worldwide. The family Chironomidae includes over 10,000 species, which are distributed from the tropics to the Arctic in lakes, streams and puddles. Unfortunately, the larval stage of Chironomids, commonly collected in aquatic sampling surveys, possesses relatively few morphological characteristics useful for identification. Molecular approaches are now being used for identification and taxonomic resolution in many animal taxa. This study was the first time in Turkey for Chironomidae. In this study, species of larval Chironomids were collected from 15 lakes of Turkey. Species were characterized morphologically and 13 Tanypodinae, 1 Prodiamesinae, 61 Chironominae and 30 Orthocladiinae species were identified. Mitochondrial gene, Cytochrome c oxidase subunit I (COI), has been used as phylogenetic marker in *Cricotopus* species from 15 lakes of Turkey to identify the relationship between *Cricotopus* species.

# Within-lake productivity affects the role of methane as a carbon source for benthic and planktonic food webs in Lake De Waay, The Netherlands

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The analysis of the carbon isotopic composition of animal tissues provides insights into the carbon sources and the structure of aquatic food webs. In lake ecosystems biogenic methane can provide a significant carbon source for some autotrophic microorganisms (i.e. methanotrophic bacteria). Chironomid larvae and planktonic Cladocera play an important role in the transfer of this methanogenic carbon from these microbes to higher trophic levels, as indicated by the depleted  $\delta^{13}\text{C}$  signature typical of biogenic methane that has been reported from some chironomid and water flea samples. In De Waay, a small dyke-breach lake in The Netherlands, we observed a strong seasonal variation in  $\delta^{13}\text{C}$  of chironomid larvae (-28.4 to -39.0‰), *Daphnia* (Cladocera) (-26.5 to -44.3‰) and *Daphnia* ephippia (resting eggs, -37.9 to -49.4‰). Such low  $\delta^{13}\text{C}$  values indicate that besides food sources consisting of carbon of photosynthetic origin, chironomids and *Daphnia* in Lake De Waay also ingest methanogenic carbon. Diatom assemblages in the lake sediments showed that De Waay experienced a distinct eutrophication during the 20th century and the Mn:Fe ratios in the sediments revealed that this eutrophication was associated with an increase of hypoxic and anoxic conditions during stratification. Fossilized head capsules of chironomid larvae and ephippia of *Daphnia* from these sediments were characterized by more negative  $\delta^{13}\text{C}$  values (-36 to -42‰) during the more productive phase of the lake. This implies that during the high productivity phase both the benthic and planktonic parts of the food web received higher contributions of methanogenic carbon than during periods of lower productivity. Our results show that in small, nutrient-rich lakes such as De Waay a change in trophic state can lead to distinct changes in the source of carbon assimilated by aquatic invertebrates and incorporated into the lacustrine food web.

## Endogenic bacteria facilitate chironomids survival in polluted habitats

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Chironomids (Chironomidae) are the most widely distributed insects in freshwater. *Chironomus* are considered pollution tolerant, however, little is known about their protective mechanisms in contaminated environments. Females deposit egg masses at the water's edge, each egg mass containing hundreds of eggs embedded in a gelatinous matrix. Chironomid egg masses serve as a reservoir for *Vibrio cholerae* (Broza & Halpern, 2001, Nature) as well as for *Aeromonas* pathogenic species. In order to determine the diversity of bacterial communities (other than *V. cholerae* and *Aeromonas*) associated with chironomid egg masses and larvae, we used culture dependent and independent (PCR-DGGE, cloning and 454-Pyrosequencing) methods. A great similarity was found between the bacterial communities of different egg masses that were sampled during a five months period. This implies that those bacteria are stable residents in this niche. Using cloning and Pyrosequencing methods it was found that ca. 40% of the bacterial species that inhabit the egg masses and the larvae had a potential ability to detoxify pollutants. Koch's postulates were applied to test whether bacteria play a role in protecting chironomids from toxicants. When *Chironomus transvaalensis* larvae that were alcohol sterilized, were incubated with 0.05mM Pb, they did not survive. However, the control larvae without

lead or with lead and without sterilization, survived. A bacterial species (*Chromobacterium* sp.) was isolated from the unsterilized control larvae that survived in the presence of lead. The addition of this bacterium to the sterilized larvae with lead resulted in the larval survival. Similar results were obtained when the experiment was repeated with 0.05mM Cr as the toxicant. We conclude that the bacteria that inhabit chironomids have a role in protecting them from pollutants. These findings could open new horizons in our understanding of the relationships between bacteria and insects.

## **Two new species of *Hudsonimyia* Roback, 1979 (Diptera: Chironomidae: Tanypodinae) from Neotropical Region**



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Non-biting midges of the genus *Hudsonimyia* Roback are small dipterans found in stream shallow water slowly flowing over granite outcrops covered with algae, moss and detritus. The genus comprises two species described and it was erected based on *H. karelena* from the eastern United States. In this study, two new species are described and generic diagnosis to pupa is emended. The larvae were collected in streams from Atlantic forest and mountain areas in Brazil. The holotypes material is deposited in the Museu de Zoologia da Universidade de São Paulo (MZUSP), São Paulo. The pupa diagnose must be emended as follows: thoracic horn with basal lobe present or absent. The male of *H. sp. 1* resembles *H. sp. 2* distinguishing by head having uniserial temporal setae frontally and number of prealar setae. The pupa of *H. sp. 1* is similar to the pupa of *H. sp. 2*, but can be separated by shagreen on tergites arranged in slightly rows and inner margins of anal lobe without spinules. The larva of *H. sp. 1* seems to be closely related to *H. sp. 2* and *H. karelena* differentiating by maculation on the distal margin, occupying about 1/4 length of head in *H. sp. 1*. The male of *H. sp. 2* resembles *H. sp. 1* distinguishing by head having multiserial temporal setae frontally and number of prealar setae. The female of *H. sp. 2* differs from other *Hudsonimyia* species by gonapophysis VIII broadly rounded, without setae. The pupa of *H. sp. 2* is similar to the pupa of *H. sp. 1*, but can be separated by shagreen on tergites arranged in slightly arched rows and inner margins of anal lobe with spinules. The larva of *H. sp. 2* seems to be closely related to *H. karelena* except for procercus ratio (L/W), higher in *H. sp. 2*.

## **A new neotropical species of *Larsia* Fittkau, 1962 (Chironomidae: Tanypodinae) from *Aechmea distichantha* Lem. (Bromeliaceae) phytotelmata**

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The cosmopolitan genus *Larsia* Fittkau 1962 is known by seven species in the Neotropical region (Ashe & O'Connor 2009, Oliveira & Silva 2011). Immatures of *Larsia* were found in lentic and lotic environments. The immatures of a new species of *Larsia* were collected in phytotelmata in northeastern Argentina. The new species was collected from the water held by the leaf axils of the bromeliad *Aechmea distichantha* Lem. and reared to adult in laboratory. *Aechmea distichantha* is a bromeliad with an erect rosette

of arching strap-shaped leaves, distributed in the deciduous, semideciduous and evergreen forests in southern Brazil, Bolivia, Paraguay, Uruguay and northern Argentina. The pupa of the new species bears an unusual thoracic horn for the genus, being this character very useful to distinguish this new species from other *Larsia* species. The thoracic horn is long and narrow, lacks a corona and the respiratory atrium is slightly rugose. The male of *L. angusticornis* shares the abdominal coloration pattern with *L. lyra* Sublette, *L. marginella* Malloch and *L. gelhausi* Oliveira et Silva. As in *L. fittkaui* Sublette et Sasa and *L. labartheae* Serpa-Filho the dorsomedial lobe of the female gonapophysis VIII is short and curved. The antennal length / mandible length ratio of *L. angusticornis* is the smallest for the genus. The larval cephalic setation of the new species fits with the generic description by Kowalyk (1985), but the setae S9, S10 and VP are arranged in line.

## **Short comment on Chironominae (Insecta, Diptera) from Distrito Federal, Brazil**

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It is believed that great part of Brazilian chironomid fauna remains unknown. To help to solve this issue, a preliminary study of the Chironomidae present in a stream from the core Brazilian area was proposed. The aim of the present study was to give a first record of the Chironominae assemblage at Sarandi stream, Brazilian Cerrado. Sarandi stream spring is at Embrapa Cerrados (Planaltina-DF) and runs towards Mestre D'Armas River which belongs to Paraná River Basin. Using a D-hand net, two replicates were taken from the stream on October/2009. The samples were placed in plastic devices and transported to the laboratory where the material was processed and the Chironominae specimens were slide mounted, counted and identified into genera. Sixty-six individuals were sampled, comprising 11 genera. Chironomini was the richest in number of genera besides Tanytarsini showed the higher abundance of individuals (66.7%). *Rheotanytarsus* and *Caladomyia* were the most abundant. More studies will be conducted in order to identify the chironomid fauna from other aquatic environments of the Distrito Federal region.

## ***Ablabesmyia americana* and *Rheotanytarsus distinctissimus* are valid species**

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The species *Ablabesmyia americana* Fittkau, 1962 has been considered a junior synonym of *A. monilis* (Linnaeus, 1758) and *Rheotanytarsus distinctissimus* (Brundin, 1947) has been treated as junior synonym of *R. pellucidus* (Walker, 1848). Analysis of partial COI gene sequences as well as adult male morphology of North American *R. pellucidus* and *R. distinctissimus* from northern Europe revealed differences sufficient to treat these as separate species. Similar differences were observed between the North American *A. americana* and the European *A. monilis*. Both *R. distinctissimus* and *A. americana* should therefore be treated as valid species.

# The response of chironomid assemblages to mineral richness gradient in the Western Carpathian spring fens

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Water chemistry, especially mineral richness determined mainly by bedrock, represents the main gradient in Western Carpathian spring fens. It shapes the aquatic environment of spring fens as it determines vegetation cover and influences substratum composition, and may have a direct effect on aquatic organisms as well. In Western Carpathians, spring fens covering a whole range of mineral richness (basicity) gradient from mineral-poor, *Sphagnum* fens to mineral-rich fens with tufa formation can be found. Within this study we asked a question whether, and to what extent, do chironomid assemblages respond to the mineral richness gradient. Chironomid larvae were collected from two contrasting habitats (standing- and flowing-water part of the fens) at 17 sites three times: in spring, summer and autumn in order to collect most of the chironomid species inhabiting the fens. The samples from the three seasons were pooled together leading to 17 samples from each of the two habitats. Preliminary results indicate that although neither total abundance nor taxa richness did change along the mineral richness gradient, the main variation in the chironomid assemblage taxonomic structure could indeed be found on the gradient of mineral richness. Moreover, the taxonomic composition of chironomid assemblages of flowing-water habitats responded to this gradient more strongly than the assemblage of standing-water habitats. We hypothesize that this discrepancy might have been caused by higher vulnerability of standing-water habitats to desiccation or overheating. The shift in the assemblage taxonomic structure along the mineral richness gradient could be documented by responses of individual taxa: e.g. *Macropelopia* sp. and *Paratendipes nudisquama* preferred acidic, while *Parametriocnemus stylatus* and *Neostempellina thienemanni* preferred basic conditions. The abundance of *Natarsia* sp. and *Rheocricotopus effusus* peaked at medium basicity. As substratum was strongly related to water chemistry, it might have been one of the driving forces behind the assemblage shift.

## Notes on the genus *Nilodosis* Kieffer from East Asia, with emphasis on the microhabitat ecology

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Larval materials of genus *Nilodosis* collected from China, Japan and Korea were compared. The result shows no significant differences exist in the morphology across such a large region. It seems that there is one widespread species, but never common, occurring in East Asia. The larva is a mud-sandy bottom dweller, associated with a layer of silt and organic matter in the upper surface. Based on the cleft mentum and strong curved mandible, it may belong to the of semi-psammorheophilic predator guild. All sampling sites show that larvae prefer relatively clear waterbodies, either in the littoral zone of lakes or the potamals of larger rivers, with maximum depth of 5 meters. Due to co-evolution under similar aquatic environments, further survey of associated adults is urgently needed to clarify the “conspecific hypothesis”.



# Is the Holocene chironomid-inferred temperature at the Plešné Lake (the Bohemian Forest, Czech Republic) reliable?

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The Holocene and Late Glacial mean July temperature has been inferred from subfossil chironomid assemblages at the Plešné Lake (Czech Republic) using the transfer function developed for the Swiss Alps (Heiri et al., 2003). The inferred Holocene July air temperature varied from 10.6 to 14.0°C. The oldest Holocene samples of the Preboreal period (10.3–9 cal ka BP) produced a temperature range of 10.6–12.2°C and showed decreasing trend, whereas Boreal (9–8 cal ka BP) temperature varied between 11.4–12.4°C with an increasing tendency reversing around 8 cal ka BP. The Late Holocene temperature demonstrates increasing trend with a reverse in period of 3–2 cal ka BP and exhibits large fluctuations overall. The quantitative temperature reconstruction apparently does not follow the common trend of the maximum temperature recorded in the early Holocene and subsequent cooling, as was documented from other sites throughout the northern hemisphere (Larocque & Hall, 2004). Over the period ca. 6–5 cal ka BP, the greatest afforestation in the lake catchment resulted in the climax forest as a source of organic matter for the lake. Since ca. 2 cal ka BP, the pollen analysis showed a first distinct human impact on the original vegetation. The high organic matter input and human activities near the lake could lead to the oxygen deficiency/ anoxia in the lake. Decreasing trends of Mn:Fe molar ratio in the sediment suggest the oxygen depletion could be the explanatory factor that has overridden the effects of temperature on chironomid community.

## Chironomid as Palaeoenvironmental proxy: where, when, and which environmental variables can be inferred?

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Palaeoenvironmental proxies are widely used to reconstruct past changes in a variety of environmental variables through the use of inference models. A major challenge lies in validating and interpreting the reconstructions. Chironomids were first used to infer changes in summer air and water temperature, but were later adopted to infer past changes in hypolimnetic anoxia, chlorophyll a, total phosphorus, salinity, water depth, stream flow, and distance to littoral vegetation. All these environmental variables are significant predictors of particular training-set assemblage composition. However, what confidence can we have that any significant predictor variable can be reconstructed from past species assemblages? Most training-sets are designed to maximise variation of the environmental variable of interest and to minimise the variation in other variables. Similarly, sites selected for reconstructions need to be sensitive to changes in the environmental variable of interest, but be less influenced by changes in potentially confounding variables. Here, we employ a new numerical method to test if quantitative environmental inferences from several Norwegian and Swedish sites using chironomids explain more of the variation in assemblage composition than reconstructions of random environmental variables.

By using this method, we aim to determine which sites are good for palaeoenvironmental inferences, which environmental variables can be inferred, and whether some periods give more reliable results along specific environmental gradients than others. For Holocene sequences, preliminary results suggest that inferred temperature is significantly different from random variables at 40% of the sites. Inferences of lake depth and total organic carbon are significant different from random at 30% of the sites. For Late Glacial sequences, both inferred pH and inferred temperature are significantly different from random at 67% of the sites.

## Revision of the Neotropical *Corynoneura* Winnertz, preliminary results

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The genus *Corynoneura* Winnertz was revised by Fu et al. (2009) and according to the authors 66 species of the genus are known worldwide. Considering the Neotropical region, 10 species of *Corynoneura* are known so far: two from Guatemala, one from Argentina and seven from Brazil. The aim of this paper is to increase the knowledge of the group in the Neotropical region. Nine new species were already found, six were collected in the south and southeastern Brazil, one is from Costa Rica and two from Chile. Females of three species and pupa of five species are known, only one species has a known larva. The ten species already described for the region are revised and a preliminary key for the males of *Corynoneura* found in the Neotropical region is also presented.

## Phylogeny of Diamesinae inferred from mtDNA sequences

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Partial mitochondrial DNA-sequences (Cox2 and 16S) were retrieved from Diamesinae species including *Diamesa*, *Lappodiamesa*, *Linevitshia*, *Pagastia*, *Potthasia*, *Pseudodiamesa*, *Pseudokiefferiella*, *Protanypus* and *Sasayusurika*. Bayesian estimates of tree topologies and branch lengths with different models of evolution indicate somewhat modest levels of phylogenetic signal with low credibility of some nodes. Puzzling results were obtained with representatives of the latitarsis and cinerella groups, respectively, possibly suggesting lineage sorting or hybridization of closely related species in sympatry. Putative *Diamesa aberrata* is either para- or polyphyletic. However, the results also support some previously published phylogenetic hypotheses: 1) In accordance with Serra-Tosio's (1973) ideas, *Diamesa* is a relatively derived genus and *Diamesa permacra* is near the root of the *Diamesa* clade. 2) *Diamesa ruwenzoriensis* belongs to a more recent clade, lending support to the proposal (Willassen and Cranston, 1986) that *Diamesa* colonised Africa from Eurasia. 3) As suggested by Willassen (1985) *Diamesa nivicaavernicola* is the sister of the *davisi* group and the morphologically somewhat cryptic species of this group are more genetically divergent than some of the *Diamesa* with conspicuously different male genitalia. Tentative datings of tree nodes were based on various assumptions, including calibration with Australian and S. American *Paraheptagyia*.

## **A review of the genus *Tudayusurika* from Japan (Chironomidae: Orthocladiinae)**

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The genus *Tsundayusurika* was erected by Sasa in 1985 for a single species *T. fudosecunda* collected from the shore of Lake Fudo, southern Kyusyu, Japan. The genus is distinct from any other orthoclad genera by the following combination of characters: antennal flagellum composed of 10 flagellomeres in female; in male genitalia, anal point very short but wide, transparent; basal lobe of gonocoxite large, rounded posteriorly and strongly sclerotized; in female genitalia, 9T completely divided into two tergites by a membrane running along the median longitudinal line, cercus large, rhombic and directed posteriorly. Up to date, 3 species of the genus *Tudayusurika*, *T. multiannulata* Tokunaga, *T. fudosecunda* Sasa and *T. yufunivea* (Sasa), are known from Taiwan and Japan. As pointed out by Cranston et al. (1989), it becomes clear that the first species is a senior synonym of the last species based on the study of the type material. Furthermore we can recognize that *Kuroyonyusurika kuroheius* Sasa, 1996 is also a junior synonym of *T. multiannulata*. Recently, we could have an opportunity to examine the 3rd species belonging to the genus *Tsundayusurika* collected from Yambaru, Okinawa Island, Japan. Although the species is not clearly separable from *multiannulata* in the male, the female is quite distinct from the latter by having the longitudinal membranous area on 9T wider than that of the latter.

## **Ecological variables influencing the distribution of subfossil chironomid assemblages in the lakes from the middle and lower reach of Yangtze River and its paleolimnological application**

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Previous studies have shown chironomids to be excellent indicators of nutrient level of the lakes from the middle and lower reaches of Yangtze River and training sets have been developed in order to allow these changes to be reconstructed quantitatively from subfossil sequences. Here we present the results of the enlarged training set from this region. The 60-lake chironomid-TP calibration set from the middle and lower reaches of the Yangtze River represents a significant expansion of the first calibration set we assembled which was based on 30 lakes. Data from these lakes indicate that the nutrient gradient was the major factor affecting the distribution of chironomid across these sites. A chironomid-TP inference model was developed by applying an optimal two-component WA-PLS model and this model could be used to produce quantitative records of past water quality for lacustrine sites across the middle and lower reaches of Yangtze River, which has important implications for future lake management and ecological restoration. In order to determine baseline conditions (pre-impact) and recent changes to lakes on the middle reach of the Yangtze River, China, the sediment cores from three typical lake (Wushan Lake, Taibai Lake and Longgan Lake) were extracted covering the last ca 150 years. The history of eutrophication based on the changes in chironomid fauna of the lake sediments was studied.

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