CHIRONOMID RESEARCH THROUGH PANDEMICS, RECENT ADVANCES

Richard Cornette[1*], Valeria Lencioni[2] & Peter Langton[3]

[1]*Institute of Agrobiological Sciences, NARO, Tsukuba, Japan.*
E-mail: cornette@affrc.go.jp
[2]*MUSE-Museo delle Scienze, Research and Museum Collections office, Trento, Italy.*
E-mail: valeria.lencioni@muse.it
[3]*Coleraine, Co.Londonderry, Northern Ireland.*
E-mail: langtonph@gmail.com
[*]*Corresponding author*

Abstract

After 5 blank years since the last symposium and 3 years of COVID-19 pandemics, the 21st International Symposium on Chironomidae (ISC2022) was held for the first time online from the 4th to the 7th of July, 2022. The symposium gathered a total of 45 presentations organized around the major topics of ecology, taxonomy, genomics, phylogeny, and physiology. We introduce here a general overview of the studies presented during the symposium, together with the few papers published in the present proceedings special issue.

Introduction

Chironomids (non-biting midges) belong to the family Chironomidae, which encompasses a diverse group of insects found in various aquatic and terrestrial habitats worldwide. Chironomidae constitute one of the most diverse families of insects, comprising more than 7,290 described species (Lencioni et al. 2023, Stur and Ekrem 2020) and a species richness estimated to be over 10,000 species worldwide (Cranston 1995). Despite their small size, chironomids play crucial roles in aquatic ecosystems, acting as indicators of environmental health, participating in nutrient cycling, and serving as a vital food source for many organisms (e.g., Hirabayashi et al. 2018, Orendt 2018, Tokeshi 1995), even in the riparian zone (Hågvar et al. 2016, Paetzold et al. 2005). Over the past few years, scientific investigations have shed light on the life history, behavior, and physiological adaptations of chironomids. Researchers have delved into their intricate mating systems, larval development, and unique strategies for survival in extreme habitats (e.g., Cornette et al. 2015, Lee et al. 2006). Additionally, studies have explored the genetic diversity and evolutionary relationships within the family Chironomidae, enhancing our understanding of their evolutionary history and taxonomic classification (Cranston et al. 2012).

The present special issue reports selected topics presented at the 21st International Symposium on Chironomidae (ISC2022). The previous international symposium was organized by Valeria Lencioni in Trento, Italy (Lencioni et al. 2018). During the final forum, it was decided that the next symposium would be scheduled in Tsukuba, Japan in July 2021 (Cornette 2019). However, the COVID-19 pandemic and Tokyo Summer Olympic Games postponed the symposium to summer 2022 (Cornette 2021). Contrary to our expectations, the pandemic was still plaguing the world in early 2022 and Japan's government closed its borders, forcing the organizing committee to shift to an online version of the 21st International Symposium on Chironomidae (Cornette 2022). For the first time of its 58 years' history, the International Symposium on Chironomidae was held online between the 4th and the 7th of July, 2022, using a combination of the LincBiz platform and Zoom video conference system (Fig. 1). ISC2022 gathered 70 participants from 21 countries over the world. Unfortunately, due to the war in Ukraine, we were not able to welcome our Russian colleagues. Delegates shared their recent works through 27 oral presentations and 18 posters. The Honorary Thienemann Lecture was presented by Valeria Lencioni as a synthesis of two decades of studies on cold-adapted chironomids, mainly from the European Alps: "Response of chironomids to climate change: evidence on cold, heat and chemical tolerance of cold-adapted species". Oral sessions were organized around four major fields: ecology, taxonomy, genomics, phylogeny, and physiology. The in memoriam section paid homage to our dear colleagues Len C. Ferrington Jr. (1948-2021), Tadashi Kobayashi (†2020), Bernard Serra-Tosio (†2021) and Patrick (Paddy) Ashe (1954-2022), who deceased just before the symposium (Bouchard et al. 2021, Murray 2022). Finally, the taxonomy workshop, which was traditionally an occasion to exchange specimens, techniques and views about taxonomy, was chaired by Elisabeth Stur. It was a challenge to make the taxonomy workshop online, however the upload of specific discussion topics and microphotographs of specimens allowed fruitful exchanges on chironomid taxonomy.

In spite of the limitations of an online meeting, after a 5-year blank and pandemic restrictions, ISC2022 was a necessary symposium to gather again the chironomid scientist community and share recent advances in their research. We present here an overview of the symposium content with special attention to the papers of ISC2022 proceedings published in this special issue of the CHIRONOMUS Journal of Chironomidae Research.

ISC2022 and special issue contents

A large majority of the topics presented at the 21st International Symposium on Chironomidae were focused on biomonitoring and chironomid autoecology, mainly adaptations in a rapidly changing environment. In this context, Valeria Lencioni, who has been monitoring the adaptation of chironomids in cold streams and alpine environments for decades, was naturally commissioned to present the Honorary Thienemann Lecture. In her lecture, after a presentation of alpine kryal environment and *Diamesa* spp. diversity, she demonstrated the influence of global warming and glaciers melting on the loss of *Diamesa* species richness and colonization by less cold-tolerant species. Most studies referred to *Diamesa tonsa* and *Pseudodiamesa branickii*, selected as model species to investigate the cold adaptation, thermal tolerance and chemical resistance in chironomids (Lencioni et al. 2015, Lencioni et al. 2021). She narrated a story that started with the study of the physiology (e.g., basal metabolism under oxygen and thermal stress) and then the molecular basis of cold resistance in these species, known to have a different autecology, thus occupying different ecological niches. The different level of stenothermy was related to the different vulnerability to climate change of these species. For example, both species were freeze-tolerant, but *D. tonsa* was shown to be more stenothermic and adapted to low temperatures in the kryal zone (Lencioni et al. 2015). De facto, *D. tonsa* is also more threatened by extinction than *P. branickii* in cold habitats where cases of local extinction were already found. Lipidome analysis showed that in both species cell membrane composition was similar, with a high proportion of polyunsaturated fatty acids, which is congruent with cold adaptation. Especially, *D. tonsa* showed a higher phosphatidylethanolamine/phosphatidylcholine ratio and a lower sterols content, which were interpreted as allowing higher membrane fluidity and adaptation to membrane deformations due to freezing (Trenti et al. 2022). This membrane structure might be also related to the unexpected high chemical resistance of *Diamesa* larvae to pesticides, pharmaceuticals and other xenobiotics (Lencioni et al. 2021). During their investigation of the heat shock

Figure 1. Group photo on Zoom video conference system at the final forum of ISC2022.

response of *D. tonsa* under heat stress, Valeria Lencioni's team discovered a new molecular regulatory mechanism involving a heat shock protein 70 (hsp70) pseudogene that apparently acted as a putative long noncoding RNA sequestrating ribosomes and thus controlled post-transcriptionally HSP70 protein expression during the transition between intermediate and acute heat stress (Bernabò et al. 2020). An interesting discovery was the presence of antifreeze proteins, glucose and sucrose as the main cryoprotectants in such species (a topic still little investigated in freshwater insects). As a conclusion, although not yet considered a model organism sensu stricto, *D. tonsa* emerged as one of the better documented species for the physiological and molecular mechanisms of extreme cold adaptation, along with the Antarctic midge *Belgica antarctica* (Lopez-Martinez et al. 2009). Its presence and abundance in freshwater habitats can be considered the best bioindicator of 'glaciality' in all biogeographic regions where the genus *Diamesa* is present.

Ecological studies on chironomids constituted the majority of presentations during ISC2022, with monitoring reports from environments with different anthropogenic influence, from remote natural reserves to urban landscapes. For example, a 14 years survey of tufa barriers in Plitvice lakes national parks with minimal anthropogenic impact revealed the evolution of dipteran community structures in relation to climate stressful events such as droughts (Pozojevic et al. 2023). Concerning the Chironomidae community structure, a shift was observed from detritivorous and active filter feeders to rheophilic passive filter feeders such as species belonging to the genus *Rheotanytarsus* (Doric et al. 2023). Another study in a mountain stream from eastern Croatia yielded new Diamesinae species in Croatia, *Diamesa* cf. *insignipes* (Kieffer, 1908) and *Boreoheptagyia legeri* (Goetghebuer, 1933), *B. legeri* being recorded for the first time at relatively low altitude. Similarly, monitoring of chironomid pupal exuviae of Iceland and Faroe Islands allowed the identification of new genera for the Icelandic fauna: *Conchapelopia*, *Parakiefferiella* and *Paratanytarsus*. Concerning the fauna of Faroe Islands, seasonal monitoring of the dominant species in the streams, *Tvetenia calvescens* (Edwards 1929) was described and is reported in the present special issue (Hansen et al., 2023). An expedition in the remote area of Pico da Neblina in northern Brazil enabled the investigation of Chironomidae communities thriving in streams and madicolous environments at elevations ranging from 100 to 2800 m a.s.l.. At the generic level similar communities were observed in both madicolous and stream biotopes but comparisons at species level are ongoing and new species have been described (Shimabukuro et al. 2021). In another tropical stream located in Puerto Rico, an annual monitoring study indicated relatively low taxa richness and verified the hypothesis that tropical streams show longer and less synchronous chironomid emergence periods throughout the year, although a trend to higher emergence rates was observed during the dry season. This study is reported in detail in the following special issue (Bouchard and Ferrington, 2023). Similarly, annual monitoring of 13 Chironomidae species (Mohammadi et al. 2021) in the Queshlagh river, Kurdistan, Iran, showed that emergence occurred from June to October. Highest Chironomidae diversity was observed in June, with a peak of emergence for 7 species in July, showing high seasonality in this region with a continental climate. Another long-term study on Chironomidae production in the Chikuma river, Japan, estimated an average annual secondary production of 21.49 g of ash-free dry weight /m^2/year, with Orthocladiinae and Diamesiinae the dominant taxa. In comparison to the diversity observed in rivers and streams, few Chironomidae species colonized the marine environment and among them, *Ainuyusurika tuberculatum* (Tokunaga) was found associated with sea lettuce on the shores of Kume island in Japan (Kimura et al., 2023). Salinity is not restricted to the marine environment. For example, continental Nebraska sandhills include permanent and ephemeral lakes with variable salinity ranging from freshwater to hyposaline (Hayford and Baker 2011). A study of Chironomidae in these lakes showed decreasing taxa richness with salt concentration and identified indicative species for subsaline and hyposaline lakes, respectively.

Concerning environments with increased anthropogenic influence, a study on reservoir lakes in Texas showed that aquatic macrophyte heterogeneity was correlated with increased Chironomidae genera/species richness, emphasizing the importance of ecological diversity in lake restoration practices. Another study focusing on the effect of dams on the biodiversity of rivers in Lithuania detected an apparent slightly higher Chironomidae diversity in non-dammed rivers, suggesting that dams influence the geomorphometric characteristics of the rivers.

Along with increasing urban influence, a study on Dallas airport drain water streams focused on the Chironomidae diversity at different flow regimes and if family/subfamily level identification allowed sufficient discrimination between the investigated sites: genus level identification showed strongest correlations with watershed characteristics. Similarly, a study on stormwater retention ponds along an urban gradient based on genus/species level Chironomidae identification from pupal exuviae clearly separated ponds based on changes in water quality. Further into urban areas, similar Chironomidae identification from pupal exuviae showed higher diversity in man-made lakes, compared to extreme habitats, such as fountains (Čerba and Hamerlik 2022). This study showed the high adaptability of Chironomidae to urban environments.

The studies on Chironomidae taxonomy generate the necessary fundamental information for the ecological surveys cited above. In the taxonomy sessions of ISC2022, Masaru Yamamoto studied the movability of the gonostylus on the gonocoxite within the subfamily Chironominae and defined five types ranging from fused gonocoxite and gonostylus to articulated gonostylus, namely *Pseudochironomus-Harnischia-Biwatendipes* type, *Chironomus* type, *Stictochironomus* type, *Nilodosis* type and *Shangomyia* type, respectively. Another study focused on the loss and re-acquisition of mouth parts in relation to blood feeding during the evolution of Chironomidae. A revision of Japanese *Dicrotendipes* genus confirmed the 11 reported species and suggested distinct clades for *D. inouei* inhabiting freshwater and brackish water, respectively. New Chironomidae species were described during the symposium: *Orthocladius* (*Orthocladius*) *montisdei* sp. n. and *O.* (*O.*) *queyrassianus* sp. n. from continental France (Moubayed et al. 2022), *Limnophyes knispelae* sp. n. and *L. sartorii* sp. n. from the Swiss Alps, and *Bryophaenocladius adigensis* sp. n. from Trentino, Italy. The last 3 species are described in the present special issue (Moubayed and Lods-Crozet, 2023; Moubayed and Lencioni, 2023). Chironomid identification to the species level often requires the expertise of specialists and the number of such qualified taxonomists is declining nowadays. In order to overcome this problem of qualification and time spent for identification, an attempt of deep learning on mounted specimens allowed the first automatic identification of Chironomidae, although the genera *Chironomus* and *Tvetenia* showed some inconsistencies (Milosavljević et al. 2023). During the last decade, DNA barcoding emerged as a powerful tool for the monitoring of Chironomidae in the field (Chua et al. 2023). However, the necessity of accurate annotation was pointed out in a survey of the European Chironomidae barcode data in the Barcode of Life Data System (BOLD), showing some technical mismatches, but also inconsistencies caused by taxonomic ambiguities or based on actual biological differences. Thus, DNA barcode of *Tanytarsus gracilentus* (Holmgren, 1883) allowed the identification of 3 distinct clades, raising the possibility of cryptic species. Similarly, a DNA barcode survey of Chironomidae in lake Skadar basin (Montenegro/Albania) validated the high identification efficiency of the database (98.6%) and confirmed previously postulated species synonyms, namely *Cricotopus glacialis* (Edwards, 1922) and *Cricotopus sylvestris* (Fabricius, 1794) (Gadawski et al. 2022). Another study associating DNA barcode and nuclear Single Nucleotide Polymorphism (SNP) on Japanese *Nanocladius shigaensis* and *N. asiaticus*, Chironomidae with phoretic larvae living on other aquatic insects (Inoue et al. 2015), confirmed different clades of *N. shigaensis* concordant with the association to different species of host Plecoptera.

Along with DNA barcoding, next generation sequencing techniques allowed unprecedented advances in the genetic and molecular analyses in Chironomidae. For example, the genome skimming technique allowed the reconstruction of complete mitogenomes and thousands of nuclear single orthologue genes for several species of Chironomidae. This approach is promising for resolving the relationships between different Chironomidae taxa and case studies placed Prodiamesinae within Orthocladiinae, or showed that *Cladotanytarsus*, *Neozavrelia* and *Pontomyia* were nested within *Tanytarsus* (Li et al. 2022, Lin et al. 2022, Lin et al. 2022). *Clunio marinus* is a marine chironomid, whose adult emergence is controlled by circalunar clock and tides times. The draft genome of *C. marinus* was published in 2016 (Kaiser et al. 2016) and recent work using Assay for Transposase-Accessible Chromatin (ATAC-seq) and RNA-seq predicted the regulatory network of genes involved in the circalunar clock. The sleeping chironomid *Polypedilum vanderplanki* is a desiccation-tolerant species, whose larvae can survive almost complete desiccation in an ametabolic state known as anhydrobiosis (Cornette and Kikawada 2011). The draft genome of *P. vanderplanki* was deciphered about 10 years ago (Gusev et al. 2014) and recent advances allowed a chromosome-level assembly of the *P. vanderplanki* genome, suggesting that the 4th chromosome acted as an evolutionary "sandbox" allowing the acquisition of anhydrobiosis through high degree of subfunctionalization in paralogous anhydrobiosis genes (Yoshida et al. 2022). Such high-quality genome information for *P. vanderplanki* allowed the development of genome editing techniques for the expression of useful proteins in the cultured cell line Pv11, derived from the sleeping chironomid and also showing desiccation-tolerance (Miyata et al. 2021, Miyata et al. 2022, Watanabe et al. 2016). Another report on *Chironomus ramosus*, a species tolerant to a certain level desiccation, suggested that trehalose and glucosamine could interact with chitin metabolism and facilitate the recovery of larvae after exposure to desiccation stress (Thorat et al. 2017).

Many species of Chironomidae live in extremely acidic hot springs and high-quality genome and transcriptome analysis of two Japanese acid-tolerant species, *Chironomus acerbiphilus* from lake Katanuma and *Polypedilum* sp. cf. *tamanigrum* from Kusatsu hot springs was reported during the symposium. Both species inhabit acidic waters (pH2) and the study identified transcription factors and F-type ATPase as putatively associated with acid adaptation. These results are congruent with those of a previous transcriptome analysis of another acid-tolerant species, *Chironomus sulfurosus* (Fujii et al. 2021). A study of high temperature tolerance and HSP gene expression in *C. sulfurosus* was presented at the symposium.

Molecular studies also focused on hemoglobin genes and a phylogenetic analysis suggested that Tanypodinae might have different hemoglobin components, compared to other Chironomidae. The analysis also separated clusters of Chironomus species in correlation with adaptation to stagnant waters or running waters. Another phylogenetic study suggested that the larval hemoglobins of chironomid midges show sequence and structure similarities with a specific annelid hemoglobin. The investigation of benthic diptera larvae showed that the larvae of some mosquito species, such as *Armigeres* or *Tripteroides*, also expressed hemoglobins that were up-regulated in response to anoxia, as in Chironomus, suggesting a respiratory function.

Chironomidae are also widely used for toxicity tests of pollutants and especially *Chironomus riparius* is a model species recognized by OECD for testing of chemicals (Weltje et al. 2010). During the symposium, an evaluation of aquatic pollution caused by tire rubber microparticles showed that these microparticles disrupt the pathway of oxidative stress and that rubber leachate induced higher toxicity than microparticles in *C. riparius* (Carrasco-Navarro et al. 2021). For adding supplementary information to ecotoxicology tests, histological characterization of *C. riparius* larvae defined the normal tissue architecture in unstressed conditions. On this basis, histological alterations due to magnetic nanoparticles exposure were investigated. The study showed that nano-Fe3O4 exposure affected the digestive system, inducing intracellular vacuolization of midgut cells and significant reduction of the brush border length of epithelial cells (Stojanovic et al. 2021). Similarly, real-time bioimaging experiments targeting zinc ions with a fluorescent probe showed that toxic zinc ions were concentrated in the midgut and sometimes in the hindgut after 24-48h exposure. All these reports confirm that the digestive tract is a major barrier for various pollutants and nanoparticles, but in turn this tissue is also particularly sensitive to toxic compounds. The gut of Chironomidae is also a tissue hosting various fungi from the order Harpellales, usually associated with aquatic insects. A preliminary survey of Japanese Harpellales inhabiting Chironomidae digestive tracts was presented and 3 species new to Japan were identified, increasing the number of Japanese Harpellales species to 11 in total (Sato 2022). A checklist of Harpellales associated with Chironomidae and of their respective hosts is included in the present special issue (Sato, 2023).

Finally, concerning Chironomidae rearing and development, a study was presented on *Chironomus riparius* aiming to optimize mass rearing for aquaculture food. The optimal growth temperature was about 18ºC and the degree days for each larval instar and pupae was determined at this temperature. Similarly, investigation of *P. vanderplanki* development at optimal temperature (27ºC) determined the length of each larval instar, varying from 2-3 days for the first instar to 7-17 days for the fourth instar. The study also showed that ovarian development was not complete at the emergence of adult females and suggested that maturation of the spermatozoa in the spermatheca would be needed for efficient fertilization of the egg masses. In contrast, optimal rearing temperature for the Antarctic midge *Belgica antarctica* was determined at 4ºC (Yoshida and Goto 2023) and in these conditions some last instar larvae had eggs, suggesting that ovarian development occurred earlier than adult emergence.

Overall, ISC2022 gathered chironomid workers after five blank years. During the symposium, most presentations focused on ecology and biomonitoring in relation to global warming, anthropogenic influence and water pollution. New sequencing techniques and big data analysis has allowed significant progress in the fields of phylogeny, genomics and adaptative physiology. On the other hand, the small proportion of presentations about taxonomy and systematics, and the loss of our dear colleagues in the field emphasize the importance of their work for sustaining the constantly forward-moving field of chironomid research.

List of the papers in the special issue

Bouchard, R.W. and Ferrington, L.C. Temporal partitioning of Chironomidae emergence in an insular, tropical rainforest stream.

Hansen, L.J., Kreiling, A.K. and Gíslason G.M. Seasonal variation in the Chironomidae (Diptera) communities of two Faroese streams

Kimura, G., Nakamoto, A., Uezu, T. and Kawai, K. Chironomidae collected at the seashore in Kume Island.

Moubayed, J. and Lencioni, V. *Bryophaenocladius adigensis* sp. n., a new species from the Italian Alps (Diptera: Chironomidae, Orthocladiinae).

Moubayed, J. and Lods-Crozet, B. *Limnophyes knispelae* sp. n. and *L. sartorii* sp. n., two new crenophilous species from the Swiss Alps (Chironomidae, Orthocladiinae).

Sato, H. A new association between Harpellales, insect-gut inhabiting fungi, and Chironomidae in Japan with an updated list of Harpellales documented from Chironomidae

Acknowledgments

We thank all members of the organizing committee who made possible the online version of ISC2022 in spite of various troubles and also all members of the scientific committee who made thorough reviews of all the presentation abstracts. We are also grateful to NARO (Tsukuba, Japan) for the financial support that helped smooth organization of the symposium.

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Article submitted 4. September 2023, accepted by Torbjørn Ekrem 12. September 2023, published 29. December 2023.