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Front page layout: Chironomid in title from photograph by Steve Marshall, Graphic design by Kolbjørn Skarpnes, NTNU Information Division.

Front page photo: *Chironomus acerbiphilus* by Richard Cornette. Logo design by Sachiko Shimura, NARO, Tsukuba, Japan.

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CHIRONOMID RESEARCH THROUGH PANDEMICS, RECENT ADVANCES

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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

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Temporal partitioning of Chironomidae emergence in an insular, tropical rainforest stream

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Abstract

Annual water temperature variation strongly influences larval growth of aquatic insects in streams located in temperate regions or at high elevations, which produces cohorts with highly synchronized emergence periods and short average annual durations of emergence. Studies of Chironomidae in tropical streams indicate that species in these habitats have longer average durations of emergence due to reduced annual variation in water temperature. We used emergence trap data collected over one year from Quebrada Prieta (El Verde Field Station, Puerto Rico) to test the prediction that chironomids of an insular rainforest stream should have longer average annual durations of emergence than chironomids in both temperate streams and mainland streams in continental tropical regions. Taxa richness was relatively low with twenty-eight Chironomidae taxa collected from Quebrada Prieta. Emergence patterns of the most common taxa demonstrated some seasonally with the highest emergence generally occurring during the dry season (January through April). The estimated average emergence duration of Chironomidae in Quebrada Prieta was 205 days/species/year, which was greater than estimates of average durations for chironomids of three streams in Pennsylvania, USA (70 days/species/year), 6 streams in Minnesota, USA (89 days/species/year), and four streams in Guanacaste National Park in northwestern Costa Rica (116 days/species/year). The emergence duration for the chironomid community in Quebrada Prieta was most similar to another tropical, mountain stream in the Democratic Republic of the Congo (255 days/species/year). Although many taxa emerged throughout the one-year sampling period, some taxa in Quebrada Prieta had distinct emergence peaks. This demonstrates that although environmental conditions are stable enough to allow for emergence throughout the year for many species, there is some seasonality in the development of chironomid taxa in this tropical stream. These results are consistent with predictions that invertebrates in tropical streams will have longer, less synchronous emergence periods than species in temperate regions with high annual fluctuations in temperature. However, this research is also similar to several other studies that have identified seasonal emergence peaks in tropical chironomids which demonstrates a need to better understand the exogenous cues that affect these patterns.

Introduction

Temporal partitioning of emergence is commonly documented in Chironomidae from aquatic habitats located in temperate regions or at high elevations where this group has been more thoroughly studied (Armitage 1995). Coffman and de la Rosa (1998) quantified patterns of emergence in three temperate streams and four tropical streams, and developed testable hypotheses and predictions related to the patterns of temporal duration of emergence that may operate over large geographic scales. Their results documented longer average durations of emergence and greater degrees of overlap in emergence among species in tropical streams when compared with streams at higher latitudes. Wolda (1988) also observed reduced seasonality in temporal patterns in duration of emergence for several terrestrial insect species in the tropics but concluded that studies of seasonal patterns of insects in tropical areas were relatively rare and generally focused only on one or a few taxa in a restricted geographic area. Consequently, he recommended that studies of longer duration and dealing more broadly with local faunas would provide a better understanding of factors related to the timing of life history features and their seasonality in insects. Although reduced seasonality appears to be typical for tropical midge communities, several studies on Chironomidae have identified seasonal changes in emergence or abundance of midges in tropical habitats (e.g., Lehmann 1979, Ramírez and Pringle 1998, Sonoda and Trivinho-Strixino 2000, da Silva et al. 2009, Kranzfelder and Ferrington 2016). However, most of these studies only report on seasonal changes in richness or abundance of the total chironomid community and not individual species. Regardless, there is evidence that seasonal exogenous cues do influence chironomid emergence patterns in tropical habitats, but there is a need for additional research to document and determine the factors that affect these patterns.

The aquatic insect fauna of Quebrada Prieta in the Caribbean National Forest in Puerto Rico was the focus of a comprehensive emergence and phenological study in 1990 and 1991 (Masteller and Buzby 1993). The climatic seasonality of weather conditions of the Caribbean National Forest is low, with air temperatures often varying as much on a daily basis as on an annual basis. There is a weak dry season from January through April (Masteller and Buzby 1993) and leaf fall is weakly seasonal although it occurs continuously through the year (Weigart 1970). Ferrington et al. (1993) determined the taxonomic composition and annual patterns of species richness and abundance of the chironomid community but did not make quantitative comparisons of the durations of emergence and temporal overlap of the fauna. Based on the model provided by Coffman and de la Rosa (1998), we developed predictions regarding temporal durations and overlap of emergence of chironomids and re-analyzed the data from Ferrington et al. (1993) to determine if the patterns conformed to our predictions. In addition, we compare our results for Quebrada Prieta with the values presented by Coffman and de la Rosa (1998) for three temperate streams in western Pennsylvania (USA) and four mainland tropical streams in Guanacaste National Park (Costa Rica) and by Bouchard (2007) for six temperate streams in Minnesota (USA). We also re-analyzed emergence data published by Lehmann (1979) for the Kalengo River in the Democratic Republic of the Congo to extend our comparisons to another mainland tropical stream.

Quebrada Prieta experiences very minimal seasonality in terms of temperature, photoperiod, incident solar radiation, rainfall, and changes in the input of allochthonous organic matter. Consequently, we predicted that chironomid species in Quebrada Prieta would emerge continuously throughout the year with no or only minimal peaks in emergence. In addition, we predicted the durations of emergence would be broader on average than species of Chironomidae for streams in western Pennsylvania and Minnesota, which experience greater seasonality for all of these parameters. We also predicted that durations of emergence for chironomids in Quebrada Prieta would be greater compared to species emerging from the four streams in Guanacaste National Park and the Kalengo River where species richness is higher. Finally, we predicted that there would not be a strong relationship between community dissimilarity as the interval between samples increased. As a result, average annual change in emergence should be less than that measured for streams in the two areas investigated by Coffman and de la Rosa (1998) and the Kalengo River in the Democratic Republic of the Congo (Lehmann 1979).

Materials and Methods

Site description

The main study site, Quebrada Prieta, is located in the Luquillo Mountains of northeastern Puerto Rico within the Luquillo Experimental Forest (El Yunque National Forest). It is a second-order, steep-gradient stream with an average slope of approximately 20% in the vicinity of the sample site. The stream originates at approximately 600 meters above sea level and flows into Quebrada Sonadora at approximately 310 meters elevation. The emergence trap was located at 390 meters elevation at the approximate coordinates of 18.32°, -65.81°. The stream is roughly 1.5 kilometers long and it is fully contained within the El Yunque National Forest. The predominant substrates at the study site were bedrock, large boulders, and cobbles, with finer substrates of sand and silts in pools. Quebrada Prieta flows through tabonuco forest at the sample site, which is the predominant forest type at this level of elevation, gradient, and slope stability (Snyder et al. 1987). Compared to temperate habitats, Quebrada Prieta is exposed to limited seasonality, although there is some seasonality in water temperature, rainfall, and input of allochthonous organic matter. Mean monthly water temperatures from a nearby stream (Quebrada Sonadora) ranged from approximately 20-23 ºC with higher temperatures from June through October (Fig. 1a). However, this range is much lower than is typically observed in surface-water fed streams in temperate regions. Rainfall is more variable in the Luquillo Mountains, but there is a weak dry season from January through April (Masteller and Buzby 1993; Fig. 1b). Although most trees drop their leaves throughout the year, there is some seasonality with increased leaf fall from April through July and decreased leaf fall from December through March (Masteller and Buzby 1993). Maps and more information about the stream, including water chemistry and the surrounding riparian setting, are available in Masteller and Buzby (1993).

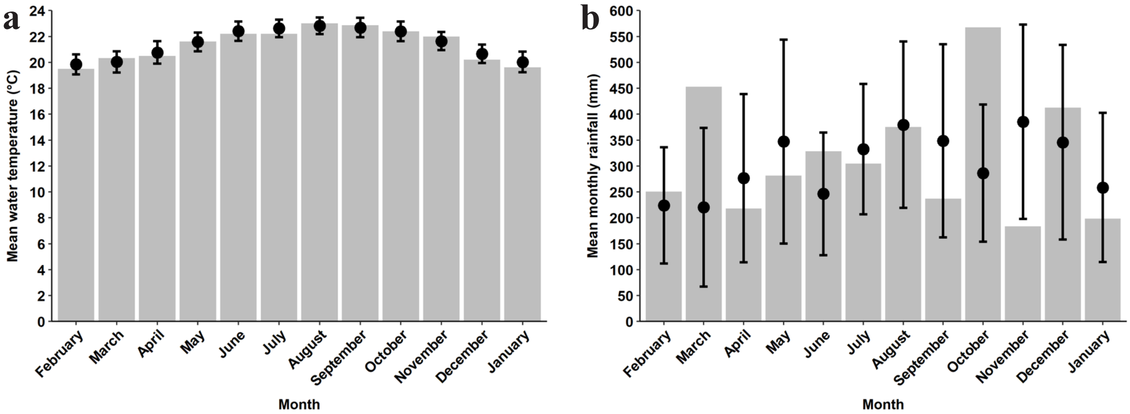

Figure 1. Average monthly water temperature (a) and rainfall (b) from stations near the sample site on Quebrada Prieta. Circles are average values of water temperature (1983-2018) and rainfall (1975-2021) with error bars representing standard deviation. Grey bars are data for February 1990 through January 1991. Water temperature data are from a downstream station in Quebrada Sonadora (McDowell 2021) and rainfall data are from the El Verde Field Station (Ramirez 2021).

Sampling Methodology and Specimen Processing

Samples of emerging aquatic insects were collected for one year with an aspirator from a walk-in emergence trap ($4m^2$) suspended over Quebrada Prieta (Fig. 2). Additional details on the trap design are in Masteller and Buzby (1993). Emerging insects were collected 2-3 times weekly from February 1990 through the end of January 1991. Insects were preserved in 70% ethanol, identified to genus or species, and enumerated. Selected specimens of each taxon were cleared and slide mounted in Euparal using the method described by Schlee (1966).

Analyses

Counts of each taxon were aggregated by month due to the relatively small number of specimens collected in each sample. This also allowed comparison with other studies of chironomid emergence. Community level emergence patterns (abundance and taxa richness) and taxon specific patterns were assessed against seasonal patterns to identify emergence patterns that may be related to exogenous cues. Emergence patterns were categorized based on the number of emergence peaks, the timing of these peaks in relation to seasonal rainfall patterns, and the synchronicity of emergence peaks. Durations of emergence for each species were calculated by dividing the number of months each taxon was collected by 12 and multiplying this value by 365 days. This approach was similar to the method used by Coffman and de la Rosa (1998) to estimate durations of emergence. Bray-Curtis dissimilarity coefficients were calculated between all 66 pairs of monthly samples. The individual and average dissimilarity values were graphed as a function of the number of days between the two months being compared. Monthly emergence totals published by Lehmann (1979) were analyzed in a similar manner for comparison with another tropical habitat. All statistical analyses and plots were generated using R 4.0.4 (R Core Team, 2021). Bray-Curtis dissimilarity coefficients were calculated using "vegdist" function in the "vegan" package (Oksanen et al., 2020).

Figure 2. Emergence trap on Quebrada Prieta.

Table 1. Chironomidae collected from Quebrada Prieta (Puerto Rico) with estimated emergence durations and emergence categories. Emergence categories: Wet = taxa with an emergence peak during the wet period; Dry = taxa with an emergence peak during the dry period; Aseasonal = taxa with multiple emergence peaks which did not correspond to a seasonal rainfall period; and Rare = taxa sampled in low numbers which did not permit emergence pattern categorization.

Taxon	Total Count	Number of months collected	Estimated emergence duration	Emergence Pattern
Chironomidae				
Tanypodinae				
Ablabesmyia	38	10	304	Wet
Djalmabatista	2	2	61	Rare
Labrundinia	111	11	335	Aseasonal
Larsia	273	12	365	Dry
Pentaneura	183	12	365	Aseasonal
Procladius	1	1	30	Rare
Orthocladiinae				
Corynoneura	8	6	183	Rare
Cricotopus	103	12	365	Aseasonal
Diplosmittia	40	3	91	Dry
Limnophyes	65	11	335	Dry
Parametriocnemus	83	12	365	Dry
Thienemanniella	4	3	91	Rare
Unknown orthoclad genus # 1	2	2	61	Rare
Unknown orthoclad genus # 2	7	3	91	Rare
Chironominae				
Chironomini				
Fissimentum	5	4	122	Rare
Paratendipes reidi Freeman, 1957	26	9	274	Aseasonal
Polypedilum sp. 1	4	3	91	Rare
Polypedilum sp. 2	21	7	213	Rare
Polypedilum sp. 3	6	3	91	Rare
Polypedilum sp. 4	8	5	152	Rare
Polypedilum sp. 5	3	2	61	Rare
Stenochironomus cf. *innocuus* (Williston, 1896)	12	4	122	Rare
Stenochironomus sp. 1	463	12	365	Wet
Xestochironomus furcatus (Johannsen, 1938)	6	5	152	Rare
Xestochironomus cf. *nebulosus* Sublette & Wirth, 1972	623	12	365	Dry
Tanytarsini				
Rheotanytarsus	1	1	30	Rare
Tanytarsus sp. 1	211	12	365	Wet
Tanytarsus sp. 2	43	10	304	Dry

Results

Total taxa richness in Quebrada Prieta was low with twenty-eight species of Chironomidae from three subfamilies identified from 137 sample events over the one-year period the stream was monitored (Table 1). As noted in Ferrington et al. (1993), the most common trophic category was xylophages (47% of individuals). The 28 species of Chironomidae from Quebrada Prieta had an estimated average duration of 205 days based on the aggregated monthly data. Chironominae species had the shortest average estimated emergence duration at 193 days/species/year (d/sp/yr). Tanypodinae had the longest average emergence duration (243 d/sp/yr) and Orthocladiinae were intermediate at 198 d/sp/yr. Fifteen taxa in the study each had cumulative abundances that were less than 1% of total annual emergence and were considered rare taxa (Table 1). Rare taxa were present in seven or fewer months and their monthly abundance was low with fewer than 10 individuals collected in any month. Accordingly, the estimated annual durations of emergence for rare taxa were short. When rare taxa were deleted from the analyses, the estimated emergence durations were 323 d/sp/yr for all common taxa and 342, 289, and 355 d/sp/yr, respectively, for Tanypodinae, Orthocladiinae, and Chironominae.

The seven most abundant taxa occurred in all months and accordingly, had the longest estimated durations of emergence (Table 1). These taxa included *Larsia*, *Pentaneura*, *Cricotopus*, *Parametriocnemus*, *Stenochironomus* sp. 1, *Xestochironomus* cf. *nebulosus*, and *Tanytarsus* sp. 1, and all of these taxa had estimated emergence durations of 365 d/yr when samples were composited at monthly intervals. Some seasonal patterns in abundance and richness of the overall chironomid community were observed with declining abundance and taxa richness from January or February and an increase from July through December or January (Fig. 3). These patterns were not as evident as those typically observed in temperate streams and were largely the result of changes in a small number of abundant taxa. Many of the rare taxa were sporadically collected and did not substantially contribute to the community-level emergence patterns (Table 2). Examination of monthly emergence patterns for taxa comprising at least 1% of total abundance did indicate that some had seasonal emergence patterns (Figs. 4-6). Some taxa had emergence peaks that suggested these taxa were univoltine, bivoltine, or multivoltine. Species were divided into the following emergence patterns: (1) peak emergence during the wet period (*Ablabesmyia*, *Larsia*, *Stenochironomus* sp. 1, and *Tanytarsus* sp. 1), (2) peak emergence during the dry period (*Diplosmittia*, *Limnophyes*, *Parametriocnemus*, *Xenochironomus* cf. *nebulosus*, and *Tanytarsus* sp. 2), and (3) aseasonal emergence with one or more emergence peaks (e.g., *Labrundinia*, *Pentaneura*, *Cricotopus*, *Paratendipes reidi*). Taxa categorized as emerging during either the wet or dry periods had a single, broad emergence peak that corresponded to seasonal rainfall patterns. Aseasonal taxa were those that had multiple emergence peaks which did not correspond to seasonal rainfall patterns. Despite seasonal timing and the observation of emergence peaks, most taxa had moderate emergence throughout the year with one or more peaks in emergence. However, some taxa had highly synchronized emergence with a single, distinct peak in emergence and low emergence throughout the rest of the year (e.g., *Diplosmittia*, *Xenochironomus* cf. *nebulosus*, and *Tanytarsus* sp. 2). All three of these taxa had an emergence peak in January at the beginning of the dry season.

Forty-five species of Chironomidae were collected

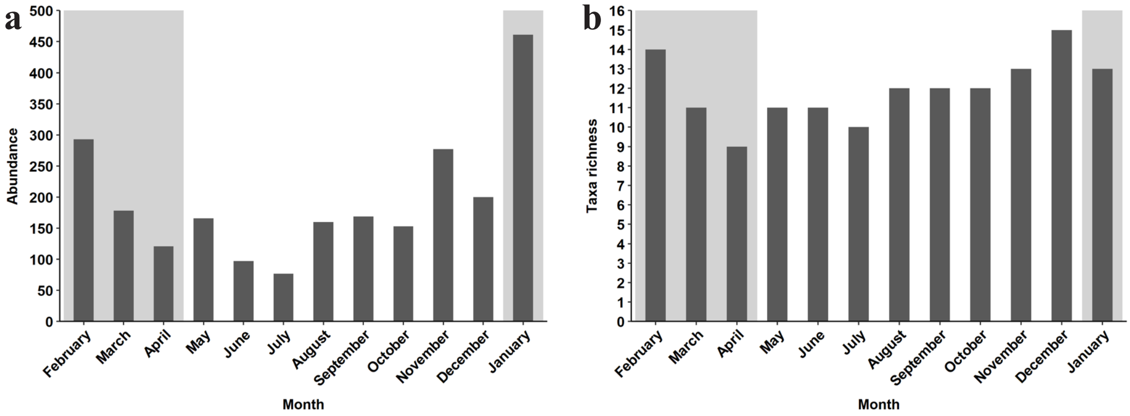

Figure 3. Monthly total abundance (a) and taxa richness (b) of emerging Chironomidae from Quebrada Prieta. Shaded area indicates dry season.

over a one-year period from the Kalengo River by Lehmann (1979) (Table 3). On average, the chironomid community from this stream had an estimated average duration of 255 d/sp/yr. Tanypodinae species had the shortest average estimated emergence duration of 225 d/sp/yr. Orthocladiinae averaged 243 d/sp/yr and Chironominae averaged 281 d/sp/yr. Thirteen taxa each had abundances of at least 1% of the total abundance and all of these abundant taxa emerged during every month. In total, twenty-one taxa (47% of total richness) were collected every month.

Dissimilarities between samples from Quebrada Prieta and the Kalengo River were low although dissimilarity was higher in Quebrada Prieta (Fig. 7). For all 66 pairs of samples, the average dissimilarity among any two months was 0.462 (Quebrada Prieta) and 0.277 (Kalengo River). When considered as a function of the number of months between composited monthly samples, there was

Table 2. Monthly emergence of individual Chironomidae taxa (abundance coding: white = 1, light gray = 2-4, medium gray = 5-9, dark gray = 10-24, black = >25).

Taxon	Month											
	F	M	A	M	J	J	A	S	O	N	D	J
Tanypodinae												
Ablabesmyia	5		2	3	2		4	3	7	2	4	6
Djalmabatista	1				1							
Labrundinia	11	2	7	35	6	5	15	7	4	8	11	
Larsia	15	11	1	11	14	11	28	31	44	36	30	41
Pentaneura	15	21	7	13	17	11	16	38	9	19	10	7
Procladius									1			
Orthocladiinae												
Corynoneura	1				1		1			1	1	3
Cricotopus	9	6	9	13	6	3	17	8	5	10	1	16
Diplosmittia	1			1								38
Limnophyes	5	9	15	3	1		3	6	3	4	9	7
Parametriocnemus	12	14	19	5	3	1	6	1	3	2	3	14
Thienemanniella			1		2			1				
Unknown Orthoclad Genus 1	1		1									
Unknown Orthoclad Genus 2									3		3	1
Chironominae												
Chironomini												
Fissimentum	2	1				1			1			
Paratendipes reidi	1	1	1	1		1	4	10			2	5
Polypedilum sp. 1				1					1	2		
Polypedilum sp. 2		2		1	2	2			8	2	4	
Polypedilum sp. 3	4	1								1		
Polypedilum sp. 4	1					2	1	3	1			
Polypedilum sp. 5										1	2	
Stenochironomus cf. *innocuus*	5	4	1					2				
Stenochironomus sp. 1	171	64	24	22	11	11	6	15	10	68	40	21
Xestochironomus furcatus			1	1			1				2	1
Xestochironomus cf. *nebulosus*	20	38	25	47	27	22	32	25	23	67	45	252
Tanytarsini												
Rheotanytarsus						1						
Tanytarsus sp. 1	9	1	6	5	1	3	21	19	30	52	30	34
Tanytarsus sp. 2	4	3	1	4	3	3	5			2	3	15

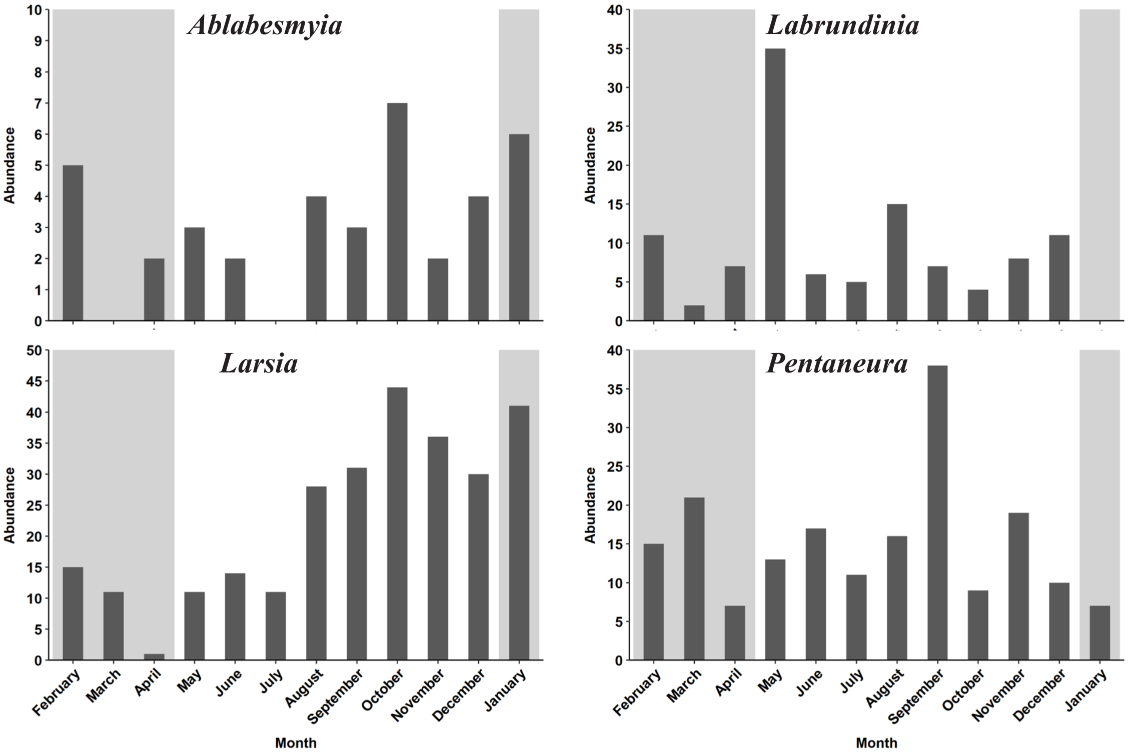

Figure 4. Monthly emergence of abundant Tanypodinae taxa (>1% of total abundance). Shaded area indicates dry season.

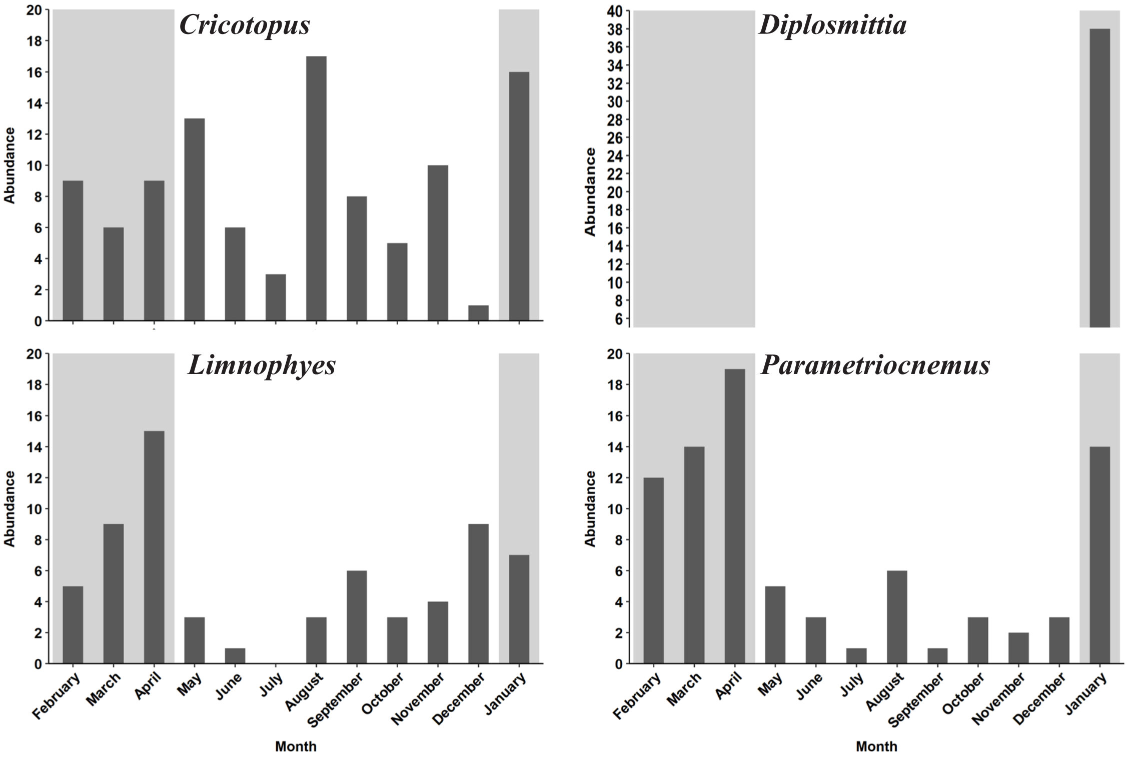

Figure 5. Monthly emergence of abundant Orthocladiinae taxa (>1% of total abundance). Shaded area indicates dry season.

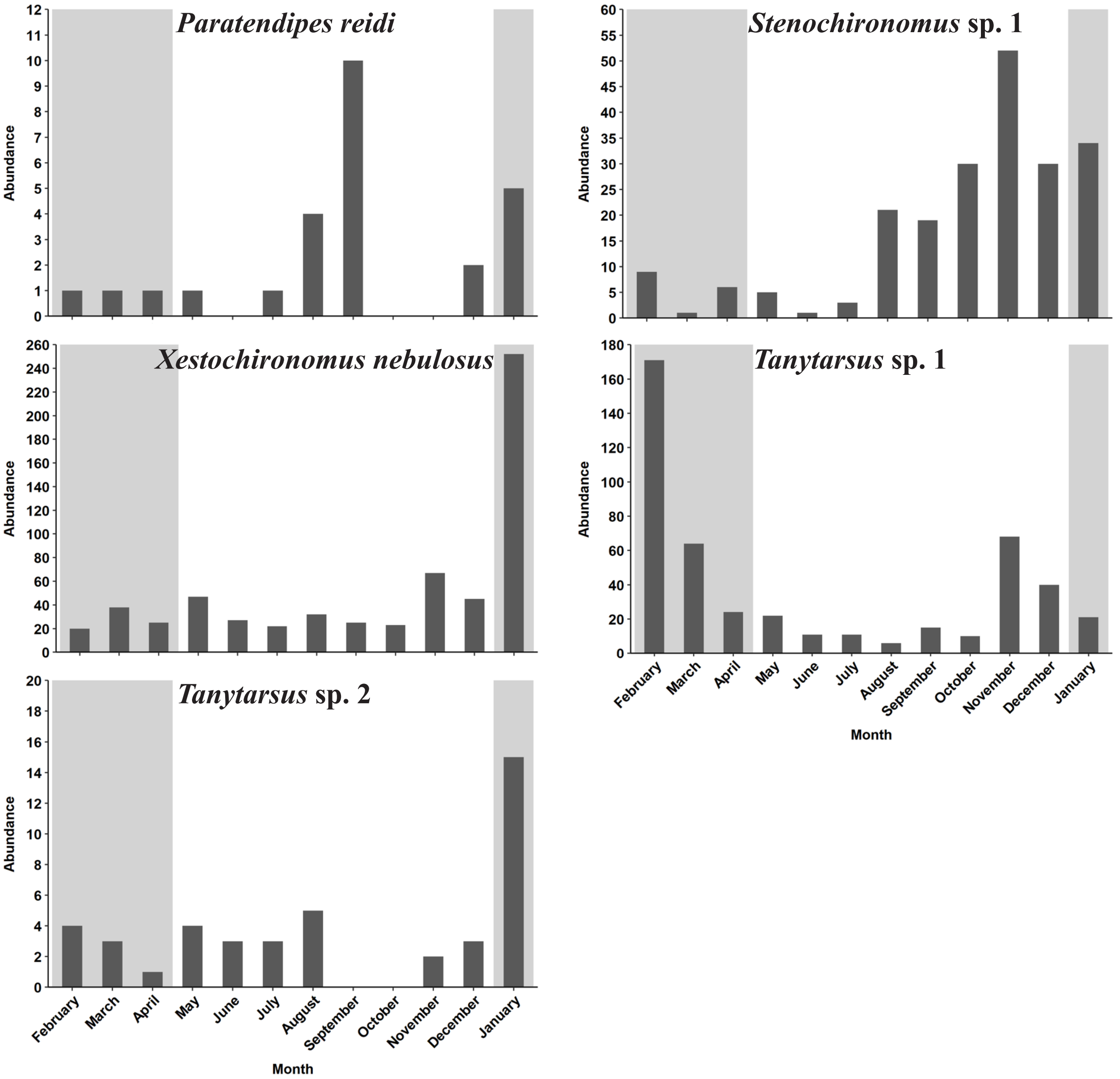

Figure 6. Monthly emergence of abundant Chironominae taxa (>1% of total abundance). Shaded area indicates dry season.

a gradual increase in dissimilarity as the time between samples became greater. However, this increasing trend was only observed when the gap between samples was 1 to 4 (Kalengo) and 1 to 7 (Quebrada Prieta) months. Overall, dissimilarity in both streams was lower than that observed for temperate streams in Coffman and de la Rosa (1998) and Bouchard (2007).

Discussion

Quebrada Prieta is an insular, topical stream and as a result ecological theories predict that this habitat will have a Chironomidae community with low taxa richness (MacArthur and Wilson 1967) and taxa which emerge continuously throughout the year (Coffman and de la Rosa 1998). Based on the model by Coffman and de la Rosa (1998), we predicted that the strongly reduced climatic seasonality in the Caribbean National Forest, would result in longer emergence durations of chironomids in Quebrada Prieta than in temperate habitats. The low taxa richness was expected to reduce temporal niche partitioning which would further lengthen emergence durations such that they exceeded those in other tropical habitats with greater species richness. Finally, emergence patterns of midges in Quebrada Prieta were predicted to be largely aseasonal in contrast to the emergence patterns of many temperate taxa. Some of these predictions were supported by adult emergence data collected over one year from Quebrada Prieta. As expected, midge emergence occurred year-round and many of the most common taxa were present throughout the year. However, when examined in more detail, seasonal emergence patterns in abundance were apparent for the total chironomid community and for some taxa. Overall, chironomid taxa richness and abundance of emerging chironomids increased

Table 3. Chironomidae collected from the Kalengo River (Democratic Republic of the Congo; see Lehmann (1979)).

Taxon	Total count	Number of Months collected	Estimated Emergence duration
Tanypodinae			
Cantopelopia robacki Lehmann, 1979	1	1	30
Conchapelopia zairensis Lehmann, 1979	86	12	365
Larsia africana Lehmann, 1979	31	10	304
Nilotanypus comatus (Freeman, 1953)	179	12	365
Paramerina sp. 1	3	2	61
Orthocladiinae			
Bryophaenocladius brincki (Freeman, 1955)	1	1	30
Bryophaenocladius kalengoensis Lehmann, 1979	1	1	30
Cardiocladius hessi Freeman, 1956	82	9	274
Corynoneura dewulfi Goetghebuer, 1935	394	12	365
Cricotopus (*C.*) *albitibia* (Walker, 1848)	567	12	365
Cricotopus (*C.*) *flavozonatus* (Freeman, 1953)	168	7	213
Cricotopus (*C.*) *harrisoni* Freeman, 1956	39	7	213
Cricotopus (*M.*) *kisantuensis* Goetghebuer, 1934	2	2	61
Eukiefferiella kivuensis Lehmann, 1979	118	9	274
Limnophyes natalensis (Kieffer, 1914)	125	12	365
Nanocladius (*N.*) *jannae* Lehmann, 1979	3554	12	365
Nanocladius (*N.*) *ortsi* Lehmann, 1979	227	12	365
Parakiefferiella ephippium (Freeman, 1956)	1342	12	365
Parametriocnemus scotti (Freeman, 1953)	363	12	365
Paratrichocladius micans (Kieffer, 1918)	1670	12	365
Paratrichocladius pierretti Lehmann, 1979	1	1	30
Pseudorthocladius bernadetti Lehmann, 1979	41	8	243
Pseudosmittia subtrilobata (Freeman, 1956)	2	2	61
Pseudosmittia topei Lehmann, 1979	5	4	122
Rheocricotopus capensis (Freeman, 1953)	18	10	304
Smittia subnigra Freeman, 1956	2	2	61
Thienemanniella fuga Lehmann, 1979	185	12	365
Thienemanniella safi Lehmann, 1979	138	9	274
Tvetenia calvescens (Edwards, 1929)	56	12	365
Chironominae			
Chironomini			
Cryptochironomus incertus Lehmann, 1979	3	2	61
Demicryptochironomus zairensis Lehmann, 1979	55	12	365
Microtendipes kahuziensis Lehmann, 1979	118	11	335
Microtendipes numerous Lehmann, 1979	17191	12	365
Polypedilum aethiops Lehmann, 1979	27	4	122
Polypedilum brunneicornis Kieffer, 1911	855	12	365
Polypedilum majiis Lehmann, 1979	1071	12	365
Polypedilum melanophilus Kieffer, 1911	211	12	365
Stenochironomus spatuliger Kieffer, 1922	14	6	182

Tanytarsini			
Cladotanytarsus/*Tanytarsus* (3 spp.)	1848	12	365
Rheotanytarsus montanus Lehmann, 1979	530	12	365
Rheotanytarsus ororus Lehmann, 1979	5	3	91
Rheotanytarsus Samaki Lehmann, 1979	91	11	335
Tanytarsus luctuosus Freeman, 1958	8	3	91

through the wet season and declined through the dry season. Patterns of individual taxa were more variable than the overall community with some taxa increasing emergence during the dry or wet seasons while others indicated possible patterns of bi- or multi-voltinism. This contradicts predictions that tropical communities would largely be aseasonal due to the lack of strong seasonal cues (Coffman 1989, Coffman and de la Rosa 1998). The apparent seasonality of emergence peaks of some tropical midge species raises questions regarding which exogenous cues or environmental drivers are important for these patterns.

One of the strongest seasonal patterns in tropical systems is precipitation and this has been suggested as a possible important factor for the phenology of many tropical insects (Wolda 1988). In Quebrada Prieta, the overall midge community increased in abundance and richness through the wet season and declined during the dry season. However, it should be noted that even though long-term rainfall averages indicate a regular, weak wet-dry season pattern in the Caribbean National Forest, actual rainfall patterns vary year-to-year. This variability was apparent during the study sampling period (1990-1991) when March (dry period) had the second highest monthly rainfall total. Regardless, there appears to be a direct or indirect effect of precipitation on midge emergence in Quebrada Prieta which is supported by other research. Several other studies have noted changes in tropical midge richness or abundance patterns with most of these studies determining that chironomid abundance was greatest during the dry period (Ferrington et al. 1993, Coffman and de la Rosa 1998, Ramírez and Pringle 1998, Sonoda and Trivinho-Strixino 2000, da Silva et al. 2009, Kranzfelder and Ferrington 2016). Lower abundances of aquatic insects during the wet season have been proposed to be the result of high precipitation events causing spates that disturb habitats and dislodge invertebrates (Ramírez and Pringle 1998, da Silva et al. 2009). In Quebrada Prieta, abundance on average was higher during the dry season (January through April; 263 individuals/month) compared to the wet season (May through December; 162 individuals/month). However, there was an overall decline in emergence abundance through the dry season followed by increasing abundance through the wet season in Quebrada Prieta. In contrast, Lehmann (1979) identified seasonal patterns in chironomid emergence from a continental, tropical stream with increasing emergence through the dry season and declining emergence in the wet season. Regardless, the available research indicates that there is a general pattern of higher emergence in tropical habitats during the dry season and this is at least anecdotally related to the impact of spates on aquatic invertebrates.

Although, most studies of tropical chironomids report emergence patterns at the community level, more interesting patterns may emerge at the spe-

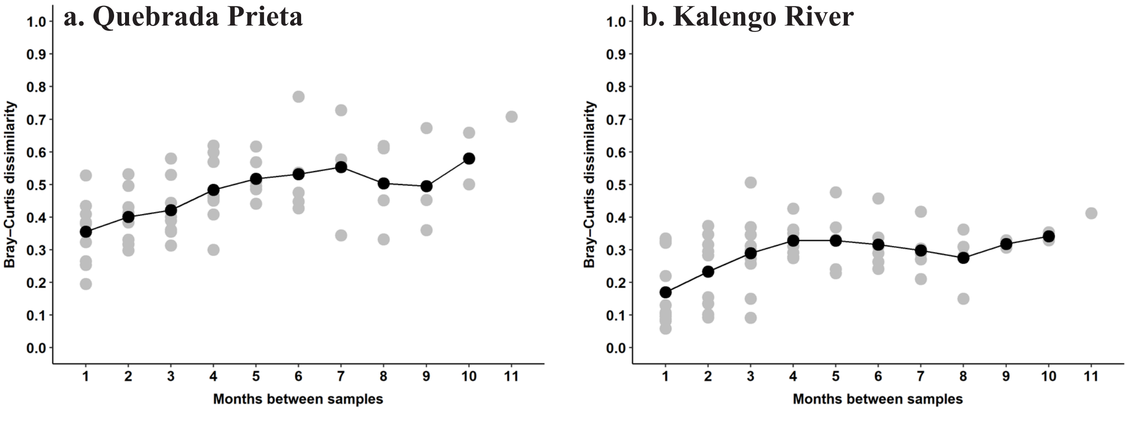

Figure 7. Dissimilarities (Bray-Curtis) between monthly samples of emerging Chironomidae for (a) Quebrada Prieta (USA; Puerto Rico) and (b) Kalengo River (Democratic Republic of the Congo). Grey circles are individual paired months and black circles are the average dissimilarities of all pairs of samples separated by the same number of months.

cies level. In contrast to the general pattern of increasing chironomid emergence during the dry season, Strixino and Sonoda (2006) determined that a species of *Tanytarsus* had higher emergence during the wet period when rainfall and temperatures were higher. Lehmann (1979) determined that several species had seasonal emergence patterns and these patterns differed between species with some increasing emergence in the dry period whereas other increased during the wet period. The presence of different voltinism types have been identified among emerging tropical chironomid species (Arpellino et al. 2022). Rare taxa (<1% of total individuals) in Quebrada Prieta showed limited seasonality which may be the result of a failure to detect these taxa. Therefore, is it possible that rare taxa also have seasonal emergence patterns, but the sampling effort was not sufficient to measure these patterns. However, seasonal emergence patterns were apparent for many common taxa (>1% of total individuals) in Quebrada Prieta with these taxa showing different seasonal patterns of emergence. It should be noted that although possible seasonality was identified for many taxa, emergence peaks were not as strong as those observed for many temperate midge species. This is likely due weaker seasonal patterns in Quebrada Prieta compared to temperate regions. Low temperatures and freezing of aquatic habitats greatly reduce or halt emergence of chironomids in temperate habitats in the winter. In contrast, the specific factors influencing the phenology of individual chironomid species are often difficult to determine due to weaker exogenous cues in tropical habitats.

Taxa emerging from Quebrada Prieta were divided into three emergence pattern types including: (1) emergence during the wet period, (2) emergence during the dry period, and (3) aseasonal emergence with one or more emergence peaks. Most surprising was the highly synchronous emergence peaks of three taxa, but apparent emergence patterns were also observed for other common taxa and suggest that the phenologies of many species were structured by exogenous cues. For example, there is indirect evidence that stream discharge was important as there was an increase in *Limnophyes*, a genus generally considered to be semiaquatic, during the dry season. In general, the taxa that had their highest emergence during the dry period may be responding to the reduced disturbance caused by spates during this period. However, there were also taxa that had emergence peaks during the wet period. Possible factors that may be influencing this emergence pattern could be higher temperatures or increased leaf fall. Average monthly water temperatures in this region only vary 3 °C over the course of the year so the input of allochthonous matter may be more probable. Increased leaf fall generally begins at the end of the dry season and extends into the wet period. The increased emergence of these taxa through the wet season could reflect patterns of allochthonous matter inputs and the utilization of this resource as it conditions in the stream during the wet season. However, the variable timing of emergence peaks for other taxa in Quebrada Prieta suggest that different exogenous cues may be important for these taxa. However, for the taxa with aseasonal emergences patterns, no obvious exogenous cues could be assigned to these patterns.

The study of midge emergence in Quebrada Prieta supports the work of other studies (e.g., Sonoda and Trivinho-Strixino 2000) which indicate that in tropical midge communities, emergence of the most common taxa are continuous throughout the year with apparent pulses in emergence that may correspond to exogenous cues. However, other than noting the correspondence of some chironomid emergence peaks to seasonal precipitation or leaf fall patterns, we were unable to explicitly link these emergence patterns to specific exogenous factors. This was complicated by the fact that temperature and leaf fall patterns in the Caribbean National Forest largely covaried with precipitation patterns. Other studies of tropical aquatic communities have indicated that seasonal patterns of temperature, rainfall, photoperiod, and lunar period can result in synchronized emergence patterns (e.g., Fryer 1959, Arpellino et al. 2022). Although Siqueira et al. (2008) identified some peaks in emergence of tropical chironomids, these pulses could not be linked to specific exogenous factors and the authors hypothesized that this could be the result of "chaotic" emergence patterns or to a failure to measure important exogenous factors. Further study of the phenology of aquatic communities are needed, particularly research to better determine the factors that influence these patterns. Specifically, it would be interesting to determine if seasonal emergence patterns are linked to measurable abiotic factors or if emergence pulses are more stochastic. This would require multiple years of sampling coupled with detailed measurement of possible emergence cues.

Although the apparent seasonal emergence patterns of some chironomid taxa contradicted our expectation that the emergence patterns of midges in Quebrada Prieta would be aseasonal, our results do support the predictions for estimated durations of emergence based on the hypothesis by Coffman and de la Rosa (1998). An average emergence duration of 205 d/sp/yr in Quebrada Prieta was higher than those estimated for temperate communities (Table 4; 61-120 d/sp/yr). In comparison to tropical chironomid communities, the estimated emergence duration in Quebrada Prieta was greater than that for Costa Rican chironomid communities (110-126 d/sp/yr) and less than chironomids from a river in the Democratic Republic of the Congo (255 d/sp/yr; Table 4). Similarity between monthly samples also followed predicted patterns with overall low dissimilarity between samples which slightly increased as the interval between samples became greater. A similar pattern was observed in a headwater stream in Brazil by Siqueira et al. (2008). These results further demonstrated that although seasonal patterns measured as peaks in emergence are present in tropical stream habitats, they are weaker than those observed in temperate habitats. This pattern is likely due to the strong thermal ranges in temperate habitats compared to tropical habitats, but other seasonal cues may also be important.

The long emergence durations for Chironomidae in Quebrada Prieta and the Kalengo River were considerably higher than other tropical habitats in Costa Rica. If we can accept that methodical differences were not the cause of this difference, it raises the question as to why emergence duration is approximately double in in Quebrada Prieta and the Kalengo River. Our expectation was that monthly dissimilarity in the Kalengo River would exceed the values for Quebrada Prieta due to the continental habitat of the Kalengo River and the associated greater taxa richness. However, the dissimilarities between monthly samples for the Kalengo River were consistently low, both in terms of the annual average dissimilarity and among all combinations of months regardless of the time interval between the monthly pairs. Although the Kalengo River had nearly double the taxa richness of Quebrada Prieta, both habitats are unremarkable for tropical habitats with only 46 and 28 taxa, respectively. In comparison, taxa richness in the Costa Rican streams ranged from 142-151 species (Coffman and de la Rosa 1998). Although the seasonality of all habitats is reduced relative to temperate regions, it can be hypothesized that in communities that are lower in species richness, the degree of temporal spacing of lifecycle dynamics, including larval growth, emergence duration,

Table 4. Average emergence durations for tropical and temperate Chironomidae communities. The average sampling interval is based on either the average number of days between samples (Coffman and de la Rosa (1998)) or the aggregation unit (i.e., months) for multiple samples (this study, Lehmann (1979), and Bouchard (2007)). *These studies are based on adult emergence traps whereas the remaining studies are based on pupal exuviae sampling. #Biweekly sampling intervals were used in Bouchard (2007) and these samples were aggregated into monthly samples for the present study.

Locality	**Emergence duration**	**Average sampling interval***	**Source**
			Tropical streams
Quebrada Prieta, Puerto Rico, USA	205	30	This study*
Kalengo River, Democratic Republic of the Congo	255	30	Lehmann (1979)*
Quebrada Las Yegüitas (Maritza), Costa Rica	110	23	Coffman and de la Rosa (1998)
Quebrada Las Yegüitas (Orosí), Costa Rica	126	28	Coffman and de la Rosa (1998)
Rio Tempisquito Sur, Costa Rica	118	28	Coffman and de la Rosa (1998)
Rio Tempisquito, Costa Rica	111	16	Coffman and de la Rosa (1998)
			Temperate streams
Powdermill Run (Moul), USA	71	17	Coffman and de la Rosa (1998)
Powdermill Run (Headquarters), USA	61	18	Coffman and de la Rosa (1998)
Linesville Creek, USA	77	9	Coffman and de la Rosa (1998)
Chub Creek, USA	91	30#	Bouchard (2007)
Credit River, USA	120	30#	Bouchard (2007)
Sunrise River, USA	84	30#	Bouchard (2007)
Cedar Creek, USA	71	30#	Bouchard (2007)
Rock Creek, USA	85	30#	Bouchard (2007)
Rush Creek, USA	84	30#	Bouchard (2007)

and overlap, is less than it is in more diverse chironomid communities that experience similar degrees of seasonality. Perhaps the higher biological diversity can only be achieved by greater degrees of temporal isolation among growing larvae, thus reducing competition for limiting food resources, rather than finer degrees of food resource sharing among larvae of species with overlapping periods of growth. In general, studies of niche partitioning among tropical aquatic insect communities are needed to understand how these communities are structured by biotic and abiotic factors.

Although predicted differences in emergence duration were apparent between tropical and temperate studies of chironomid emergence, differences in sampling methodology can complicate these patterns and these effects should be considered. Our data and those of Lehmann (1979) were derived from single emergence traps at one stream site. In contrast, Coffman and de la Rosa (1998) and Bouchard (2007) collected surface-floating pupal exuviae to determine the phenology of emerging species. There are two main issues that may impact the comparability between emergence trap and pupal exuviae data: 1) habitat types sampled and 2) the relative sampling effort. Use of an adult emergence trap is more restrictive in that only adults from the area covered by the trap are collected. The method of collecting surface-floating pupal exuviae, by comparison, provides samples of exuviae that are left behind by adults emerging from a larger stream area, perhaps as much as several meters to several decimeters or more upstream. Although our emergence trap effectively captured adults of species that live in microhabitats covered by the trap, the trap did not extend over the entire adjoining pool habitats. However, it is possible that some individuals came from upstream habitats when pupae in the process of emerging were carried downstream as the adults were eclosing. The effect of sampling methodology may also be impacted by changes in the habitat when stream discharge changes during trap deployment. This possibly impacted emergence patterns in Quebrada Prieta as some taxa associated with the stream margins became more common during the dry period (e.g., Limnophyes). Alternatively, the increase in these taxa may have been the result of stream-wide changes caused by seasonal patterns. In contrast, sampling surface-floating pupal exuviae result in collections of specimens of exuviae for species that occur in a wide variety of microhabitats, including species living at different depths, substrates, and habitat types (Kranzfelder et al. 2015). Consequently, the use of an emergence trap could have resulted in several species that are common in pools showing up as rare in our collections, and only being collected on a few sample dates. This would cause our estimates of the average emergence duration for all chironomids to be lower than if taxa from pools were more efficiently collected. Lehmann (1979) also used an emergence trap, but the trap area was more than double that of the trap used in Quebrada Prieta which may explain the longer emergence durations. As a result, we cannot rule out the effects of sampling method differences on estimates of emergence durations and community dissimilarity between months.

Coffman and de la Rosa (1998) discussed another important aspect associated with estimating emergence duration and drew attention to the importance of how one interprets the significance of intervals when individual species are not present in samples. Gaps in detecting a species over one or more sample dates could either reflect sampling error or could indicate a period between emergence of successive generations, and these gaps require that a judgment be made regarding two alternatives. If a gap reflects a sampling error, counting the sample as a period when the species is not emerging results in a shorter estimated annual duration of emergence. Alternatively, counting a gap as a sampling error when it actually represents a period of no emergence between successive generations will result in an over-estimate of the annual emergence duration. In our study, the estimate derived from analysis of monthly data may miss small gaps in emergence of species and means that species with short, synchronous generation times may appear to have continuous, asynchronous emergence. Consequently, the estimates based on monthly data may miss seasonal patterns or over-estimate emergence durations. By compositing our data according to monthly intervals, we minimize the significance of shorter gaps in appearance of a species in the samples, and only accept larger gaps as indications of breaks in emergence between successive generations.

The research in Quebrada Prieta and from several other studies demonstrate that taxa in tropical chironomid communities largely emerge throughout the year. Rare taxa in tropical habitats are an exception as they often appear to have sporadic emergence patterns, but this may be due to a failure to detect these species when they are in fact present. Although adults of common species are present year-round, there are apparent pulses of emergence which may correspond to seasonal, exogenous cues. Based on this study and several others, there is evidence that some degree of seasonality occurs and may in fact be common in tropical midge communities. Most commonly, increased emergence is associated with the dry season indicating that spates increase stream disturbance and lower larval midge densities. However, some tropical midge taxa had higher emergence during the wet season (*Ablabesmyia*, *Larsia*, *Stenochironomus* sp. 1, and *Tanytarsus* sp. 1 (this study); *Tanytarsus obiriciae* Strixino and Sonoda (Strixino and Sonoda 2006)). Variable timing of emergence peaks may correspond to differences in emergence cues between species (e.g., discharge, temperature, photoperiod, lunar period, food resources) or this may reflect stochastic emergence patterns among these species. Most studies of tropical insect emergence are limited to a single year and to understand if regular, seasonal cues are important or if emergence peaks are stochastic, long-term studies of emergence is needed. Seasonality of insect activity in tropical habitats are likely to be widespread (Wolda 1988), but there continues to be limited detailed studies of the ecology and phenology of tropical chironomids. The ability to describe and model these patterns is of interest for the conservation of these species as they face threats including climate change, habitat loss, and water quality degradation.

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SEASONAL VARIATION IN THE CHIRONOMIDAE (DIPTERA) COMMUNITIES OF TWO FAROESE STREAMS

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Abstract

Seasonal variation of freshwater invertebrate communities is strongly influenced by abiotic factors including temperature and precipitation which, in turn, are predicted to be affected by climate change. It is important to study these effects, not least since they may affect higher trophic levels and ecosystem dynamics.

Our aim with this study was to compare the seasonal variation of the community composition of Chironomidae (Diptera) in two streams in the Faroe Islands and to see how this related to abiotic factors. Additionally, we studied the life cycle of *Tvetenia calvescens* (Edwards 1929), the dominant species in the streams. For this purpose, Chironomidae larvae were collected from two adjacent streams of different sizes in the Faroe Islands at regular intervals over the course of 15 months.

We found that *Tvetenia calvescens* was the most abundant species in the streams, followed by *Eukiefferiella minor* (Edwards 1929). The community composition varied in different months and between the two streams and was shaped by water temperature and flow velocity.

Larval densities were generally higher in the smaller stream than in the larger stream, but densities were not correlated with water temperature, monthly temperature amplitude or flow velocity.

We found that in both streams *Tvetenia calvescens* was bivoltine with adult emergence in May/June and September/October.

Introduction

Aquatic invertebrate communities are subject to seasonal variation in various biotic and abiotic factors. These variations are reflected at the higher trophic levels feeding on invertebrates (Kreiling et al. 2021) and aquatic invertebrates can constitute an important part of the diet of surrounding terrestrial fauna (Gratton et al. 2008). In this way, seasonal variation may affect not only the ecology of the aquatic system but also the surrounding terrestrial ecosystem.

Temperature is commonly cited as influencing seasonal variation in aquatic invertebrate communities (Soulsby et al. 2001, Bottazzi et al. 2011, Kreiling et al. 2021). However, it might not be the water temperature per se which determines community composition but rather the temperature variability, i.e., the daily or annual amplitude of the temperature. The invertebrate communities in Alpine spring brooks, for example, change due to a decreased annual variability in water temperature along an altitudinal gradient from lowland to high alpine springs (von Fumetti et al. 2017).

The basis of community changes are the responses of species to biotic and abiotic factors. One well known response is the growth and development rate of Chironomidae larvae, which is influenced by temperature (Taylor 1981, Pinder 1986, Reynolds and Benke 2005) and can determine whether a species produces one or several generations annually (Oliver 1971). Even populations of the same species inhabiting different water bodies in close vicinity may show variation in their voltinism, as a study on *Chironomus pseudothummi* Strenzke 1959 in two small ponds in England showed (Smith and Young 1973). It is likely that fluctuations in temperature can induce variation in voltinism, both in neighbouring and within the same water body (Tokeshi 1995, Hannesdóttir et al. 2012). Other variables such as variations in food supply, water depth, or photo period may likewise influence the voltinism of chironomid species (Tokeshi 1995).

In the Faroe Islands, streams are subject to a high temperature variability in addition to an altitudinal temperature gradient. The Faroe Islands are an island group consisting of 18 mountainous islands situated at 62° N, 7° W in the North Atlantic Ocean. Streams in the Faroes have very small catchment areas, typically between 0.1 and 1 km2 and the main stream channels reach only lengths from several hundred meters to 7.3 km for the longest river (Hansen and Gíslason 2010). Most of the stream channels are situated below 200 m asl, and more than three quarters are on sloping surfaces, i.e., fast flowing mountainside channels. The basaltic bedrock is mostly impermeable to water. At the same time, precipitation is high with some places having an average annual rainfall of more than 4000 mm (Hansen and Gíslason 2010). Consequently, discharge of streams fluctuates greatly between high water masses after heavy rainfalls and falling dry during the rare drier periods (Hansen and Gíslason 2010). Despite originating in springs, the Faroese streams are highly influenced by precipitation and show thus characteristics of run-off streams in which water temperature follows the ambient air temperature (Kiilerich 1928, Hansen and Gíslason 2010). As the dark streambeds can absorb energy from the sun, water temperature in shallow streams can occasionally even reach several degrees above ambient air temperature (Hansen and Gíslason 2010).

Despite the numerous streams, ponds and lakes in the Faroes only few studies have been conducted which focus on freshwater ecosystems (Christoffersen 2002). The aquatic invertebrate fauna of the Faroe Islands is species poor, as a result of the stochastic distribution of freshwater fauna on the North Atlantic Islands (Gíslason 2005, Gíslason 2021). Whereas in most parts of the world, a large proportion of the invertebrate fauna are EPT (Ephemeroptera, Plecoptera, and Trichoptera) taxa, Ephemeroptera and Plecoptera as well as Odonata are entirely absent in the Faroese streams (Hansen and Gíslason 2020). Trichoptera are present with 20 species belonging to seven families (Hansen and Gíslason 2020). By far the most species-rich taxon in Faroese freshwaters is Chironomidae (Diptera) with 72 recorded species in 42 genera (Gíslason 2005, unpublished data).

The Faroe Islands are predicted to be affected by climate change (Pachauri et al. 2015). In this regard, it is important to study the effects of temperature and precipitation on freshwater invertebrate communities, especially since Chironomidae have been shown to be excellent bioindicators (Molineri et al. 2020) and freshwater invertebrate communities are being used to evaluate freshwater quality (Begum et al. 2022).

The aim of this study was to compare the seasonal variation in chironomid community composition and the life cycle of *Tvetenia calvescens* (Edwards 1929) as the dominant species in two adjacent streams differing in their environmental characteristics, namely water temperature and flow velocity. We proposed the following predictions:

I. We expect variation in the density and community composition of chironomids within each stream

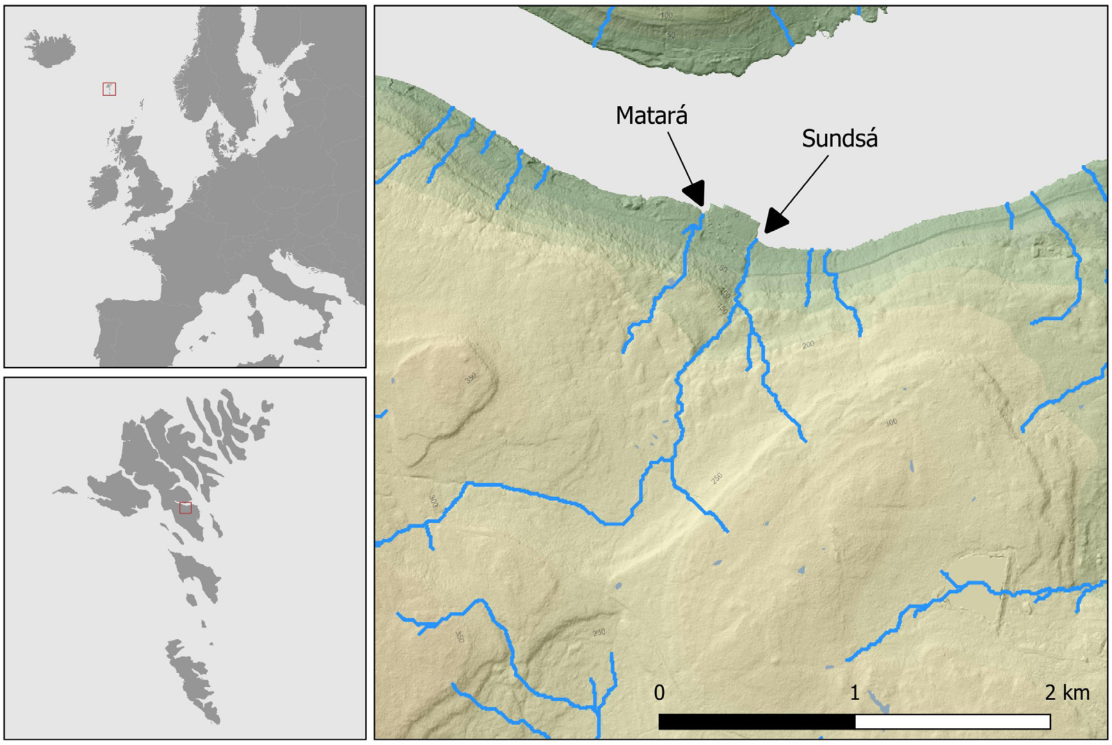

Figure 1. Map of the Faroe Islands with the location of the two sampled streams, Matará and Sundsá.

throughout the year, in accordance with seasonal variation of water temperature and flow velocity.

II. We expect differences in the chironomid community of the two streams. The community of the larger stream should be more stable (i.e., less seasonal variation), due to less variation in temperature and flow velocity.

III. We expect differences in the life cycle of *Tvetenia calvescens* in the two streams. One generation or a more stretched out life cycle in the smaller and less stable stream compared to two generations or a shorter life cycle in the larger and more stable stream.

Material and Methods

The two study streams are located on the island Streymoy in the Faroe Islands, situated on a slope above the main road and about 8 kilometres north of the capital Tórshavn. Although the streams are just 350 m apart in neighbouring water basins, they differ in size, average discharge, and substrate. The smaller stream, Matará (Lat. 62.047937N, Long. -6.849097W), has a main channel length of 1000 m, a maximum width of 15 m, runs from 230 m asl to the sea, and has an average discharge of 10 L/s. The larger stream, Sundsá (Lat. 62.045890N, Long. -6.843184W), has a main channel length of 3800 m, a maximum width of 20 m, runs from 350 m asl to the sea, and has an average discharge of 200 L/s (Fig. 1). Both streams are relatively steep at the sampling locations leaving nearly naked bedrock as substrate which to some degree is covered by moss and diatoms. Matará, the smaller stream, has a larger proportion of moss and diatom cover and at one point within the sampling stretch a depression which can contain some sand.

From both streams, benthic invertebrates were collected monthly between March 2009 and May 2010. At each sampling date, invertebrates were collected at ten locations along each stream, using a Surber sampler with an area of 0.0196 m^2 and a mesh size of 200 µm. Invertebrates were stored in 70 % ethanol. To describe the physico-chemical properties of the two streams, water temperature, pH, flow velocity, depth, and conductivity were measured at each location at the time of sampling. Conductivity was measured using EcoSense EC300 (YSI Environmental 2003) and pH was measured using Orion 230A (Orion Research Inc. 2000). Water temperature was measured with both the pH and conductivity meter, and the mean was used in cases where values differed between measurements. Data on air temperature was obtained from a nearby (1 km) weather station operated by Landsverk (the Faroese public building and transportation authority).

The water temperature in Sundsá and Matará, and the air temperature next to the two streams was measured once every hour by TinyTag temperature loggers from May 2010 to May 2011. The loggers were unavailable to us from the start of the study which explains the mismatch between the invertebrate sampling and the temperature data by a year. The monthly amplitude of the air temperature was calculated by averaging the differences between the daily minimum temperature and the daily maximum temperature over a month.

The invertebrates in the Surber samples were sorted under a dissecting stereomicroscope and counted. From each Surber sample a random subsample of approximately 50 chironomid larvae were mounted on glass microscope slides and fixed in Hoyer's mounting medium (Anderson 1954), unless the number of individuals in the sample was close to or less than 50 in which case all chironomid larvae were mounted. Mounted Chironomidae were identified, and the total number for each taxon in the sample was calculated based on its proportion in the subsample. Identification to lowest possible taxonomic level was done under a Leica DMLB compound microscope (Leica Microsystems, Mannheim, Germany). Chironomid larvae were identified using keys by Wiederholm (1983) and Schmid (1993). Before mounting, the larval head widths were measured under a Leica MZ6 stereo microscope (Leica Microsystems, Mannheim, Germany) with 0.025 mm accuracy using a graticule ocular in the microscope. The head capsule widths were compared with those found by Schmid (1993) and based on these the larvae were grouped to instars.

Due to resource limitations, we were unfortunately not able to process Chironomidae in the samples for all the months. Thus, we included only eight months for Sundsá and 12 for Matará. For the same reason, not all ten Surber samples collected at each sampling time were processed. In some instances, invertebrates were identified from only every second Surber sample. The average of all processed Surber samples per month and stream was used to characterize density and community composition of Chironomidae larvae over the year.

Statistical analysis was done using the software R (version 4.2.1, R Core Team 2022). Correlations between environmental variables were assessed by pairwise scatterplots and correlation coefficients using the function chart.Correlation in the package PerformanceAnalytics (Peterson and Carl 2020). Furthermore, the variance inflation factors (VIF) were obtained using the function corvif (Zuur et al. 2009). All resulting VIF values were below 5, indicating that there was no collinearity between variables, and thus all environmental variables were retained for analysis. The correlation between density of Chironomidae larvae and water temperature, monthly temperature amplitude, and flow velocity was tested with linear regressions (function lm in the stats package).

The differences in the chironomid community composition between the two streams and among sampling months were summarized with Bray-Curtis distances. The dependency of those differences on the explanatory variables (water temperature, average monthly temperature amplitude, conductivity, pH, depth, flow velocity, sampling month, and stream) were analysed by running a permutational multivariate analysis of variance (adonis2 function in the vegan package; Oksanen et al. 2022). The ordination of samples based on the Bray-Curtis distances, and the association of the environmental variables to the main axes were visualised with nonmetric multidimensional scaling (NMDS) using the functions metaMDS and envfit in the vegan package.

Results

Water temperature varied from 2°C in winter to 18°C on sunny summer days. The water temperature of both streams followed ambient air temperature (Fig. 2). Average monthly amplitudes in air temperature ranged from 2.5°C in August 2009 to 4.2°C in April 2010. The water temperature in the smaller stream Matará was more stable over the year with an average of 9.3±3.9°C, whereas Sundsá had an average temperature of 10.0±4.4°C (Table 1). The flow velocity in Matará was also more stable with an average velocity of 0.8 ±0.2 m s^{-1} whereas Sundsá had an average flow velocity of 1.0±0.4 m s^{-1} (Table 1).

Average chironomid densities ranged from 117 individuals/m^2 in Sundsá in September 2009 to 7,331 individuals/m^2 in Matará in May 2010. Densities were higher in the smaller stream Matará than in the larger stream Sundsá for all months except May 2009. Density of Chironomidae larvae was not correlated with water temperature (adjusted R-squared = 0.1653, F-statistic = 3.971, p-value = 0.066, Fig. 3), monthly temperature amplitude (adjusted R-squared = 0.0036, F-statistic = 1.069, p-value = 0.3149) or flow velocity (adjusted R-squared = 0.1350, F-statistic = 3.030, p-value = 0.107).

We found a total of 23 chironomid taxa, identified to genus or species level (Table 2). In some cases, congeneric species were combined under a higher taxonomic level to be able to include individuals into the analysis for which identification to species level was not possible, e.g., early instars (Table 2). This resulted in 20 taxa being included in the analysis. Both streams were dominated by chironomids of the subfamily Orthocladiinae, with *Tvetenia calvescens* being the most abundant species, followed by *Eukiefferiella minor* (Edwards 1929). Chironomid communities differed between Matará and Sundsá, with a higher proportion of *Tvetenia calvescens*, *Synorthocladius semivirens* (Kieffer 1909), and *Rheocricotopus effusus* (Walker 1856) in Matará (Fig. 4) and a higher proportion of *Diamesa* spp. Meigen 1835 and *Orthocladius frigidus* (Zetterstedt, 1838) in Sundsá (Fig. 5). *Eukiefferiella claripennis* (Lundbeck 1898), *Limnophyes* sp. Eaton 1875, *Trissopelopia* sp. Kieffer 1923, and *Macropelopia* sp. Thienemann 1916 were found in Matará but not in Sundsá (Table 2).

Chironomid community composition was shaped by water temperature, conductivity and flow ve-

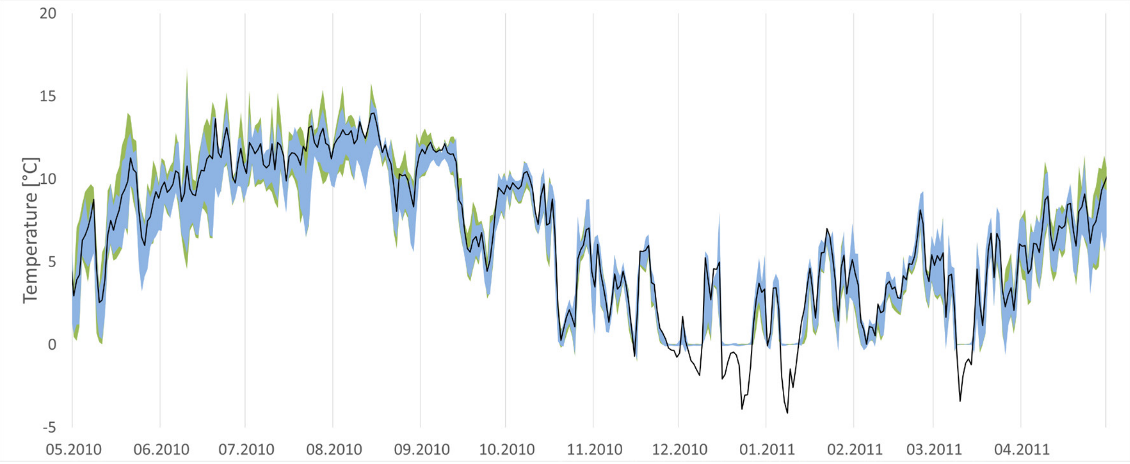

Figure 2. Daily temperature changes in Sundsá and Matará over a year. Water temperature of Sundsá (in green) and Matará (in blue) follow air temperature (black).

Table 1. Attributes of the two studied streams.

Stream	Basin (km^2)	Maximum altitude (m asl)	Sample altitude (m asl)	Longitude	Latitude	Length (m)	Maximum width (m)	Average velocity ($m\ s^{-1}$)	Average water temperature (°C)
Matará	0.38	230	40	62.048	-6.849	1000	15	0.8	9.3
Sundsá	3.49	350	60	62.046	-6.843	3800	20	1	10. 0

locity and differed between the two streams (Table 3, Fig. 6). Although month was not a significant variable in the analysis, there was seasonal variation in the chironomid communities in both Matará (adjusted R-squared = 0.0.270, F-statistic = 2.99, p-value = 0.002) and Sundsá (adjusted R-squared = 0.0.274, F-statistic = 3.88, p-value = 0.001). However, communities were more similar in different months within a stream than in the same month between streams, with the exception of November 2009 in Matará and September 2009 in Sundsá, which group with the other stream, respectively (Fig. 6a). *Rheocricotopus effusus*, *Cricotopus* sp. van der Wulp 1874 and the Tanypodinae *Trissopelopia* sp. and *Macropelopia* sp. were associated with relatively high temperatures and high conductivity, whereas *Diamesa* spp., *Pseudodiamesa* sp. Goetghebuer 1939 and *Eukiefferiella minor* (Edwards, 1929) were associated with high flow velocity (Fig. 6b).

Life cycle patterns of *Tvetenia calvescens* were similar in the two streams (Fig. 7). The measurements of head capsule widths did not allow for a clear separation into instars I and II, and II and III. However, instar IV did clearly separate from instar III. Despite some overlap it seems that there are two generations with adult emergence in May/June and September/October in both streams (Fig. 7).

Discussion

Community composition of chironomids in the two streams varied throughout the year as we had predicted (I.) and was associated with water temperature. Density, on the other hand and contrary to our prediction, was not influenced by water temperature. Neither temperature amplitude nor flow velocity affected density or community composition.

We had furthermore predicted (II.) that there were differences in the chironomid communities between the two streams, which was supported by our results. Larval densities were much higher in the smaller stream (Matará) than in the larger stream (Sundsá) and community composition in the samples differed more between the two streams than between sampling months. We had expected that the community of the larger stream would be more stable than that of the smaller stream but found the opposite.

Lastly, we predicted (III.) differences in the life cycle of the dominant chironomid species, *Tvetenia calvescens*, in the two streams. This hypothesis was not supported by our results, showing the same life cycle pattern in both streams.

Although Chironomidae was the dominant taxon in the invertebrate communities of the streams, their

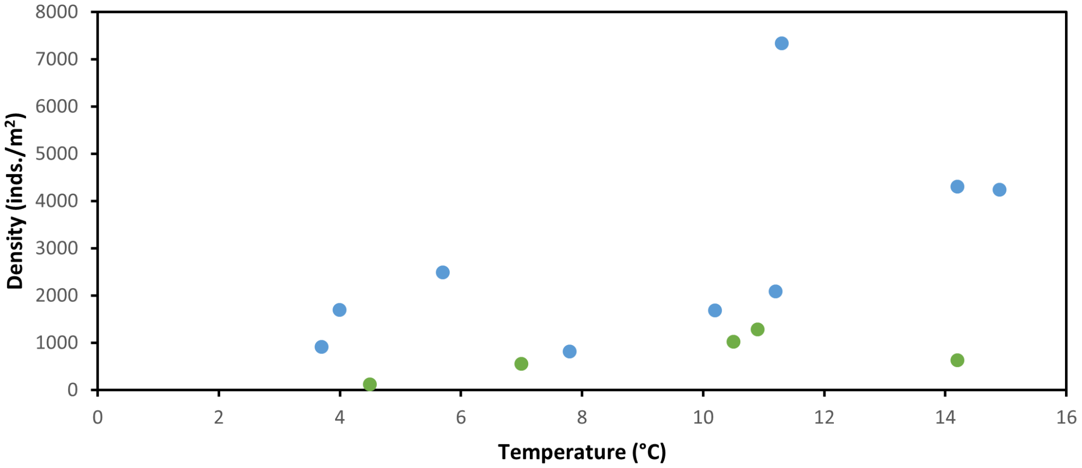

Figure 3. Average density of chironomid larvae per sampling occasion in relation to water temperature. The larger stream Sundsá in green and the smaller stream Matará in blue dots. Standard Deviation lies between 141 and 5944 but is not shown for the sake of clarity.

Table 2. Chironomid species found in Matará and Sundsá in this study. Species acronyms indicate the taxa included in the analysis and are used in Figures 4, 5, and 6.

Species	**Acronym**	**Matará**	**Sundsá**
Podonominae			
Parochlus kiefferi (Garrett 1925)	Pak	x	x
Tanypodinae			
Trissopelopia sp. Kieffer 1923	Tri	x	
Macropelopia sp. Thienemann 1916	Mac	x	
Diamesinae			
Diamesa spp. Meigen 1835	Dia	x	x
Diamesa bohemani/*zernyi* species group			
Diamesa bertrami/*latitarsis* species group			
Pseudodiamesa sp. Goetghebuer 1939	Pse	x	x
Orthocladiinae			
Chaetocladius spp. Kieffer 1911	Cha	x	x
Chaetocladius dentiforceps species group			
Chaetocladius piger species group			
Cricotopus sp. van der Wulp 1874	Cri	x	x
Eukiefferiella claripennis (Lundbeck, 1898)	Euc	x	
Eukiefferiella minor (Edwards, 1929)	Eum	x	x
Eukiefferiella sp. Thienemann 1926	Euk	x	x
Limnophyes sp. Eaton 1875	Lim	x	
Metriocnemus eurynotus (Holmgren, 1883)	Mee	x	x
Metriocnemus sp. van der Wulp 1874	Met	x	x
Orthocladius spp. van der Wulp 1874	Ort	x	x
Orthocladius frigidus (Zetterstedt, 1838)			
Orthocladius oblidens (Walker, 1856)			
Rheocricotopus sp. Thienemann & Harnisch 1932	Rhe	x	x
Synorthocladius semivirens (Kieffer 1909)	Syn	x	x
Thienemanniella sp. Kieffer 1911	Thi	x	x
Tvetenia calvescens (Edwards 1929)	Tve	x	x
Chironominae			
Tanytarsus sp. van der Wulp 1874	Tan	x	x
Micropsectra sp. Kieffer 1909	Mic	x	x

densities were low compared to densities of chironomid larvae in streams elsewhere (Nolte 1991). Both streams have a solid basaltic streambed with little loose material and aquatic vegetation, as is typical for streams on slopes in the Faroes (Hansen and Gíslason 2010). The scarcity of sand, gravel, submersed macrophytes and mosses, and therefore limited microhabitats and suitable substrate, might explain the low chironomid densities (Ilmonen and Paasivirta 2005) The recorded difference between the streams, with higher density in Matará, can be attributed to the higher moss and diatom cover offering more microhabitats for the chironomids. However, densities were comparable to those in glacial and non-glacial high mountain streams with similarly little aquatic vegetation (Lods-Crozet et al. 2001, Lencioni and Rossaro 2005). Neither water temperature, monthly temperature amplitude or flow velocity influenced the chironomid density. Similarly, densities of Chironomidae larvae in glacial streams in different regions of Europe varied a lot between sites, seemingly not dependent on water temperatures and flow velocities (Lods-Crozet et al. 2001).

Despite the close vicinity of the streams and overall similar environmental conditions, the chironomid communities differed. Both streams were inhabited by typical cold-stenothermal taxa such as

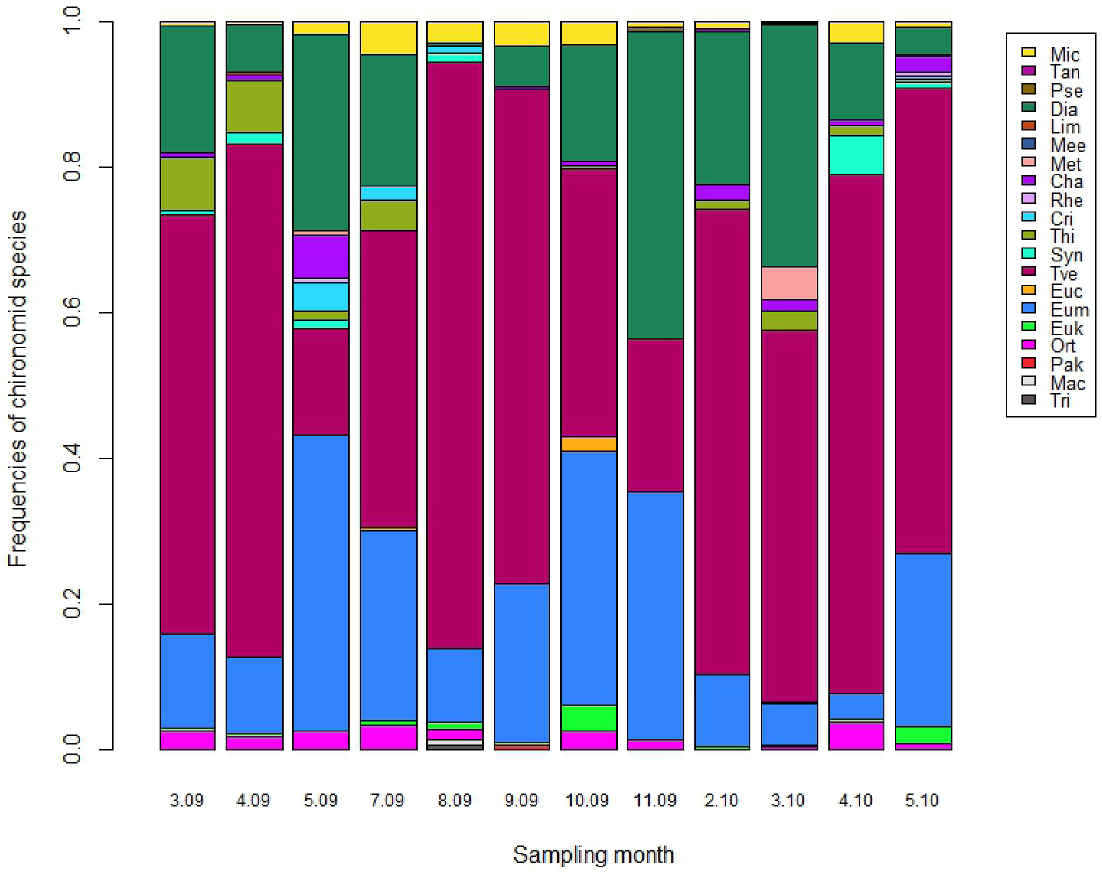

Figure 4. Seasonal variation of the chironomid community of Matará. Bar labels refer to month and year. Species acronyms as in Table 2.

Diamesa spp. and *Orthocladius frigidus* and taxa adapted to high flow velocity like *Tvetenia calvescens*, *Synorthocladius* sp., and *Rheocricotopus effusus*. However, the former two taxa were relatively more abundant in the larger stream Sundsá whereas the latter three were more abundant in the smaller stream Matará. In addition to these differences in relative abundances of taxa, four species were exclusively found in the smaller stream Matará, including the only two species of Tanypodinae, *Trissopelopia* sp. and *Macropelopia* sp., found in the study. Thus, even streams which are similar in geographical location and environmental variables can harbour different communities which consequently might be differently affected by seasonal changes. The relatively more stable environmental conditions (i.e., water temperature and flow velocity) of the smaller stream Matará compared to the larger stream Sundsá was reflected in a more stable Chironomidae community with less seasonal variation in its composition. Matará also had a slightly higher taxa richness than Sundsá. This supports the point that small water bodies can contribute much to regional biological diversity and ecosystem services (Biggs et al. 2017).

Water temperature strongly influenced separation of chironomid communities in the ordination plot. Similar results were found in comparable studies of aquatic communities, for example in streams in the Cairngorm Mountains, Scotland (Soulsby et al. 2001) and in boreal springs in Finland (Virtanen et al. 2009). Likewise, flow velocity contributed to the variance in the ordination and was slightly higher in the larger stream, Sundsá.

Except for larvae of *Rhyacophila dorsalis* (Curtis 1834) (Trichoptera) present in both streams, there is a distinct absence of large predators in the two streams studied. The Tanypodinae recorded from one sample in Matará (Table 2) accounted for less than 0.5% of the individuals in that sample and can not be expected to have an influence on the overall community. Common predators in aquatic habitats in the Faroes are water beetles in the family Dytiscidae (Hansen and Kreiling 2022) as well as Brown trout (*Salmo trutta* Linnaeus 1758) and

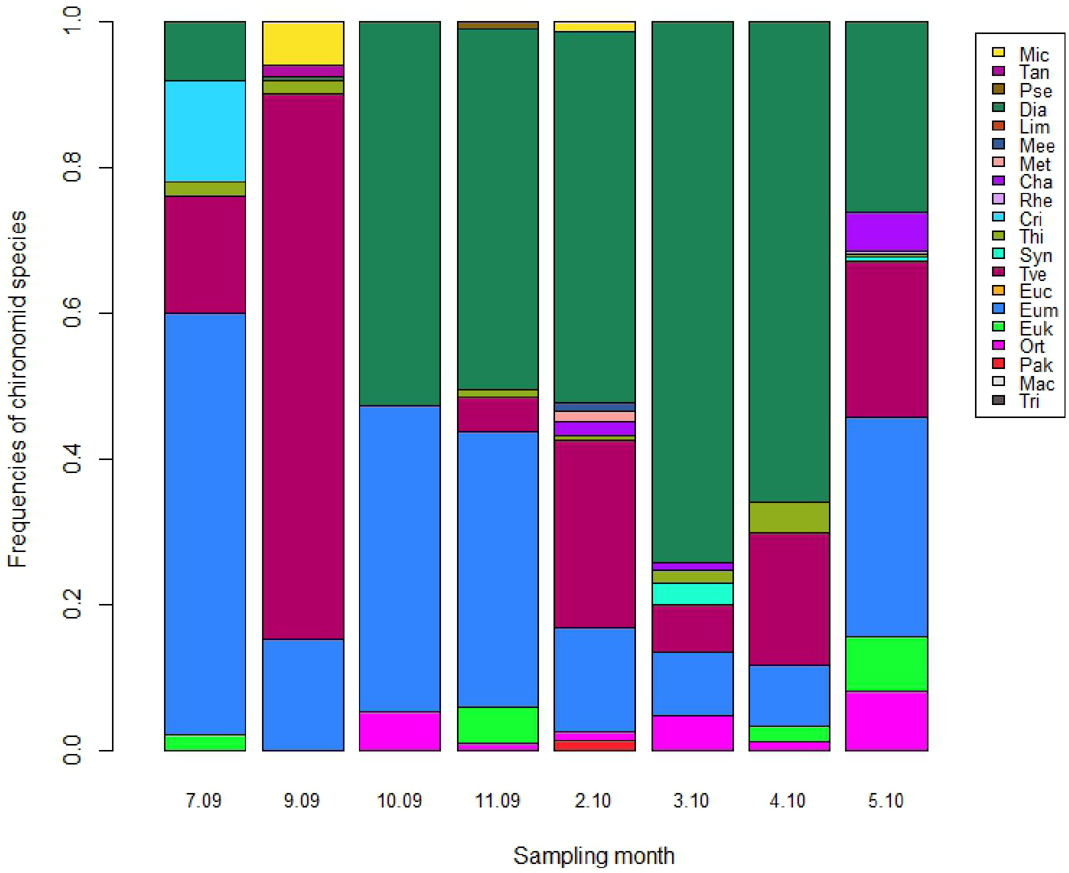

Figure 5. Seasonal variation of the chironomid community of Sundsá. Bar labels refer to month and year. Species acronyms as in Table 2.

Three-spined stickleback (*Gasterosteus aculeatus* Linnaeus 1758) (Christoffersen 2002). These taxa are, however, absent in steep and shallow streams such as Matará and Sundsá. Amphibia and Odonata which are common aquatic predators elsewhere in Europe, are not natively occurring in the Faroes at all. The observed seasonal variation and between-stream differences in the chironomid community composition is thus not likely to be a result of selective predation via top-down regulation (Kreiling et al. 2021).

In both streams, the tube-building *Tvetenia calvescens* (Orthocladiinae) was the dominant Chironomidae species and showed similar life cycle pattern with two generations per year. Based on the occurrence of instar IV larvae, adult emergence supposedly happened during May in the first generation with over-wintering larvae and in July for the second, shorter summer generation. In a study on seasonal variation of Chironomidae in a small stream in Germany, Nolte (1991) found the highest densities of *Tvetenia* larvae in June. In that stream, there seemed to have been only one generation with over-wintering larvae which showed up early in the year in the samples (Nolte 1991). Unfortunately, we lack data for Matará and Sundsá in June in our study but considering abundances in months for which data is available, *Tvetenia* abundances in both streams peak in May and August/September (Figs. 4 and 5). It is documented that Chironomidae develop slower and have longer life cycles in colder environments - even stretching one generation over seven years in extreme cases (Butler 1982) - whereas the same species can complete several generations a year in temperate regions (Pinder 1986). Although far from being this extreme, the uni- versus bivoltinism of *Tvetenia* in Nolte's (1991) compared to our study could be due to differences in stream water temperatures. In the present study, average stream temperatures (9.3°C and 10.0°C) were higher than in the small stream (7.6-8.2°C) investigated by Nolte (1991). Up to five generations a year have been recorded for the species from English rivers (Tokeshi 1986).

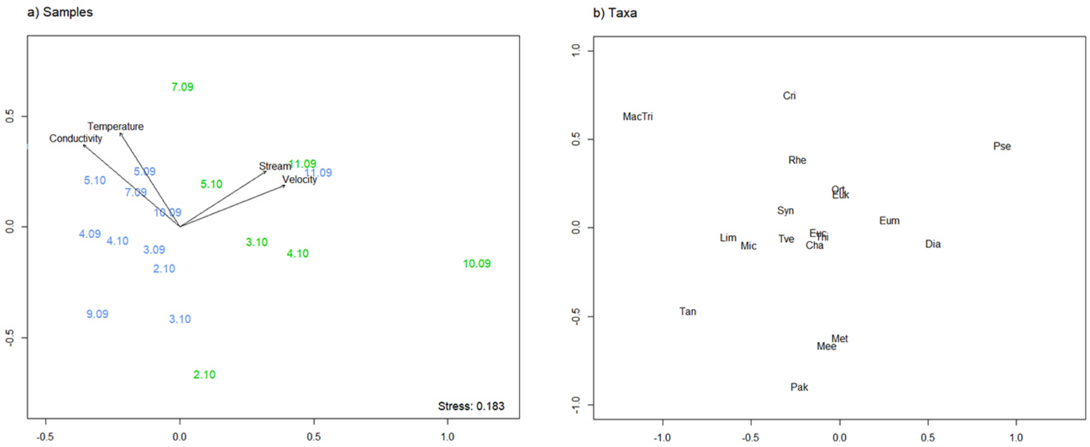

Figure 6. Non-metric multidimensional scaling (NMDS) plots illustrating the distribution of (a) communities and (b) Chironomidae taxa in the larger stream Sundsá (green) and smaller stream Matará (blue). Significant associations ($p < 0.05$) of the environmental variables to the axes are shown with arrows. Taxa acronyms are listed in Table 2.

Although we did not attempt to analyse life cycles of other species, we can conclude a few general seasonal patterns. Larvae of *Micropsectra* sp. were most abundant in late summer. *Metriocnemus* spp. was mainly found in winter and spring which corresponds with the findings of Nolte (1991). Abundances of *Eukiefferiella* minor peaked in May but larvae were present in fairly even numbers all year round. *Diamesa* spp. reached their highest abundances early in the year, in February and March and then again as a presumably second generation in July. Both observations concur with Nolte's study, which found an even distribution for *Eukiefferiella* across the year and an early occurrence for *Diamesa* (Nolte 1991). Likewise, emergence of *Eukiefferiella* minor were observed all year round in a 13.3°C warm spring-fed stream in Iceland whereas in a colder (5.3°C) neighbouring stream emergence occurred mainly in May (Hannesdóttir et al. 2012).

Lotic communities have been shown to respond to climatic variables which can cause changes at the ecosystem level (Jourdan et al. 2018). Temperature and precipitation are impacted by climate change (Pachauri et al. 2015) and our results show that these variables (precipitation through flow velocity) shape Chironomidae communities. This study did not aim to directly relate freshwater invertebrate dynamics to climate change but considering that Chironomidae are the most abuntant benthic invertebrates in streams, climate change can affect both freshwater and terrestrial ecosystems in the Faroe Islands.

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Table 3. Dependence of chironomid community composition on environmental variables. Asterisks behind p-values indicate level of significance as *<0.05, **<0.01.

Variable	NMDS1	NMDS2	r^2	p-value
Stream	0.742	0.671	0.472	0.045 *
Month	0.550	0.835	0.416	0.067
Temperature	-0.460	0.888	0.642	0.003 **
Temperature amplitude	-0.988	-0.156	0.215	0.288
Conductivity	-0.699	0.715	0.743	0.002 **
pH	-0.492	-0.871	0.033	0.846
Depth	-0.218	0.976	0.089	0.647
Velocity	0.880	0.475	0.507	0.029 *

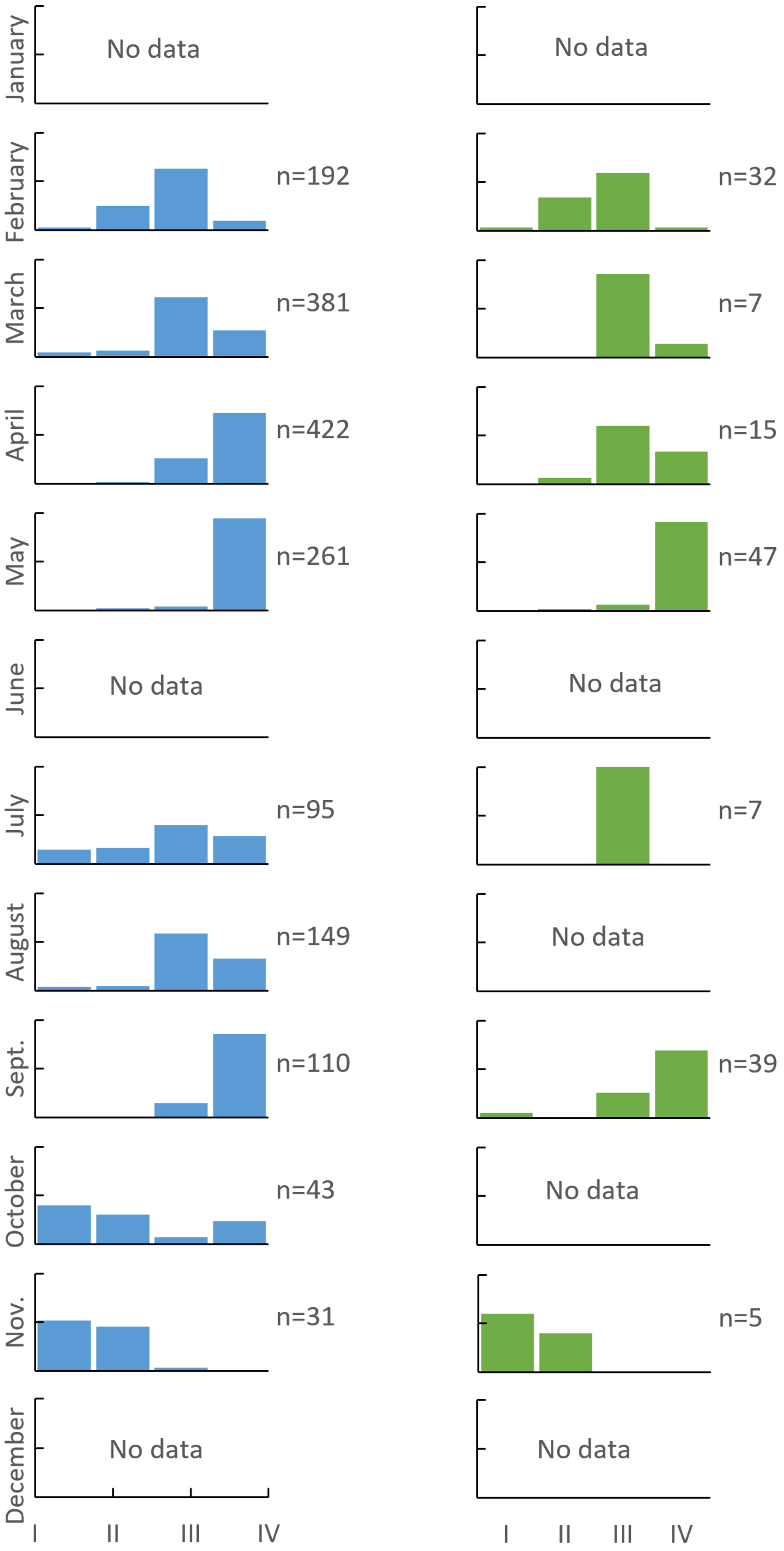

Figure 7. Relative abundance of *Tvetenia calvescens* life stages (instars I, II, III and IV) in the smaller stream Matará (blue) and larger stream Sundsá (green). Black lines indicate emergence of adults.

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CHIRONOMIDAE COLLECTED AT THE SEASHORE IN KUME ISLAND, JAPAN

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Abstract

Local residents and tourists of Kume Island suffer from the biting of *Leptoconops taiwanensis* (Lien, Lin & Weng 1998) (Ceratopogonidae), a species known as asa-mushi (sea lettuce bug). Because *L. taiwanensis* bites tend to occur while harvesting sea lettuce, some locals mistakenly believe that *L. taiwanensis* inhabits the sea lettuce. The objectives of the current study are to (i) identify the chironomid species inhabiting the sea lettuce, (ii) determine the distribution of the larvae, and (iii) perform a faunistic investigation of Chironomidae at Shinri Beach. Only one male chironomid species, *Ainuyusurika tuberculatum* (Tokunaga, 1940), emerged from the samples taken from the beach. The density of chironomid larvae was the highest in the presence of sea lettuce. A total of 53 males were collected using light traps, of which we identified males of seven genera and seven species belonging to three subfamilies. No *L. taiwanensis* were collected in this study.

Introduction

Females of the genus *Leptoconops* (Ceratopogonidae) are particularly vicious biting midges that attack humans in many parts of the world (Kettle 1962). Local residents and tourists of Kume Island suffer from the biting of *Leptoconops* (*Leptoconops*) *taiwanensis* (Lien, Lin & Weng 1998), a species that is known as asa-mushi (sea lettuce bug) in Japanese, because it appears during the sea lettuce (*Monostroma nitidum* Wittrock, 1866) gathering season (Okinawa Prefecture 2022) from January to the end of March. Specimens of *Leptoconops*, are commonly found in arid habitats where the larvae burrow in sandy soil or clay (Mullen and Hribar 1988). Because bites of *L. taiwanensis* tend to occur while harvesting sea lettuce, some locals mistakenly believe that this species inhabits the sea lettuce. Motivated by this, and our observation that larvae of Chironomidae were present in the sea lettuce at Shinri Beach, we wanted to survey the chironomid species in sea lettuce. There is almost no information on midges on Kume Island other than the report by Kawai et al. (2011). The objectives of the current study are thus to (i) identify the chironomid species inhabiting the sea lettuce, (ii) determine the distribution of the larvae, and (iii) perform a faunistic investigation of Chironomidae at Shinri Beach.

Materials and Methods

Study site

Shinri Beach is located in front of the Cypress Resort hotel on the western coast of Kume Island. This 3 km white sand beach is popular for its shoals and great view of the sunset. Adults of *L. taiwanensis* have a biased distribution around Shinri Beach (Okano et al. 2012). The sampling site (26°21'N, 126°42'E) was located near the airport at the edge of the beach (Fig. 1).

Chironomid sampling

Samples of chironomid larvae were collected on 8 March 2019. Larvae were taken from the seabed by pushing petri dishes (9 cm in diameter, 1 cm in depth) into the substratum, digging into the sand with a plasterer's trowel, and inverting the dishes upon removal (Hirabayashi et al. 2004). Each dish was covered with a cover slip, and the whole was sealed with insulating tape. There were four kinds of sampling habitats: always underwater, exposed (to the air) at low tide, high water mark, and terrestrial (always above sea water). On each sampling occasion, three samples were taken from a sand area apparently free of sea lettuce (underwater, sand exposed at low tide, and high-water mark), three samples where sea lettuce was present (un-

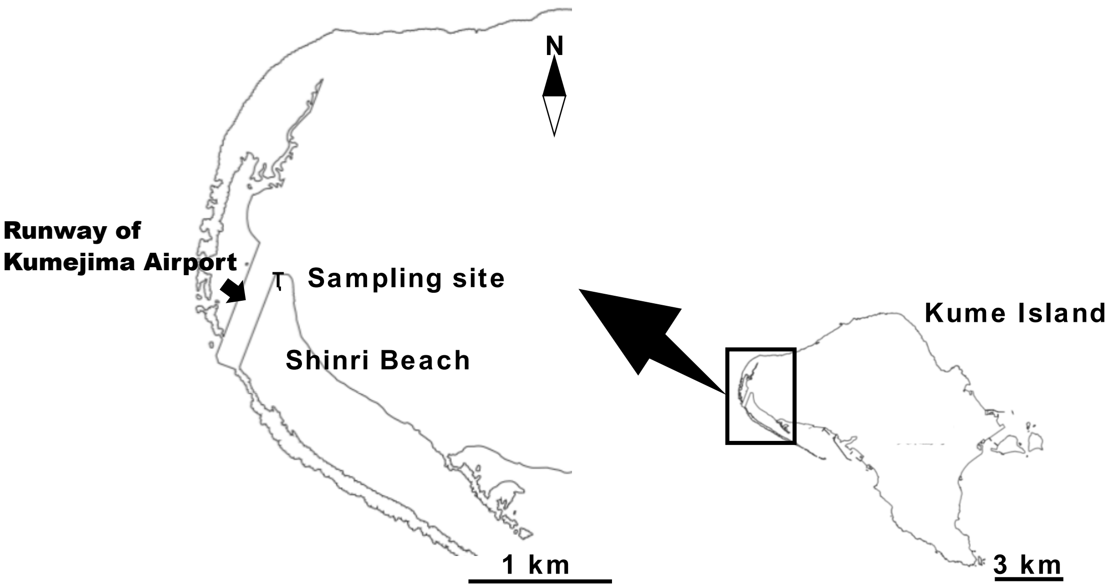

Figure 1. Map of Shinri Beach with the locations of the sampling site (●). This map is based on public survey maps published by the Geospatial Information Authority of Japan.

derwater and sand exposed at low tide), and three samples where terrestrial vegetation was present.

Estimates of chironomid larval density and larval rearing

The samples were transported to our laboratory under cool conditions the following day. In the laboratory, the dishes were unsealed, the chironomid larvae were picked out, then counted under a binocular microscope.

For larval rearing, seabed substrates were collected with petri dishes in without sea lettuce area and sea lettuce areas on 8 March 2019. The two substrate samples were mixed in a plastic bag and transported to our laboratory under cool conditions on the same day. The samples were submerged in water and kept at room temperature with aeration treatment using an air pump. Temperature of the rearing water was monitored every hour during rearing with a thermistor thermos recorder and averaged 22.0 ± 2.0 °C. No additional food was supplied during rearing because the substrate samples considered to contain enough detritus as food for the chironomid larvae. Emerging adults were checked and collected every day for 23 days until 31 May 2019.

Light trap

The abundance of adult chironomid midges from Shinri Beach was investigated with UV light and white LEDs using pan traps. The traps were set up at Shinri Beach in front of the Resort Hotel and were located about 10 m apart from each other. The pan traps were in place from the 6th to the 8th of March 2019. The pans were replaced with new ones every morning (at about 09:00) and replaced the position of the UV light and white LEDs was switched each period. Light intensities measured using HSR-OE1000 (Opto Research Corp.) for each LED were 2.0×10^{11} photons $\cdot$ m^{-2} $\cdot$ s^{-1} for both UV and white light.

Identification of species and data analyses

Male adults were mounted on slides with gum chloral and their species were identified following the taxonomical keys of Wiederholm (1989), Sasa and Kikuchi (1995), Saether et al. (2000), and Langton and Pinder (2007). Females were excluded from the analysis as they could not be identified to species level. Larval density was analyzed using a multiple comparison test (Tukey's honestly significance difference (HSD) test). All data were analyzed using a computer program package (SPSS Ver. 11.5.1J, SPSS Japan Inc., Tokyo, Japan).

Results and Discussion

The density (mean ± S.D.) of chironomid larvae ranged from 0 to 69,251.1 ± 10,491.6 individuals/m^2. A significant difference was found in the density of chironomids, based on the substrate type. The density of chironomid larvae was the highest in the sea lettuce exposed at low tide ($p < 0.001$, Tukey's HSD test). Sea lettuce did not affect the larval density in sea sediment that was not exposed at low tide ($p > 0.05$, Tukey's HSD test). On the other hand, this species was not distributed on terrestrial substrate. These results suggest the length of exposure of the sediment to sea water has an effect on the distribution of chironomid larvae. We would like to know the undiscovered habitats of the *L. taiwanensis*, but we have not been able to find them from the sea sediment with or without sea lettuce.

A total of six males emerged from the substrate samples. The only species found was *Ainuyusurika tuberculatum* (Tokunaga 1940).

A total of 53 males were collected using light traps. We identified seven genera and seven species belonging to three subfamilies of meles. Chironominae was the most abundant subfamily, accounting for 92.5 % of the males, followed by Telmatogetoninae (5.7 %) and Orthocladiinae (1.9

Table 1. Chironomid species collected at the sampling site using light traps.

Taxon	3/6–3/7		3/7–3/8		Total	Percent male (%)
	UV	White	UV	White		
Chironominae						
Chironomini						
Ainuyusurika tuberculata	3		19	9	31	58.5
Chironomus okinawanus				2	2	3.8
Dicrotendipes enteromorphae				1	1	1.9
Tanytarsini						
Yaetanytarsus iriomotensis	11		2		13	24.5
Tanytarsus sp. nr. *boodleae*	2				2	3.8
Orthocladiinae						
Smittia littoralis			1		1	1.9
Telmatogetoninae						
Thalassomya japonica		1	1	1	3	5.7
Male	16	1	23	13	53	100
UV				39		
White				14		

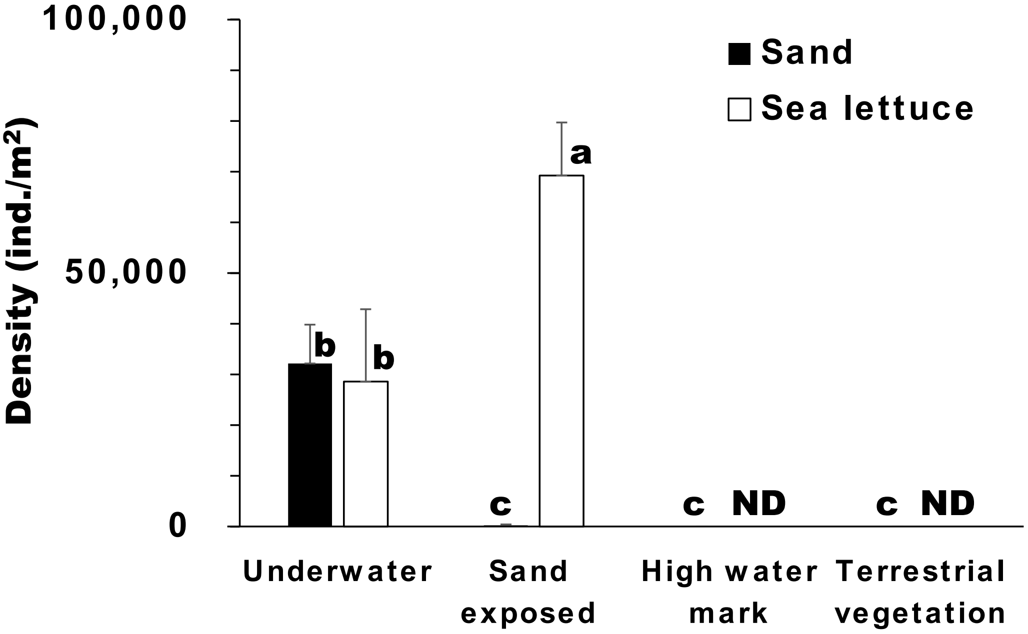

Figure 2. Larval density at each sampling point. Data are presented as mean ± S.D. Different lower-case letters indicate significant differences between groups. ND = No data.

%). The most abundant species, *A. tuberculatum* (58.5 %), *Yaetanytarsus iriomotensis* (24.5 %) and *Thalassomya japonica* (5.7 %), composed 88.7 % of the total males. No *L. taiwanensis* adults were collected in the present study.

Ainuyusurika tuberculatum and *Y. iriomotensis* were the most widely collected species from the Honshu to Yaeyama Islands obtained from sea substrate samples (Kawai et al. 2011). In the present study, *A. tuberculatum* and *Y. iriomotensis* were dominant species, and they were attracted UV lights. Residents may be exposed not only to *L. taiwanensis* adults but also to a seasonal abundance of chironomid midges, especially *A. tuberculatum* and *Y. iriomotensis*. In the present study, adults of *Y. iriomotensis* were collected at several sampling sites using light traps and sweeping nets, but their emerging adults were collected from only one sampling site of the sea substrate samples. These results suggest that it was more difficult for larvae of *Y. iriomotensis* than for larvae of *A. tuberculatum* to develop into adults under the same rearing conditions. The abundance of emerged adults from larval rearing seems to be an underestimation of the habitat range as well as the density for some species (Inoue et al. 2008).

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Bryophaenocladius adigensis sp. n., a new species from the Italian Alps (Chironomidae, Orthocladiinae)

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Abstract

Bryophaenocladius adigensis sp. n., is diagnosed and described based on two male adults material collected in the Sardagna stream, near the city of Trento (Northern Italy). Although the male of *B. adigensis* sp. n. shows some morphological affinities with other *Bryophaenocladius* species (*B. aestivus*, *B. flexidens*, *B. muscicola*, *B. subvernalis* and *B. thaleri*), it exhibits a combination of unique characters that make it a different species: palpomere 3 with 3 typical sensilla coeloconica; absence of antepronotal setae; antennal ratio= 0.86; tergite IX and anal point without lateral expansion; aedeagal lobe typically sub-oval; virga consisting of 2 curved unequal spines; distal part of gonocoxite with a vertical row of setae; inferior volsella, long nose-like shaped, distal part spatulate with 2 characteristic pre-apical setae, median part bare. Currently, about 42 *Bryophaenocladius* species are reported from Europe, of which only 11 are known from Italy. Consequently, the description here of *B. adigensis* sp. n. increases the total number in the genus to 12 from this country. Based on type-locality features, we can consider *B. adigensis* sp. n. as typical of mountain streams fed mainly by groundwater.

Introduction

An extensive material collected with a sweep net in the Sardagna stream, a tributary of the River Adige the city of Trento (Northern Italy), revealed the presence of a new species of the genus *Bryophaenocladius*, *Bryophaenoladius adigensis* sp. n. The genus *Bryophaenocladius* was stated by Thienemann in 1934 with *Orthocladius muscicola* Kieffer, 1906 as type species. To date, more than 100 species have been recorded all over the world of which 42 in Europe and 11 in Italy (Rossaro et al. 2019), now 12. Based on a consistent literature on *Bryophaenocladius* species from Europe and neighbouring areas (Brundin 1947, 1956; Strenzke 1957; Sæther 1973; Sasa 1985, 1996; Tuiskunen and Lindeberg 1986; Armitage 1987; Caspers and Reiss 1987; Cranston and Armitage 1988; Cranston et al. 1989; Sasa and Okazawa 1992; Willassen 1996; Sasa and Suzuki 2000, 2001; Kaczorowska and Gilka 2002; Makarchenko and Makarchenko 2006, 2009, 2012; Langton and Pinder 2007; Du et al. 2011; Ashe and O'Connor 2012; Sæther and Spies 2013; Rossaro et al. 2019; Moubayed and Lods-Crozet 2022; Moubayed and Langton 2023). *B. adigensis* sp. n. can be considered to be local biogeographic element. Taxonomic remarks on related *Bryophaenocladius* species, with comments on the ecology and geographical distribution of the new species are given.

Material and methods

Material is composed of 2 male adults collected in the Sardagna stream (Northern Italy), using a sweep net, and then preserved in 80-85% ethanol. The methodology of mounting and conservation of the holotype and paratype material is provided in Moubayed and Langton (2019, 2023). Morphological terminology and measurements follow those of Sæther (1980) and Langton and Pinder (2007).

Results

Bryophaenocladius adigensis sp. n.

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Type material

Holotype: 1 male adult, Italy, Trentino Province, River Adige, Sardagna stream, 45° 26' 58.78" N, 10° 58' 52.7" E, 194 m a.s.l., leg. J. Moubayed and P. Ashe, 11.VII.2017. Mounted on 1 slide, deposited in the entomological collection of the MUSE-Museo delle Scienze, Trento, Italy (Accession number: cINV0017).

Paratype: 1 male adult, same locality and data as for holotype, deposited in the collection of the first author.

Etymology

The species 'adigensis' is named after the second longest river in Italy, the Adige River, which crosses the Trentino-Alto Adige and Veneto Regions in northern Italy and of which the Sardagna stream is a tributary.

Diagnostic characters

Head. Antenna 910 μm long, last flagellomere 420 μm long, AR 0.86; clypeus smoothly sub-rectangular to square-like shaped, with 6 setae in 3 rows; palpomere 3 with 3 characteristic sensilla coeloconica. Thorax. Lobes of antepronotum not gaping, lateral antepronotals absent; acrostichals 17 starting close to scutum. Wing. Squama with 9 setae; veins: R4, located on proximal half; remaining veins bare; squama with 9 short setae in 1 row. Legs. Sensilla chaetica on tibia and tarsomeres ta_1-ta_5 of PI, present only on tarsomeres of PII-PIII. Abdomen. Tergite IX without anal tergite bands, dorsal margin bent downwards; anal point triangular, without lateral expansions. Virga composed of 2 curved unequal spines. Phallapodeme well-developed, aedeagal lobe ellipsoid, basal expansion short. Gonocoxite with characteristic sclerotization on basal junction; setiferous dorsal area with 6 stout setae on distal part, median part bare. Inferior volsella long nose-shaped, spatulate apically and bearing 2 characteristic pre-apical setae. Gonostylus without posterior projection, posterior margin distinctly rounded; crista dorsalis present (visible in lateral view).

Description

Male adult

(n = 2; Figs 1A-L)

Medium sized species. Total length (TL) 2.45 mm. Wing length (WL) 1.70 mm. TL/WL = 1.44. General colouration contrasting pale brown to dark brown-blackish. Head dark brown to blackish, antenna pale brownish; thorax contrasting brown to dark brown with blackish mesonotal stripes, scutellum brownish with blackish margins; legs uniformly brownish; abdomen brownish, anal segment contrasting from pale brown to dark brown.

Head. Eyes bare. Frontal area without tubercles; margins of vertex slightly thinner at base; coronal triangle (Fig. 1A) smoothly heart shaped, coronal 4; temporals 9 including 6 inner and 3 outer verticals. Antenna 13-segmented, 910 μm long, last flagellomere 420 μm long, AR 0.86; antennal groove reaching segment 3. Clypeus (Fig. 1B) smoothly sub-rectangular to square-shaped, nearly equal sides (each of 85 μm long), with 6 setae in 3 rows. Palp 5-segmented, palpomere 3 (Fig. 1C) with 3 sensilla clavata and 3 sensilla coeloconica; length (in μm) of segments: 35, 48, 75, 75, 135; segments 3 and 4 subequal.

Thorax. Antepronotum well developed, antepronotal lobes (Fig. 1D) not gaping, dorsal part distinctly thinner, not pointed, all antepronotals typically absent; acrostichals 17 in 1–2 rows, starting close to antepronotum; dorsocentrals consist of 7 not decumbent setae in 1 row; prealars 3; humeral pit absent; supraalars absent; scutellum with 6 uniserial setae (3 on each side of the midline).

Wing. Brachiolum with 1 seta; subcosta overreaching fork of radius; costal expansion 55 μm long. Membrane with coarse punctuation. Distribution of setae on veins: R, 4, located on proximal half; remaining veins bare; squama with 9 short setae in 1 row.

Legs. Sensilla chaetica on: tibia and tarsomeres ta_1–ta_5 of PI, only on tarsomeres of PII-PIII. Length (in μm) and proportions of leg segments as in Table 1 (n = 1).

Abdomen. Hypopygium in dorsal and ventral view as in Figs 1F-G (1F, dorsal; 1G, ventral with tergite IX and anal point omitted). Tergite IX in dorsal and lateral view (Figs 1F, H: 1F dorsal, 1H, lateral), broadly sub-circular with rounded posterior margin; dorsal margin without hump, regularly arched when viewed laterally; anal tergite bands absent (Figs 1F, H); presence of 8 setae close to the posterior margin (4 on each side of anal point). Laterosternite VIII with 14 lateral setae (7 on each

Table 1. Male adult of *Bryophaenocladius adigensis* sp. n. Length (μm) and proportions of prothoracic (PI), mesothoracic (PII) and metathoracic (PIII) legs (n = 1).

	fe	ti	ta1	ta2	ta3	ta4	ta5	LR	BV	SV	BR
PI	610	635	415	340	235	155	90	0.66	2.03	2.97	2.60
PII	645	605	285	185	135	105	85	0.47	3.0	4.39	3.0
PIII	680	740	330	235	190	120	100	0.45	2.71	4.30	3.20

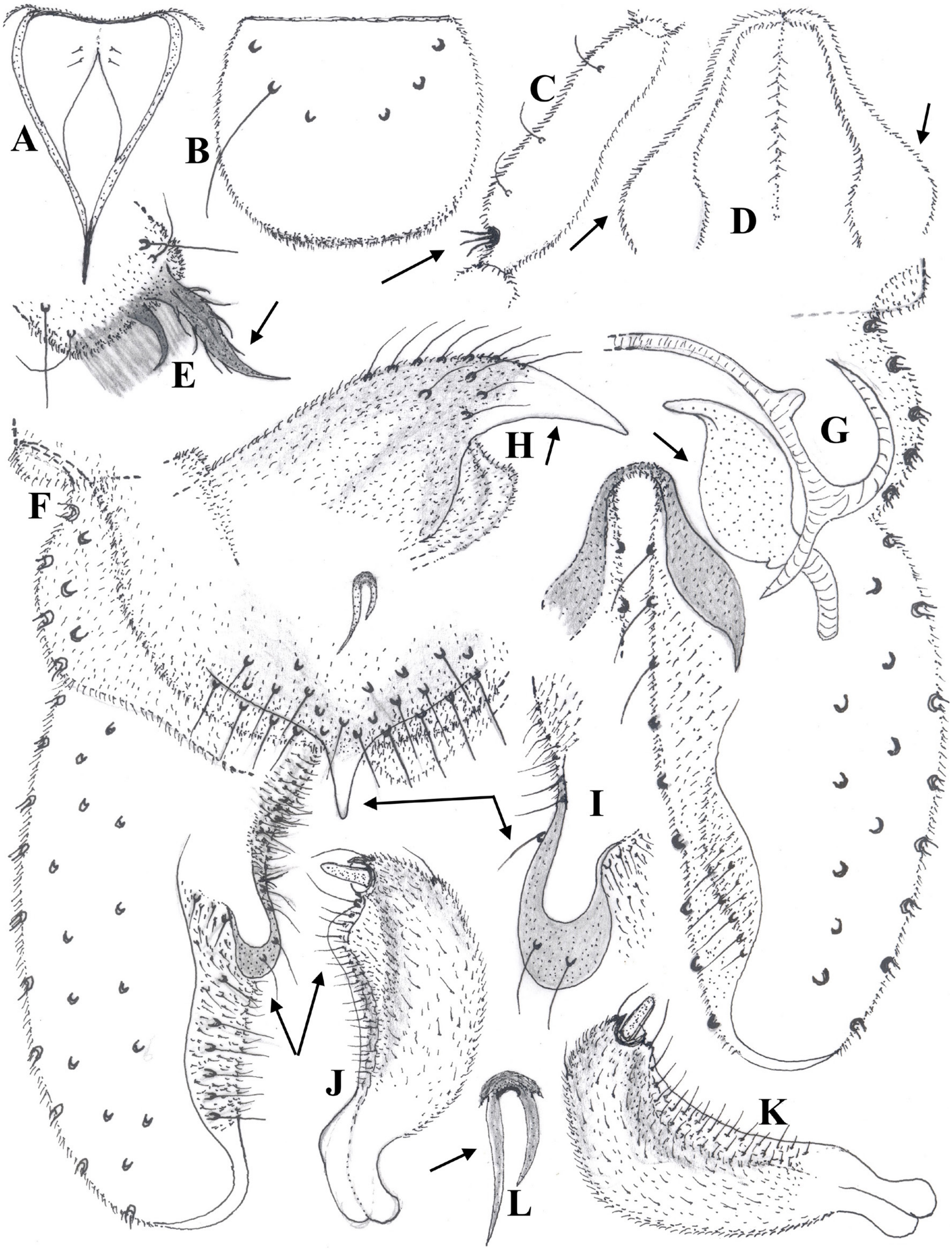

Figure 1. Male adult of *Bryophaenocladius adigensis* sp. n. A) coronal triangle; B) clypeus; C) palpomere 3; D) lobes of antepronotum; E) tibial spurs of PIII; F-G) hypopygium in dorsal and ventral view; H) tergite IX and anal point, lateral; I) inferior volsella, right side; J-K) gonostylus at right and acute angle; L) virga. The arrows indicate some distinguishing characters.

side). Anal point in dorsal and lateral view (Figs 1F, H) 15 µm long, 40 µm maximum width at base, triangular and short, apex rounded; lateral expansions absent; with about 16 setae. Virga (Figs 1F, L) consists of 2 curved spines, about 35 and 20 µm long. Transverse sternapodeme not projecting, rounded; lateral sternapodeme short. Phallapodeme well-developed, basal expansion short; aedeagal lobe large, distinctly ovoid to ellipse-like. Gonocoxite (Figs 1F-G) 180 µm long, 65 µm maximum width, rounded apically; superior volsella low lobe shaped; ventral side (Fig. 1G) with characteristic short sclerotization on basal junction, distal part with a characteristic setiferous lobe, inner margin with 9-10 stout setae; setiferous dorsal area well developed, only proximal and distal parts densely covered with setae, median area bare, distal part bearing 6 characteristic setae in a vertical row. Inferior volsella (Figs 1F, I) about 65 µm long, 15-20 µm maximum width; long nose-like shaped, strongly bent downwards; apical part spatulate with 2 distinct pre-apical short setae, median area bare. Gonostylus (Figs 1J-K) about 75 µm long and 25 µm maximum width, without posterior projection; anterior side covered with short and long fine setae; posterior margin distinctly rounded; crista dorsalis (Fig. 1J) low lobe-like, clearly visible in lateral view; megaseta well-developed. HV = 3.27; HR = 2.40.

Female adult, pupal exuviae and Larva:

unknown.

Faunal data and taxonomic remarks

Based on recently published faunal data on known *Bryophaenocladius* species from Europe (Tuiskunen and Lindeberg 1986; Armitage 1987; Caspers and Reiss 1987; Willassen 1996; Kaczorowska and Gilka 2002; Langton and Pinder 2007; Ashe and O'Connor 2012; Sæther and Spies 2013; Moubayed and Lods-Crozet 2022, Moubayed and Langton 2023), currently, there are about 42 known species from Europe, of which only 11 are reported from Italy (Rossaro et al. 2019). Consequently, the description here, of *B. adigensis* sp. n., increases the total number in the genus to 12 from this country.

Although some morphological similarities are observed between *B. adigensis* sp. n. and other related members of the genus, namely *B. aestivus* (Edwards, 1929), *B. flexidens* (Brundin, 1947), *B. muscicola* (Kieffer, 1906), *B. subvernalis* (Edwards, 1929) and *B. thaleri* Willassen, 1996, a combination of some morphological distinctive characters found in the male adult are highlighted in the following differentiating characters, which will separate the new species from other related congeners: coronal triangle (Fig. 1A) heart-shaped; clypeus (Fig. 1B) smoothly square-like; palpomere 3 (Fig. 1C) with 3 typical sensilla coeloconica; antepronotals (Fig. 1D) absent; tergite IX and anal point (Figs 1F, H) without lateral expansion; anal tergite bands absent; anal point (Figs 1F, H) triangular; aedeagal lobe of phallapodeme (Fig. 1G) typically sub-oval; virga (Figs 1F, L) consisting of 2 curved unequal spines; gonocoxite with characteristic sclerotization on basal junction, distal part with a vertical row of stout setae on both dorsal and ventral sides; distal part of gonocoxite (Fig. 1 F) with a vertical row of setae; inferior volsella (Figs 1F, I), long nose-like shaped, distal part spatulate with 2 characteristic pre-apical setae, median part bare.

Ecology geographical distribution

Male adults of *B. adigensis* sp. n. were collected around the riparian zone of Sardagna stream, at 194 m a.s.l., about 50-100 m upstream of the

Figure 2. Type-locality of *Bryophaenocladius adigensis* sp. n.. The Sardagna stream (left), the confluence between Rio Sardagna and the Adige River (right).

confluence with the Adige River in Trento (Fig. 2). Sardagna is a mountain stream, fed mainly by groundwater, its source is located in the Sardagna village, from which it waterfalls down to Trento. The stream is characterised by hard and basic water, with a conductivity of 320-415 µS/cm and a pH of 8.0-8.6, and chilly waters, with a temperature ranging from 7 to 12-14°C from spring to autumn. Enriched substratum with submerged and emergent bryophytes, humus, deciduous wood and tree bark present the riparian zone of the stream are believed to represent the most favourable microhabitats for the larvae, as typical for larvae of this genus known to be semi-terrestrial in groundwater-fed streams (Lindegaard 1995, Lencioni et al. 2011).

Geographical distribution of *B. adigensis* sp. n. is currently restricted to its type-locality.

Acknowledgements

The adults of *B. adigensis* sp. n. were coughed in July 2017 during the XX Symposium on Chironomidae held in Trento, Italy, by the first author and Patrick Ashe to whose memory the authors dedicate this work. Environmental data and photos of the stream were taken by Francesca Paoli (MUSE).

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LIMNOPHYES KNISPELAE SP. N. AND *L. SARTORII* SP. N., TWO NEW CRENOPHILOUS SPECIES FROM THE SWISS ALPS (CHIRONOMIDAE, ORTHOCLADIINAE)

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Abstract

Limnophyes knispelae sp. n and *L. sartorii* sp. n. are diagnosed and described based on material collected in the high Alpine valley of the Rhône river and the cirque of Macun in the Swiss National Park (alt. 1800-2616 m a.s.l.). The first new species is described only as male adult, while the second as male adult and pupal exuviae. Main distinguishing characters found in the male adult of *L. knispelae* sp. n. *L. sartorii* sp. n. are: clypeus shield-like shaped; humeral pit typically circular with 3-4 or 9 lanceolate setae; virga strong spine-like; preepisternals vary from 4-6 to 4-8. The pupal exuviae of *L. sartorii* sp. n. is characterized by having frontal apotome domed; thoracic horn reduced to nose-like tubercle; posterior area of tergites II-VI with 1-7 rows of minute hooks; segment VIII with 5 lateral setae; inner posterior margin of anal lobe straight.

A combination of the latter relevant morphological characters has allowed us to consider each of the two new species as a local biogeographic representative of the Swiss Alps. Currently, the genus *Limnophyes* Eaton, 1875 is represented in Switzerland by 15 species. Consequently, the description of *L. knispelae* sp. n and *L. sartorii* sp. n. increases the total number in the genus to 17 known valid species from this country. Discussions and a differential diagnosis on the two new species are given, in which some morphological affinities and distinguishing characters with other related congeners are detailed and highlighted. Comments on the ecology and geographical distribution of the new species are also provided.

Introduction

In general, larval populations of the genus *Limnophyes* Eaton, 1875 occur mainly in semiterrestrial, terrestrial to riparian habitats encountered in wetlands (pools, peat bogs, wet meadows) or in bordering parts of streams and rivers. On the basis of knowledge provided on the taxonomy, geographical distribution and ecology of the known *Limnophyes* species from Europe (Brundin 1947, 1956, Sæther 1975, Cranston 1979, Sæther 1980, Cranston et al. 1989, Sæther 1990, Langton 1991, Wang and Sæther 1993, Lindegaard 1995, Langton and Moubayed 2001, Langton and Pinder 2007, Moubayed-Breil and Ashe 2011, Moubayed-Breil 2013, Ashe and O'Connor, 2012, Sæther and Spies 2013), the genus comprises about 35 known valid species.

In this paper, two new *Limnophyes* species (*L. knispelae* sp. n and *L. sartorii* sp. n.) are described on the basis of material recently collected in the Swiss Alps (high Alpine valley of the Rhône River and the cirque of Macun in the Swiss National Park, alt. 1800-2616 m a.s.l.). *L. knispelae* sp. n is described only as male adult, while *L. sartorii* sp. n. is described as both male adult and pupal exuviae.

Methods and materials

Examined material of *L. knispelae* sp. n and *L. sartorii* sp. n. was collected using Malaise trap and drift nets. Male adults were preserved in 80-85% ethanol and cleared of musculature in 90% lactic acid (head, thorax, abdomen and anal segment) for about 60 to 80 minutes. The specimens were checked under a binocular microscope after 20 minutes to determine how the clearing was progressing. When clearing was complete, the specimens were washed in two baths of 70% ethanol to ensure that all traces of lactic acid were removed. The studied material was mounted in polyvinyl lactophenol. Before the final slide mountings in dorsal view, the hypopygium including tergite IX and anal point, the gonocoxite and the gonostylus, were viewed ventrally and laterally, in order to examine and draw all the necessary details of the species, from both sides. For a better examination of the specific features of the hypopygium, the anal point and tergite IX were removed and the hypopygium was illustrated in a lateral view separately. Remaining part of the abdomen and the halters are preserved in 85% ethanol for an eventual DNA analysis. Morphological terminology, abbreviations and measurements follow those of Sæther (1980) and Langton and Pinder (2007) for the imagines and Langton (1991) for pupal exuviae.

Results and Descriptions

Limnophyes knispelae sp. n.

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Material examined

Holotype, Switzerland. 1 male adult, Malaise trap, leg. B. Lods-Crozet. Rhône River, upper stream (46°34'17.421" N, 8°22'49.460"E); alt. 1800 m a.s.l., 24.IX.1998. Environmental data of water (after Knispel and Castella 2003): crystalline water, conductivity range: 2.9-17.8 µS/cm, mean: 10.5 µS/cm; temperature (max 3.9 °C; mean, 2.3 °C).

Paratypes. 3 male adults, leg. B. Lods-Crozet, same date and locality as for holotype.

Holotype (mounted on 1 slide; GBIFCH00617929) and 1 paratype (mounted, each on 1 slide) are deposited in the collections of the Musée cantonal de Zoologie, Palais de Rumine, 6 place de la Riponne, CH-1014 Lausanne (MZL), Switzerland. 2 paratypes, male adults are deposited in the collection of the senior author.

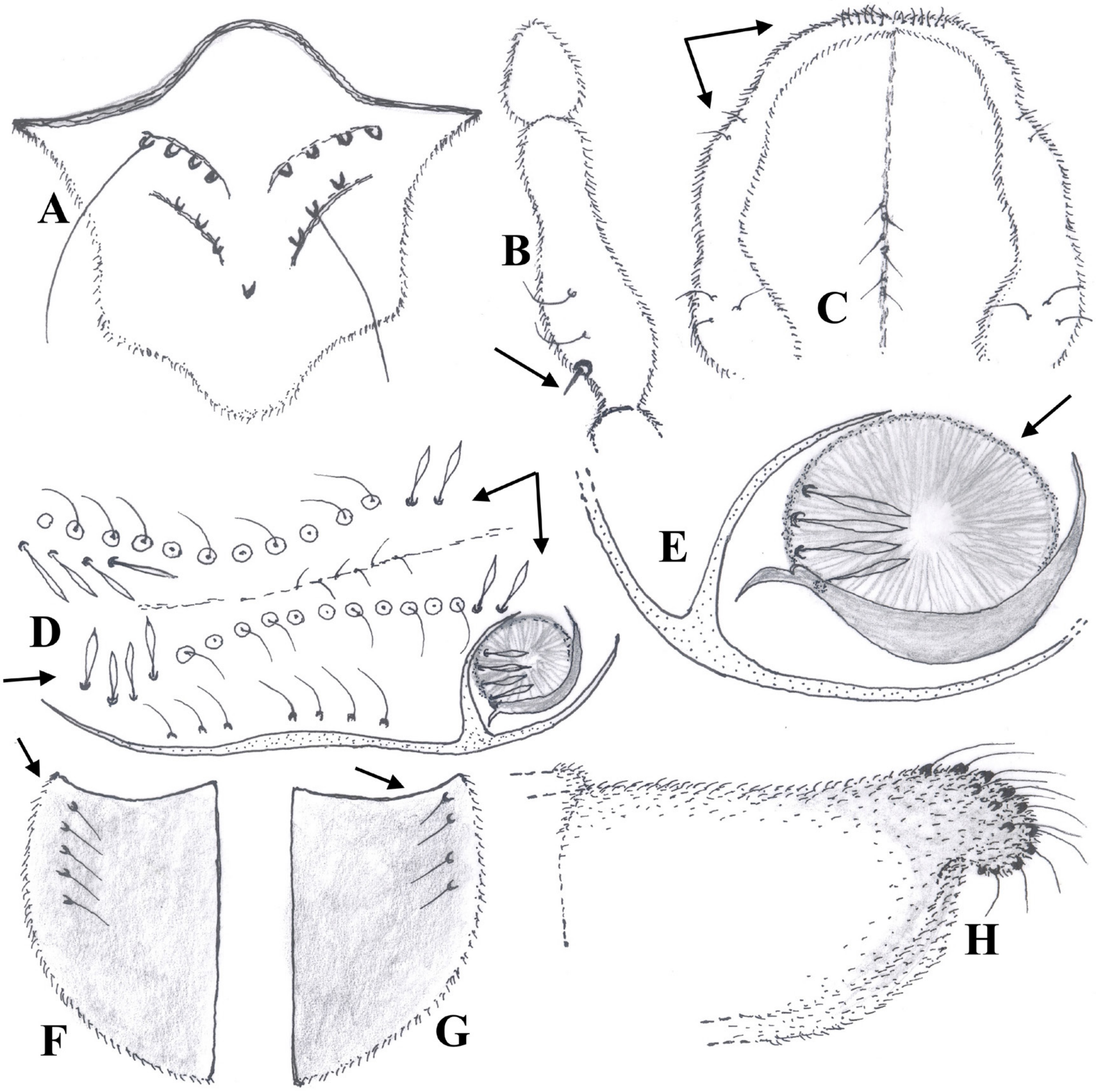

Figure 1. Male adult of *Limnophyes knispelae* sp. n. A) clypeus; B) palpomere 3; C) lobes of antepronotum; D) humeral pit with dorsocentrals and prealars; E) humeral area with humeral pit; F-G) distribution pattern of preepisternals; H) tergite IX and anal point in lateral view. The arrows indicate some distinguishing characters.

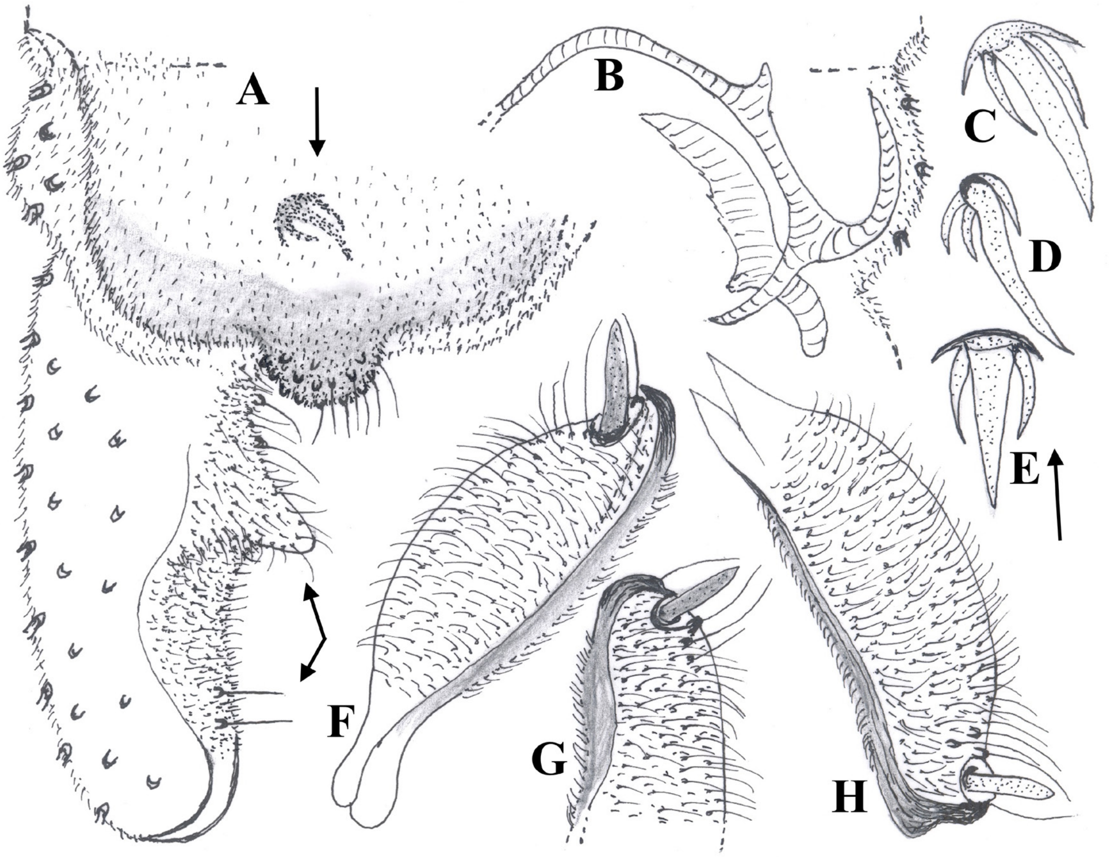

Figure 2. Male adult of *Limnophyes knispelae* sp. n. A) hypopygium in dorsal view; B) apodemes; C-E) virga, three different aspects; F) gonostylus at acute angle; G) distal part of gonostylus; H) gonostylus at obtuse angle. The arrows indicate some distinguishing characters.

Etymology

The name '*knispelae*' is given in honour to Sandra Knispel, who did her PhD thesis on an alpine glacier-fed alluvial system (Rhône River basin, Swiss Alps). She keeps working as consultant in aquatic ecology and is a Swiss specialist on Plecoptera.

Diagnostic characters

Male adult of *L. knispelae* sp. n can be distinguished from some related congeners on the basis of the following combination of characters. Head. Temporals 6 (3 inner and 3 outer verticals); clypeus shield-like shaped, with 15 setae; palpomeres 3 with 2 sensilla clavata and 1 pin-like sensilla coeloconica; antenna 770 µm long, last flagellomere 320 µm long, AR 0.82. Thorax. Lobes of antepronotum not gaping, sinuous medially, antepronotals (6 dorsal, 2 median, only 3 lateral); acrostichals 4; dorsocentrals 17 including 11 decumbent setae located medially and 6 lanceolate setae (2 located proximally and 4 distally); humeral pit typically circular, basal part consists of a characteristic sclerotized crescent-like bearing 4 lanceolate humerals in 1 row; preepisternals 4-5 located on anterior part. Wing, squama with 3 setae. Legs. Sensilla chaetica abundant on tarsomeres ta_1-ta_5 of PI-PIII, present only on apical part of tibiae. Abdomen. Tergite IX without hump. Anal point well-developed, downwardly bent. Virga consists of 3 stout spines, median one much longer. Transverse sternapodeme rounded, orally projecting; aedeagal lobe of phallapodeme slender. Gonocoxite with 2 characteristic setae on distal part. Inferior volsella triangular. Gonostylus linearly elongate, apex well-sclerotized and inwardly curved; anterior side well domed, densely covered with short setae; megaseta well developed, located pre-apically.

Adult male

(n = 4; Figs 1A-H, 2A-H)

Total length, TL 3.25 mm. Wing length, WL 1.55 mm; TL/WL = 2.10. General colouration pale brown to dark brown; head and antenna dark brown. Thorax brown with dark brown mesonotal stripes; humeral pit contrasting brown to dark brown. Legs brown. Abdomen brownish, anal segment brown to dark brown.

Head. Eyes bare, frontal tubercles absent; coro-

Table 1. Lengths (in µm) and proportions of leg segments of *Limnophyes knispelae* sp. n., adult male (n=1)

	fe	ti	ta_1	ta_2	ta_3	ta_4	ta_5	LR	BV	SV	BR
PI	565	675	330	215	190	95	85	0.49	0.68	3.76	1.60
PII	615	510	265	160	110	75	85	0.52	3.23	4.25	2.00
PIII	650	705	385	195	165	95	85	0.55	3.22	3.52	2.35

nals 4; temporals 5 including 3 inner and 2 outer verticals, postorbitals absent. Antenna 775 µm long, last flagellomere about 300 µm long, apex clubbed, apical seta absent, antennal groove reaching segment 3, AR 0.63. Clypeus (Fig. 1A) 100 µm long, 140 µm maximum width at base and 70 µm at median part; typically shield-like shaped, smoothly rounded distally, apex rounded; with 15 setae in 3 rows. Palp 5-segmented; segments 1-2 fused; palpomeres 2-3 (Fig. 1B), palpomere 3 with 2 distal sensilla chaetica and 1 pin-like pre-apical sensilla coeloconica; length (in µm) of segments: 25, 35, 85, 75, 135; fourth segment shorter than the third.

Thorax. Lobes of antepronotum (Fig. 1C) not gaping, with sinuous edges; dorsal antepronotals 12 (6 on each side), median antepronotals 2, lateral antepronotals atypical (consist of 3 setae); acrostichals 6 in 1 row, starting at some distance from lobes of antepronotum. Half area of thorax with dorsocentrals as in Figs 1D-E; dorsocentrals 17 including 2 lanceolate setae (located proximally), 11 decumbent setae (located medially) and 4 lanceolate setae (located distally). Humeral pit typically circular (diameter, about 75 µm), with a characteristic crescent-like inner edge where 9-10 lanceolate humerals are located in 1 row. Prealars 7 in 1 row, located in 2 groups (4 proximal and 3 distal); supraalars (Figs 3G-K) vary from 4 to 5 setae located on anterior side; scutellum with 6 setae in 1 row.

Wing. Brachiolum with 1 seta; membrane with dense punctuation visible at 200X; subcosta overreaching the fork of radius, costal extension 40 µm long. Distribution of setae on veins: R, 5-6, located on proximal part; remaining veins bare; squama with 3 setae.

Legs. Length (in µm) of tibial spurs: PI, 50; PII, 25; PIII, 55; pseudospurs absent. Only few sensilla chaetica present apically on tibia of PI-PIII, are abundant on tarsomeres ta_1-ta_5. Length (in µm) and proportions of leg segments as in Table 1.

Abdomen. Hypopygium in dorsal view as in Fig. 2A; apodemes as in Fig. 2B. Tergite IX (Figs 1H, lateral; Fig. 2A, dorsal) broadly rectangular, posterior margin rounded laterally; dorsal side lacking hump. Anal point (Fig. 1H, lateral; Fig. 2A, dorsal) 30 µm long, 75 µm maximum width at base, distinctly semicircular, with 17-19 setae. Laterosternite IX with 10-12 setae (5-6 on each side). Sternapodeme and phallapodeme as in Fig. 2B; transverse sternapodeme rounded, orally projecting; phallapodeme slender, pointed basally. Virga (Figs 1C-E, 3 aspects) 30-35 µm long, composed of 3 unequal stout spines, median one much longer. Gonocoxite (Fig. 2A) 240 µm long, distal half with 2 stout inwardly projecting setae; apex broadly rounded. Superior volsella weakly-developed. Inferior volsella (Fig. 2A) triangular, apex rounded. Gonostylus (Figs 1F-H, 3 aspects) about 100 µm long, 15-18 µm maximum width, apex well-sclerotized, pointed and inwardly turned over; anterior side strongly swollen, densely covered with short setae; anterior area densely covered with short curved setae; crista dorsalis absent; megaseta well-developed. HV = 3.25; HR = 2.40.

Female adult, pupa and larva: unknown.

Limnophyes sartorii sp. n.

https://zoobank.org/34BA34DB-8A35-4FFD-B4FC-2593BC1DA575

Material examined

Holotype, Switzerland. 1 male pharate adult, collected by drift net, leg. B. Lods-Crozet. Macun cirque (Swiss National Park), streamlet and rheocrenes, left shore of Immez Lake (46°43’39.678”, 10°07’55.764”E); alt. 2616 m a.s.l.; 27.VII.2013.

Pupal exuviae: 1 male, same data and locality as for holotype.

Paratypes. 2 male adults (1 captured by Malaise trap and 1 by drift net as a male pupa), leg. B. Lods-Crozet. Same data and locality as for holotype.

Environmental data from inlet of Immez Lake (Lods-Crozet et al. 2012; Robinson et al. 2016): crystalline water, conductivity 5.9 µS/cm; temperature (min-max, 3.9-19.5 °C; mean, 11.6 °C), pH 6.7.

Holotype (mounted on 1 slide; GBIFCH00617930) and 1 paratype (mounted on 1 slide) is deposited in the collections of the ‘Musée cantonal de Zoologie, Palais de Rumine, 6 place de la Riponne, CH-1014 Lausanne (MZL), Switzerland. 1 paratype, male adult, mounted on 1 slide is deposited in the collection of the senior author.

Etymology

The name '*sartorii*' of the new species is given in honour to Michel Sartori, who is retired after being head director of the museum of Lausanne for more than 20 years. He keeps working as taxonomist on Ephemeroptera worldwide.

Diagnostic characters

Male adult of *L. sartorii* sp. n is easily distinguished from all members of the genus *Limnophyes* by the shape and structure of its humeral area and distribution pattern of dorsocentrals. Additional differentiating characters are also summarized as follows: antenna 770 µm long, last flagellomere 320 µm long, AR 0.82; clypeus shield-like shaped, with 15 setae; palpomeres 3 with 2 sensilla clavata and 1 pin-like sensilla coeloconica; lobes of antepronotum gaping, sinuous medially antepronotals (3 dorsal, 2 median, only 3 lateral); acrostichals 6; dorsocentrals 25-27 including 10-11 decumbent setae located medially and 15-16 lanceolate setae (5-6 located proximally and 10 distally); humeral pit typically circular, basal part consists of a characteristic sclerotized crescent-like bearing 9 lanceolate humerals in 1 row; preepisternals vary from 4-7 setae located on anterior side. Wing, squama with 3-5 setae. Legs. Sensilla chaetica abundant on tarsomeres ta_1-ta_5 of

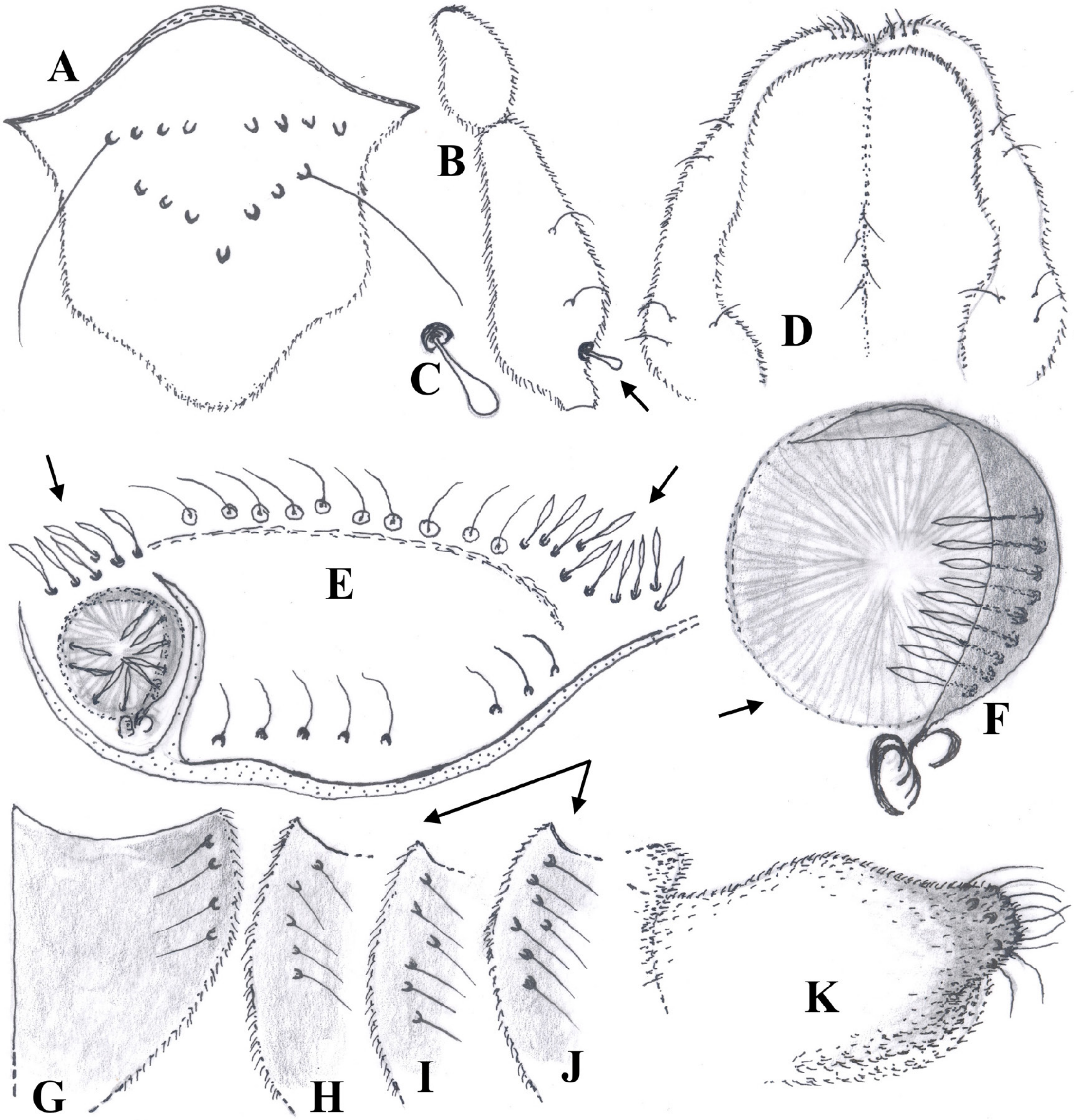

Figure 3. Male adult of *Limnophyes sartorii* sp. n. A) clypeus; B-C) palpomere 3 and sensilla coeloconica ; D) lobes of antepronotum; E) humeral pit with dorsocentrals and prealars; F) humeral pit; G-J) distribution pattern of preepisternals; K) tergite IX and anal point in lateral view. The arrows indicate some distinguishing characters.

PI-PIII, present only on apical part of tibiae. Abdomen. Tergite IX broadly rectangular, with a low dorsal hump. Anal point semicircular, much larger at base, with 16-18 setae. Virga present, consists of 1 stout long spine. Transverse sternapodeme atypically straight; aedeagal lobe of phallapodeme typically enlarged. Gonocoxite with 3 distinct setae on distal part. Inferior volsella digitiform with 1 stout

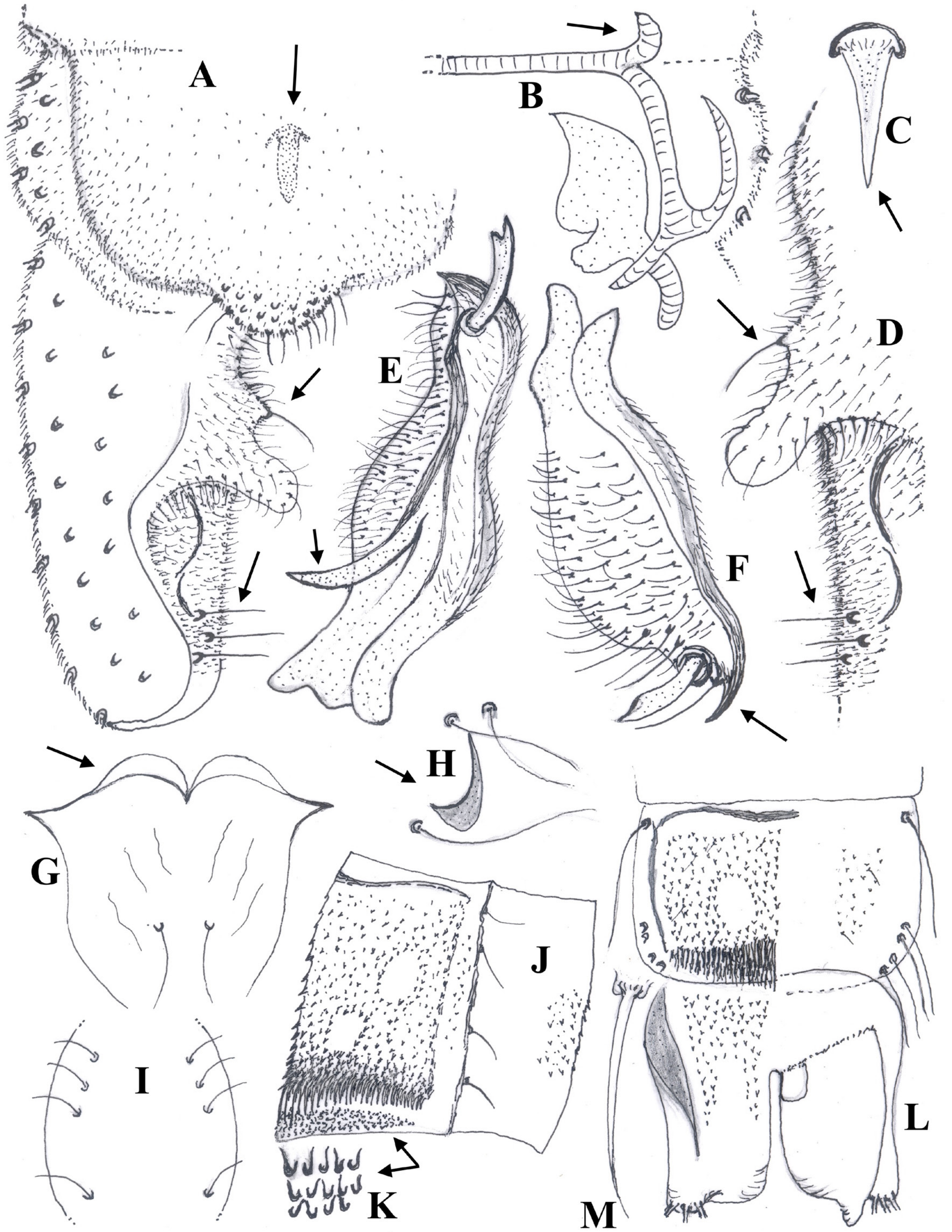

Figure 4. Male adult and pupal exuviae of *Limnophyes sartorii* sp. n. Male adult: A) hypopygium in dorsal view; B) apodemes; C) virga, other aspect; E) gonostylus at acute angle; F) gonostylus at obtuse angle. Male pupal exuviae: G) frontal apotome; H) thoracic horn (reduced) and precorneals; I) dorsocentrals Dc_1-Dc_4; J) segment VI in lateral view; K) details of caudal transverse rows of small hooklets; L) segment VIII and anal lobe in dorsal and ventral view; M) anal macroseta. The arrows indicate some distinguishing characters.

seta located proximally on a characteristic projecting expansion. Gonostylus narrowing distally, well domed medially and pointed apically; anterior side densely covered with setae; megaseta well developed, located pre-apically.

Adult male

(n = 3; Figs 3A-K; 4A-F)

Total length, TL 3.45 mm. Wing length, WL 1.60 mm; TL/WL = 2.16. General colouration contrasting from pale brown to dark brown; head, thorax and antenna brown. Thorax brown with dark brown mesonotal stripes; humeral pit contrasting brown to dark brown, inner part distinctly dark brown. Legs uniformly brown. Abdomen brownish, anal segment contrasting brown to dark brown.

Head. Eyes bare, frontal tubercles absent; coronal setae 4; temporal setae 6 including 3 inner and 3 outer verticals, postorbitals absent. Antenna 770 µm long, last flagellomere about 320 µm long, apex clubbed, apical seta absent, antennal groove reaching segment 3, AR 0.82. Clypeus (Fig. 3A) 125 µm long, 130 µm maximum width at base, typically shield-like shaped, smoothly rounded distally, apex rounded; with 15 setae in 3 rows. Palp 5-segmented; segments 1-2 fused; palpomeres 2-3 (Figs. 3B-C), palpomere 3 with 2 sensilla chaetica and 1 characteristic spatulate pre-apical sensilla coeloconica; length (in µm) of segments: 25, 45, 80, 70, 125; segment 3 longer than the fourth.

Thorax. Lobes of antepronotum (Fig. 3D) slightly gaping, with sinuous edges; dorsal antepronotals 6 (3 on each side), median antepronotals 2, lateral antepronotals only 3; acrostichals 4 in 1 row, starting at some distance from antepronotum. Half area of thorax with dorsocentrals as in Figs 3E-F, dorsocentrals 25-27 including 5-6 lanceolate setae (located proximally), 10-11 decumbent setae (located medially) and 15-16 lanceolate setae (5-6 proximal, 10 distal). Humeral pit typically circular (diameter, about 70 µm), with a characteristic crescent-like inner edge with 9 lanceolate humerals in 1 row. Prealars 8 in 1 row, located in 2 groups (5 proximal and 3 distal); supraalars (Figs 3G-K) vary from 4 to 7 setae located on anterior side; scutellum with 6 setae in 1 row.

Wing. Brachiolum with 1 seta; membrane with dense punctuation visible at 200X; subcosta not reaching the fork of radius, costal extension 35-40 µm long. Distribution of setae on veins: R, 4-5, located on proximal part; remaining veins bare; squama with 3-5 setae.

Legs. Length (in µm) of tibial spurs: PI, 45; PII, 25; PIII, 50; pseudospurs absent. Only few sensilla chaetica present apically on tibia of PI-PIII, are abundant on tarsomeres ta_1-ta_5. Length (in µm) and proportions of leg segments as in Table 2.

Abdomen. Hypopygium in dorsal view as in Fig. 4A; apodemes as in Fig. 4B. Tergite IX (Figs 4A) broadly rectangular, lateral margins nearly vertical, posterior margin straight with rounded angles; dorsal side with a weak median hump as shown in lateral view (Fig. 3K). Anal point (Fig. 4A, dorsal; Fig. 3K, lateral) 30 µm long, 75 µm maximum width at base, semicircular, with 16-18 setae. Laterosternite IX with 12-14 setae (6-7 on each side). Sternapodeme and phallapodeme as in Fig. 4B; transverse sternapodeme atypically straight, lateral expansion well-developed (horn-like); phallapodeme with a large aedeagal lobe, projecting medially. Virga (Figs 4A, C) 15-20 µm long, composed of 1 cylindrical to an acute triangular stout spine. Gonocoxite (Figs 4A-D) 145 µm long, distal half with sclerotization and 3 stout inwardly projecting setae; apical part broadly rounded. Superior volsella weakly-developed. Inferior volsella (Figs 4A, D) about 25-30 µm long, 15 µm maximum width, consists of a digitiform lobe; proximal margin with a prominent small expansion bearing a curved characteristic seta. Gonostylus (Figs 4E-F, 2 aspects) 80 µm long, 5-7 µm maximum width, slightly narrowing distally, posterior part projecting with horn-like backward process clearly visible on Fig. 4F (obtuse angle); anterior and posterior margins sinuous, medially swollen; anterior area densely covered with short curved setae; crista dorsalis absent; megaseta robust and well-developed, occasionally bifid apically. HV = 4.44; HR = 1.81.

Pupa

(n = 1, pharate male; Figs 4G-M)

Total length 3.55 mm. General colouration of exuviae as in other Limnophyes, almost entirely transparent, wing sheath and cephalothorax with slight yellowish to pale tinge, apophyses and genital sac

Table 2. Lengths (in µm) and proportions of leg segments of *Limnophyes sartorii* sp. n., adult male (n = 1)

	fe	ti	ta_1	ta_2	ta_3	ta_4	ta_5	LR	BV	SV	BR
PI	535	605	310	195	125	75	85	0.51	3.02	3.68	2.20
PII	520	535	250	140	105	75	80	0.47	3.26	4.22	2.40
PIII	565	620	355	185	160	75	85	0.57	3.05	3.34	2.00

distinctly brownish.

Cephalothorax. Frontal apotome (Fig. 4G) broadly triangular, frontal tubercles domed; frontal setae 35-40 µm long, markedly thin. Antepronotals 3 including 1 median (95 70 µm long) and 2 subequal lateral antepronotals 60-65 µm long. Thoracic horn and precorneals (Fig. 4H), thoracic horn reduced, long lobe-like shaped, precorneals 50, 55 and 85 75 µm long. Dorsocentrals (Fig. 4I) consist of 4 subequal setae 25-30 µm long, inserted in 2 groups (3 proximal, 1 distal); Dc_1, Dc_2 and Dc_3 located close together in one longitudinal row; distance between Dc_1, Dc_2 and Dc_3 about 15 µm, Dc_3 and Dc_4 are 45 µm distant.

Abdomen. Tergite I bare. Tergites II-VIII densely covered with chagrin, spinulae and spinous points becoming progressively more elongated at the posterior margin of each tergite. Posterior transverse rows of small hooklets, restricted to tergites II-VI, consist of 1-7 rows of minute hooks (Fig. 4J) progressively increasing on tergites VI-VIII. About 50 to 75 long spines on posterior part II-VIII are longer medially and decreasing in size laterally; chaetotaxy of segments VI and VIII as in figures 4J and 4L. Paratergites II-VII armed with minute spines, are bare on tergite VIII (Fig. 4L). Sternites I-II/III bare; sternites III/IV-V with scattered anterior patch of spinulae located on median and lateral areas, which become more extensive on sternites VI-VIII. Lateral setae (LS) on segments I-VII: I (2), II-VII (4); segment VIII (Fig. 4L) with 5 LS including 1 long anteriorly and 4 little shorter caudally of 80-85 µm long. Apophyses weakly-developed. Anal segment (Fig. 4L); anal lobe 210 µm long, 200 µm maximum width; much wider in proximal half, narrowing distally; outer caudal margin lobe-like, inner part typically straight. Genital sac (Fig. 4L) about 200 µm long, overreaching tip of anal segment by 25-30 µm; apex ending with a characteristic tubercle. Anal macrosetae (Fig. 4M) 210 µm long, not curved apically.

Female adult and larva: unknown.

Discussion

Systematics

A large material of Orthocladiinae, recently collected between 1996 and 2018 in Switzerland (mostly in the Swiss Alps (Lods-Crozet 2012, Lods-Crozet et al. 2001; Knispel and Castella 2003, Robinson et al. 2016) has been identified by the first author. This enabled us to generate a list of 15 species belonging to the genus Limnophyes Eaton, 1875 namely: *Limnophyes angelicae* Sæther, 1990; *L. asquamatus* Soegaard Andersen, 1937; *L. bidumus* Sæther, 1990; *L. brachytomus* (Kieffer, 1922); *L. difficilis* Brundin, 1947; *L. edwardsi* Sæther, 1990;

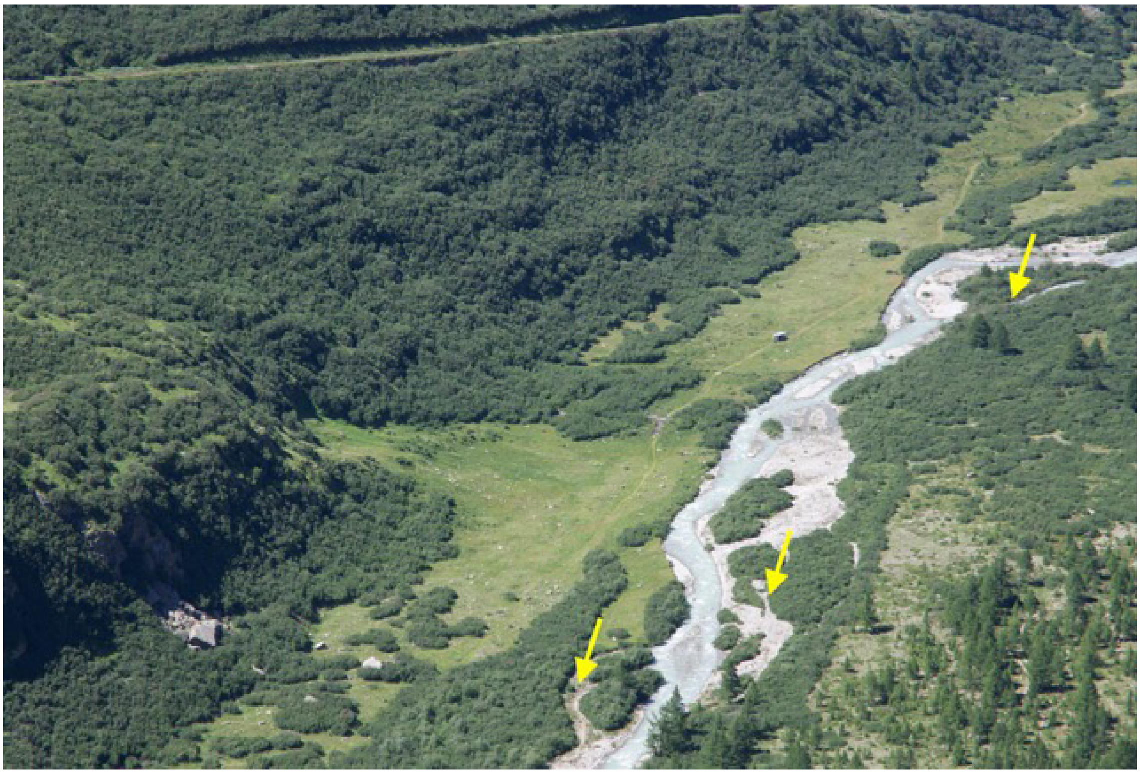

Figure 5. Type-locality of *Limnophyes knispelae* sp. n. at the upper basin of the Rhône river (Gletschboden, Central Swiss Alps, alt. 1800 m; photo J.L. Lods).

L. gelasinus Sæther, 1990; *L. gurgicola* (Edwards, 1929); *L. habilis* (Walker, 1856); *L. minimus* (Meigen, 1818); *L. natalensis* (Kieffer, 1914); *L. ninae* Sæther, 1975; *L. pentaplastus* (Kieffer, 1921); *L. pumilio* (Holmgren, 1869) and *L. punctipennis* (Goetghebuer, 1919). Consequently, the description of the two new described species increases the total number in the genus of known *Limnophyes* species from Switzerland from 15 to 17. A combination of some common morphological characters found in the male adult (clypeus shield-like shaped; humeral pit typically circular with 3-4 or 9 lanceolate setae; virga strong spine-like; preepisternals vary from 4-6 to 4-8) indicate that these two new species are related and separate from other *Limnophyes*. Although phylogenetic analyses should be conducted to confirm if species groups in *Limnophyes* are evolutionary coherent entities, we hypothesize that they form a group defined by a set of morphological characters.

Morphological differences between the male adult of the two new species and that of morphologically similar congeners (namely: *L. asquamatus*, *L. bidumus*, *L. cranstoni* and *L. difficilis*) are: An unusual shape of the humeral pit, which is atypically circular; atypical distribution pattern of dorsocentrals and humerals; the preepisternals on anterior side of preepisternum vary from 4 to 7.

The pupa of *L. sartorii* sp. n. can be separated from those of other members of the genus by having a reduced thoracic horn, which is markedly long lobe-like shaped; the morphological details of armament on tergites; and the characteristic shape of anal lobe and genital sac. However, since the pupa of *L. knispelae* is unknown it is at present not possible to determine if these are characters are shared between the two species.

Ecology and geographical distribution

Several male adults of the two new described species were collected in riparian habitats bordering the upper and down basins of glacial springs and cold streams over the Swiss Alps. They belong to the crenophilous community of species as documented by Lindegaard (1995) and Lencioni et al. (2000). Enriched soil in humus, deciduous woods and bark trees on the margins of rivulets and streams, represent the most favourable microhabitats for larval populations. Emergence of adults is observed between July and October.

Figure 6. Type-locality of *Limnophyes sartorii* sp. n. at the inlet of the Immez Lake (Macun cirque, Eastern Alps, Swiss National Park, alt. 2616 m; photo J.L. Lods).

L. knispelae sp. n. and *L. sartorii* sp. n. belong to the Helvetico-Alpine elements, which are considered as local biogeographical representatives of the Swiss Alps. Their geographical distribution is currently restricted to their type-locality, which is delimited by: the Alpine streams in the high valley of the Rhône river (central Swiss Alps, alt. 1800 m, Fig. 5) for *L. knispelae* sp. n.; the Macun cirque (Swiss National Park, eastern Swiss Alps, alt. 2616 m, Fig. 6) for *L. sartorii* sp. n. Moreover, these two new species can be expected to occur in other similar mountainous areas situated in Switzerland and neighbouring countries (Italy, France, Germany, Austria, Poland).

Associated semiterrestrial species encountered with *L. knispelae* sp. n. and *L. sartorii* sp. n. include: *Hydrosmittia brevicornis* (Strenzke, 1950); *H. oxoniana* (Edwards, 1929); *Limnophyes bidumus* Sæther, 1990; *L. difficilis* Brundin, 1947; *L. gelasinus* Sæther, 1990; *L. habilis* Walker, 1856; *L. pumilio* (Holmgren, 1869); *Pseudosmittia albipennis* (Goetghebuer, 1921); *P. angusta* (Edwards, 1929); *Smittia alpicola* Rossaro & Lencioni, 2000; *S. aterrima* (Meigen, 1818); *S. contingens* Walker, 1856; *S. edwardsi* Goetghebuer, 1932 ; *S. foliosa* (Kieffer, 1921); *S. leucopogon* (Meigen, 1804); *S. nudipennis* (Goetghebuer, 1913); *S. paranudipennis* Brundin, 1947; *S. pratorum* (Goetghebuer, 1927); *S. reissi* Rossaro & Orendt, 2001.

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A NEW ASSOCIATION BETWEEN HARPELLALES, INSECT-GUT INHABITING FUNGI, AND CHIRONOMIDAE IN JAPAN WITH AN UPDATED LIST OF HARPELLALES DOCUMENTED FROM CHIRONOMIDAE

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Abstract

Harpellales (Zoopagomycotina) is a fungal order of which species inhabit the intestine of aquatic arthropods by attaching their thalli to the host's gut lining. Harpellales consists of 270 species with over half found in Chironomidae larvae. The aim of this research is to document a species, *S. pedifer*, new to Japan with a chironomid host association and to list all Chironomidae-commensal species of Harpellales with host and country information.

Introduction

Harpellales is an order of Zoopagomycotina. In the old taxonomy, it belonged to Trichomycetes in Zygomycota. Species in Harpellales inhabit the digestive tracts of aquatic arthropods: mainly juveniles of Ephemeroptera, Plecoptera and Diptera, a few species in larvae of Trichoptera, and one species from both Coleoptera and Isopoda (Lichtwardt et al. 2001).

Infection begins with the ingestion of spores. After the germination in the gut, young thalli attach to the gut lumen with holdfasts. Thalli develop and produce spores in the intestine. Spores released from the anus to the environment will be the inocula for the new infection. Each genus of Harpellales has site specificity to the midgut (peritrophic membrane) or hindgut cuticle and young thalli attach to the defined site on the lumen. Species in this group live as commensals in the host gut and show no pathogenicity to the host insects. Only one exception, *Smittium morbosum*, has been reported to have a pathogenicity to kill the host mosquito larvae (Sweeney 1981).

Many species of Harpellales have been described from Diptera larvae. Chironomidae is an important group among host insects, hosting many species of Harpellales. Most studies of Harpellales focus on taxonomy and phylogeny, while research on ecological aspects is limited and mainly studied in species associated with Simuliidae (Taylor et al. 1996, Beard and Adler 2002, Nelder et al. 2010, Suyama et al. 2018), and in infection experiments in Culicidae (Williams and Lichtwardt 1972, Sato and Aoki 1989, Vojvodic and McCreadie 2007). Though Harpellales is an organism associated with host insects, many species have been described without exact host identification. The aim of this paper is to document a new association between Harpellales and Chironomidae in Japan and to provide an updated global list of Chironomidae-associated species of Harpellales and their chironomid hosts.

Materials and Methods

Chironomidae larvae (Orthocladinae sp.) were collected in a thin film of water flow beside Yoro River, close to Awamata Fall (Yoro Fall), Chiba prefecture, at 150m above sea level, in Feb. 2014 and Mar. 2015. Host insects were dissected to observe Harpellales. The larvae were individually placed on glass slides and the head and end of their abdomens were removed with a razor blade. The digestive tracts were pinched and removed from the abdomen with sharp forceps. Gut content was rinsed in a drop of water on the glass slide. The cleaned gut tube was then observed in water mounted on a new glass slide. Specimens were examined with a Nikon Optiphoto II microscope with a differential interference contrast apparatus or a Nikon Eclipse 2 microscope with a phase contrast apparatus (Nikon, Tokyo, Japan). Observations were conducted with ×10, ×20, ×40, and ×100 oil objective lenses in both microscopes. Photo images were taken with the Nikon DS-Fi 3 digital photo system (Nikon). After the observation, the mounting water was replaced by lactophenol, and the coverslips were sealed with nail polish. Vouchers are kept in the collection of the insect management laboratory, Department of forest entomology, Forestry and Forest Products Research Institute. Specimen ID: 140224-1. 140225-1 and 4, 150211-1, and 3.

Research achievements accumulated to date on Harpellales are presented in a website (Lichtwardt et al. 2001, revised in 2019). In this article, the information on this website is combined with the findings by Kim (2019), Sato (2020), and Sato (2022), and the fungal species recorded from Chironomidae are presented.

Results

New association between Harpellales and Chironomidae in Japan

A *Stachylina* sp. was recognized as new to Japan. The genus *Stachylina* attaches to the peritrophic membrane of host insects (midgut-specific genus), having a single simple (non-branched) thallus with two to dozens of spores. The morphological characters of the specimens fit the diagnosis of genus *Stachylina*. The *Stachylina* species has a thallus 56–107.5 µm in length and 6.3–9.3 µm in width, and produces two to eight long ellipsoid spores, 19.9–30.0 × 6.5–11.2 µm (25.5 ± 2.7 × 8.4 ± 1.3, average ± SD, N=45), without a collar and with an appendage. The basal part of the thallus penetrates through the peritrophic membrane by footlike cell apex (Fig. 3).

Species number detected from Diptera among Harpellales

Harpellales hold 270 species. Diptera hosts the highest number of Harpellales species of all insect hosts (188 Harpellales species in total). Chironomidae larvae are hosts to just over half of the known species of Harpellales (139 species from 10 genera, Figs 1, 2). The two largest genera, *Smittium* and *Stachylina*, each have 85 and 39 species respectively documented from Chironomidae larvae (Table 1, 2). Other minor genera with chironomid hosts constitute the remaining 15 species in 8 genera (Table 3).

Smittium aggregatum (Fig. 4) is shown, recently reported in Japan by Sato (2022), as an example of the genus *Smittium* which is the most common genus in association with Chironomidae. Species in *Smittium* attach to the hindgut cuticle specifically, having branched hyphae producing several spores at the distal end of the hyphae.

Distribution

The distribution of Chironomidae-associated Harpellales species is included in Tables 1-3. There are 28 countries with records, the most species are recorded from the United States and Canada, followed by France and Spain. In Japan, 10 species in three genera have been reported as associated with Chironomidae (*Stachylina grandispora*, *St. longa*, *St. magna*, *St. nana*, *St. penetralis*, *St. prolifica*, *St. subgrandis*, *Smittium aggregatum*, *Sm. elongatum*, *Trichozygospora chironomidarum*) (Lichtwardt et al. 1987, Sato 2020, 2022). *Smittium simulii* has been recorded form both Simuliidae and Chironomidae in Japan (Lichtwardt et al. 1987).

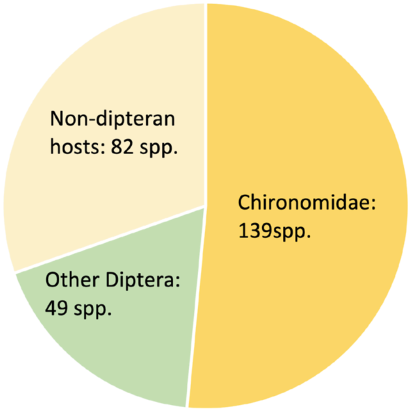

Figure 1. Total number of species in Harpellales described from Chironomidae and other hosts (N=270 species.)

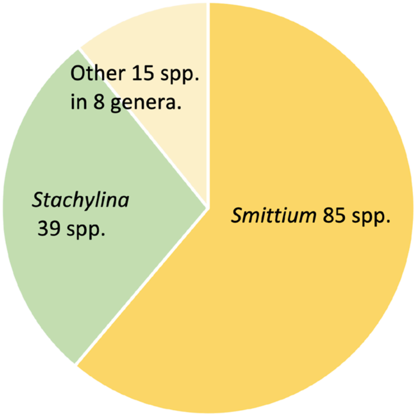

Figure 2. The species number in the two major genera of Harpellales described from Chironomidae (N=139 species)

Host identification and correspondence between fungi and hosts

At present, 23 species and 38 genera of Chironomidae have been identified as hosts of Harpellales. The most frequent host genera are *Cricotopus* (17 species in 4 genera of Harpellales), *Chironomus* (14 spp. in 3 genera), *Diamesa* (9 spp. in 3 genera), *Orthocladius* (8 spp. in 2 genera), *Polypedilum* (7 spp. in 2 genera), and *Tanytarsus* (6 spp. in 3 genera).

Of the 139 Chironomidae-commensal species, 69 species (about half) have hosts identified to genus or species, and 57 species (41 %) to family. *Smittium* has seven hosts identified to species and 35 to genus. *Stachylina* has seven hosts identified to species and 10 to genus (Table 4).

In the genus *Smittium*, 23 species are known from

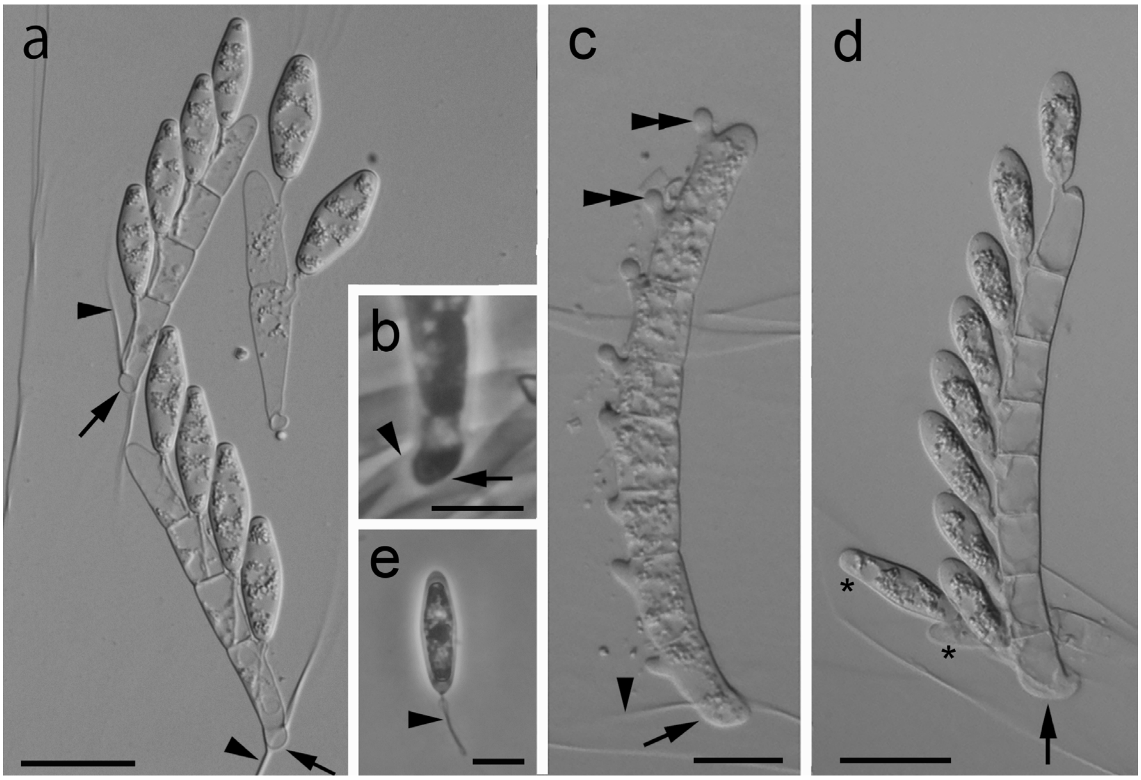

Figure 3. *Stachylina pedifer*. a. Mature thalli in the peritrophic membrane. Arrows: foot-like shaped basal part penetrating the peritrophic membrane. Arrowheads: folding peritrophic membrane. Also, the same symbols mean the same meaning in figs b, c, and d. b. Higher magnification of the basal part of a thallus. c. Young thallus producing spores. Double arrowheads: spore initial. d. Almost mature thallus with eight spores. *: Spores of adjacent thallus. e. Detached spore. Arrowhead: appendage. Scales. a: 20 µm. b–e: 10µm. Specimen ID. a and b: 140224-1. e: 140225-4. Photos c and d are taken from temporary slides. a, c and d: water-mounted. b and e: Lactophenol-mounted.

multiple chironomid taxa. In particular, *Smittium phytotelmatum* has been recorded from more than 5 taxa (Table 1). In the genus *Stachylina*, six species have been recorded from multiple host species. For example, *St. grandispora* is known from seven host taxa (Table 2). *Furculomyces boomerangus* has been recorded from four host taxa (Table 3).

There have been several records of Chironomidae species as a host of multiple species of Harpellales. The first case is the simultaneous infection of two Harpellales species to one individual larva of Chironomidae: *Boreoheptagyia lurida* was infected with *Stachylina pedifer*, and *Smittium dimorphum* (Lichtwardt and Williams, 1983), *Chironomus oppositus* group, and *Tanytarsus* near *inextentus*, both host species also with *Stachylina grandispora*, and *Furculomyces boomerangus* (Lichtwardt and Williams, 1990), and Tanitarsini sp. infected with *St. subgrandis*, and *Sm. aggregatum* (Sato 2022). The second case is without the description of simultaneous infection from the same collection. *Chironomus plumosus* complex was recorded as host of *St. euthena* and *Sm. typhellum* (Manier and Coste 1971), and *Tanytarsus* near *inextentus* was recorded as a host of *Sm. paludis*, and *F. boomerangus* (Lichtwardt & Williams 1990). The reported fungal species were derived from the same collection in the above two hosts' cases. However, the papers do not reveal if there were simultaneous infections or not.

The third case is from independent collections: *Brillia* cf. *longifurca*, host for *St. lentica* and *St. acutibasilaris* (White and Lichtwardt 2004); *Chironomus oppositus*, host for *F. boomerangus* and *F. westraliensis* (Lichtwardt and Williams 1990, 1992); and *Psectrotanypus varius*, host for *St. euthena* and *Sm. bisporum* (Manier and Coste 1971).

Discussion

The *Stachylina* species, derived from Orthocladinae sp., has a foot-like basal part that penetrates the peritrophic membrane. This morphology is the most prominent characteristic of *Stachylina pedifer*. Judging other morphological characteristics (thallus length, spore sizes, with an appendage, and without a collar) showed no remarkable differences from the original description of *St. pedifer* by Lichtwardt and Williams (1983). This species is therefore identified as *Stachylina pedifer* and is

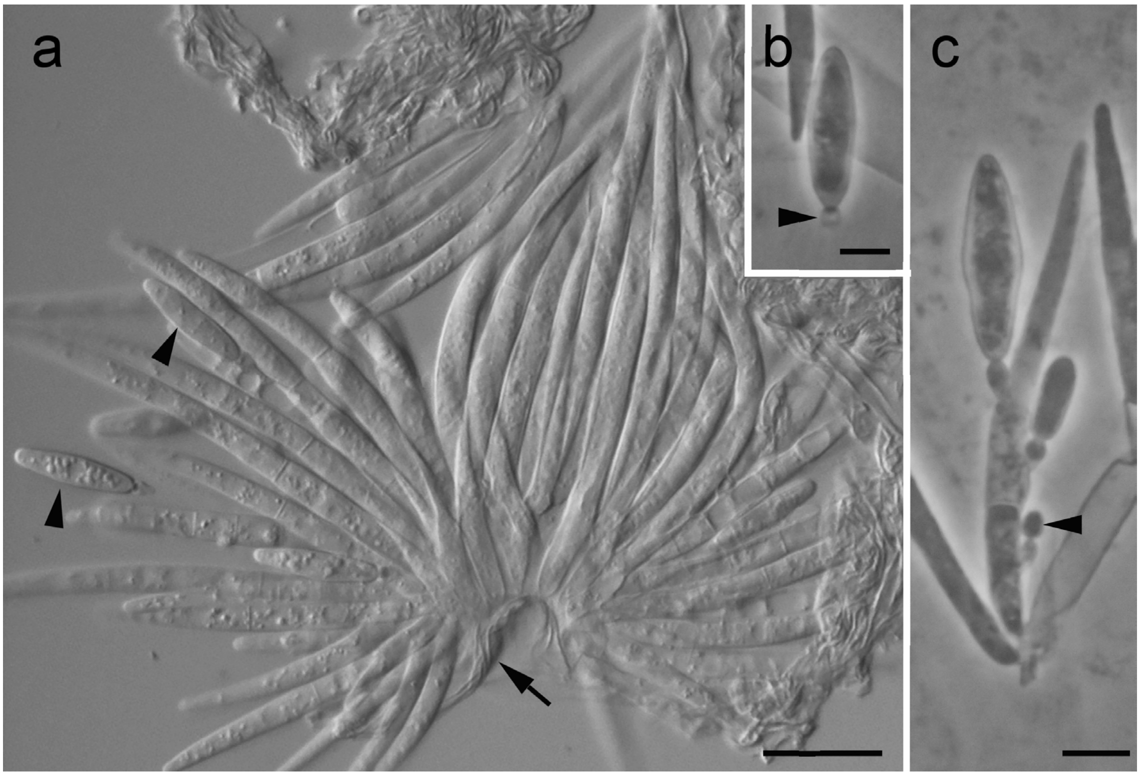

Figure 4. *Smittium aggregatum*. a. Thallus in the hindgut. Arrow: Hindgut cuticle (folded when dissected). Arrowheads: spores. Hyphal aggregation at the basal area of the thallus is the feature of this species. b. Detached spore. Arrowhead: Collar. Appendage is not reported in this species. c. Spore production. Arrowhead: The youngest spore. Scales. a: 20 µm. b–c: 5µm. Specimen ID. a: TNS-F-89237. b and c: TNS-F-89234. National Museum of Nature and Science, Tokyo. Host: larvae of Tanytarsini collected at Enzan Takahashi, Yamanashi, 1300m above sea level. a: water-mounted. b and c: Lactophenol-mounted.

the first record of this species in Japan. The known host insects of this fungus are *Boreoheptagyia lurida* (Diamesinae) (Lichtwardt and Williams 1983, White and Lichtwardt 2004) and *Eukiefferiella* sp. (Orthocladiinae) (Valle 2007).

Stachylina grandispora, *Smittium culicis* and *Sm. simulii* have been recorded from more than 10 countries (Tables 1 and 2). This distribution is of biogeographical interest. Furthermore, since some species of chironomids are found in severe environments, it is of interest to know whether Harpellales can adapt to such extreme conditions. For example, *Smittium incrassatum* has been collected in Greenland (Kobayasi et al. 1971).

The same fungal species would be detected from different host species in different countries. It is important to consider the environmental commonalities between the collection sites. *Trichozygospora chironomidarum* was reported in Japan from larvae of *Orthocladius* sp. (Sato 2020). The larvae were collected from the algal mass attached to the surface of rocks under fast-running water. The habitat was similar to the place of the original description (Lichtwardt 1972). The fungus was originally described from the United States infecting *Orthocladius* spp. and *Cricotopus* sp. larvae (Lichtwardt 1972). Subsequently, the species was recorded in Sweden from *Orthocladius* (*Euorthocladius*) and *Diamesa valkanovi* (Moss & Lichtwardt 1977, Lichtwardt et al. 2001), Switzerland, and England (no host information in these two countries) (Lichtwardt et al. 2001). The British record, however, needs to be re-examined (shortage of specimens). In the United States, this fungus was collected in the summer at about 2000 m above sea level. The Swiss site is also at about 2000 m above sea level, and the Swedish site is within the Arctic Circle. The Japanese collection site is the southernmost and is at 190 m above sea level. The specimens were collected in winter, however, and the habitat conditions in which this species is recorded may indicate that it is cold adapted.

The simultaneous association of multiple Harpellales species is also known in blackflies (Simuliidae). Especially, multiple fungal cohabitations have been known in the hindgut. However, cohabitation in the hindgut has not been reported in

Table 1. Host insects of *Smittium* based on Lichtwardt et al. (2001), Strongman and Xu (2006), Strongman et al. (2010), Wang et al. (2010), Strongman and Wang (2015), Kim (2019), Strongman and White (2019), and Sato (2022). All members except one of *Smittium* species are recorded from dipteran larvae (mainly Chironomidae). The exception: *S. foliocistum* from Trichoptera. *Hosts other than Chironomidae. # Possible distribution (Lichtwardt et al. 2001). AM: Armenia. AR: Argentina. AU: Australia. BR: Brazil. CA: Canada. CH: Switzerland. CL: Chile. CN: China. CR: Costa Rica. DK: Denmark (Greenland). DO: Dominican Republic. EN: England. ES: Spain. FR: France (including Crozet isl.) . JP: Japan. KR: Korea. MX: Mexico. NO: Norway. NZ: New Zealand. RU: Russia. SE: Sweden. TN: Tunisia. US: United States.

	Species	Host	Distribution
1	*Smittium aciculare*	Chironomidae, Simuliidae*	AU, MX
2	*Smittium acutum*	Chironomidae	US
3	*Smittium adaiosporum*	Chironomidae	CA
4	*Smittium aggregatum*	Tanitarsini	CA, JP
5	*Smittium alpinum*	*Diamesa* spp., other Chironomidae	CA, CH, FR, SE, US
6	*Smittium ampliboja*	Chironomidae, Simuliidae*	CA
7	*Smittium angustum*	*Cricotopus* sp.	AU
8	*Smittium annulatum*	Simuliidae*	CR
9	*Smittium arcticum*	Chironomidae	US
10	*Smittium arvernense*	*Smittia* sp.	FR
11	*Smittium basiramosum*	*Polypedilum* sp.	AR
12	*Smittium biforme*	*Diamesa aberrata*, possibly *D. bertrami*	NO
13	*Smittium bisporum*	*Psectrotanypus varius*	FR, MX#
14	*Smittium brasiliense*	*Paratendipes* sp., *Polypedilum* sp., Simuliidae*	BR, MX
15	*Smittium brevisporum*	*Cricotopus* spp. and other Chironomidae	CA, ES
16	*Smittium bulbosporophorum*	Diamesinae, Orthocladiinae	ES
17	*Smittium bullatum*	*Nanocladius forsythi*	NZ
18	*Smittium caribense*	Chironomidae	DO
19	*Smittium caudatum*	*Orthocladius* (*Orthocladius*) sp., *Cricotopus* sp., *Hydrobaenus* sp.	US
20	*Smittium cellaspora*	*Sympotthastia* sp. and possibly other Chironomidae	AR, US
21	*Smittium chinliense*	Tipulidae*	CN
22	*Smittium chironomi*	Orthocladiinae	EN#, FR
23	*Smittium colboi*	Chironomidae	CA
24	*Smittium coloradense*	*Orthocladius* (*Orthocladius*) sp., *Eukiefferiella* sp., *Cricotopus* sp., Simuliidae[3]	NO, US
25	*Smittium commune*	*Microtendipes* sp., *Cricotopus* (*Cricotopus*) sp., *Orthocladius* (*Orthocladius*) sp., *Phaenopsectra* sp.	US
26	*Smittium compactum*	Chironomidae	AU
27	*Smittium cryptancora*	Chironomidae	CA
28	*Smittium culicis*	Chironomidae, Culicidae[3], Simuliidae[3], Psychodidae[3],Stratiomyiidae[3], Thaumaleidae[3]	AR, AU, CA, CN, ES, FR, NO, NZ, TN, US
29	*Smittium culicisoides*	Chironomidae, Simuliidae[3]	AU#,CR, FR,
30	*Smittium cylindrosporum*	*Paraheptagyia* sp., *Paraheptagyia cinerascens*, *Cricotopus* sp.	AR, CL
31	*Smittium delicatum*	*Chironomus alternans*, *Cladopelma* sp., *Phaenopsectra* sp.	AU, US
32	*Smittium dimorphum*	*Boreoheptagyia lurida*	US
33	*Smittium dipterorum*	Chironomidae, Simuliidae*	CR, DO, MX
34	*Smittium ditrichosporum*	Chironomidae	CA
35	*Smittium elongatum*	*Diamesa* near *nivoriunda*, possibly *Cricotopus* sp.	AU, JP, US

36	*Smittium esteparum*	*Cricotopus* sp., *Eukiefferiella* sp.	AR
37	*Smittium fasciculatum*	Chironomus sp.	CR
38	*Smittium fastigatum*	Chironomidae	AU
39	*Smittium fecundum*	*Psectrocladius* sp.	ES, US
40	*Smittium foliocistum*	Trichoptera*	CA
41	*Smittium fruticosum*	*Cricotopus* sp.	AU
42	*Smittium georgense*	Chironomidae	CA
43	*Smittium gigasporus*	*Pagastia* sp.	US
44	*Smittium gracillis*	*Chironomus* sp. Diamesinae	CA, ES
45	*Smittium gravimetallum*	*Dicrotendipes fumidus*	US
46	*Smittium gronthidium*	Chironomidae, Simuliidae*	CA
47	*Smittium guttisporum*	Chironomidae	CA
48	*Smittium hecatei*	*Cricotopus* spp. and Diamesini	CN, ES
49	*Smittium heterosporum*	*Sympotthastia* spp., *Potthastia* spp. (Diamesinae), *Cricotopus bicintus* (Orthocladiinae)	ES
50	*Smittium imitatum*	Simuliidae*	AR, CL
51	*Smittium incrassatum*	Chironomidae	DK
52	*Smittium inexpectans*	Orthocladiinae	ES
53	*Smittium insolitum*	Chironomidae	CA
54	*Smittium insulare*	Chironomidae, Culicidae*	CA
55	*Smittium kansense*	*Hydrobaenus* sp., *Orthocladius* (*Orthocladius*) sp.	US
56	*Smittium lentaquaticum*	*Chironomus* sp.	US
57	*Smittium longisporum*	*Cricotopus* sp. and other Chironomidae	SE, US
58	*Smittium macrosporum*	Chironomidae	US
59	*Smittium magnosporum*	Thaumaleidae*	AU
60	*Smittium manierei*	Chironomidae	FR
61	*Smittium megazygosporum*	*Syncricotopus rufiventris*	FR, KR
62	*Smittium microsporum*	*Tanytarsus* sp., possibly *Cricotopus* sp.	AU, MX
63	*Smittium minutisporum*	Culicidae*	CN, US
64	*Smittium morbosum*	Culicidae*	AM, AR, AU, IT, JP, RU
65	*Smittium mucronatum*	*Psectrocladius sordidellus*, *Psectrocladius* sp. *Psectrocladius limbellatus*	CA, FR, NO, US
66	*Smittium naiadis*	Chironomidae	CN
67	*Smittium nodifixum*	Chironomidae	CN
68	*Smittium orthocladii*	*Orthocladius* spp., *Diamesa* sp., and other lotic Chironomidae	FR, US
69	*Smittium ouselii*	*Eukiefferiella* sp.	US
70	*Smittium paludis*	*Tanytarsus* near *inextentus*	AU
71	*Smittium papillum*	Chironomidae	CA
72	*Smittium parvum*	*Cricotopus* sp.	CR
73	*Smittium pavocaudatum*	Chironomidae	CA
74	*Smittium peculiare*	Chironomidae	CA
75	*Smittium pennelli*	Simuliidae*	US
76	*Smittium perforatum*	*Orthocladius* (*Euorthocladius*) sp. *Diamesa* sp.	US
77	*Smittium petilum*	Chironomidae	CA
78	*Smittium phytotelmatum*	*Chironomus* sp., *Polypedilum* spp., *Tanytarsus* sp., and other Chironomini and Tanitarsini	AR, CR

79	*Smittium precipitiorum*	*Orthocladius fuscimanus*, *Diamesa* sp.	NO
80	*Smittium prostratum*	Orthocladiinae	ES
81	*Smittium pseudodimorphum*	Diamesinae	ES
82	*Smittium pusillum*	*Procladius* sp.	FR
83	*Smittium radiculans*	Simuliidae*	CA
84	*Smittium rarum*	Chironomidae	NZ
85	*Smittium rupestre*	Orthocladiinae	AU, CN
86	*Smittium scrobense*	Chironomidae	CA
87	*Smittium shaanxiense*	Chironomidae	CN
88	*Smittium simulatum*	Tipulidae*	CL
89	*Smittium simulii*	Chironomidae, Simuliidae*, Culicidae*, Tipulidae*	AU, CA, CN, EN, ES, FR, JP, KR, MX, NO, NZ, SE, US
90	*Smittium sparsum*	Chironomidae	CA
91	*Smittium tipulidarum*	Tipulidae*	US
92	*Smittium tronadorium*	*Paraheptagyia* sp., *Cricotopus* sp.	AM#, AR, MX
93	*Smittium tynense*	Chironomidae	CA
94	*Smittium typhellum*	*Chironomus* sp. (*plumosus* complex)	FR
95	*Smittium urbanum*	Chironomidae	AR
96	*Smittium verticillatum*	Chironomidae	CA

Table 2. World records of *Stachylina* spp. based on Lichtwardt et al. (1987), Lichtwaradt et al. (2001), Misra and Tiwari (2002), Hapsari et al. (2009), Strongman et al. (2010), Wang et al. (2010), Strongman and Wang (2015), Sato (2020). All *Stachylina* species have been recorded from larvae of Diptera (mainly Chironomidae). *Hosts other than Chironomidae. (+) New record from Japan in this study. # Possible misidentification. AR: Argentina. AU: Australia. BR: Brazil. CA: Canada. CH: Switzerland. CL: Chile. CN: China. CR: Costa Rica. DO: Dominican Republic. EN: England. ES: Spain. FR: France (including Crozet Isl.). IN: India. JP: Japan. MX: Mexico. NO: Norway. NZ: New Zealand. PT: Portugal. SE: Sweden. TH: Thailand. US: United States

	Species	**Host identification**	**Records**
1	*Stachylina abundans*	Chironomidae	CA
2	*Stachylina acutibasilaris*	*Brillia* cf. *longifurca*	NO
3	*Stachylina brevicellaris*	Chironomidae	CA
4	*Stachylina ceratopogonidarum*	Ceratopogonidae*	CL
5	*Stachylina chironomidarum*	Chironomidae	IN, US
6	*Stachylina dolichospora*	*Riethia* sp.	AU
7	*Stachylina euthena*	*Chironomus plumosus* complex, *Psectrotanypus varius*	ES, FR
8	*Stachylina extensiva*	Chironomidae	CA
9	*Stachylina forantipes*	Chironomidae	CA
10	*Stachylina grandispora*	*Chironomus hawaiensis*, *C. zealandicus*, *Chironomus* spp., *Cricotopus*, *Polypedilum*, *Paratendipes*, *Tanytarsus*	AR, CA, DO, EN, ES, IN, JP, MX, AN, SE, US
11	*Stachylina gravicaudata*	*Paralauterborniella* sp.	CN, US
12	*Stachylina infrequens*	Chironomidae	AU
13	*Stachylina jujuyensis*	Chironomidae	AR
14	*Stachylina lentica*	*Chironomus* sp., *Brillia* cf. *longifurca*, *Polypedilum* sp. *Metriocnemus eryngiotelmatus*	AR, NO
15	*Stachylina litoralis*	*Telmatogeton japonicus*, Simuliidae*	CA, FR
16	*Stachylina longa*	*Tanytarsus* sp.	FR, JP

17	*Stachylina lotica*	Psychodidae*	AR, US
18	*Stachylina macrospora*	*Diamesa* sp., *Syndiamesa macronyx*	FR, IN, US#
19	*Stachylina magna*	Chironomidae	JP, US
20	*Stachylina manicata*	*Polypedilum* sp. and/or *Pseudochironomus* sp.	US
21	*Stachylina minima*	Orthocladiinae	AR, NZ
22	*Stachylina minuta*	Chironomidae	FR
23	*Stachylina nana*	Chironomidae	CA, CN, R, JP, TH, US
24	*Stachylina paludosa*	*Chironomus* sp., *Polypedilum* sp.	CR
25	*Stachylina paucispora*	*Cricotopus* sp.	BR, MX
26	*Stachylina pedifer* (+)	*Boreoheptagyia lurida*, *Eukiefferiella* sp.	CA, CN, ES, JP, NO, US
27	*Stachylina penetralis*	*Diamesa* and other Chironomidae	AR, CA, CH, CN, ES, FR, JP
28	*Stachylina philoricola*	Blephariceridae*	JP
29	*Stachylina platensis*	Chironomidae	AR
30	*Stachylina pleurospora*	Chironomidae	ES, PT
31	*Stachylina prolifica*	*Chironomus* sp.	CN, ES, JP
32	*Stachylina queenslandiae*	Chironomidae	AU
33	*Stachylina robusta*	*Psectrocladius* sp.	ES, US
34	*Stachylina serpula*	Chironomidae	CA
35	*Stachylina somnisimilis*	Chironomidae	CA
36	*Stachylina stagnicola*	Chironomidae	CA
37	*Stachylina stenospora*	*Phaenopsectra* sp.	US
38	*Stachylina subgrandis*	Chironomidae, Tanitarsini sp.	CA, CN, JP
39	*Stachylina tanysoma*	Chironomidae	CA
40	*Stachylina thaumaleidarum*	Thaumaleidae*	AU
41	*Stachylina tianensis*	Chironomidae	CN
42	*Stachylina uranus*	Chironomidae	CA
43	*Stachylina zeppelin*	Chironomidae	CA

Table 3. Host insect of Harpellales associated with Chironomidae except the genera *Smittium* and *Stachylina* based on Lichtwardt et al. (2001), Strongman and Xu (2006), Strongman et al. (2010), Wang et al. (2010), Strongman and Wang (2015), Kim (2019), Strongman and White (2019) and Sato (2020). *Zancudomyces culisetae* has wide host range of insect groups other than Chironomidae. # Possible distribution because of specimen shortage (Lichtwardt et al. 2001). AR: Argentina. AU: Australia. BR: Brazil. CH: Switzerland. CN: China. DO: Dominican Republic. EN: England. FR: France (including Crozet isl.). JP: Japan. MX: Mexico. NZ: New Zealand. SE: Sweden. US: United States.

	Species	Host identification	Records
1	*Austrosmittium aussiorum*	near *Kiefferophyes*, *Paratrichocladius pluriserialis*	AU
2	*Austrosmittium biforme*	Orthocladiinae	AU
3	*Austrosmittium kiwiorum*	Orthocladiinae	NZ
4	*Austrosmittium lenticum*	*Corynoneura* sp.	AR
5	*Austrosmittium norinsulare*	Orthocladiinae	NZ
6	*Austrosmittium patagonicum*	*Parochlus* sp.	AR
7	*Dacryodiomyces oklahomensis*	*Cricotopus* sp.	US
8	*Furculomyces boomerangus*	*Aspectrotanypus maculosa*, *Chironomus oppositus*, *Procladius paludicola*, *Tanytarsus* near. *inextentus*	AU

9	*Furculomyces septentrionalis*	*Chironomus* sp.	US
10	*Furculomyces westraliensis*	*Chironomus oppositus* group	AU
11	*Klastostachys reflexa*	*Cryptochironomus* sp.	US
12	*Sinotrichium chironomidarum*	Chironomidae	CN
13	*Stachylinoides arctata*	*Thienemanniella* sp.	AR
14	*Trichozygospora chironomidarum*	*Cricotopus* sp., *Diamesa valkanovi*, Orthocladius sp.	CH, EN#, JP, SE, US
15	*Zancudomyces culisetae*[2]	Chironomidae, Culicidae, Simuliidae, Psychodidae, Ceratopogonidae, Ephemeroptera	US, JP, AU, NZ, BR, AR, FR, DO, MX

Table 4. Number of species in Harpellales at each identified chironomidae rank.

Taxa	**species**	**genus**	**tribe**	**subfamily**	**family**	**total**
Smittium	7	35	1	6	36	85
Stachylina	7	10	1	2	19	39
other	4	6	0	3	2	15
total	18	51	2	11	57	139

Chironomidae. Lichtwardt (1972) reported two or more fungal species associated with the hindgut of blackfly larvae among a decade of collections in the USA. In Japan, a fungal species in the midgut and two species in the hindgut have been reported (Sato 2013, Suyama et al. 2018).

Fixed point observations are effective to reveal host-associate correspondence. By repeating the collection effort of the same host species at a fixed point from 2003 to 2017, four species associated with *Simulium japonicum* have been recognized in Japan (Sato and Degawa 2003, Sato and Orihara 2013, Suyama et al. 2017, 2018). Moreover, the periodical collection at fixed points has revealed seasonal fluctuation in the prevalence of each associating Harpellales in one host species (Suyama et al. 2018), and also the difference of prevalence between sympatric other host species (Taylor et al. 1996, Beard and Adler 2002, Nelder et al. 2010). Researches using fixed point are expected for Chironomidae-associated Harpellales.

The large number of fungal species recorded from multiple hosts suggests that the host specificity of Harpellales in Chironomidae may not be strict. On the other hand, the host identification rank for 57 Harpellales species is still family level. At the same time, many species of Harpellales have been recorded from only one country where they were described. As new hosts are identified, and also as new ecological information is derived, more detailed discussions of the fungus-host relationship are allowed. The interrelationship between fungi and insects is expected to be elucidated through the accumulation of records from different countries and regions by entomologists and mycologists.

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