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Feeding time. Photo: Michel Noël.

CHIRONOMUS Journal of Chironomidae Research

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

Front page photo: European robin (*Erithacus rubecula*) with a beak-full of chironomids. Photo: Michel Noël.

Editorial

Progress in a time of change

Delta, Omicron, vaccination, variant, booster, super-spreader, mask mandates, asymptomatic, quarantine, shelter-in-place, lockdown, social distance, remote work, hybrid, Zoom. All are “keywords” that, over the past two years (whether we like it or not!), have become part of our everyday vernacular and are recognizable around the world. Change and progress could also be used to define the past two years. When considering change, the pandemic has certainly brought about many disruptions and unwanted changes to our work and home lives. In terms of work, most, if not all, of us have faced some combination of cancelled meetings or conferences, closures to our offices or labs, delayed or reduced funding, and limited opportunity to collaborate in-person with colleagues. These changes have resulted in the need for us to acclimate quickly to new working and living conditions. Like many of you, for many weeks after the onset of the pandemic, I was faced with very minimal access to my lab space – this was problematic as I, along with my students, were several weeks into a study involving live chironomids. Given we were working with living organisms, we were fortunately granted special access, albeit only one of us could be in the lab at any given time, and only for limited duration. I’m sure many of you have similar stories.

Despite the necessary changes, we’ve come a long way, and perhaps it’s worthwhile to reflect on some of the progress associated with the last several months. While in-person gatherings have been limited, many of us have quickly learned to better utilize virtual environments that allow us to stay connected and continue to effectively collaborate with and learn from one another. Even though this virtual world may not be as desirable as face-to-face, many of us have benefitted immensely from virtually “meeting” with or collaborating in real-time on the same digital document with colleagues in distant parts of the world –or, even with those right down the hall! And, even though mask requirements and the progress associated with vaccines has allowed us to loosen up some restrictions, we can continue to use and build off of these virtual work and meeting environments, more effectively utilizing and fostering our networks.

Staying on top of current research publications, such as those included in our journal, has been another great way we have been able to stay connected to one another. We celebrate the close of the year with a compilation of excellent publications which highlight change and progress that has occurred in our chironomid community over the past year. As for change, our community unfortunately lost two long-standing colleagues/friends in 2021. We take time to remember and celebrate the lives of Dr. Len Ferrington Jr. (Bouchard et al. 2021) and Dr. Clive Pinder (Cranston 2021). The passing of these experts certainly leaves holes in our chironomid community, however their legacy will live on; we hope you take a moment to read their tributes and reflect on their many contributions. The research articles included here are a great example of progress in terms of new knowledge shared. Specifically, this volume of *CHIRONOMUS* includes work that describes longevities of Icelandic Chironomidae (Nyquist et al. 2021), descriptions of new species within the genera *Pseudorthocladius* (Lods-Crozet and Moubayed-Breil 2021) and *Dicrotendipes* (Lin and Qui 2021), taxonomic research that stresses the placement of *Propsilocerus* within the Prodiamesinae (Baranov 2021), as well as work that documents chironomids known from Brazil (Sonada and Ambiente 2021).

We hope you enjoy learning from these contributions. Here’s to a healthy year ahead filled with positive change and continued progress!

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LONGEVITIES OF ADULT CHIRONOMIDAE (DIPTERA) FROM TWO STREAMS IN ICELAND

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Abstract

Mean annual air temperatures are predicted to increase by several degrees in the Arctic. This increase in temperature will likely impact organisms adapted to current conditions. Studies of longevities of winter-active Chironomidae from cold, groundwater-fed streams in Minnesota demonstrate that winter-emerging species are long-lived as adults when incubated at cold or sub-freezing air temperatures post emergence. It is unknown if this holds for species emerging in sub-arctic latitudes during warm months, or if warm air temperatures affect the adults of species emerging from cold and geothermally heated streams. The goal of this study was to determine the effects of exposure to air temperatures predicted by climate change models on the longevities of Chironomidae emerging from both cold and geothermally heated larval environments. Chironomidae were trapped at emergence from two groundwater-fed streams with contrasting (warm and cold) thermal regimes at the Hengladalir valleys in the Hengill alpine geothermal area in southwestern Iceland over 4 days in July 2018. Adults (N=102) were randomly divided into two treatments and incubated at either 20°C or 6°C for 28-32 days to determine influence of source stream and incubation temperature on adult longevity. These temperatures were selected to simulate current and predicted water and air temperatures that adult chironomids are likely to be exposed to with climate change. Adults incubated at 6°C survived longer than adults incubated at 20°C irrespective of source stream. All adults incubated at 20°C died within 1-5 days post-emergence. Longevities of taxa incubated at 6°C ranged from 13 to more than 32 days. Species of *Diamesa* exhibited the greatest longevities, with 41.7% surviving more than 28-32 days post-emergence at 6°C. We were unable to determine maximum longevity for *Diamesa* within the 6°C treatment because surviving adults had to be sacrificed at the

termination of the experiment on day 32. However, the minimum longevity we report is similar to longevities determined for other *Diamesa* species and indicates that *Diamesa* species in Iceland are also long-lived under cold air temperatures. We suggest there may be a difference in longevity between sex, with females living longer than males at cold temperatures. By contrast, we found that both sexes of *Diamesa* and all other chironomid taxa that we tested are susceptible to warm air temperatures regardless of stream of origin. Therefore, warming climatic temperatures could potentially lead to shortened adult lifespans for these sub-arctic taxa.

Introduction

Although most species of Chironomidae are known to have a short adult lifespan (Ferrington and Berg 2019), three species of the subfamily Diamesinae, *Diamesa mendotae* Muttkowski, 1915, *Diamesa kohshimai* Sæther et Willassen, 1987 and *Diamesa yalavia* Sæther et Willassen, 1987, have survived for at least 30 days when adults are incubated at cool temperatures (Ferrington et al. 2010, Kohshima 1984). All three long-lived species actively grow and develop as larvae in cold water, then pupate and emerge as adults into climates characterized by air temperatures that often dip below freezing or are at subfreezing temperatures for extended periods (Bouchard and Ferrington 2009, Kohshima 1984). What is not known is whether these three species represent isolated instances of extreme adaptations to cold, or if other cold-adapted species of chironomids that emerge in winter or occur in cold climates have extended longevities as adults. Also unknown is whether extended adult longevity is restricted to the genus *Diamesa*, or if this characteristic is common for Diamesinae or species of other subfamilies that develop in cold water in concert with Diamesinae (Soszyńska-Maj et al. 2015).

A significant number of studies discussing the life history of winter-active chironomids have been conducted in Minnesota. The Driftless region of southeastern Minnesota has a high concentration of groundwater-fed streams where the groundwater approximates mean annual air temperature (8-9.5°C) (Anderson and Ferrington 2013, Bouchard and Ferrington 2009, Krider et al. 2013). Winter-emerging populations of chironomids are commonly found in the unfrozen stream reaches fed by groundwater. Winter emergence in these streams consists mostly of species of Orthocladiinae and Diamesinae (Nyquist et al. 2020), and historical records indicate that *Diamesa* are active on snow in Minnesota (Hansen and Cook 1976). Adults emerge in water temperatures between 0° to 10°C and can be found walking and mating on snow at air temperatures well below 0°C, including days when air temperatures do not exceed -8°C (Ferrington et al. 2010). In laboratory studies, adults of *D. mendotae* live between 10-30 days when held at 6°C after collection from snowbanks near streams (Ferrington et al. 2010). This species also survives over 60 days when incubated at sub-freezing temperatures (Anderson et al. 2013, Mazack et al. 2015). The extended longevity of adult *Diamesa* has been hypothesized as an adaptation to increase the probability of finding a mate and successfully reproducing under the harsh conditions of winter in Minnesota (Ferrington 2019).

The Hengladalir valleys of the Hengill alpine geothermal area in southwestern Iceland has been a focus of ecological research on dynamics of groundwater-fed streams of differing temperatures (Friberg et al. 2009, Gudmundsdottir et al. 2011, Hannesdottir et al. 2013, O’Gorman 2016, Woodward et al. 2010). Numerous springs in this valley have contrasting thermal regimes ranging from 22.0 to 6.6°C during the summer. These contrasting thermal regimes emanate from cold groundwater sources and snowmelt, or groundwater that is warmed by contacting volcanically heated bedrock and soils (Friberg et al. 2009, Hannesdottir et al. 2013). The springs are within 1 meter to several hundred meters of each other (Gudmundsdottir et al. 2011, O’Gorman 2016, Woodward et al. 2010) and have similar riparian vegetation along their spring runs. The chironomid fauna of the streams are well documented and include *Diamesa* and other cold-water genera including *Eukiefferiella* and *Orthocladus* (Hannesdottir et al. 2013). The streams also have populations of more eurythermal species, such as *Micropsectra atrofasciata* (Kieffer, 1911), that are common in the groundwater-fed streams that have geothermally heated

groundwater sources (Hannesdottir et al. 2013). The varying thermal regimes and species compositions provides opportunity to test the longevities of chironomid species among predominant subfamilies whose larvae grow in contrasting thermal regimes but emerge as adults into similar air temperature conditions. This system is of interest in the context of climate change since warming thermal regimes are expected as mean annual air temperatures continue to increase in the arctic (Woodward et al. 2010). Warming thermal regimes of water may affect development times of larvae, and increased air temperatures may alter adult longevity after emergence. Therefore, studying the longevity of taxa emerging from contrasting thermal regimes and comparing results to what has been previously discovered for cold-adapted genera in other geographic areas can provide critical insight into how climate warming will impact cold-adapted taxa at large spatial scales.

Goals for this research were to (1) determine longevities of adults emerging from two Icelandic groundwater-fed streams with contrasting water temperature conditions, when incubated at two contrasting air temperatures, and (2) determine if longevities of *Diamesa* species emerging in summer at Hengill are similar to longevities documented under controlled conditions for *D. mendotae* emerging during winter from streams in Minnesota (Ferrington et al. 2011).

Methods and materials

Site description: Two groundwater-fed streams were selected for study in the Hengladalir valleys of the Hengill alpine geothermal area located approximately 30 km east of Reykjavík (64°03’N: 021°18’W). These streams are in close proximity to one another (at some places ~ 2m apart) but have contrasting thermal regimes (Fig. 1). The cold stream, IS7, is fed by a cold groundwater source and maintains a mean summer temperature of 6.6±1.3°C (5.4±1.4°C mean annual temperature), whereas the warm stream, IS8, is geothermally heated, with a mean summer temperature of 22.0±0.8°C (21.3±1.0°C mean annual temperature) (Hannesdottir et al. 2013). The streams flow over similar terrain and both streams flow into the larger River Hengladalsá (Fig. 1).

Field collection and lab study: Uninjured and recently emerged adult chironomids were collected using 8 close-weave muslin emergence traps, 3 on IS7 and 5 on IS8; additional traps were placed on IS8 since previous research on IS8 indicated that this stream has lower numbers of emerging adults compared to IS7 (Gisli Mar Gislason personal

observation, July 2018). Collections were made daily from 5-8 July 2018 at approximately the same time each day (1000-1300). Adult chironomids were collected individually into one-dram snap top vials using an aspirator and transported to the laboratory in a cooler. A small amount of stream water (approximately 10-20% the volume of the vial) was added to each vial during collection to prevent desiccation. Adults were sorted by sex to have equal numbers of each sex within each treatment, and then were randomly assigned to one of two treatment groups for incubation at either constant 6°C or constant 20°C. Adults were kept in total darkness except when assessed for survival, as stipulated in the protocol used by Ferrington (2019) in previous studies of longevity (Ferrington, unpublished lab protocol). No food was provided because adult chironomids are non-feeding. The two temperatures were selected to approximate conditions of the larval habitats, and air temperatures that adults could be exposed to after emergence. Air temperatures during the summer in southern Iceland range from approximately 6°C (World Weather Information Service 2020) to more than 10°C (Degenhardt and Ólafsson 2019). Thus, our 6°C treatment simulated coolest summer air temperatures. Based on the most extreme climate warming models, a 10°C increase in mean air temperature is a possibility for the arctic (Bintanja and Andry 2017). Therefore, the 20°C treatment simulates temperatures that adults could be exposed to in the most severe climate scenario. We chose the lower temperature treatment to provide continuity with protocols of adult longevity experiments conducted during winter in Minnesota

at 6°C (Ferrington, unpublished standard operating procedure).

Individual adults were randomly assigned to incubation treatments using physical attributes but could not be unambiguously differentiated to genus until after they died and were preserved. Consequently, specimens were not always evenly assigned by genus to treatment groups. Adults were assessed daily for survival using the protocol outlined in Ferrington (2019). Minimal handling was employed to prevent any influence on longevity. Individuals not exhibiting physical movement when the vials were tapped were judged as dead and preserved in 90% EtOH. The experiment was terminated at 32 days. All individuals still alive on day 32 were sacrificed by preserving them in 90% ETOH. Depending on the date of collection, the surviving adults preserved on day 32 of the experiment were 28-32 days post-emergence.

Adults were identified to genus following preservation using Weiderholm (1989). Longevities of *Diamesa* were analyzed at the genus-level because females could not be unambiguously differentiated to species. Data for both male and female *Diamesa* were summed to estimate longevity. Males of Orthocladiinae and Tanytarsini were also identified and analyzed at genus-level. However, not all Orthocladiinae and Tanytarsini females could be reliably identified to genus, and therefore the females are treated at subfamily and tribe levels, respectively, and analyzed independent of the totals for males.

Temperature effects on longevity of *Diamesa* adults were analyzed using a generalized linear mixed-effects model (GLME) with a Poisson distribution from the lme4 package (Bates et al. 2015) in RStudio Desktop Version 1.1.423 (R Core Team 2017). Only data for *Diamesa* from IS7 were analyzed since there was only one *Diamesa* individual collected from IS8. Residuals for the GLME were more evenly distributed after removing outliers (3 individuals that were dead when checked at day 1 post-collection). Visualization of treatment effects was performed using ggplot2 (Wickham 2016).

Mean longevity was calculated based on time from retrieval in the emergence traps to death in the laboratory for all individuals that died during incubation (as in Ferrington et al. 2010, Anderson et al. 2013, and Mazack et al. 2014). Survival estimates for *Diamesa* were also calculated using the Kaplan-Meier nonparametric estimation approach (Hintz, NCSS Version 2007.lnk). This approach was only employed for *Diamesa* because over half of the adult *Diamesa* collected survived for the du-

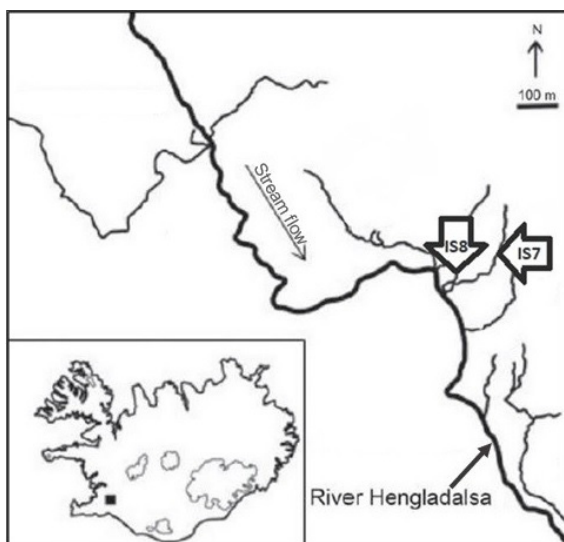


Figure 1. Map of research streams IS7 and IS8 within the Hengill alpine geothermal area, Iceland (inset) (adapted from Hannesdottir et al. 2013).

ration of the study. For this analysis, adults dying during the first 24 hours were coded as left censored, those dying after 24 hours but before day 32 were coded as interval censored, and adults surviving to day 32 were coded as right censored (Klein and Moeschberger 1997).

Results

Of the total of 135 adults collected, 10 individuals were removed from the analyses: 3 adults could not be reliably identified, 3 adults died in transit or upon arrival to the laboratory, 1 adult was removed because there was an error in recording the behavior during observation, and 3 adults were outliers for the longevity analysis. Twenty-three adults (18%) incubated at 6°C survived the duration of incubation (21 of these were *Diamesa*) and had to be sacrificed on termination of the experiment on

day 32. Therefore, longevity of 102 adults were determined for identifiable specimens that died during the experiment. Tables 1 and 2 indicate the mean longevity of each taxon collected based on treatment. However, for the taxa which had individuals that survived and needed to be sacrificed upon termination of the experiment, an asterisk shows the mean longevity computed here, which should be considered as a minimum estimate (Table 1).

Emergence from IS7 (95 adults, 7 taxa) exceeded emergence from IS8 (7 adults, 5 taxa) resulting in uneven numbers of adults available for incubation. All specimens incubated at 20°C had reduced longevity regardless of taxon and stream origin (Tables 1 and 2). Sixty-two *Diamesa* were collected from IS7 (Table 1) and 1 from IS8 (Ta-

Table 1. Mean longevity of taxa collected from stream IS7 that died during the two temperature treatments. (* See text for corresponding Kaplan-Meier nonparametric estimates of longevity). The longevity for *Diamesa* is a minimum longevity estimate since 21 adults survived the incubation and had to be sacrificed.

Taxon	Stream	Incubation Treatment	Total Incubated	Number Died	Longevity of Individuals That Died
<i>Diamesa</i>	IS7 (Cold)	6°C	33	12	19.9*
<i>Eukiefferiella</i>	IS7 (Cold)	6°C	2	1	18.0
<i>Micropsectra</i>	IS7 (Cold)	6°C	5	5	19.8
Orthocladiinae	IS7 (Cold)	6°C	6	5	18.2
<i>Orthocladius</i>	IS7 (Cold)	6°C	3	3	13.0
<i>Thienemanniella</i>	IS7 (Cold)	6°C	8	8	20.8
<i>Diamesa</i>	IS7 (Cold)	20°C	29	29	3.8*
<i>Eukiefferiella</i>	IS7 (Cold)	20°C	3	3	5.0
<i>Micropsectra</i>	IS7 (Cold)	20°C	3	3	3.7
Orthocladiinae	IS7 (Cold)	20°C	19	19	3.0
Tanytarsini	IS7 (Cold)	20°C	1	1	4.0
<i>Thienemanniella</i>	IS7 (Cold)	20°C	6	6	3.2
Total			118	95	

Table 2. Mean longevity of taxa collected from stream IS8 that died during the two temperature treatments.

Taxon	Stream	Incubation Treatment	Total Incubated	Number Died	Longevity of Individuals That Died
<i>Eukiefferiella</i>	IS8 (Warm)	6°C	2	2	22.5
Tanytarsini	IS8 (Warm)	6°C	2	2	11.5
<i>Diamesa</i>	IS8 (Warm)	20°C	1	1	2.0
<i>Micropsectra</i>	IS8 (Warm)	20°C	1	1	1.0
Orthocladiinae	IS8 (Warm)	20°C	1	1	5.0
Total			7	7	

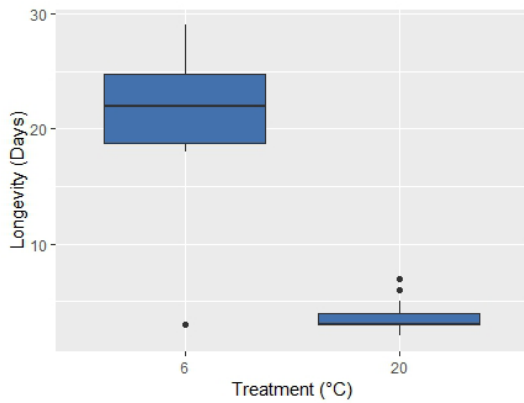


Figure 2. Comparison of *Diamesa* longevity from stream IS7 between treatments, based only on individuals (males and females together) that died during the incubation period. Results are shown for 12 individuals in the 6°C treatment and 29 in the 20°C treatment. The black bars indicate median longevity of adults that died.

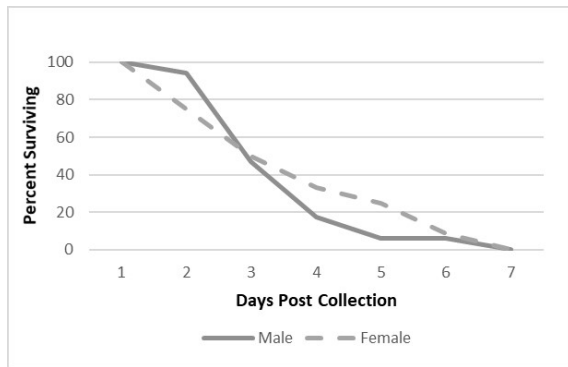


Figure 3. Percent of surviving adult females (n=12) and males (n=17) of *Diamesa* versus days post collection incubated at 20°C.

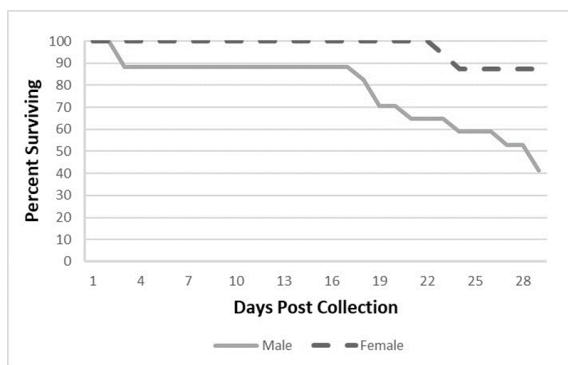


Figure 4. Percent of surviving adult females (n=16) and males (n=17) of *Diamesa* versus days post collection incubated at 6°C. Results are shown for all *Diamesa*, including individuals that did not die during the experiment but were sacrificed at the end of the experiment on day 32.

ble 2). Twenty-five Orthocladiinae were identified from IS7 (Table 1). Only one individual identified to Orthocladiinae was collected from IS8 (Table 2). Fourteen individuals of *Thienemanniella* were collected from IS7 (Table 1). However, none were collected from IS8 (Table 2). Within the 6°C treatment, only 12 *Diamesa* died naturally by day 28; 21 survived and were preserved on day 32 of the study (Table 1). In addition, one individual of the subfamily Orthocladiinae and one *Eukiefferiella* incubated in the 6°C treatment survived to the end of the experiment and both were preserved on day 32 (Table 1).

The mean longevity of *Diamesa* in the 20°C treatment was 3.8 days, whereas the mean longevity of individuals held at 6°C was 19.9 days, equating to an 81% reduction in longevity at the higher incubation temperature. Longevities were significantly different between treatments ($P < 0.0001$, Z value = -13.14) (Fig. 2); however, this test was based on 12 individuals that died naturally within the 6°C treatment and, therefore, indicates a minimum longevity estimate. Forty-seven percent of females and 50% of males survived to day 3 in the warm treatment, but all died by day 7 (Fig. 3). The Kaplan-Meier nonparametric estimates of 50% survival (regardless of sex) in the warm treatment was 3.6 days (95% CI = 3.3-4.3 days), and 3.0 days (95% CI = 2-4) for 75% survival. For the cold treatment, 88% of females survived to day 28; whereas 53% of males survived to day 28 (Fig. 4). In contrast to results for incubation at 20°C, the corresponding Kaplan-Meier nonparametric estimate of longevity for 75% percent survival of *Diamesa* adults in the cold treatment was 24 days (95% CI = 18-29).

Discussion

Longevities and cold adaptation: This is the first study of adult longevity for summer-emerging chironomids in relation to thermal regime of parent streams at the Hengill alpine geothermal area. Previous studies of chironomids have demonstrated the influence of thermal regime on emergence, taxonomic composition and abundances (e.g., Hannesdottir et al. 2013), but influences on other aspects of the life history, especially responses to temperature by adults, need to be better understood for comparative life-history analyses and refining hypotheses relating more generally to global warming. Our study is especially noteworthy because summer emergence from the cold, groundwater-fed streams at Hengill consisted of several chironomid genera, including *Diamesa* and *Orthocladius*, that characterize emergence during winter in several mid-latitudes of the northern hemisphere

(Nyquist et al. 2020), where species of *Diamesa* have been shown to be long lived after emergence when incubated at cool temperatures (Anderson et al. 2013, Ferrington et al. 2010, Mazack et al. 2014). Although we were not able to determine the maximum longevity for *Diamesa* because of time constraints associated with our experimental design, we are able to demonstrate that summer emerging species of *Diamesa* and *Orthocladius* are long-lived, potentially indicating that longer adult life spans are common among Diamesinae, and even cold-adapted Orthocladiinae, including species of *Orthocladius* and *Eukiefferiella*. For instance, even though fewer in number, adults of *Thienemanniella* had longevities comparable to the *Diamesa* that died naturally, although only by less than a day. The other Orthocladiini collected also had longevities comparable to the *Diamesa* that died naturally at 6°C.

Although fewer adults were collected from the warm spring run, the general trend of greater longevity when incubated at 6°C appears to hold regardless of the stream origin for the taxon. We conclude that adults emerging from both cold and geothermally heated springs are relatively cold adapted during the summer and able to survive longer periods of cold air temperatures regardless of the thermal regime that the immature stages experience. For example, *Micropsectra*, a genus more commonly found in the warm streams (Hannesdottir et al. 2013) at Hengill, exhibited longevities similar to *Diamesa* within the cold treatment.

Long duration of the adult stage of cold-adapted midges was first documented by Kohshima (1984) for a “new species of midge” collected on snow and ice of Yala Glacier in Nepal, where adults were active at air temperatures down to -16°C. Although only measured once, brachypterous adults collected at 5000-5600 meters elevation and incubated in plastic cups under field conditions displayed sex-related differences in longevities, with 96% of females surviving for the 35-day duration of the experiment. All males, however, died during the test interval. In the original paper by Kohshima (1984), the adults were presumed to represent a single species. Specimens sent by Kohshima to Sæther and Willassen (1987) for identification were determined to be *Diamesa*, but the collections contained two undescribed species, subsequently described as *Diamesa kohshimai* Sæther et Willassen and *Diamesa yalavia* Sæther et Willassen. The ratios of the two species in the experiment by Kohshima (1984) were not determined, but females of both species can be assumed to be among those that survived the 35-day duration of the experiment.

We collected too few females in this study to determine statistically significant differences in longevity between the sexes. However, graphically (Figure 4), our results from the 6°C treatment suggest that females may live longer than males under cold temperatures. This interpretation is consistent with the conclusions of long adult portion of the life cycle by Kohshima (1984). However, future work is needed to confirm whether this difference is significant and holds for *Diamesa* and other cold-adapted taxa at Hengill.

Winter-emerging species of Chironomidae in unfrozen, temperate streams in the United States show adaptations to cold similar to those observed by Kohshima (1984). Ferrington (2000) found that species of Orthocladiinae and Diamesinae dominate winter emergence, as was generalized by Armitage (1995) for temperate zones of the northern hemisphere, and that species of both subfamilies emerge and are active as adults at sub-freezing temperatures. Adults of *D. mendotae* have been observed to supercool to $-19.7 \pm 0.58^\circ\text{C}$ with some individuals able to supercool to -25.8°C (Bouchard et al. 2006). Species of *Diamesa* were among the most cold-adapted of 51 species of chironomids that emerged as adults during the coldest months of the year in Kansas (Ferrington 2000). Thus, it is not surprising that we found the *Diamesa* within our study to be long-lived under cold temperatures. However, other taxa collected from IS7, such as *Eukiefferiella* and *Orthocladius*, are also part of the winter-emerging, cold-adapted fauna of Kansas and Minnesota (Nyquist et al. 2020), and Anderson et al. (2011) documented that winter emergence of *Diamesa* and several species of Orthocladiinae were restricted to small intermittent or groundwater-dominated stream reaches in Kansas. Thus, the long lifespans of Orthocladiinae under cold temperatures in our study is consistent with the evidence for cold adaptation in subarctic Orthocladiinae found by other studies.

Climate change implications: The mean air temperature during emergence (July 5-8, 2018) was 4.3°C and the mean air temperature for the summer of 2018 (June 1-August 31) was 5.7°C (temperature data from Skardsmyrarfjall Weather Station, Iceland). However, the summer of 2018 was atypically cool. Air temperatures typically average around 10.7°C from June through August for the south coast of Iceland (Degenhardt and Ólafsson 2019). Therefore, our cold treatment reflects cool summer temperatures that adults may be exposed to, and indicate that adult chironomids are likely to be naturally long lived at 6°C during the summer since even summer temperatures do not typically

reach 20°C. However, predicted changes in Arctic mean annual air temperature due to climate change range from 4.2 to 8.3°C based on different climatic scenarios (Stocker et al., 2013) with some models predicting up to a 10.6°C increase in temperature based on intermodel standard deviation (Bintanja and Andry 2017). Thus, air temperatures in the arctic could approach 20°C as climate change proceeds, and the longevity of adults incubated at 20°C provide insight into how climate change could potentially alter the lifespans of chironomids in Iceland and other Arctic regions. Although oviposition rates and fecundity were not assessed in this study, they may also be altered by warming temperatures and, thus, warrant further investigation.

Although capable of living an extended period as adults in cool conditions, exposure to warm air temperatures appears to affect the behavior and longevity of cold-adapted species. For instance, Kohshima (1984) commented that adults collected from the Yala Glacier in Nepal were “very sensitive to a temperature as high as that of the human hand” and “became hyperactive for a few seconds, but were paralysed within about 20 s” when held in a human palm. Presumably, the rapid heating could affect adult longevity. We found reduced longevity of *Diamesa* when incubated at 20°C and the warm treatment had a negative effect on longevity of all taxa. This effect on adults suggests that cold-adapted chironomids could be extremely vulnerable to increasing air temperatures associated with global warming, especially if reduction of the adult phase of the life cycle reduces their probability of securing a mate and reproducing successfully.

Future research directions: Previous determinations of longevity for adults of *D. mendotae* were based on adults collected from snow adjacent to trout streams, and it was originally presumed that they represented recently emerged individuals (Anderson et al. 2013, Ferrington et al. 2010). However, subsequent findings indicate that specimens incubated in snow, or at controlled sub-freezing temperatures, can survive in vials for extended periods. Thus, we suggest some individuals collected from snow in these earlier studies could have emerged several or many days before being collected, especially if they congregate in protected areas such as under snow that is drifted and overhangs some of the unfrozen stream bed. If this is the case, estimates in previous literature must be re-interpreted as minimum longevity estimates, and actual longevity could be substantially longer. Future research should be conducted to

quantify differences in longevity between trapped individuals and individuals collected off snow.

Adults used in this study were trapped at emergence, and the traps were emptied daily, thus the ages of adults (post-emergence) did not exceed one day at the beginning of the incubation periods. Consequently, the long durations of survival of adults reported for this study accurately represent length of time survived up to and including 32 days, and therefore, the difference between survival estimates of *Diamesa* collected at Hengill relative to studies of *D. mendotae* in Minnesota may largely reflect difference in collection methods rather than difference that corresponds to the different species involved. Therefore, we suggest the use of emergence traps in future studies of *D. mendotae* to provide more comparable estimates of species-level difference in adult longevity.

Winter-active *Diamesa* have been observed emerging under subzero temperatures on the River Laxá in Northern Iceland (Gislason personal observation, 2019). However, the life histories of cold adapted winter-emerging species have not been extensively documented for these populations. In addition, no previous studies have quantitatively measured adult longevity of specimens collected from snow or documented how prevalent winter emergence is in Iceland. Thus, winter-active chironomids in Iceland may also possess long lifespans as adults similar to *D. mendotae*. Emergence of *Diamesa* in winter on the River Laxá provides an opportunity for future research related to climate change models and other aspects of adaptation to cold in life cycles of chironomids. For example, here we demonstrate that summer emerging *Diamesa*, whose larvae inhabit cold, groundwater-fed streams at Hengill, are long lived and can survive beyond 29-32 days when incubated at 6°C in the laboratory. These results are comparable to the longevity of *D. kohshimai* and *D. yalavia* from Nepal (Kohshima 1984) and *D. mendotae* in Minnesota (Ferrington et al. 2010) which has winter activity facilitated by freeze resistance (Bouchard et al. 2006). Therefore, we expect that the winter-emerging species in Iceland may also survive for extended periods at sub-freezing air temperatures if they also can depress freezing points similar to *D. mendotae* (Carrillo et al. 2004).

Conclusion: Chironomid adults in this study emerged from both cold and geothermally heated springs and are cold adapted during the summer. Air temperatures in the arctic could approach 20°C as climate change proceeds, and the longevity of adults incubated at 20°C provide insight into how

climate change could potentially alter the lifespans of chironomids in Iceland and other Arctic regions. Future research efforts should be directed toward investigating effects of climate warming on winter-active chironomids in Iceland, since there is evidence for reduced lifespans under warm temperatures for both summer emerging Icelandic species and shared taxonomic groups which are winter-emerging in the subarctic.

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***PSEUDORTHOCLADIUS IMMEZENSIS* SP. N., A NEW RELICT SPECIES INHABITING THE MACUN HIGH-ALPINE STREAM, SWISS ALPS (DIPTERA: CHIRONOMIDAE)**

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<http://zoobank.org/C44EAFE1-C247-405C-9B81-6CBC6CDC1C16>

Abstract

Male adult of *Pseudorthocladius immezensis* sp. n is diagnosed and described based on material collected in the high alpine cirque of the Macun area (Immez Lake basin, alt. 2616 m a.s.l.). A combination of morphological characters found in the male adult has allowed us to consider this new species as a local biogeographical representative of the eastern part of the Swiss Alps: coronal suture with lateral extension, low antennal ratio (AR 0.30); distal half of wing hairy; anal point broadly triangular with a characteristic enlarged base; inner apical margin of gonocoxite truncate; superior volsella large lobe-like; inferior volsella double, dorsal lobe nose-like and distinctly bent apically; virga present, with 4 spines; crista dorsalis absent. A differential diagnosis is given, in which some distinguishing morphological characters are summarised. Comments on the ecology and geographical distribution of the new species are also provided.

Introduction

The relatively harsh environmental conditions, such as long winters, thick snow and ice cover and low temperatures prevail in alpine freshwaters. These limiting conditions contribute to the settlement of a highly specialized fauna (especially insects) (Ward 1994). Moreover, the insular nature of alpine regions constrains the distribution of species colonizing and inhabiting alpine streams and lakes (Hieber et al. 2005). In alpine headwaters, Chironomidae dominate most stream assemblages in terms of abundance (Lods-Crozet et al. 2001, Ilg and Castella 2006, Robinson et al. 2016, Alther et al. 2019). In the remote area of the high-alpine cirque of Macun (Swiss National Park), rare and climate sensitive species colonize spring-fed streams, ponds and lake shores (Lods-Crozet et al. 2012). New chironomid species have been re-

cently described from here (Moubayed-Breil and Lods-Crozet 2018).

Larval populations of the genus *Pseudorthocladius* Goetghebuer, 1943 include exclusively rheophilic species mainly encountered in lotic habitats (small waterfalls in particular) delimited by the upper and middle basins of cold mountain streams. Based on knowledge provided on the taxonomy, geographical distribution and ecology of the known *Pseudorthocladius* species from Europe and the Palaearctic Region (Goetghebuer 1938, Brundin 1947, 1956, Albu 1966, Lehmann 1971, Caspers and Siebert 1981, Sæther and Sublette 1983; Cranston et al. 1989, Ashe and O'Connor 2012, Moubayed 1990, Schnell 1991, Stur and Sæther 2004, Langton and Pinder 2007, Makarchenko and Makarchenko 2012, Sæther and Spies 2013, Ren et al. 2014, Moubayed-Breil 2020), worldwide, there are 53 valid species, 10 of which are reported from Europe and only two from Switzerland: *P. berthelemyi* Moubayed, 1990 and *P. curtistylus* (Goetghebuer, 1921).

In this paper, *P. immezensis* is described as new to science and its morphology compared to the morphologically similar *P. cristagus* Stur & Sæther, 2004; *P. curtistylus*; *P. filiformis* (Kieffer, 1921) and *P. pilosipennis* Brundin, 1956. A differential diagnosis that highlights morphological characteristics and relevant distinguishing characters is given.

Material and methods

Male adults of *P. immezensis* sp. n were collected using a Malaise trap, 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 halteres 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).

Results

Pseudorthocladus immezensis sp. n.

<http://zoobank.org/51951519-569F-44D8-B0F0-0DB2370A1D21>

Material examined. Holotype, Switzerland. 1 male adult, Malaise trap, leg. B. Lods-Crozet. Macun cirque, streamlet and rheocrenes, left shore of Immez Lake (46°43'39.678" N, 10°07'55.764" E); alt. 2616 m a.s.l., 27.VII.2013. Environmental data from inlet of Immez Lake (after Lods-Crozet et al. 2012): crystalline water, conductivity 5.9 µS/cm; temperature (min-max, 3.9-19.5 °C; mean, 11.6 °C), pH 6.7.

Paratype. 1 male adult, leg. B. Lods-Crozet, same date and locality as for holotype.

Holotype (mounted on 1 slide; GBIFCH 00597051) is deposited in the collections of the 'Musée cantonal de Zoologie, Palais de Rumine, 6 place de la Riponne, CH-1014 Lausanne (MZL), Switzerland. Paratypes, Switzerland. 1 male adult, mounted on 1 slide, is deposited in the collection of the senior author.

Etymology. The name '*immezensis*' refers to the Immez Lake basin where the type-material was collected.

Diagnostic characters

P. immezensis sp. n. can easily be distinguished from other related species by the following combination of characters. Head. Base and median part of coronal suture with atypical lateral and median extensions; temporal setae 10-11; antenna 1280 µm long, last flagellomere about 300 µm long,

apical seta absent, AR 0.30. Sensilla coeloconica absent on palpomere 3. Clypeus sub-trapezoidal, with 8 setae. Thorax. Lobes of anteprenotum in contact; humeral pit half ellipse-like. Wing. Distal half of membrane densely covered with macrotrichia (hairy cells are: r_{4+5} , m_{1+2} , m_{3+4} ; cells cu and an bare); squama with 5 setae. Legs. Sensilla chaetica present on tarsomeres ta_1 - ta_5 of PI-PIII. Abdomen. Tergite IX broad basally, narrowed distally. Anal point markedly enlarged at base, with 17 setae mostly located close to the lateral margin. Virga with 4 spines. Gonocoxite truncate in its inner apical margin. Superior volsella, large lobe-like. Inferior volsella double: dorsal lobe nose-like with smooth inner margin; ventral lobe low. Gonostylus without posterior projection, caudal margin rounded; crista dorsalis absent.

Description

Adult male

(n = 2; Figs 1A, D, G-H, J, I, M; 2A-H)

Small to medium sized species. Total length (TL) 2.25 mm, wing length (WL) 1.35 mm; TL/WL = 1.67 (n = 1). General colouration is ranging from pale brown to dark brown; head, thorax and antenna brown; thorax brown with dark brown mesonotal stripes; legs uniformly brown; abdomen and anal segment brown.

Head (n = 2). (Fig. 1A). Eyes bare, midline of the frontal area slightly concave, frontal tubercles low; base and median part of coronal suture with outwards and inwards extensions; coronal setae present; temporal setae 10-11 including 8-9 inner and 2 outer verticals, postorbitals absent. Palp 5-segmented, length (in µm) of segments: 15, 30, 45, 48, 55; segments 1-2 fused, segment 2 bulbous; palpomere 3 (Fig. 1D) with 3 sensilla clavata, sensilla coeloconica absent. Clypeus (Fig. 1G) sub-trapezoidal, with 8 setae in 2 rows. Antenna 1280 µm long, last flagellomere about 300 µm long, apex distinctly clubbed, apical seta absent, antennal groove reaching segment 3, AR 0.30.

Thorax. Lobes of anteprenotum (Fig. 1H) thinner basally and not gaping, lateral anteprenotals 5, located apically; acrostichals 9 in 1-2 rows, starting at some distance from anteprenotum; dorsocentrals 9 in 1-2 rows; prealars 4 in 1 row; supraalars absent; humeral area (Fig. 1I) with contrasting brownish granulation, humeral pit half ellipse-like, parapsidal fork composed of micro-granulation; scutellum (Fig. 1J) broad, heart-like with 6 thin setae in 1 row.

Wing (n = 2). (Fig. 1M). Brachiolum with 1 seta; subcosta reaching the fork of radius, distribution

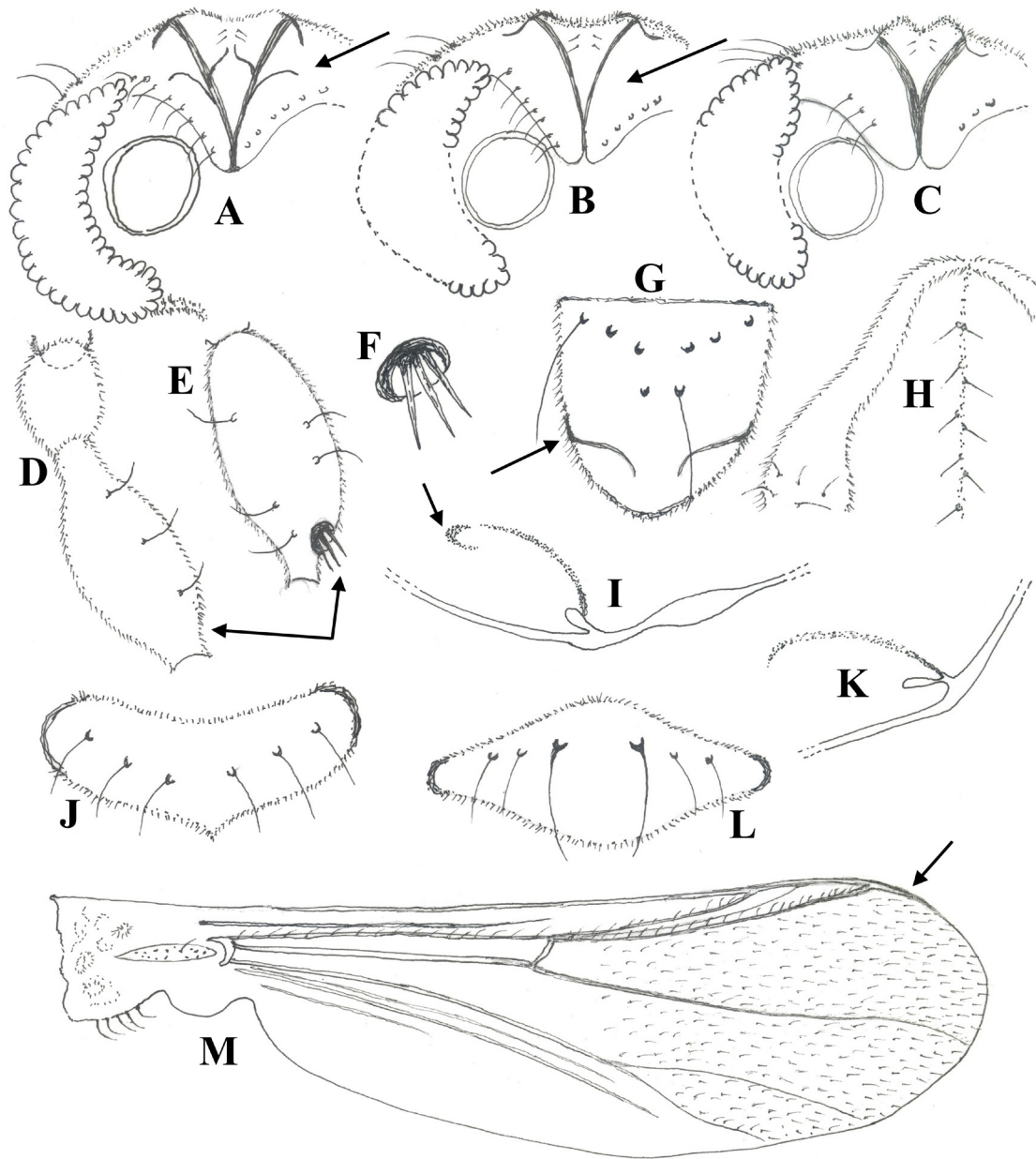


Figure 1. Male imago of *Pseudorthocladius* spp. A) Head (left side, dorsal), frontal area, vertex and temporal setae of: A) *P. immezensis* sp. n.; B) *P. curtistylus*; C) *P. sp. 1.* D) *P. immezensis* sp. n., palpomeres 2-3. E-F) *P. curtistylus*: palpomere 3 and sensilla coeloconica. *P. immezensis* sp. n.: G) clypeus; H) lobes of anteprenotum; I) humeral area; J) scutellum. *P. curtistylus*: K) humeral pit; L) scutellum. *P. immezensis* sp. n.: M) wing. The arrows indicate some distinguishing characters.

Table 1. Length (μm) and proportions of prothoracic (PI), mesothoracic (PII) and metathoracic (PIII) legs ($n = 1$).

	fe	ti	ta ₁	ta ₂	ta ₃	ta ₄	ta ₅	LR	BV	SV	BR
PI	520	525	265	170	115	65	60	0.51	3.20	3.94	2.10
PII	505	485	255	160	115	60	65	0.53	3.11	3.88	2.50
PIII	530	550	315	180	155	70	65	0.57	2.97	3.43	3.25

of setae on veins: R, 16-17; R₁, 11-12; remaining veins bare; costal extension about 25-30 μm ; distal half of membrane densely covered by macrotrichia which are clearly visible at 125-200X; hairy cells are: r₄₊₅ (150-160), m₁₊₂ (80-85), m₃₊₄ (about 40); cells cu and an bare; squama with 5 setae.

Legs ($n = 1$). Femur of PI and PIII nearly subequal (520 and 530 μm long); length (in μm) of tibial spurs: PI, 40; PII, 35 and 40; PIII, 30 and 45; longest seta of tibial comb 35 μm long. Sensilla chaetica present only on apical part of tibia of PI-PII, are more abundant on tarsomeres ta₁-ta₅. Length (in μm) and proportions of prothoracic (PI), mesothoracic (PII) and metathoracic (PIII) legs as in table 1.

Abdomen. Hypopygium ($n = 2$) in dorsal and ventral view as in Figs 2A-B. Tergite IX (Figs 2A, E) about 135-140 μm maximum width at base, occupying the entire width of segment IX, large cup-like basally, distal part subtriangular and extremely narrowing; dorsal side linear and lacking hump as shown in lateral view (Fig. 2E); setae absent on median and posterior area. Anal point (Figs 2A, E) with a characteristic enlarged base, distal part distinctly subtriangular (clearly visible in lateral view, Fig. 2E), presence of 17 setae (12 located close to the lateral margin and 5 on dorsomedian area). Laterosternite IX with 12-14 setae (6-7 on each side). Sternapodeme and phallapodeme as in Fig. 2B, transverse sternapodeme semi-circular and orally projecting, lateral expansion well developed; phallapodeme sinuous, thicker in its median part. Virga (Figs 2A, D) composed of 4 spines including 3 long and 1 short (located at base). Gonocoxite (Figs 2A-B, F) 155 μm long, 65 μm maximum width, apical part 25-30 μm wide, inner apical margin truncate. Superior volsella (Figs 2C, F) well-developed, large lobe-like as illustrated in dorsal (Fig. 2A) and lateral view (Fig. 2F). Inferior volsella (Figs 2A, C, E) about 40-45 μm long, 25 μm maximum width, consists of double lobes: dorsal one nose-like with smooth inner margin, apex distinctly bent downwards, median area with a distinct cluster of short setae; ventral one low triangular lobe. Gonostylus (Figs 2A, G-H) 70 μm long, 20 μm maximum width, without posterior projection, posterior margin rounded bearing a distinct notch distally clearly visible when viewed at acute and right angle; crista dorsalis absent. HR = 2.22. HV = 3.22.

Female adult, pupal exuvia and larva: unknown.

Differential diagnosis

Morphological differences between *P. immezensis* sp. n. and morphologically similar congeners (namely *P. cristagus*; *P. curtistylus*; *P. filiformis*; *P. pilosipennis*) are highlighted in the following differential diagnosis.

Adults of *P. immezensis* has a head with low frontal tubercles and atypical outward and inward extensions of the coronal suture (Fig. 1A), different from what is observed in *P. curtistylus* (Fig. 1B) and *P. sp. 1* (Fig. 1C) which belongs to an unnamed species known from the Mutt stream (upper Rhone basin, Switzerland, alt. 2100 m); sensilla coeloconica absent on palpomere 3 (Fig. 1D), present in *P. curtistylus* (Figs 1E-F); low antennal ratio (AR 0.30) compared to what is observed in *P. curtistylus* (0.84) and *P. cristagus* (about 1.40) (Sæther and Sublette 1983, Stur and Sæther 2004); humeral pit formed like a half ellipse (Fig. 1I), absent in *P. curtistylus* (Fig. 1K); scutellum large heart-like, with 6 similar thin setae (Fig. 1J), is horizontal diamond-like and bearing 2 stronger (occasionally 4) median setae in *P. curtistylus* and *P. filiformis* (Fig. 1L); distal half of wing densely haired (Fig. 1M), different from wings of *P. curtistylus* (Sæther and Sublette 1983, Figs 27C-D), *P. cristagus* (Stur and Sæther 2004, Fig. 1) and *P. pilosipennis* (Sæther and Sublette 1983, Fig. 26C); cells cu and an bare, while both are setose in *P. cristagus* and *P. pilosipennis*; basal part of anal point distinctly enlarged (Figs 2A, E), different from that of *P. curtistylus* (Fig. 2I), *P. cristagus* (Stur and Sæther 2004, Fig. 2) and *P. pilosipennis* (Sæther and Sublette 1983, Fig. 26D); virga with 4 spines (Figs 2A, D), is absent in *P. cristagus* and *P. pilosipennis* (Stur and Sæther 2004; Sæther and Sublette 1983); inferior volsella nose-like, bent downwards apically, with a distinct cluster of short setae on median area (Figs 2A, C), inferior volsella is bulbous in *P. cristagus* (Stur and Sæther 2004, Fig. 2) or large lobe-like in *P. pilosipennis* (Sæther and Sublette 1983, Fig. 26D); posterior margin of gonostylus rounded and bearing a notch distally (Figs 2A, G-H), gonostylus is linearly elongate and bearing a prominent outer heel in *P. cristagus* (Stur and Sæther 2004, Fig. 3); crista dorsalis absent (Figs 2A, G-H), is large, tooth-like in *P. cristagus* (Stur and Sæther 2004, Fig. 3).

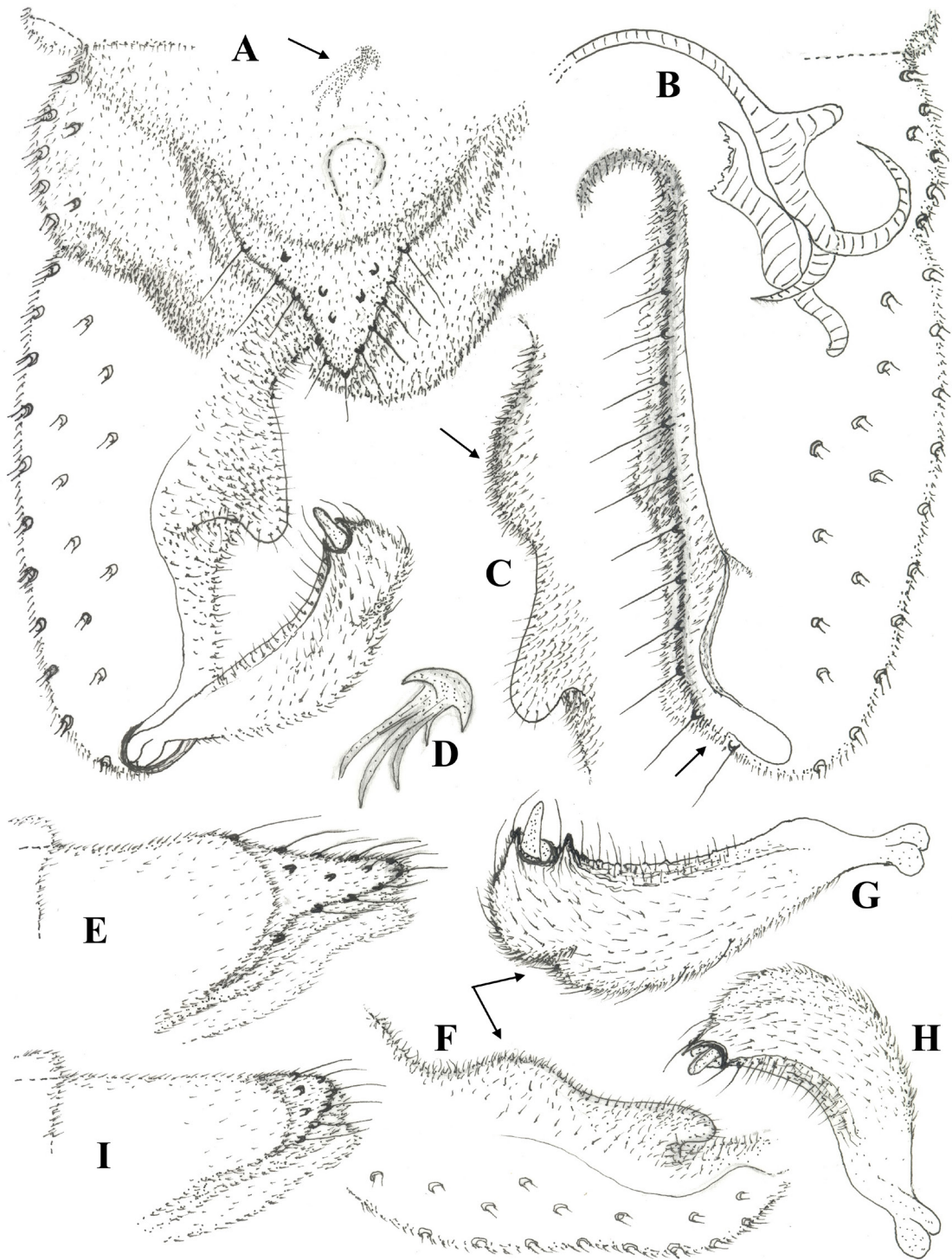


Figure 2. Male imago of *Pseudorthocladius* spp. *P. immezensis* sp. n.: A-B) hypopygium in dorsal and ventral view; C) superior and inferior volsella; D) virga; E) tergite IX and anal point, lateral; F) gonocoxite, lateral; G) gonostylus at right angle; H) gonostylus, lateral. *P. curtistylus*: I) tergite IX and anal point, lateral. The arrows indicate some distinguishing characters.

It is not feasible to provide a key to known male adult *Pseudorthocladius* from Europe until sufficient material of all species has been examined and compared.

Ecology and geographical distribution

Only one, not associated larva of the genus *Pseudorthocladius* was collected in the inflow area of Lake Immez, where bryocolous and hygropetric habitats seem to represent the most favourable aquatic microhabitat for the larval populations. The new species likely belongs to the crenophilous community of species as documented by Lindgaard (1995) as no *Pseudorthocladius* larvae were found during extensive sampling in the lake Immez and other small lakes in the area (Lods-Crozet unpublished data; Lods-Crozet et al. 2012). Such pristine lotic habitats are considered to be microrefugia and hotspots of diversity and therefore deserve much greater consideration, protection and preservation.

Geographical distribution of the new species is currently restricted to the Alpine Swiss glacial catchments including streams and lakes delimited by the Macun cirque (Swiss National Park, eastern

Swiss Alps, alt. 2616 m, Fig. 3), but *P. immezensis* sp. n. can be expected to occur in other similar mountainous areas situated in Switzerland and neighbouring countries (Italy, France, Germany, Austria, Poland).

Chironomid species encountered in the same area, and listed by Lods-Crozet et al. (2012), Lods-Crozet (2014, unpublished data, report to the scientific commission of the Swiss National Park) and Moubayed and Lods-Crozet (2018) include: *Zavrelimyia melanura* (Meigen, 1804); *Diamesa bertrami* Edwards, 1935; *D. cinerella* Meigen, 1835; *D. nowickiana* Kownacki & Kownacka, 1975; *D. vaillantii* Serra-Tosio, 1972; *Protanypus caudatus* Edwards, 1924; *Pseudodiamesa branickii* (Nowicki 1873); *Pseudodiamesa nivosa* (Goetghebuer, 1928); *Pseudokiefferiella parva* (Edwards, 1932); *Chaetocladius castellai* Moubayed-Breil, 2018; *C. macunensis* Moubayed-Breil, 2018; *C. longivirgatus* Stur & Spies, 2011; *C. lodscrozetiae* Moubayed-Breil, 2018; *C. suecicus* (Kieffer 1916); *Heleniella helvetica* Moubayed-Breil and Lods-Crozet, 2016; *H. ornatocollis* (Edwards, 1929); *Corynoneura arctica* Kieffer, 1923; *E. minor*



Figure 3. Malaise trap set up close to the inlet of the Immez Lake (Macun cirque, Eastern Alps, Swiss National Park; photo J.L. Lods). The arrow indicates the larval habitat of *P. immezensis* sp. n.

(Edwards, 1929); *Orthocladius frigidus* (Zetterstedt, 1838); *Parorthocladius nudipennis* (Kieffer, 1908); *Tokunagaia rectangularis* (Goetghebuer, 1940); *Micropsectra radialis* Goetghebuer, 1939; *Paratanytarsus austriacus* (Kieffer, 1924).

The presence of *P. immezensis* sp. n. in high mountain Alpine ranges in the Swiss Alps (above 2600 m a.s.l.) highlights and confirms that some cold and glacial high mountain enclaves can contain diversity previously unknown to science. Documentation and knowledge of this fauna is important to monitor diversity and community changes caused by global warming and climate change.

Acknowledgments

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***DICROTENDIPES SINICUS* LIN & QI, SP. N. (DIPTERA: CHIRONOMIDAE)**

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<http://zoobank.org/5CD8ED2B-7E4D-426C-8CD5-6E7681EC0688>

Abstract

The name *Dicrotendipes sinicus* was originally proposed in 2018, but the requirements of the International Code of Zoological Nomenclature were not fulfilled to make the name available. We here re-propose the name with full Code compliance and redescribe and illustrate all life stages of the species to make the name available.

Introduction

The name *Dicrotendipes sinicus* was originally proposed in Qi et al. (2018 [online], 2019 [print]), but part of the data required for making the name available appeared in the supporting information, which did not fulfill all relevant requirements of Article 8 in the International Code of Zoological Nomenclature (ICZN 1999, 2012). The name has therefore remained unavailable, which is herewith remedied by re-proposing the name in full Code compliance. Also, we take the opportunity to republish the descriptive and pictorial data for all life stages of the species.

Material and Methods

Detailed information on collecting procedures and habitat can be found in Qi et al. (2018). Morphological terminology and abbreviations in the description below follows Sæther (1980). Holotype and paratypes are deposited at the College of Life Sciences, Nankai University, Tianjin, China (NKU) except for one paratype at the NTNU University Museum, Trondheim, Norway (NTNU-VM).

Results

Dicrotendipes sinicus Lin & Qi sp. n. (Figs 1–9)

<http://zoobank.org/BFDE6B21-C90D-4A95-A5C9-B034C2787C4A>

Type material: Holotype, ♂ & Pe (NKU: J2A19), China: Zhejiang, Zhoushan, Xishan Island, sea-water ponds, 29.896°N, 122.305°E, 1 m a.s.l., 5.VI.2012, hand net, leg. X. Qi. Paratypes, China:

9♂♂ (NKU: J2A2, J2A5, J2A6, J2A7, J2A20, J2A26, J2A27, J2A29, J2A33), 1♂ & Pe (NKU: J2A4), 3♀♀ & Pe (NKU: J2A1, J2A28, J2A29), 1P (NKU: J2A22), 2Pe (NKU: J2A6, J2A24), 10L (NKU: J2A7, J2A11, J2A12, J2A13, J2A14, J2A17, J2A18) as holotype; 2♂♂ (NKU & BOLD Sample ID: XS1, XS2), 1♀ (NKU & BOLD Sample ID: XS3), 1L (NKU & BOLD Sample ID: XS4), Zhejiang, Zhoushan, Xishan Island, sea-water ponds, 29.896°N, 122.305°E, 1 m a.s.l., 5.VI.2013, hand net, leg. X. Qi; 1♂ (NTNU-VM & BOLD Sample ID: XL554), 1♀ (NKU & BOLD Sample ID: XL557), 3Pe (NKU: XL558, XL559, XL560), Zhejiang, Taizhou, Sanmen, Huaqiaozhen, marine ponds, 28.9375°N, 121.5221°E, 5 m a.s.l., 30.IV.2016, hand net, leg. B.J. Yang.

Etymology: Named after the country of the type locality, China, ‘*sinicus*’; adjective in nominative case.

Diagnostic characters: Morphological features of the new species do not fit with the generic diagnosis of *Dicrotendipes* and the remaining known genera of Chironomini. Adult male: antenna with ten flagellomeres, lacking plume; eye without microtrichia between ommatidia, without dorsomedial extension; palp with five segments, segment 4 and 5 occasionally slightly fused; brachypterous, shortened wings oar-shaped, with tuft of long setae apically; squama glabrous; acrostichals absent; mid and hind tibiae both with two spurs, and additional few small teeth; inferior volsella extremely long, strongly curved, extending beyond the apex of gonostylus; gonostylus moveable, with a few teeth distally. Adult female: antenna with six flagellomeres, without plume; palp with three segments; acrostichals usually absent, sometimes 1–5; mid tibia with two spurs and few small teeth, hind tibia with one spur and small teeth. Pupa: thoracic horn plumose, divided to numerous fine branches; basal ring larger, kidney-shaped, with two separated tracheal marks; pedes spurii B absent; segments V–VIII with 4 LT. Larva: antenna 5-segmented; mentum with rounded, trifid median tooth and six

pairs of lateral teeth, regularly decreasing in size laterally; premandible with four teeth; ventromental plates strongly curved; body without lateral or ventral tubules.

Description.

Adult male (n = 10, unless otherwise stated). Total length 2.60–4.48, 3.16 mm. Wing length 1.09–1.68, 1.29 mm. Total length/wing length 1.61–3.44, 2.48.

Coloration (Figs 1–2). Head, legs and abdomen brown. Basic color of thorax brown, with darkened patches posteriorly on scutum, scutellum and basal part on postnotum; median region of antepronotum dark brown.

Head (Fig. 2B). Eye glabrous, without dorsomedial extension. Frontal tubercles 5–15, 10 µm long, 5–20, 12 µm wide. Temporal setae 8–11, 9, including 0–3, 2 inner verticals, 1–10, 5, outer verticals and 1–3, 2 postorbitals. Clypeus with 16–26, 21 setae. Tentorium 93–135, 115 µm long, 13–25, 20 µm wide. Palp five-segmented, shortened; segment 3 without subapical sensilla; segment 4 and 5 incompletely fused in some specimens. Palpomere lengths (in µm): 25–50, 40; 40–60, 49; 80–105, 92; 25–60, 49; 30–70, 52.

Antenna (Figs 2A; 6A). Antenna with ten flagellomeres; ultimate flagellomere with several subapical sensilla (Fig. 2A). AR 0.96–1.43, 1.18. Plume absent, but surface of antenna covered with short setae and microtrichia.

Thorax (Figs 2C; 6B). Antepronotal lobes moderately developed, dorsally separated. Scutum not protruding beyond antepronotum, with tubercle. Acrostichals absent; dorsocentrals 9–14, 13; prealars 4–6, 5; scutellars 4–10, 7. Halter large (Fig. 2C).

Wing (Fig. 3A). Reduced in size, oar-shaped, bifurcated and with tuft of long setae apically; venation

indistinct in some specimens. Membrane without setae, but with numerous microtrichia. Costa not produced beyond R_{4+5} ; R_{2+3} ending close in distal 1/5 between apices of R_1 and R_{4+5} ; R_{4+5} ending distal to apex of M_{1+2} ; FCu far distal to RM, ending in proximal to apex of R_{4+5} . VR 1.46–1.83, 1.62. Brachiolum with one seta, R with 7–13, 10 setae; R_1 with 8–13, 11 setae; R_{4+5} with 0–1 seta; remaining veins glabrous. Anal lobe small. Squama glabrous.

Legs (Fig. 4A–C, G–I). Fore leg (Fig. 4A) very long relative to mid (Fig. 4B) and hind legs (Fig. 4C); apex of fore tibia (Fig. 4G) without spur; mid tibia (Fig. 4H) with two short, robust spurs and 3–7 teeth; hind tibia (Fig. 4I) with two short, robust spurs and 0–2 teeth, covered with three types of setae, short setae on inner margin, short but stout setae as spines on outer margin and long setae distally; all tarsomeres normally developed on fore leg but reduced on mid and hind legs. Claws normally developed, with a pointed spine. Pulvilli present. Lengths (in µm) and proportions of legs as in Table 1.

Hypopygium (Figs 5C, D; 6C, D). Anal tergite bands strongly developed, fused anteriorly. Anal tergite with 46–62, 50 moderately long setae medially and posteriorly. Anal point robust and broadly rounded, 73–100, 84 µm long, with several moderately long setae. Superior volsella absent. Median volsella broadly digitiform, with 8–13, 10 long setose lamellae, covered with numerous microtrichia. Inferior volsella extremely long, 240–305, 262 µm long, strongly curved, extending beyond apex of gonostylus, distally rounded, with 21–52, 42 setae and numerous microtrichia. Gonocoxite 205–340, 252 µm long. Gonostylus short, 158–195, 172 µm long, with inner margin weakly expanded for some distance in basal 5/7, distally sharply constricted, with 3–5, 4 small apical teeth; moveably inserted on gonocoxite. Transverse sternapodeme 13–23, 20 µm long, without oral projections. Phal-

Table 1. Lengths (in µm) and proportions of legs of *Dicrotendipes sinicus* Lin & Qi **sp. n.**, male (n = 10)

	fe	ti	ta ₁	ta ₂	ta ₃	ta ₄
p ₁	780-1050, 892	670-875, 750	1100-1425, 1225	690-900, 792	590-770, 680	490-640, 555
p ₂	335-570, 489	370-600, 514	115-175, 135	48-63, 54	35-53, 44	30-45, 38
p ₃	660-920, 753	540-740, 653	133-170, 151	40-65, 53	40-54, 49	35-53, 39
	ta ₅	LR	BV	SV	BR	
p ₁	200-240, 219	1.57-1.80, 1.64	1.20-1.31, 1.28	1.24-1.47, 1.34	0.79-1.54, 1.02	
p ₂	35-75, 64	0.19-0.32, 0.27	4.55-6.61, 5.69	5.97-8.97, 7.47	0.53-0.85, 0.68	
p ₃	63-75, 68	0.20-0.26, 0.23	6.34-8.11, 7.48	8.35-10.22, 9.32	0.46-1.39, 0.73	

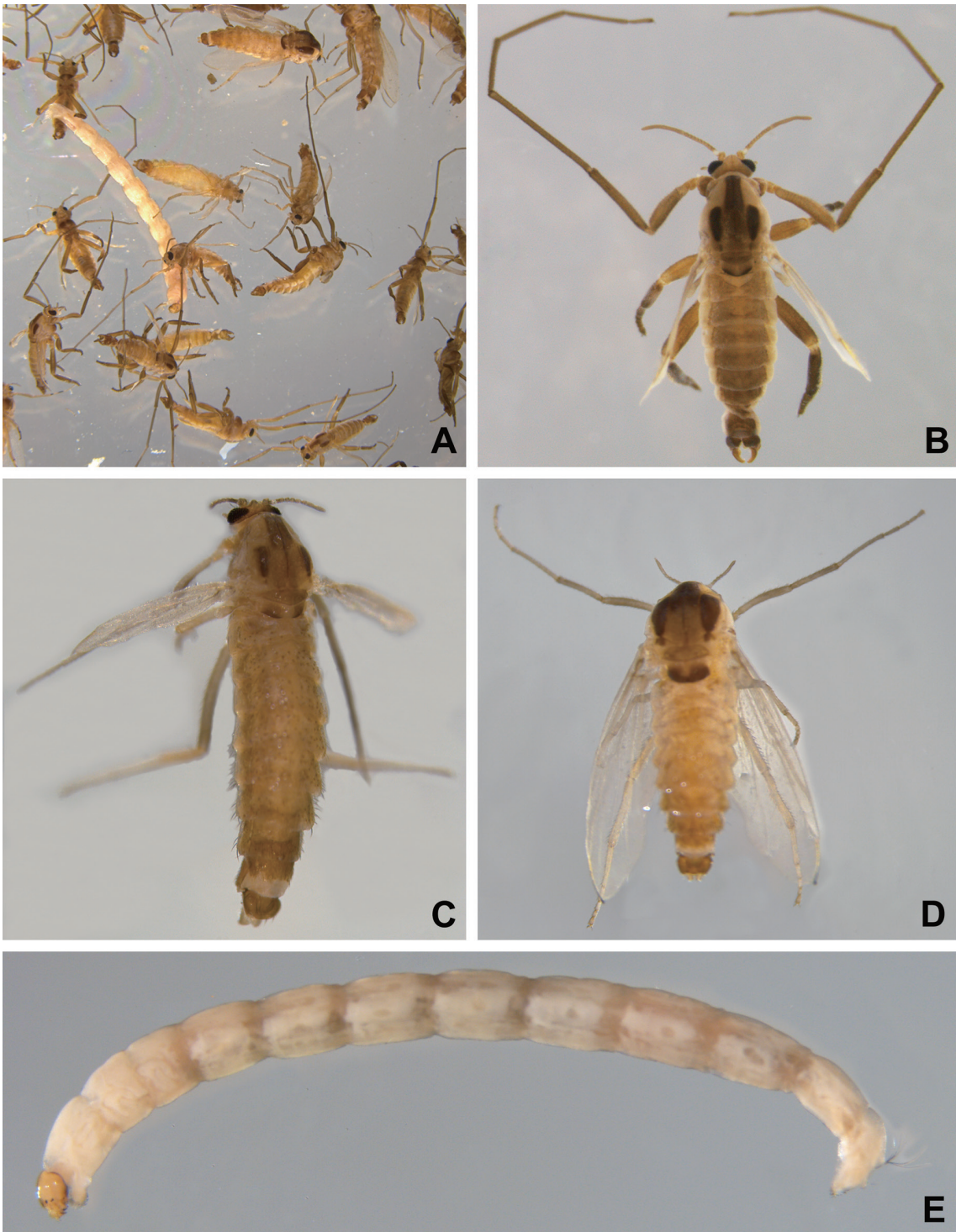


Figure 1. *Dicotendipes sinicus* Lin & Qi **sp. n.** in 85% ethanol. A, adults and a larva; B, adult male dorsal view; C-D, adult female dorsal view; E, larva lateral view. First published by Qi et al. (2018), Attribution-NonCommercial-NoDerivatives 4.0 International (CC BY-NC-ND 4.0).

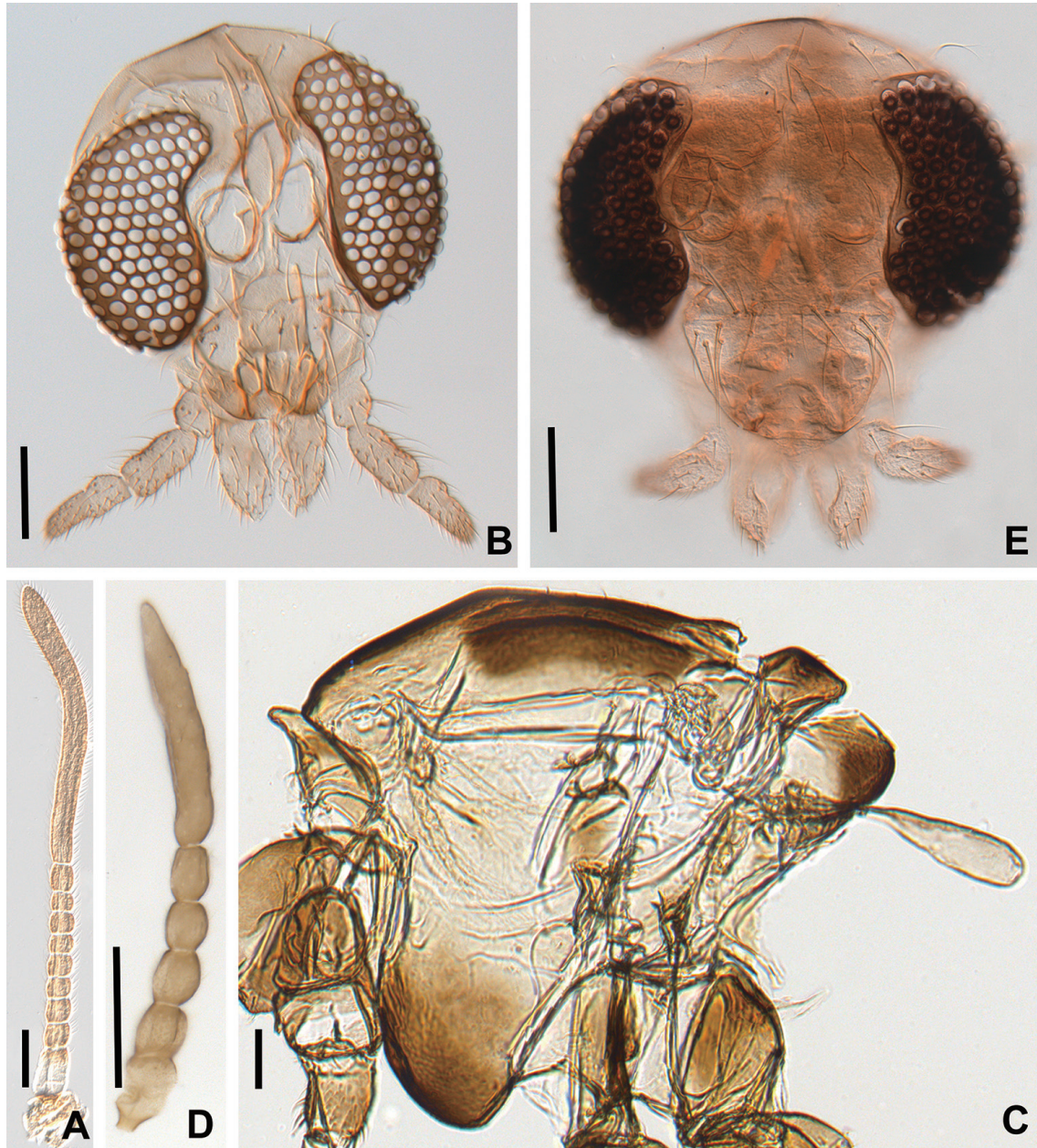


Figure 2. Antenna, head and thorax of *Dicrotendipes sinicus* Lin & Qi **sp. n.** adults. A, adult male antenna; B, adult male head; C, adult male thorax; D, adult female antenna; E, adult female head. Scale bars = 100 μm . First published by Qi et al. (2018), Attribution-NonCommercial-NoDerivatives 4.0 International (CC BY-NC-ND 4.0).

lapodeme 145–235, 170 μm long. HR 1.24–1.74, 1.46; HV 1.38–2.59, 1.85.

Adult female (n = 6, unless otherwise stated)

Total length 3.58–5.70, 4.53 mm. Wing length very variable, 1.30–2.33, 1.77 mm. Total length/wing length 1.68–4.30, 2.86.

Coloration (Figs 1C, D). Slightly darker than adult male.

Head (Fig. 2E). Eye glabrous, without extension. Frontal tubercles 8–10, 9 μm long, 18–30, 24 μm wide. Temporal setae 7–9, 8, including 1–2, 2 in-

ner verticals, 4–5, 4 outer verticals and 0–3, 2 postorbitals. Clypeus with 18–30, 21 setae. Tentorium 103–150, 127 μm long, 13–15, 14 μm wide. Palp three segmented, shortened without subapical sensilla (Fig. 2E). Palpomere lengths (in μm): 35–40, 37; 35–50, 41; 70–98, 83.

Antenna (Fig. 2D). Plume absent; with six flagellomeres. Flagellomere length (in μm): 53–70, 59; 35–63, 49; 38–50, 44; 35–45, 40; 38–50, 44; 170–200, 187; AR 0.74–0.80, 0.76.

Thorax (Fig. 7A). Anteprenotal lobes moderately developed, dorsally separated. Scutum not

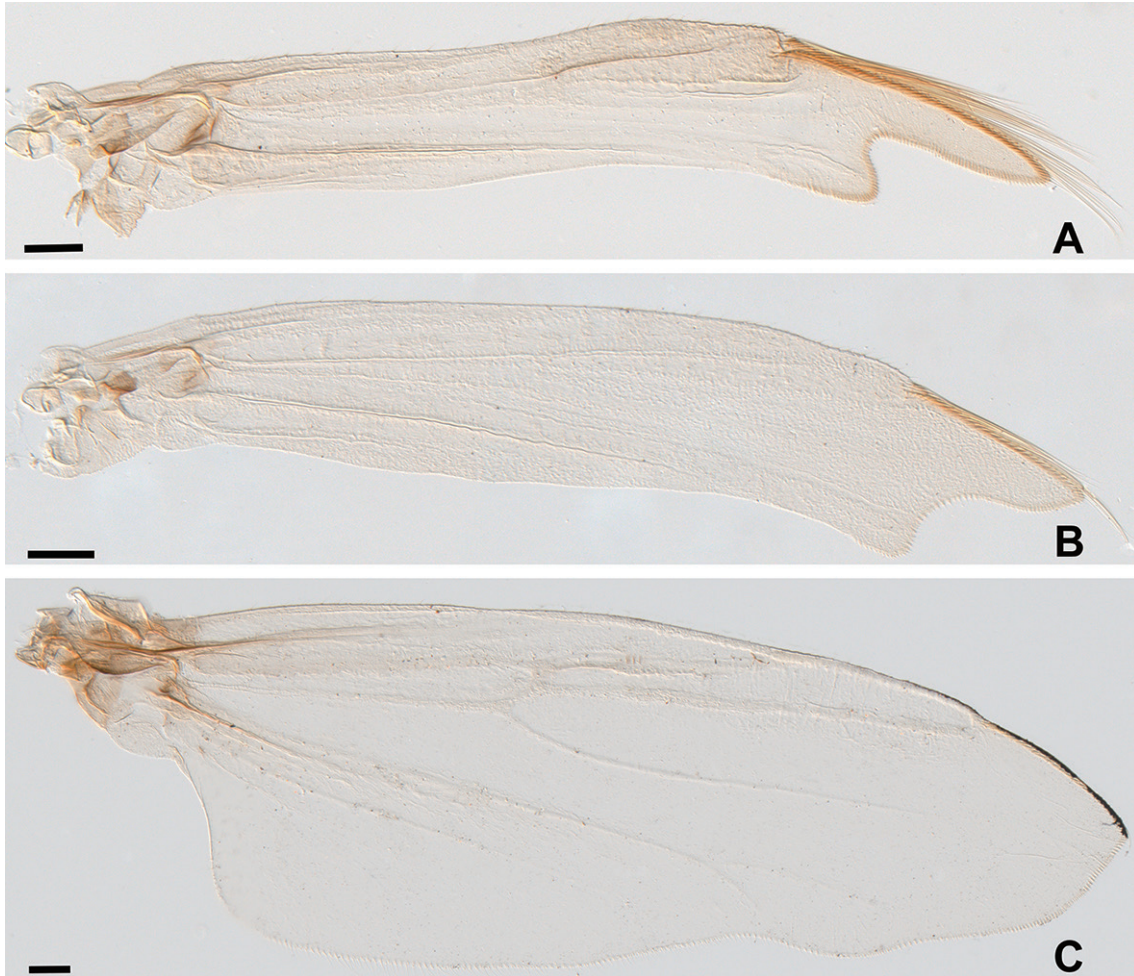


Figure 3. Wings of *Dicrotendipes sinicus* Lin & Qi **sp. n.** adults. A, brachypterous, oar-shaped wing of adult male; B, brachypterous, oar-shaped wing of adult female; C, fully developed wing of adult female. Scale bars = 100 μm . First published by Qi et al. (2018), Attribution-NonCommercial-NoDerivatives 4.0 International (CC BY-NC-ND 4.0).

protruding beyond antepronotum, with tubercle. Acrostichals usually absent, sometimes 1–5, 2 (3); dorsocentrals 10–17, 12; prealars 3–9, 6; scutellars 4–14, 10.

Wing (Figs 3B, C). Fully developed, or shortened (brachypterous females), oar-shaped, bifurcated, with tuft of long strong setae apically. Membrane with numerous microtrichia. VR 1.56–1.77, 1.67. Brachiolum with 0–1, 1 seta. R with 6–9, 7 setae; R_1 with 7–9, 8 setae; R_{4+5} with 3–7, 5 setae; remaining veins and cells bare. Squama glabrous. Anal lobe slightly developed.

Legs (Figs 4D–F, J–L). Fore leg (Fig. 4D) very similar to hind legs (Fig. 4F), mid legs (Fig. 4E) shortest; apex of fore tibia (Fig. 4J) without spur; mid tibia (Fig. 4K) with 4–13 small teeth and two short, robust spurs, 15–23, 18 and 10–20, 17 μm long; hind tibia (Fig. 4L) with one robust spur 10–15, 14 and 5–15, 9 small teeth; all tarsomeres normally developed on fore leg but reduced on mid

and hind legs. Claws normally developed, with one spine on each side. Pulvilli present. Lengths (in μm) and proportions of legs as in Table 2.

Abdomen (Fig. 7B). Number of setae on tergite I–VIII: 14–30, 19; 27–54, 40; 52–70, 62; 48–96, 64; 48–68, 57; 33–46, 39; 20–24, 22; 7–16, 13. Number of setae on sternites I–VIII: 0; 5–12, 8; 19–30, 23; 21–33, 25; 19–29, 24; 15–36, 23; 2–8, 5; 44–48, 45.

Genitalia (Fig. 7C). Tergite IX without setae; gonocoxite IX with 13–22, 19 setae. Cercus 100–185, 122 μm long, 50–110, 81 μm long, with 27–90, 45 setae. Seminal capsules oval, 165–230, 189 μm long, 110–160, 134 μm wide; spermathecal duct 180–300, 239 μm long. Notum 345–380, 365 μm long.

Pupa ($n = 8$, unless otherwise stated)

Total length 4.93–6.58, 5.68 mm. Cephalothorax brown, abdomen mostly yellow, but lateral regions

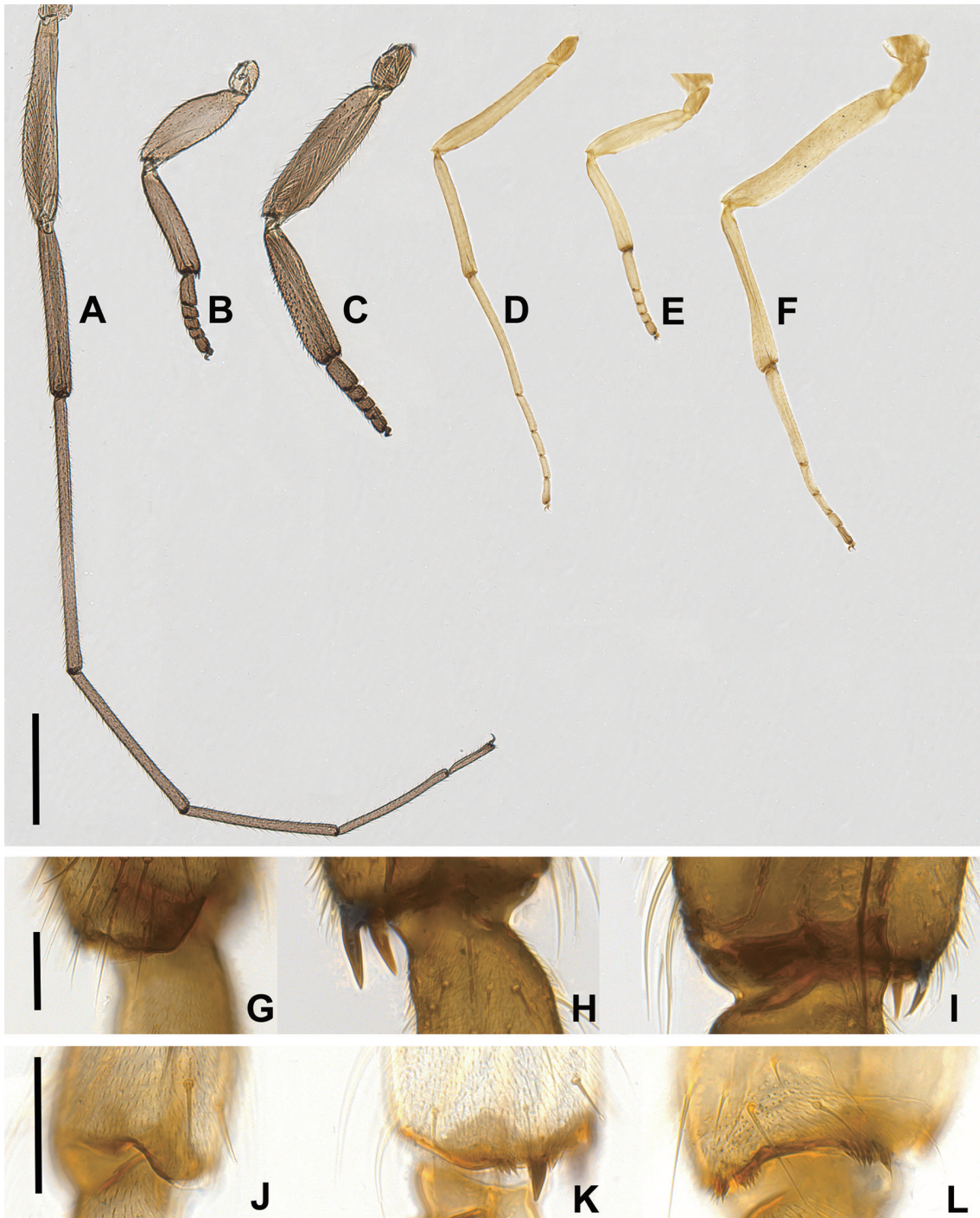


Figure 4. Legs of *Dicrotendipes sinicus* Lin & Qi **sp. n.** adults. A, male fore leg, scale bar = 500 μ m; B, male mid leg, scale bar = 500 μ m; C, male hind leg, scale bar = 500 μ m; D, female fore leg, scale bar = 500 μ m; E, female mid leg, scale bar = 500 μ m; F, female hind leg, scale bar = 500 μ m; G, part of male fore tibia, scale bar = 50 μ m; H, part of male mid tibia showing spurs and tooth-shaped combs, scale bar = 50 μ m; I, part of male hind tibia showing tooth-shaped combs, scale bar = 50 μ m; J, part of female fore tibia, scale bar = 50 μ m; K, part of female mid tibia showing spurs and tooth-shaped combs, scale bar = 50 μ m; L, part of female hind tibia showing tooth-shaped combs, scale bar = 50 μ m. First published by Qi et al. (2018), Attribution-NonCommercial-NoDerivatives 4.0 International (CC BY-NC-ND 4.0).

of segments VI–VIII and anal segment dark brown.

Cephalothorax (Figs 8A–C). Frontal setae 25–63, 41 μm long. Cephalic tubercles present (Fig. 8A). Anteprepronotum with one median seta, 38–68, 57 μm long. Two precorneals present, 30–55, 45 and 25–45, 35 μm long. Thoracic horn 450–650, 564 μm long, 25–38, 30 μm wide, plumose, divided into numerous fine branches (Fig. 8B); basal ring strongly constricted medially, with 2 separated tracheal marks (Fig. 8C). Anterior dorsocentral seta (Dc_1) 25–50, 39 μm long; Dc_2 48–85, 63 μm long; Dc_3 18–48, 34 μm long; Dc_4 30–70, 41 μm long. Distance between Dc_1 and Dc_2 30–125, 80 μm ; between Dc_2 and Dc_3 60–103, 89 μm ; between Dc_3

and Dc_4 23–40, 31 μm .

Abdomen (Figs 8D, E). Tergite I without shagreen; II–VI with continuous median, longitudinal field of uniform shagreen; VII bare; VIII with anterior pair of patches of fine shagreen; anal segment bare (Fig. 8D). Hook row continuous, 380–540, 446 μm wide, occupying about 1/2 width of segment. Conjunctions III/IV, IV/V and V/VI with transverse band of fine spinules. Vortex present on segment IV; pedes spurii B absent on segment II. Anal comb (Fig. 8E) of segment VIII dark brown, with strong and short stalk 48–77, 59 μm wide, with 4–6, 6 apical brown pointed spines and several spinules.

Table 2. Lengths (in μm) and proportions of legs of *Dicrotendipes sinicus* Lin & Qi **sp. n.**, female (n = 6)

	fe	ti	ta ₁	ta ₂	ta ₃	ta ₄
p ₁	460–670, 558	400–540, 468	365–550, 478	110–163, 146	70–105, 91	60–103, 85
p ₂	320–450, 399	300–430, 363	83–180, 148	40–60, 50	25–45, 37	35–38, 36
p ₃	580–890, 758	500–700, 606	300–450, 378	100–150, 378	83–105, 96	50–75, 62
	ta ₅	LR	BV	SV	BR	
p ₁	80–113, 97	0.91–1.06, 1.02	3.38–3.85, 3.60	2.01–2.44, 2.15	1.13–1.89, 1.47	
p ₂	50–65, 58	0.28–0.49, 0.40	4.44–5.91, 5.02	4.45–7.47, 5.35	0.75–1.11, 0.92	
p ₃	65–85, 78	0.59–0.65, 0.62	4.44–5.10, 4.74	3.46–3.79, 3.61	0.82–1.63, 1.17	

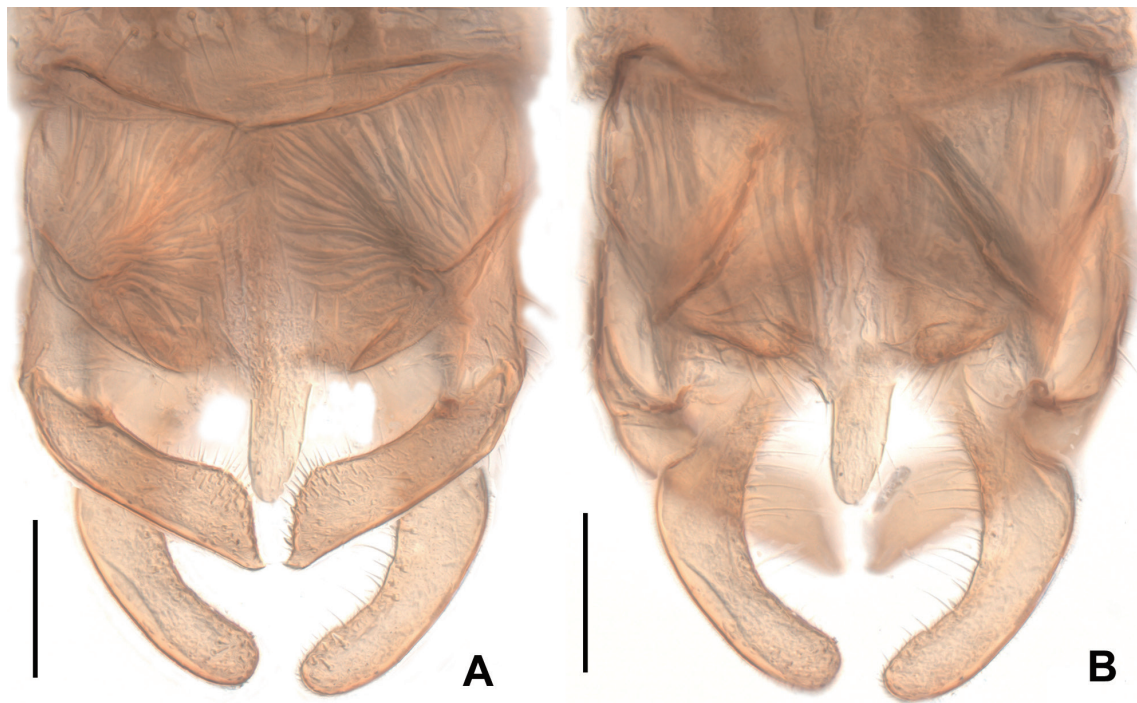


Figure 5. Adult male hypopygium of *Dicrotendipes sinicus* Lin & Qi **sp. n.** A, hypopygium dorsal view; B, hypopygium ventral view. Scale bars = 50 μm . First published by Qi et al. (2018), Attribution-NonCommercial-NoDerivatives 4.0 International (CC BY-NC-ND 4.0).

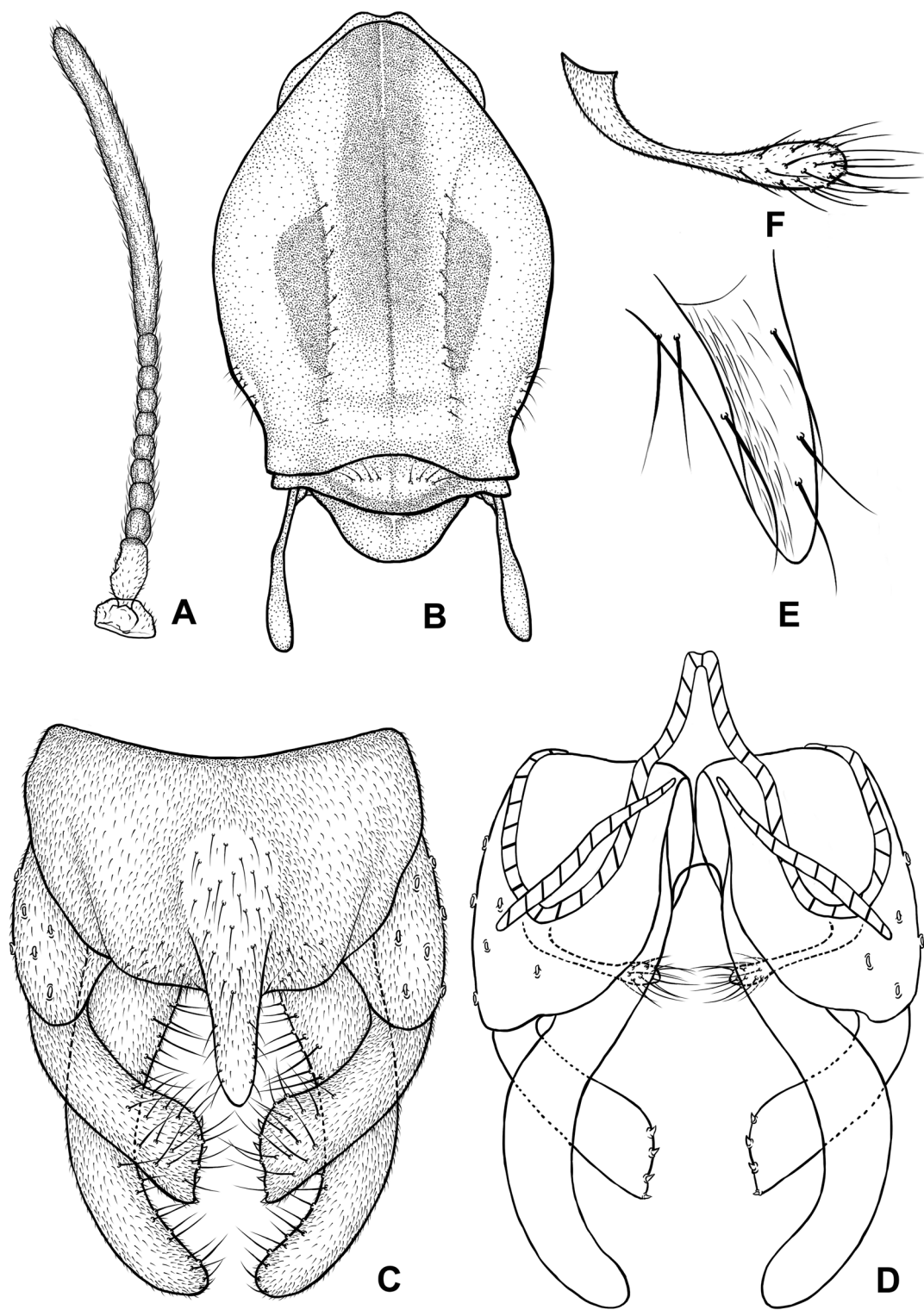


Figure 6. Adult male of *Dicrotendipes sinicus* Lin & Qi **sp. n.** A, antenna; B, thorax; C, hypopygium dorsal view; D, hypopygium ventral view; E, anal point lateral view; F, median volsella. First published by Qi et al. (2018), Attribution-NonCommercial-NoDerivatives 4.0 International (CC BY-NC-ND 4.0).

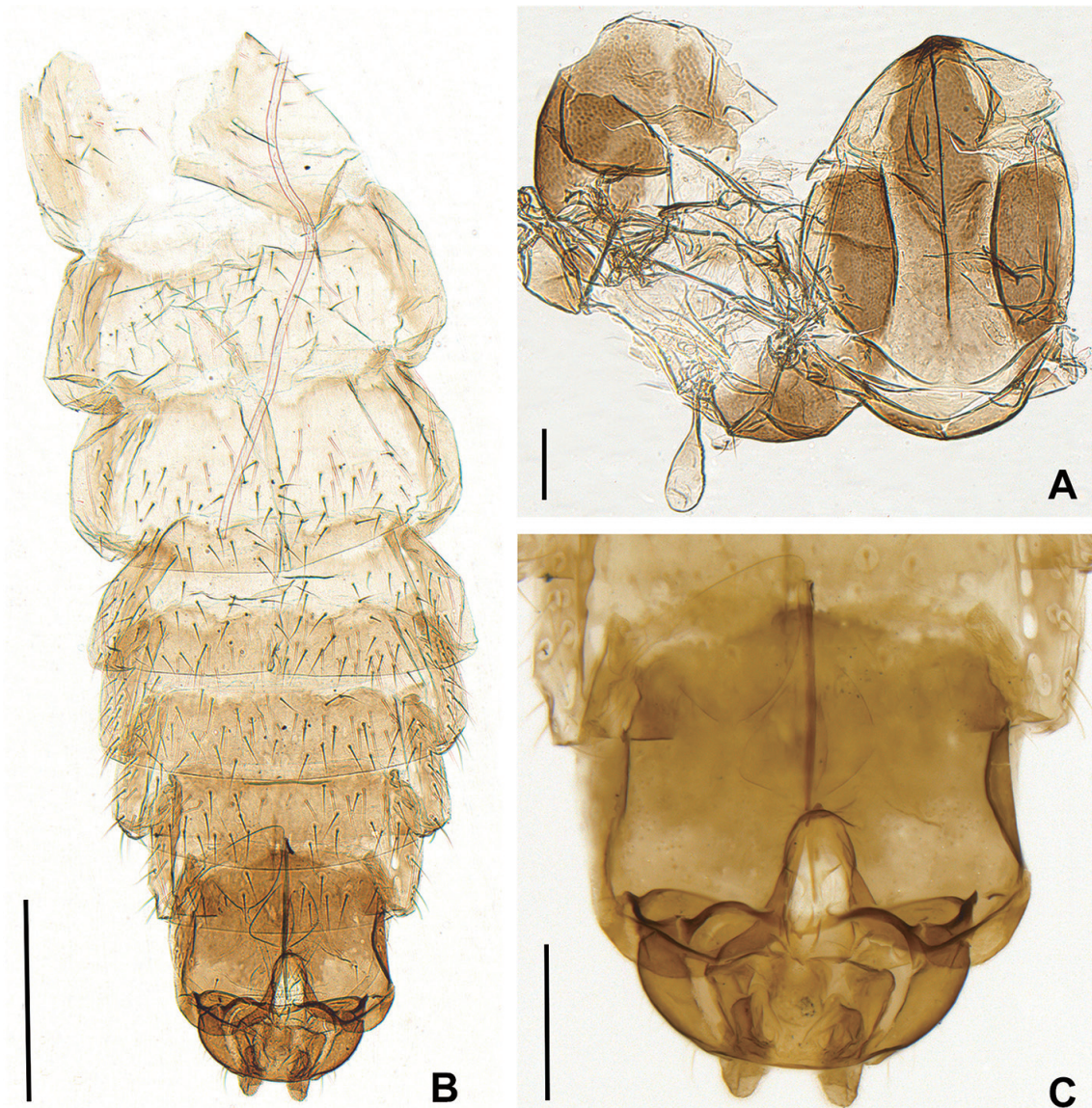


Figure 7. Adult female of *Dicrotendipes sinicus* Lin & Qi *sp. n.* A, thorax dorsal view, scale bar = 200 μ m; B, abdomen, scale bar = 500 μ m; C, genitalia, scale bar = 200 μ m. First published by Qi et al. (2018), Attribution-NonCommercial-NoDerivatives 4.0 International (CC BY-NC-ND 4.0).

Abdominal setation. Segment II–III with 2–3, 2 L setae; IV with 2 L setae; V–VIII with 4 L taeniae.

Anal lobe well-developed, with fringe of 43–57, 50 taeniae, 200–500, 393 μ m long. Genital sac of male extending beyond anal lobe.

Larva, 4th instar ($n = 6$, unless otherwise stated)

Body slender (Fig. 1E), length of 4th instar 7.63–8.40, 8.00 mm; head capsule (Fig. 9A) 400–450, 416 μ m long, 370–425, 388 μ m wide, length/width 1.05–1.09, 1.07; mental and mandibular teeth dark brown.

Dorsal surface of head. With frontal apotome, clypeus, and labral sclerite 2 present.

Antenna (Fig. 9B). With five segments. Antennal segment length (in μ m): 50–58, 53; 10–15, 12; 7–8, 8; 6–8, 8; 3–5, 4; AR 1.61–1.70, 1.62. Antennal blade 18–25, 21 μ m long. Ring organ situated in proximal 1/2 of basal segment. Seta absent.

Labrum. S I subapically plumose; S II simple; S III and S IV normally developed. Clypeal seta S3 long, simple. Labral lamella normally developed. Pecten epipharyngis simple, consisting of 3–7, 4 robust, blunt teeth. Premandible 60–83, 72 μ m long, with 4 teeth; premandibular brush well developed. Maxilla with one long lacinial chaeta, palp normally developed.

Mandible. Mandible 138–155, 146 μ m long, 45–63, 52 μ m wide, with one ventral apical tooth,

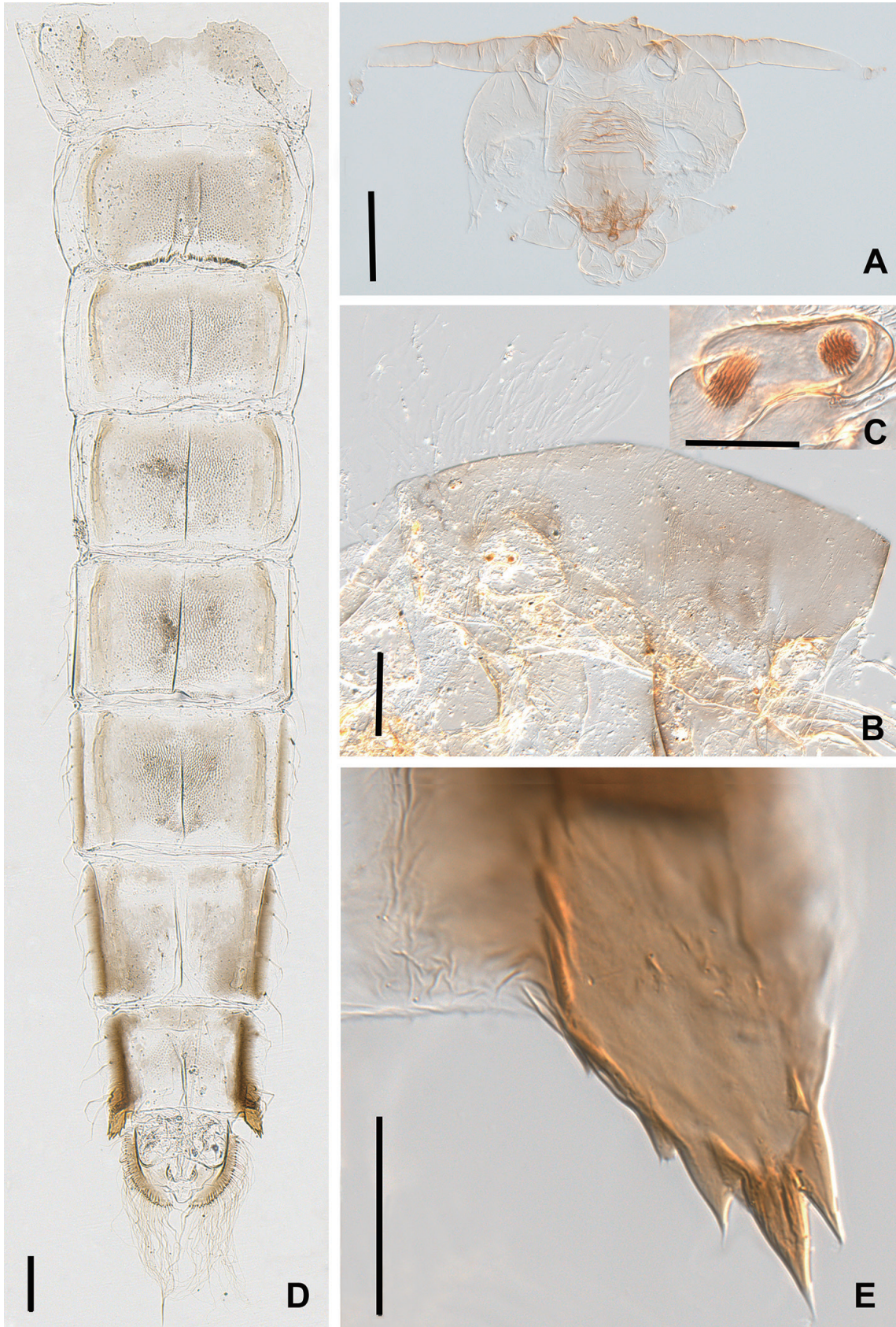


Figure 8. Pupal exuviae of *Dicrotendipes sinicus* Lin & Qi **sp. n.** A, frontal apotome and ocular field, scale bar = 200 μm ; B, thorax, scale bar = 200 μm ; C, basal ring, scale bar = 50 μm ; D, tergites, scale bar = 200 μm ; E, posterolateral comb of segment VIII, scale bar = 50 μm . First published by Qi et al. (2018), Attribution-NonCommercial-NoDerivatives 4.0 International (CC BY-NC-ND 4.0).

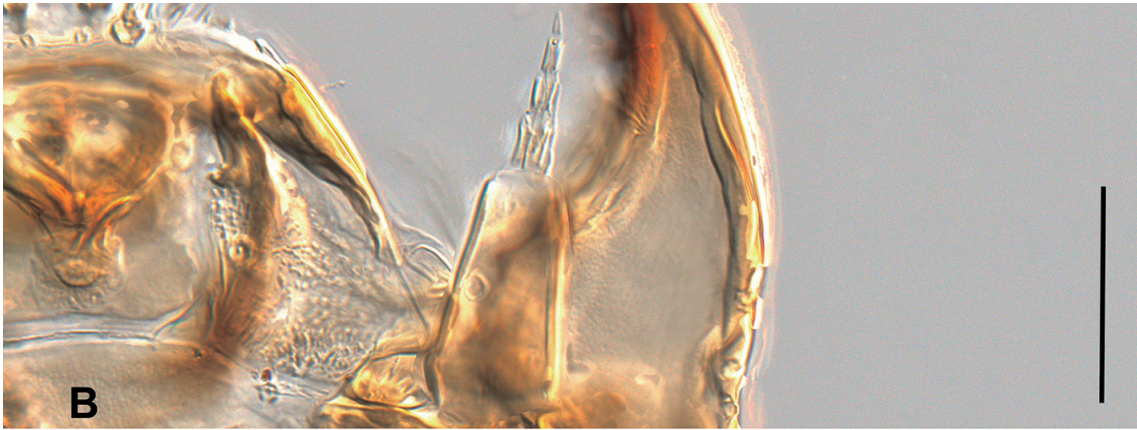


Figure 9. Larval head capsule of *Dicrotendipes sinicus* Lin & Qi **sp. n.** A, whole larval head capsule; B, larval antenna. Scale bars = 50 μ m. First published by Qi et al. (2018), Attribution-NonCommercial-NoDerivatives 4.0 International (CC BY-NC-ND 4.0).

three ventral inner teeth, one dorsal, dark apical tooth present; two outer mandibular setae present; seta subdentalis narrow, with pointed apex, 15–20, 17 μm long; seta interna well developed with three main plumose branches with long stalks. Pecten mandibularis well-developed, with eight long lamellae, the longest seta 25–48, 37 μm long.

Mentum. Mentum 115–133, 125 μm wide, with a rounded, trifid median tooth, six pairs of lateral teeth, regularly decreasing in size laterally; ventromental plates 90–108, 101 μm wide, MVR 1.19–1.37, 1.24, medially separated by about twice width of median mental tooth, with distinct striae. Postmentum 183–188, 186 μm long. Seta submentum simple, 50–70, 62 μm long.

Body. Procercus short, 18–50, 33 μm long, always bearing eight long anal setae, the longest seta 350–400, 384 μm long. Lateral and ventral tubules absent. Anal tubules of normal size, digitiform.

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Short comment on Chironomidae (Insecta: Diptera) from Brazil's Federal District

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Abstract

There is much left to learn about the diversity of Chironomidae in Brazil. To help to resolve this problem, a preliminary study of the Chironomidae present in a stream located in center of Brazil was proposed. The aim of this research was to provide a first record of the Chironomidae assemblage at Sarandi stream, in the Brazilian Cerrado. Samples were taken using a handheld D-net from the stream in October 2009. The samples were transported to the laboratory where the material was processed and the Chironomidae specimens were slide mounted, counted and identified to genera. Ninety individuals belonging to 15 genera were found, Chironomini was the richest tribe, with seven genera, while Tanytarsini showed the highest abundance of individuals (55.6%). Overall, *Rheotanytarsus* (20%), *Tanytarsus ortonii*-group (16.7%) and *Lopescladius* (14.4%) were the most abundant genera.

Introduction

The Brazilian Cerrado biome is a parallel to the African Savannah; it covers 25% of the core of the Brazilian territory (Carvalho et al. 2009) (Fig. 1) and is considered a hotspot area of biodiversity (Brasil 2006). The biome has a particular and highly significant role in Brazilian aquatic resources since it contributes to the formation of eight out of twelve of Brazil's largest river basins (Lima and Silva 2008). Estimates indicate there are 6,000 species of trees and 800 of birds. In the last decades, the Cerrado has been seen as an alternative to the Amazonian region for agriculture purposes (MMA 2007); aside from agricultural use, up to 60% of the biome is preserved (Sano et al. 2001).

Although the biology and ecology of the Chironomidae in some of the Brazilian biomes has been extensively studied (Sanseverino and Nessimian 2008, Silva et al. 2009), the composition of the aquatic entomofauna in a great part of the Cerrado remains unclear. The limited knowledge in chironomid biodiversity is not necessarily due to lack of research conducted within the Cerrado, but rather the extensive area that the biome occupies. Additionally, few focus exclusively on Chironomidae (Sonoda et al. 2009a, Sonoda et al. 2009b, Saito and Fonseca-Gessner 2014, Mazão et al. 2016). When considering the Federal District region, we notice that most studies analyze the entire aquatic entomofauna and the influence of human activities on

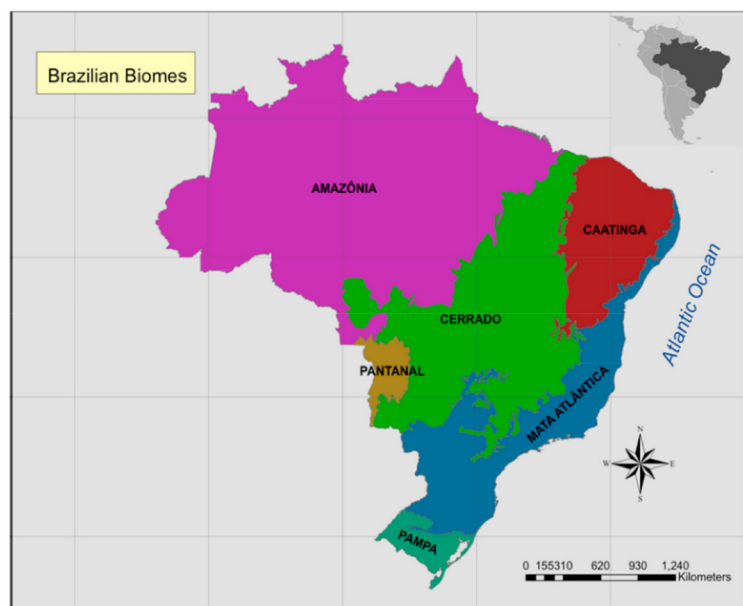


Figure 1. Map of Brazilian Biomes, Cerrado in green. From Merrick et al. (2019).

it (Silva 2007); there are few gray studies available focusing on the chironomid fauna in the Federal District in which results were not published.

While many studies registered Brazil's chironomid fauna (Oliveira and Laurindo 2011, Floss et al. 2012, De Toni et al. 2014), it is believed that only a small fraction of members of the family inhabiting aquatic ecosystems from the Neotropical Region have been described and recognized (Armitage et al. 1995, Spies and Reiss 1996, Ferrington 2008). The last update of the taxonomic catalogue of Brazilian fauna (MMA 2021) registered 632 species in Brazil, comprising 100 genera. Fittkau's remarkable Amazonian survey registered 200 species and stated that there are at least 1,000 species in the region (Fittkau 2001), illustrating the urgent need for Chironomidae biodiversity surveys.

Knowledge of the taxonomic composition of Brazilian fauna will provide key information for managers in charge of developing and maintaining conservation areas (Romero 2009) and is an additional step in the direction towards understanding the role of these aquatic organisms that play a major role in biological monitoring programs (Vondracek et al. 2005, Rodrigues et al. 2019).

The aim of this study is to provide an overview of the taxonomic composition of Chironomidae in a stream within Brazil's Federal district. Ultimately, this will improve the knowledge of Brazil's chironomid fauna.

Material and methods

The Sarandi stream spring is located in the Embrapa Cerrados compound (Planaltina-DF) and it runs towards the Mestre d'Armas River in the Paraná River Basin (Martins et al. 2002). At the sampling reach (15°35'41.5" S and 47°44'45.3" W) there were rapids, waterfalls and pools and the stream sediment was sandy with interspersed gravel and cobbles. Deposits of decaying leaves from the abundant riparian vegetation were found throughout the stream bottom.

The climate in the study region is tropical with marked hydrological periods, rainfall is concentrated from November to January and the drought period occurs mostly between June and August (Carvalho 2005). Sampling occurred before the beginning of the rainy season, in October 2009.

Chemical and physical variables of water were analyzed in field (temperature, pH, dissolved oxygen, conductivity) using a portable multiparameter device (Hanna HI 93703) and in the laboratory (nitrite, nitrate, ammonium, phosphate, fluoride, magnesium) using ionic chromatography (Compact IC 761) and UV spectrophotometry to measure total phosphorus (APHA 1998). A handheld D-net was used for sampling (0.2 mm mesh size); two replicates of 5 minutes each were taken from different habitats along the stream reach. The samples were transported to the laboratory where each sample was washed under running water over a 0.2 mm mesh sieve (Trivinho-Strixino and Strixino 1998). The material retained within the sieve was placed in clear plastic trays over a trans-illumination device and insects were sorted and preserved in 70% alcohol (Pinder 1989). The Chironomidae larvae were slide mounted, counted, and identified to genera following Trivinho-Strixino and Strixino (1995).

The community was analyzed both as raw numbers and percentage of individuals per genus. McIntosh's diversity (McIntosh 1967), Margalef's richness and McIntosh's evenness (calculated considering McIntosh diversity) were calculated. The formulae are presented below:

$$\text{Margalef's Richness: } D = (S-1)/\log N \quad \text{equation 1}$$

$$\text{McIntosh's Evenness: } E = (N-U)/[N-(N/\sqrt{S})] \quad \text{equation 2}$$

$$U = \sqrt{\sum(n_i)^2} \quad \text{equation 3}$$

$$\text{McIntosh's Diversity: } D = (N-U)/(N-\sqrt{N}) \quad \text{equation 4}$$

Where n = number of individuals from each taxon, N = total number of individuals, S = number of taxa.

McIntosh (1967) proposed equation 3, however it is extremely influenced by the sample size. Afterwards, Pielou (1969) proposed equation 4 to eliminate the negative effect of the sample size. We opted to use McIntosh's index because it better suits samples with smaller sizes as it squares abundance and also dominant taxa are weighted greater than rare ones (Semensatto Jr. 2003).

Evenness values range from 0 and 1, values closer to 1 suggest better distribution/participation among taxa.

Results

Ninety Chironomidae larvae were collected from the Sarandi stream, comprising 15 genera (Table 1). Chironomini was the most abundant tribe with seven genera, whereas Tanytarsini had a higher abundance of individuals (55.6%). Three Orthocladiini genera were present and accounted for 25.6% individuals of the entire community. Pentaneurini was present with only one genus and in low quantity.

Table 1. List of genera and corresponding percent abundance collected at the Sarandi stream.

Taxon	Percentage
Chironomini	
<i>Beardius</i> Reiss & Sublette, 1985	2.2
<i>Endotribelos</i> Grodhaus, 1987	7.8
<i>Lauterborniella</i> Thienemann & Bause, 1913	4.4
<i>Nimbocera</i> Reiss, 1972	4.4
<i>Polypedilum</i> Kieffer, 1912	4.4
<i>Stenochironomus</i> Kieffer, 1919	1.1
Tanytarsini	
<i>Tanytarsus ortonii</i> -group Lin et al., 2018	16.7
<i>Constempellina</i> Brundin, 1947	1.1
<i>Rheotanytarsus</i> Thienemann & Bause, 1913	20.0
<i>Stempellina</i> Thienemann & Bause, 1913	3.3
Tanytarsini #1 (unknown)	7.8
Orthocladiini	
<i>Corynoneura</i> Winnertz, 1846	7.8
<i>Lopescladius</i> Oliveira, 1967	14.4
<i>Nanocladius</i> Kieffer, 1913	3.3
Pentaneurini	
<i>Thienemannimyia</i> Fittkau, 1957	1.1

The most numerous genera were *Rheotanytarsus* which were the most abundant (20.0%), followed by the *Tanytarsus ortonii*-group (16.7%) and *Lopescladius* (14.4%). The *T. ortonii*-group contains species previously placed in *Caladomyia* Säwedal, 1981 (Lin et al. 2018). *Corynoneura*, *Endotribelos* and Tanytarsini #1 each represented 7.8% of the total taxa. Tanytarsini #1 did not resemble a known genus and it most likely represents a new species. Three genera, *Constempellina*, *Stenochironomus* and *Thienemannimyia*, were found in extremely low numbers, with one individual each (Table 1). We attempted to rear larvae of *Constempellina* to the adult stage, however this was unsuccessful. Our observations showed that these larvae are quite sensitive and die only a few minutes after they are taken from the stream.

Indices values were as follows: McIntosh's diversity = 0.7; Margalef's richness = 2.2; McIntosh's evenness (U) = 0.9. Water quality values registered during sampling are given in Table 2.

Discussion

Some studies on the Brazilian Federal District consider the entire aquatic insects' communities, nonetheless they identify the Chironomidae larvae only to family. This type of analysis certainly decreases the time required to execute environmental studies since identification of Chironomidae to genera is time consum-

Table 2. Water quality variable values at the sampling reach of the Sarandi stream.

Water variable	Value
pH	4.5
Conductivity ($\mu\text{S}/\text{cm}$)	6.0
Temperature ($^{\circ}\text{C}$)	21.7
Dissolved oxygen (mg/L)	7.3
Cl (mg/L)	0.23
Na (mg/L)	0.23
Ca (mg/L)	0.44
Fluoride	0.00
Nitrate	0.00
Magnesium	0.00

ing; however significant information is lost, such as the taxonomic composition, functional feeding groups, among others. In this context, a study of the same watershed examined the aquatic macroinvertebrates community composition along the Mestre d'Armas River (Silva 2007). The Chironomidae were identified to subfamily which severely limits accurate comparison with our data. They also recorded low pH levels, and this seems to be a constant among other rivers in this part of the Cerrado (Salcedo 2006, Fernandes 2007).

As the Cerrado is a biodiversity hotspot (Hopper et al. 2016), we expected to find higher taxa richness – this would certainly be the case if more streams were sampled. Fernandes (2007) conducted a study at a different stream in the same region, and registered nine taxa, eight of which were not found in our study. Combining taxa from our study and that of Fernandes (2007) yields a total of 24 taxa, increasing the richness.

Studies from other Cerrado regions have yielded higher generic richness, with 45 genera found at the State of Goiás (Mazão and Bispo 2016) and 36 at São Paulo State (Saito and Fonseca-Gessner 2014). This supports our suggestion that the true richness value in the Federal District is likely much higher than presented here.

Rheotanytarsus was documented as one of the most abundant taxa in several Brazilian studies (Sonoda et al. 2009b, Sonoda et al. 2010, Floss et al. 2012); this numerical dominance is related to agricultural land use (Sonoda et al. 2009a, Sonoda et al. 2018). Its abundance is also documented in other neotropical countries, like Trinidad (Helson et al. 2006).

At the other extreme, we found multiple rare genera, *Constempellina*, *Stenochironomus* and *Thienemanimyia*, which contributed with only one specimen for each genus. These genera usually are associated with good environmental conditions (Eintrekin et al. 2007, Saito and Fonseca-Gessner 2014). The abundance of *Stenochironomus* in Neotropical streams is quite variable as some authors report their rarity (Gonçalves Jr et al. 2007), while others have documented great abundances of this genus (Saito and Fonseca-Gessner 2014, Mazão and Bispo 2016). *Stenochironomus* larvae are important indicators for the preservation status of riparian forests, as they are known as shredders (Silva et al. 2009, Santos and Rodrigues 2015). Despite the low quantity of shredders in Neotropical communities (Boyero et al. 2011), they are good indicators of environmental impacts since their abundance decreases when riparian forests are deforested (Sonoda et al. 2009a).

Conclusion and outlook

The present report of Chironomidae in the Federal District provides critical information to the scientific community that enhances the understanding of chironomid diversity within the Cerrado and provides evidence that continued efforts should be made to add information to this list, ultimately enhancing our knowledge of the spatial occurrence and elucidating the dimensions of biodiversity richness of the Chironomidae.

Several questions remain unanswered and should be addressed in future work, such as including local seasonality patterns for specific chironomid genera, influence of the weather, hydrology and other environmental attributes, and the family's influence on litter decomposition process.

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***Prosilocerus* Kieffer, 1923 shares morphological synapomorphies with Prodiamesinae**

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Abstract

In their comprehensive analysis Cranston et al. (2012) quite unexpectedly recovered a monophyletic Orthoclaadiinae, except that *Prosilocerus* Kieffer, 1923, was recovered as a representative of Prodiamesinae. In this short communication I show that *Prosilocerus* shares morphological synapomorphies with other Prodiamesinae – namely (i) an indirect tracheal connection of the pupal thoracic horn to the adult spiracle and (ii) a parallel-sided rami of female gonapophysis IX.

Introduction

In their comprehensive analysis of molecular data, Cranston et al. (2012) quite unexpectedly recovered a monophyletic Orthoclaadiinae, despite the seeming lack of any morphological synapomorphies, but excluding *Prosilocerus* Kieffer, 1923. The placement of this genus, recovered as sister group to Prodiamesinae (Cranston et al. 2012), “can be reconciled on the basis of some morphology” listing large plates of the submentum, fringed anal lobes of the pupal abdomen and “and diverse volsellae in the male hypopygium”. However all these features, as Cranston et al. (2012) note, are, symplesiomorphies, and thus cannot corroborate the affinity of *Prosilocerus* to Prodiamesinae.

Additionally, *Prosilocerus* lacks another common symplesiomorphy of the Prodiamesinae – the MCu crossvein. Cranston et al. (2012) note that absence of this character state in *Prosilocerus* renders morphological diagnosis of the Orthoclaadiinae even more problematic. It is also notable that *Prosilocerus* have only two precorneal setae while the rest of the Prodiamesinae for which immature stages are known, have three precorneals (Sæther 1986). Notably, Sæther (1986) had used the leg sheath arrangements and MCu presence and position in his key to the Prodiamesinae pupae, but not a type of the tracheal connection.

Coffman (1979) had examined the connection between the pupal thoracic horn and the thoracic spiracle of the pharate adult as a possible phylogenetically informative character in Chironomidae. He found all examined representatives of Prodiamesinae (*Monodiamesa* Kieffer, 1922, *Odontomesa* Pagast, 1947, *Prodiamesa* Kieffer, 1906) have an indirect connection between the thoracic horn and a spiracle (Coffman 1979: p.43). This type of connection is represented by a bundle of the thin tracheoles originating from the spiracle and approaching close to the base of the thoracic horn, in contrast to the direct connection by a trachea in Podonominae and Tanypodinae, or the absence of such a connection, as in Orthoclaadiinae (Coffman 1979) (Figs 1A, B). Sæther (1977) noted that parallel rami of gonapophyses IX in the female genitalia could be

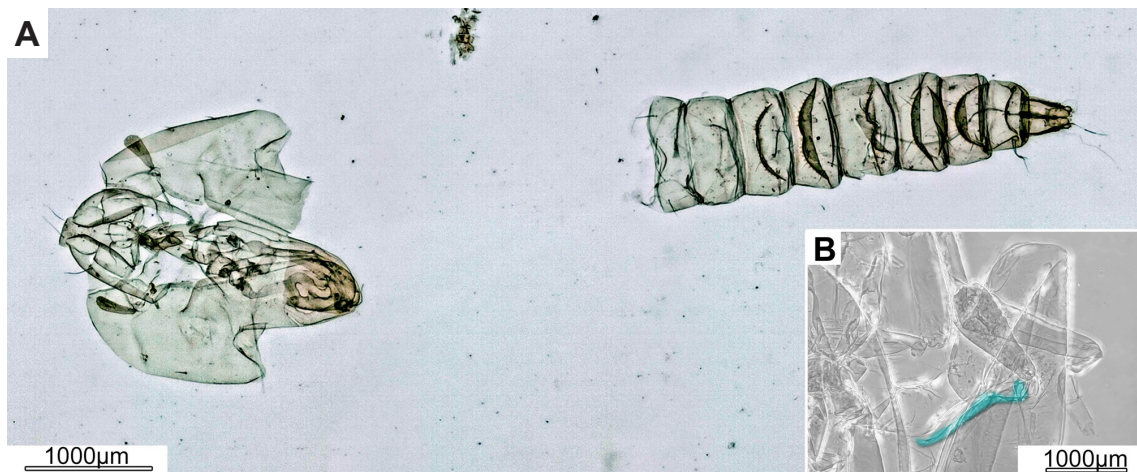


Figure 1. A. *Podochlus* sp. pupa, habitus (brightfield microscopy); B. same, thoracic horn, tracheal connection to the spiracle is marked in blue (phase contrast).

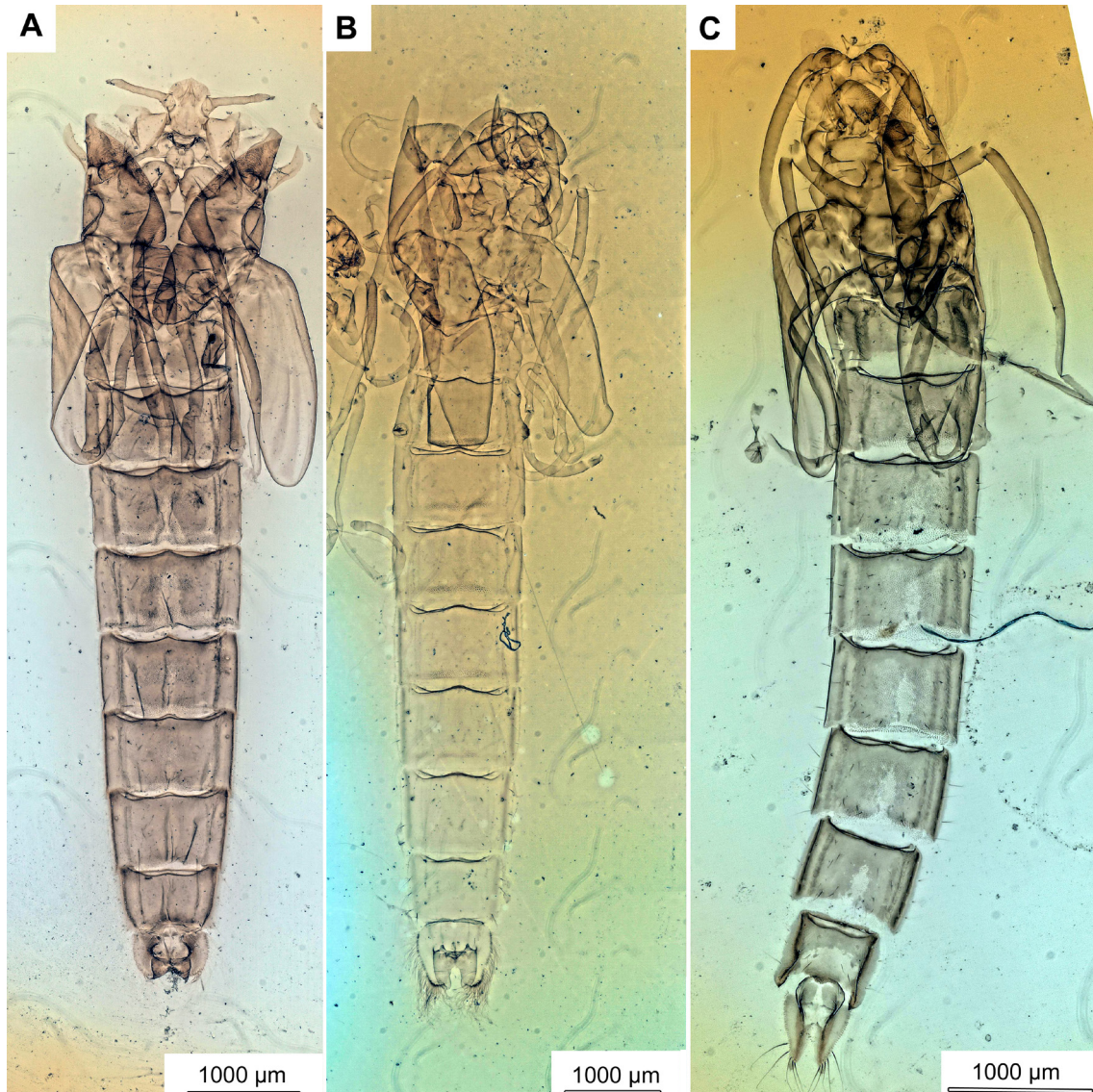


Figure 2. Pupal exuviae of Prodiamesinae. A. *Prodiamesa olivacea*; B. *Monodiamesa bathyphila*; C. *Odontomesa fulva* (brightfield microscopy).

considered as synapomorphic for the Prodiamesinae (also true for *Compteromesa* Sæther, 1981, for which only adults are known (Sæther 1985)).

In this short communication I examine the condition of the abovementioned characters in *Propiloscerus*, to assist in elucidation of its phylogenetic position.

Materials and methods

The following material was examined from the ethanol and slide collection of Zoologische Staatssammlung München (ZSM):

Propiloscerus lacustris Kieffer, 1923, from Kleiner Teufelsteich, Teichland, near Cottbus, Germany, collected in April 1962. No legator name. Numerous pupae and females preserved in ethanol.

P. lacustris pupal exuviae from Großer Plöner See, near Plön, Germany, collected by Lenz, April 24th, 1922. Canada balsam mounted pupal exuviae.

Prodiamesa olivacea (Meigen, 1818) from Schlitz, May 1st, 1985 collected by Hereberg (ZSM barcoding project number (“DIP 00254”). Numerous females.

P. olivacea from “Bäm”, Canada balsam mounted pupal exuviae, Thienemann collection, no collection date (as “*Prodiamesa praecox*”).

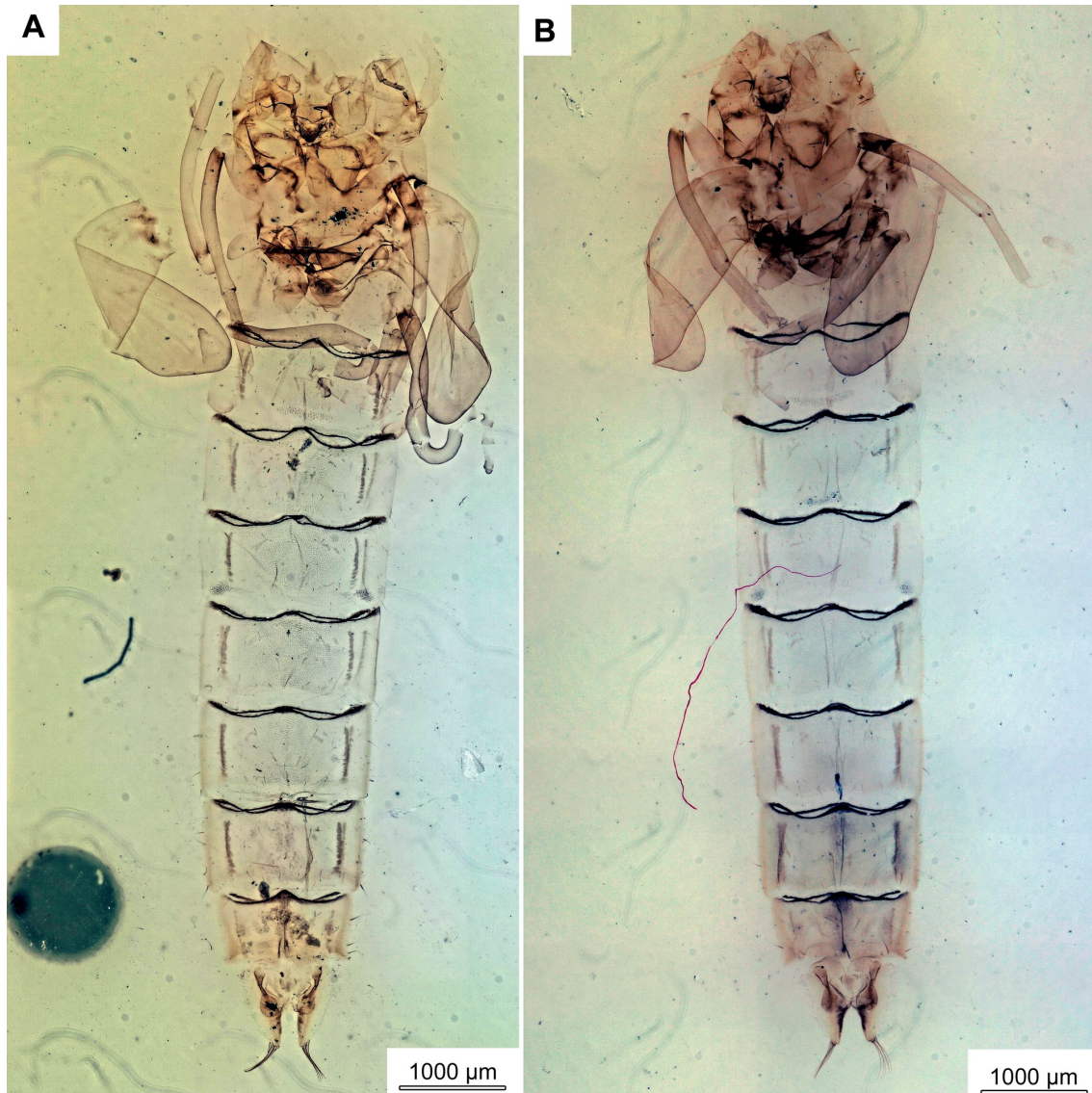


Figure 3. *Prosilocerus lacustris* pupal exuviae. A. specimen 1; B. specimen 2 (brightfield microscopy).

Monodiamesa bathyphila (Kieffer, 1918) Germany, 1937. Collected by Thienemann. Canada balsam mounted pupal exuviae.

Odontomesa fulva (Kieffer, 1919). Kalibach, Fliede, Hessen, Germany, May 1st, 1952, collected by E. J. Fittkau (# 387). Canada balsam mounted pupal exuviae.

Podochlus sp. South Chile, Lago Todos los Santos, Rio Bonito, December 5th, 1969. Collected by F. Reiss. Canada balsam mounted pupal exuviae.

Material was documented on a VHX-6000 digital microscope, following standard procedures (e.g. Haug et al. 2011). Ring light illumination was used with white background. Each image was recorded as a composite image combining images (“frames”) of different focal plains (“z-stack”) and several adjacent images to form a large panorama; processing was performed with the built-in software. Images were additionally recorded with several exposure times (HDR; Haug et al. 2011). Additionally, for the documentation of finer details, such as tracheal connections used BZX-900 fluorescent microscope, observing specimens using a brightfield, phase contrast microscopy as well as TRIC fluorescence (Haug et al. 2011). Individual photos subsequently stacked into the sharp composite using PICOLAY open software (www.picolay.de).

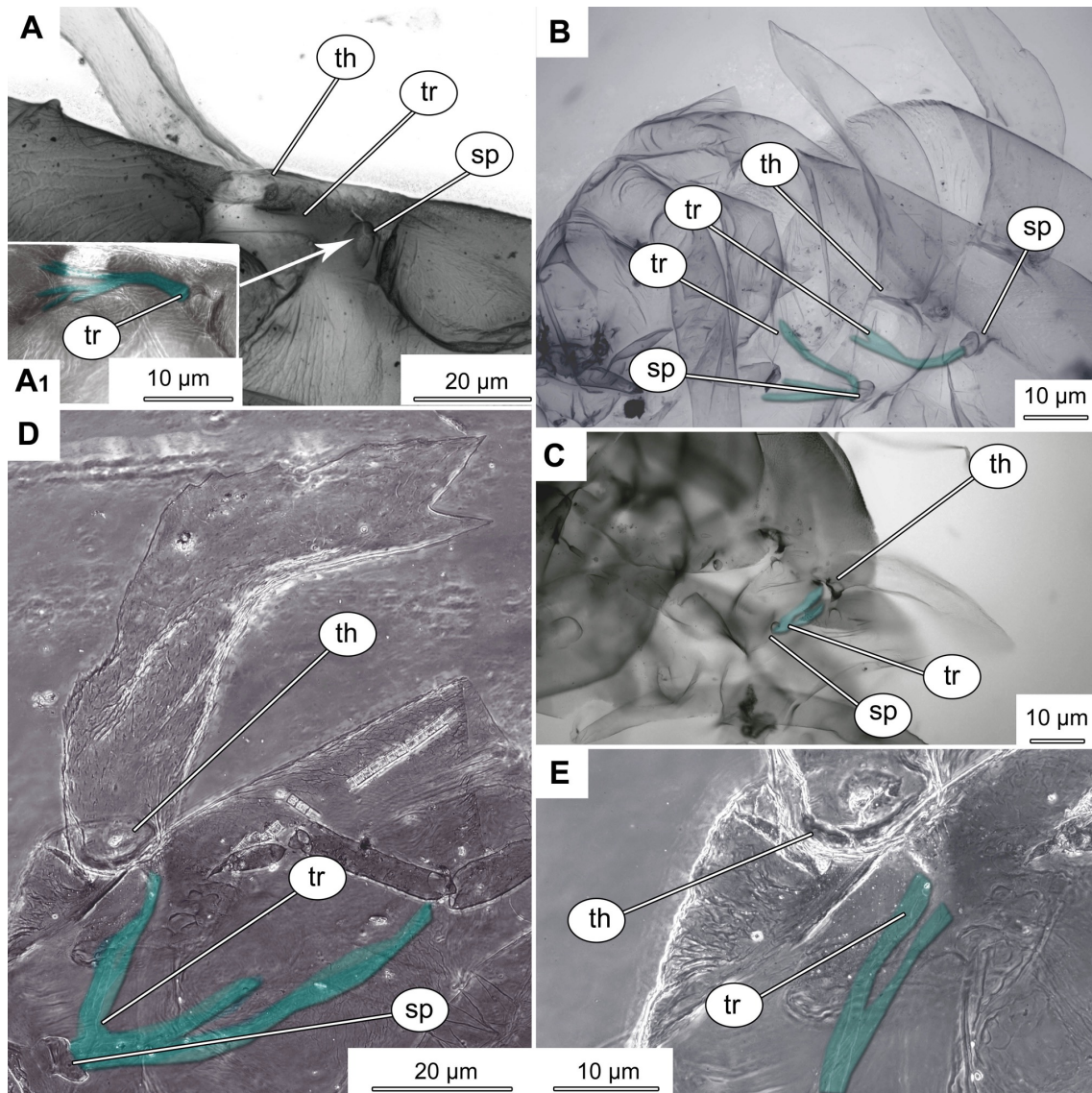


Figure 4. Indirect tracheal connections in Prodiamesinae pupae. A and A1. *Prodiamesa olivacea* (brightfield microscopy); B. *Monodiamesa bathyphila* (brightfield microscopy); C. *Odontomesa fulva* (brightfield microscopy); D, E. *Propsilocerus lacustris* (phase contrast). Abbreviations – th: thoracic horn; tr: tracheoles; sp: spiracle.

Results

My examination of the material revealed that *Propsilocerus* shares both of the abovementioned apomorphic character states with all other Prodiamesinae examined (Figs 2A-C, 3A-B, 4A-F, 5A-D).

Examination of the base of the thoracic horn and the opening of the adult spiracle in *Propsilocerus lacustris* pupae and pupal exuviae revealed a loose bundle of tracheoles extending between the opening of the spiracle and almost to the very base of the thoracic horn, as in all examined Prodiamesinae (Figs 4A-F).

In the female genitalia, the paired rami of the gonapophyses IX of *P. lacustris* were distinctly parallel, similarly to examined *P. olivacea* and in accordance with the literature concerning other Prodiamesinae (Sæther 1977: fig. 35.). It is notable that rami in *Propsilocerus* appear more weakly sclerotized than in other Prodiamesinae (Figs 5A-D, Sæther 1977: fig. 35.).

Discussion

Examination shows that *Propsilocerus* is resolved not only as an ingroup-Prodiamesinae based on the multigene phylogeny of Cranston et al. (2012) but also shares important morphological synapomorphies with Prodiamesinae, thus further cementing location of *Propsilocerus* within this monophyletic subfamily.

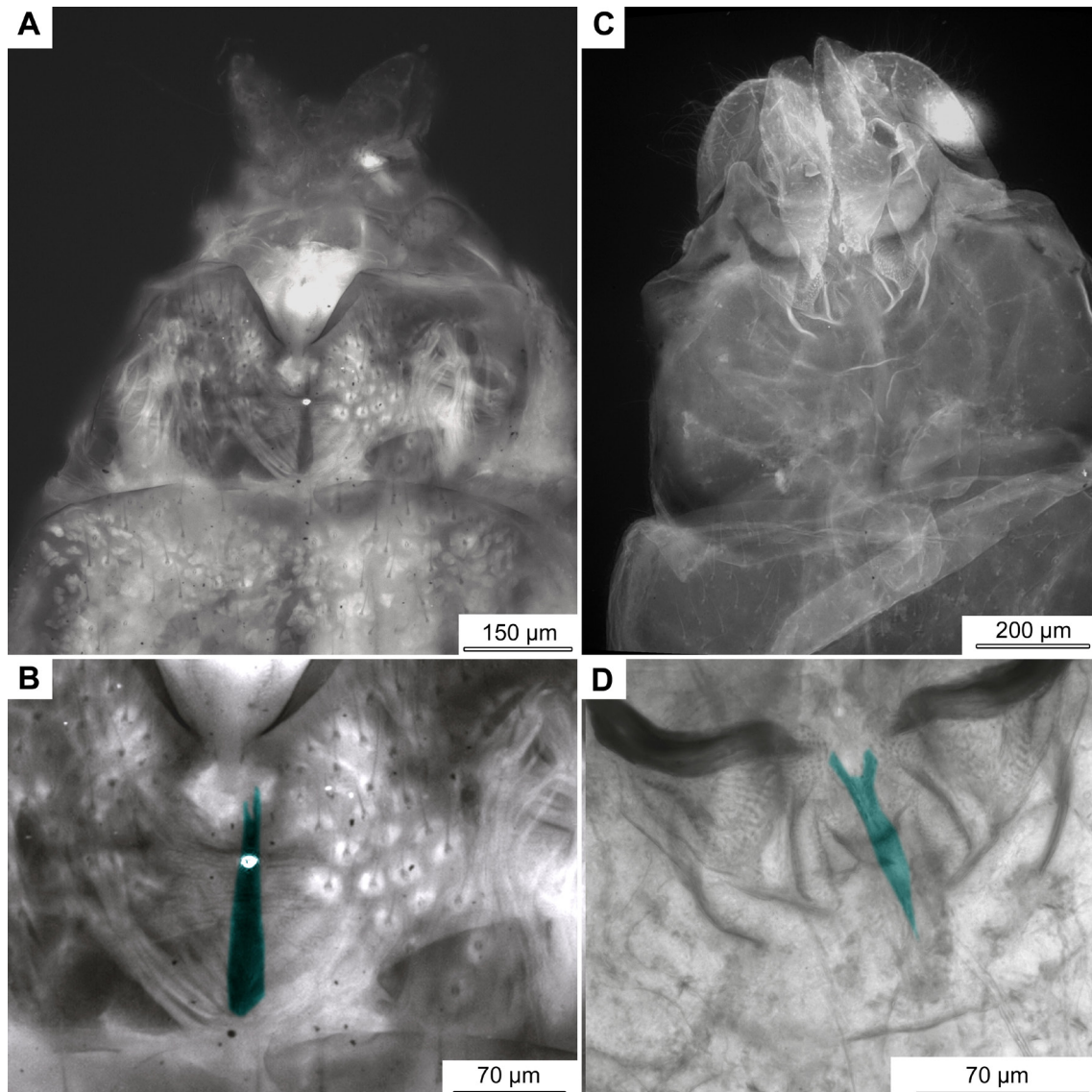


Figure 5. Female genitalia of Prodiamesina. A, B. *Prodiamesa olivacea* (TRIC Fluorescence); C. *Propsilocerus lacustris* (TRIC fluorescence); D. *Propsilocerus lacustris*. Rami of gonapophyses marked in blue (bright-field microscopy).

Status of the *Propsilocerus* as an ingroup-Prodiamesinae sheds light on the persistence of morphological analysis in the age of molecular systematics. It also shows the danger of implicit definition of the groups by plesiomorphies. For example, in most fossil Prodiamesinae (I.e. *Cretadiamesa* Veltz, Azar et Nel, 2007, *Lebanodiamesa* Veltz, Azar et Nel, 2007) their purported affinities with Prodiamesinae appears based on plesiomorphies such as the presence and location of veins R_{2+3} and MCu, rather than any meaningful synapomorphies (Veltz et al. 2007, Baranov et al. 2019). By defining fossil Prodiamesinae using such characters, we are in danger of including in Orthoclaadiinae some fossil representatives of Prodiamesinae that lack crossvein MCu. The fact that Orthoclaadiinae (as of now) still lack morphological synapomorphies (Sæther 1977; Cranston 2000; Cranston et al. 2012) confuses the matter. Future studies of extant material through all the ontogenetic stages, as well as fossils, may eventually resolve this problem, and help us to locate morphological support for the unexpected Orthoclaadiinae monophyly (Cranston et al. 2012).

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The 21st International Symposium on Chironomidae – 2022

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Due to the COVID-19 pandemic, the 21st International Symposium on Chironomidae has been postponed to summer 2022. Since several other international conferences are expected in a similar schedule, such as the International Congress of Entomology or the Congress of the International Society of Limnology, we fixed the dates of the symposium trying to avoid overlapping as much as possible.

The 21st International Symposium on Chironomidae is now expected to be held in Tsukuba, Japan, between the 3rd and the 9th of July 2022.

We hope that the COVID-19 pandemic will be under control next year and we plan to organize a physical symposium here in Tsukuba. However, we are still open to a hybrid meeting with online presentations for those who would not be able to come to Japan.

Early July in Japan is usually a wet season with daily rain, however the temperatures should be still comfortable for most participants, compared to the hottest, moist days of late summer.

We plan to organize the symposium through sessions focusing on different topics such as systematics, ecology, biomonitoring or recent advances in molecular biology. Tsukuba National Museum of Nature and Science is hosting Manabu Sasa's collection of Chironomidae specimens and we plan to make these specimens available for a Taxonomy workshop.

Basic information about Tsukuba was already published in Chironomus No32 (Cornette 2019) and we will provide further information as soon as the basic framework of the symposium is ready, but please note the dates of the Symposium in your calendars.

We wish you all good health and look forward to welcome all of you in Tsukuba next year!

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L. C (Clive) V. Pinder, 6 December 1943 - 31 July 2021. An appreciation from 'far away and long ago'

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My first professional encounter with Clive Pinder, who has died aged 77 in his beloved adopted English county of Dorset, was also a first social meeting. I recall it was in a thatched pub, the Kings Arms, Wareham, early in the 1970s. I was off the London train, newly into a PhD, having been encouraged to visit the rising guru of the study of midges - the fly family Chironomidae - at the Freshwater Biological Association's River lab. It was the first of many discussions in comparable venues from Florida to Munich, as our collaborations continued over the next 20 years. I suspect Clive, one of my examiners, would have preferred my PhD viva to have been held entirely in the East London pub close to Queen Mary College instead of making a brief celebratory visit before the 'last train home'. But be sure to recognise that this 'life-work' balance was exactly that, and with broad overlap between the two. Thus an eminent Japanese colleague Professor Mutsunori ('Nori') Tokeshi wrote recently >>*As you have warmly described, Clive and I have forged a close relationship over many years, even after I moved to Japan spending more time on marine rather than freshwater research. My year-long stay at Pinder's family home in 1979-1980 started everything: my career in science, my deep love of things British and, indeed, overall approaches to life. It is no wonder therefore that I cannot avoid thinking about him and his family since the news first came Clive was the first English gentleman that I came into contact, lively and yet measured, conscientious and full of wit, from whom I learned an awful lot, in particular the philosophy of enjoying life. Of course I also learnt science from him, but life is bigger than that, as we all know. I just cannot believe that, the next time I have an opportunity to go to my house in Essex (yes, I still have it for my frequent visits to the UK, at least before the Covid-19), I won't be able to phone him up to arrange a pub meeting.* <<

Since I agree completely with these sentiments, I'll move on to examine the role of Clive in making a difficult taxonomic group not only accessible, but mandatory in understanding freshwater ecology and allowing palaeoecologists to use midge subfossils in past climate reconstruction. His published contributions remain justifiably highly cited to the current day, although the majority were produced between 1976 and the turn of the century, when he took retirement.

Clive's educational background was of an agricultural scientist (Newcastle University), not specifically that of an entomologist or freshwater biologist. From early days in Nottinghamshire he was a naturalist, retaining a life-long interest in birds (as I do). It was at The Freshwater Biological Association (FBA) in Wareham, Dorset, a post-PhD relocation with his wife Carolyn, that Clive discovered the huge lacunae in understanding of the most diverse of freshwater organisms. The non-biting midges (chironomids) by abundance alone just had to be important in limnology. But when Clive started in 1969 the field was near inaccessible due to the diversity of the aquatic stages, the lack of associations with the named adult stages (the midges) and confusingly incompatible taxonomic schemes according to nationality and publication language of the scattered researchers. The few English publications were outdated and lacked compatibility between the USA, Japan and continental Europe. German limnologists led by August Thienemann had created a framework built on the immature-stages, but this needed major technical resources and most publications were in German. In UK at that time, the need for identification of larval and pupal immature stages was understood, but the classification was based on male adults that were previously pinned dry but needing good microscope slide mounts for modern work.

Into this morass dived Clive, provided with strong support from the FBA including an (very attractive, Nori, *pers. comm.*) artist-assistant Angela Matthews who tirelessly produced artwork for illustration of the hundreds of male genitalia - a major diagnostic feature of the adult stage. Clive argued that without stable nomenclature and regionally compatible identifications for species and genera, little progress was possible with the immature stages. Larvae were reared, slides made, publications in German and Russian were translated and a *Key to Adult Males of British Chironomidae* appeared as FBA Scientific Publication No.37 (Pin-

der, 1978), to widespread acclaim for the clarity ('user friendliness') of a modern synthesis of the British fauna. No hornet's nest was stirred up; clearly it was time for such studies to be broadened taxonomically and geographically. Plans were made for strategically located like-minded researchers with institutional support to jointly solve disparate problems in morphology and systematics, with a view to review and revise the entire northern hemisphere larvae, pupae and eventually adult males of the total chironomid biota.

Nowadays it seems incredible a research project involving up to a dozen authors could produce 3 substantial volumes in 6 years, pre-word processing and the internet. Initially we relied on typewriters and 'snail mail' and faxes for moving paper around, orchestrated by Torgny Wiederholm, an unflappable editor of all three volumes (Wiederholm, 1983 - larvae; Wiederholm, 1986 - pupae; Wiederholm, 1989 - adults). The successful plan was to gain support for the editor plus core contributors to attend official scientific meetings. Thus annual get-togethers of several days, accompanied by reams of paper assembled around several North American Benthological Society annual meetings (USA), some triennial chironomid meetings, plus hosting by labs of the Natural History Museum (London) and twice in Munich. Photographs here (Figs 1–2) are from the 1980 workshop hosted in Bavaria at the old Schloss Nymphenburg. The first shows director Sepp Fittkau with Clive and Frieder Reiss to his left and Ole Sæther to his right (Fig. 1). The second shows a typical working lunch with beer bottles, full and empty (Fig. 2) but ashtrays removed. Occupational health and safety were optional.

Chapters were allocated by preference or sometimes by coercion. Clive and Frieder adopted the speciose subfamily Chironominae in all stages. Ole provided the essential prerequisite stable morphological terminology for all stages. Sepp guided in-house support including much artwork from the Zoologische Staatssammlung. Clive and I drove to Munich from UK in my old banger and somehow got it back to UK. By the second (and final) meeting in Munich the Zoologische Staatssammlung had been relocated into a state-of-the art collection facility, with the construction and relocation directed by Fittkau. Amazing, and Sepp's chapters still came in on time.

Clive's major parallel project at this time was his 1986 'Biology of Freshwater Chironomidae' in the prestigious and highly cited *Annual Review of Entomology* series. Not only was this very timely for a burgeoning community engendering more than 700 total cites, but its authority continues with 80 cites since 2020.



Figure 1. Working session, led by (centre) Sepp Fittkau, to his right Ole Sæther, to his left Clive Pinder and Frieder Reiss (back to photographer). Photograph from Torgny Wiederholm.



Figure 2. Working lunch, also directed by Sepp Fittkau, with from his left clockwise, Clive Pinder, Frieder Reiss, Ami Reiss, Peter Cranston, unknown (hidden), Ole Sæther. Photograph from Torgny Wiederholm.

Building on the evident scientific demand for ‘more on the chironomids’ the commissioning editor for the late (and lamented) publisher Chapman and Hall, Ward Cooper asked Clive, Patrick Armitage and myself to consider writing a book on the family Chironomidae covering ‘the lot’ under a subtitle ‘The Biology and Ecology of Non-biting Midges’. Once again our employers, in Clive’s case now the Institute of Freshwater Ecology (I.F.E.) at Monk’s Wood, and mine then the CSIRO Division of Entomology in Canberra, Australia, encouraged us. Another seven contributors were lined up, and Clive covered ‘Biology of the eggs and first-instar larvae’ and ‘The habitats of chironomid larvae’ and his expertise and knowledge added much value to all other chapters. Clive brought on board Nori Tokeshi, who remained in UK at this time as lecturer at Queen Mary, to contribute three major overview chapters on ‘Life cycles and population dynamics’, ‘Production ecology’ and ‘Species interactions and community structure’. Clive was delighted that his ‘protégé’ turned these previously descriptive topics with poorly analysed data into ‘must-read’ sections of the book (Armitage *et al.* 1995).

In 2001 Clive retired from IFE, Monk’s Wood, and with Carolyn returned to Dorset to enjoy fishing in chalk streams and at the coast, thatched-roofed pubs, community volunteering in Wareham, and more recently their grandchildren. Already with a life of science well-lived behind him, one task remained, so with Peter Langton, the 1978 FBA guide to the adult males was updated as Scientific Publication #64 (Langton & Pinder, 2007). In the interval, 587 species had been recorded from Britain and Ireland, compared with 439 species in the 1978 key (did I write earlier that they were diverse?). The line illustrations were expanded to an astonishing 1400 - now much more than just the genitalia! This expansion was due in no small part to both authors’ own studies, and again required two volumes.

That the Freshwater Biological Association could add this publication to their list is a tribute to their history of sustained support for freshwater invertebrate research, including of Clive’s work. I mention here the often-derided ‘citations’ as a metric in scientific publishing, and undoubtedly the always self-effacing Clive would have pooh-poohed this, but such sustained numbers show the depth and breadth of his understanding of the field, of where it was going, and how it could be delivered better. And he never shirked the challenge to do so, with a joke, a wry smile, and ‘it’s time for the pub’. Although physically separated for 30 years by much distance (me in Australia, then California, and back in Australia), in our chosen research field Clive has been ever-present for more than half-a-century, and will remain so. Although lacking a Purbeck brew and 10,000 miles distant in covid lockdown at dusk in Australia, I raise a glass of shiraz to Clive’s memory.

Acknowledgments

Adrian Pinder, Clive's son, and erstwhile colleagues Torgny Wiederholm and Mutsunori (Nori) Tokeshi helped me with background, photographs and anecdotes respectively. Penny Gullan, John Epler and Martin Spies applied their metaphorical editor's pens over earlier drafts, with a lighter touch than Clive literally did on my thesis. I thank them all.

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Leonard C. Ferrington, Jr. (1948-2021): Chironomid cognoscente and modern-day Renaissance man

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“Let’s look at some chironomids!” Many of Dr. Leonard “Len” C. Ferrington Jr.’s students heard this phrase as he walked into the lab with an excited smile on his face. Len’s enthusiasm for chironomid research - whether it be time at the microscope or out in the field - was infectious. Whenever a student came to his office describing a new or unique observation or an unknown midge, he would always drop what he was doing to check it out and provide his input. Once he saw these discoveries, he would give you an enthusiastic, but knowing smile and then often describe the localities where he had also made a similar observation or found the same midge. Usually, any interaction with Len would branch out into an extended discussion of some ecological or taxonomic topic and ideas for future research. He always had new ideas to contribute, and certainly had no plans to retire (“Why would I want to do that?!” was a common response when asked). Sadly, Len passed away unexpectedly, on September 11, 2021, while bicycling on the Root River Trail, near Lanesboro, Minnesota.



Figure 1. Len Ferrington in Iceland in 2018 (this photo was used to announce his election in 2021 as a Society for Freshwater Science Fellow: <https://freshwater-science.org/awards-programs/sfs-fellows>). Photographer unknown.

Len made many contributions to science, particularly to the taxonomy and ecology of Chironomidae. During his 41-year academic career as a Professor and Scientist, Len was a prolific researcher who collaborated with colleagues in 52 countries on six continents. Len’s research program consisted of several areas of emphasis including: taxonomy and systematics, biodiversity, responses of aquatic insects to pollution, their roles in stream ecology, and aquatic resource sustainability, with most of his research questions centered around stories told by the Chironomidae. Although Len clearly displayed a strong focus on chironomid

Born in 1948 in Murrysville, PA, Len joined the U.S. Marine Corps in 1966 after graduating from Franklin Regional High School. He served in Vietnam as a Corporal in the 3rd Marine Division and was honorably discharged from service in 1972. During his time in Vietnam, he earned several medals, including a Purple Heart Medal, National Defense Service Medal, Vietnam Service Medal, and Vietnam Campaign Medal. He went on to study at the University of Pittsburgh where he earned a Bachelor of Science degree in Biology and Doctorate degree specializing in Entomology under William Coffman. He held tenured academic positions at both the University of Kansas, Lawrence, Kansas, USA (1980 to 2000) and the University of Minnesota, Saint Paul, Minnesota, USA (2000 to 2021).



Figure 2. Len Ferrington in Vietnam in 1967. Photographer unknown.



Figure 3. Len in Bill Coffman's lab in 1977. Photographer unknown.



Figure 4. Len with Deborah Ferrington and Ole Sæther at Kjosfossen Falls (Norway) in 1985. Photographer unknown.



Figure 5. Jim Sublette and Len chatting in Jim's lab in Arizona (USA) in 2006. Photo by Will Bouchard.

taxonomy and ecology, his research interests were diverse, and he brought his expertise and enthusiasm to other realms of freshwater biology as well, including biological monitoring, aquatic insect gut fungi, fish diets, Mecoptera diversity, and winter hardiness.

Len authored or co-authored over one hundred peer-reviewed articles, nearly thirty externally reviewed technical reports, several online resources, and multiple book chapters, all relating to the field of freshwater science; a bibliography of Len's publications follows. These include many important taxonomic contributions including the description of 4 chironomid genera, 49 chironomid species, and the re-description of 107 chironomid species. Len also described 4 genera and 12 species of trichomycetes (*sensu lato*) which include fungi and protists dwelling in the guts of arthropods. In addition to describing many species, a midge genus (*Ferringtonia* Sæther and Andersen) and two species of midge (*Odontomesa ferringtoni* Sæther and *Orthocladus ferringtoni* Saponis) were named in his honor. Notably, Len made a profound impact through his authorship in four editions of a key freshwater science resource, *An Introduction to the Aquatic Insects of North America* by Merritt, Cummins, and Berg, which many use daily to identify aquatic insects. Tracey Anderson, a graduate student of Len's, described an early edition of Merritt and Cummins as one of the few books she wore out and that it now holds a special place on her shelf.

Like many aquatic biologists, Len loved field work. Whether he was collecting in an urban stream in Kansas, a spring-fed trout stream in the middle of a Minnesota winter, or a lake on the steppes of Mongolia, he was always enthusiastic. Few researchers relish leaving the warmth of the indoors during the middle of winter in lieu of conducting field work in sub-zero temperatures. Len, however, thrived in these conditions. He found a passion for studying cold-hardy chironomids and could be described as 'cold hardy' himself, spending hours on snow banks flanking spring-fed streams, vials in hand, in search of winter-emerging insects to scoop up for studies on longevity, behavior, and diversity. Although Len did his part to contribute to the knowledge of winter-active midges in Kansas, once he relocated further north in Minnesota, his work on winter hardiness accelerated. Colleagues that visited Len and most of his students in Minnesota were treated to these winter forays to find midges. Many of his students at the University of Minnesota would find that their projects included extensive winter fieldwork, and Len's love and

fascination with working in these environments was always transferred to his students. Winter did not just mean work though, as Len would also hold full-moon, skiing parties on his lake in the winter for students and colleagues complete with chili, Bob Marley music, Malbec, and sometimes a little aquavit.

Much of his field work included the collection of chironomid pupal exuviae. He was a strong proponent of using or incorporating chironomid pupal exuviae into research and much of the research throughout his career relied to some degree on this technique. Many of his students and colleagues took the opportunity to learn from a master and became connoisseurs of surface floating pupal exuviae themselves. Barbara Hayford had this memory of sampling with Len in Tasmania: “One day as we hiked toward our collecting site, a waterfall, we observed a large pile of foam downstream on the river, deep in a ravine. Len got that look in his eye. He stopped and peered over the edge, obviously thinking of the best way down when I stopped him and pointed out that I would not be able to haul him out if he fell down the steep slope and broke his bones. Still, he hesitated, then changed his mind and continued down the path to collect at the waterfall. I think he would have collected that foam had I not been there. Although I was relieved he did not collect in the ravine, I did learn from him a lifelong passion for chironomid pupal exuviae. His excitement and enthusiasm never waned in all the years I knew him. Ultimately, that was the greatest gift he bequeathed me as a researcher.”



Figure 6. Len collecting *Diamesa* along the Kinnickinnic River (USA: Wisconsin) in 2005 for a cold-hardiness study. Photo by Will Bouchard.



Figure 7. Len with his pan and sieve, preparing to collect surface-floating pupal exuviae from a stream in Duluth (USA: Minnesota), September 2009. Photo by Alyssa M. Anderson.

Those that knew Len well also knew that when he wasn't wearing his waders (or his characteristic cowboy boots that would let all know he was coming down the hall!), he was most likely in his dance shoes. Research conversations with Len would often deviate from the subject at hand and often turn to dancing, as this was another significant passion. He could also find appropriate ways to intertwine the two topics. For example, when describing how best to walk in a stream with waders on while collecting (especially in winter, when falling in the water is not at all appealing!), Len would liken the experience to dancing, where agility, balance, and grace is of utmost importance. Len certainly excelled in this area, based on both the numerous ballroom dancing awards he received over the years and his prowess while waders were donned in the field.

Len's contributions to the scientific community extended well beyond field work and his research lab. He was a long-standing member of the Society of Freshwater Science (SFS) (formerly known as the North American Benthological Society, NABS) and served as President (1989-1990). During his tenure as NABS president, Len advocated for scientists to provide their expertise and become more involved with assisting conservation groups to develop policy. Len continued to contribute to NABS/SFS in many ways including serving and chairing the Executive Board of Directors for NABS Endowment, serving on numerous society committees and boards, and organizing several symposia at meetings. In 2021, he was elected as an SFS Fellow for his sustained excellence in contributions to freshwater science research. Len's service to SFS/NABS also included hosting and organizing the 1986 NABS meeting held in Lawrence, Kansas. Always someone that en-

joyed a joke, even an inside joke, he called the traditional 5K run at the 1986 NABS meeting the *Oreadomyia* 5K. He called it this because the route traversed Mount Oread, a 58 m high ridge upon which the University of Kansas is situated, and of course because of the genus *Oreadomyia* Kevan & Cutten-Ali-Khan. Len also hosted the XV International Symposium on Chironomidae in 2003, bringing chironomid researchers from around the world to Minnesota. Len always made it a priority to attend and bring students to professional meetings, particularly the International Symposia on Chironomidae and annual meetings of SFS/NABS. Impressively, with the exception of the 2020 meeting that was canceled due to a global pandemic, he never missed a single SFS/NABS meeting in 46 years (do note, though, that Len did participate in the modified virtual SFS event held later in the summer of 2020)! Len's service also included serving on numerous university committees at the University of Kansas and University of Minnesota. Most notable was Len's appointment as Co-Coordinator of the Environmental Science, Policy and Management undergraduate major from 2008-2011 at the University of Minnesota. Len also served as president of the Kansas Entomological Society (1984-1985) and was an editor or assistant editor for several journals including the Journal of the North American Benthological Society, Journal of the Kansas Entomological Society, and the *CHIRONOMUS* Newsletter.

In addition to his research, he was an active and well-liked professor and mentor to hundreds of undergraduate and graduate level students at the University of Kansas and the University of Minnesota. Len acted as major advisor for at least 27 graduate students (8 Ph.D. and 19 M.S.) and served as a committee member for numerous oth-

ers. His students now hold titles, such as aquatic ecologists, research scientists, aquatic invertebrate taxonomists, professors of biology, environmental educators, among many others. As an advisor, Len encouraged his students to not only focus intensely on their thesis topic, but to also become broadly trained freshwater scientists that are ready to address issues spanning from local to global importance. Len was always available to his students to answer questions, develop projects, and to simply steer them through graduate school, but he also gave students the latitude to pursue their interests. For example, one of his former students, Petra Kranzfelder, gained a passion for tropical biology after doing some research on sea turtles in Costa Rica, so he supported her interests and helped her develop both M.S. and Ph.D. projects in tropical chironomid ecology and taxonomy (even though the project had nothing to do with winter-active midges). Len's broad expertise in taxonomy, ecology, water quality, and biological monitoring and the support of his students' strengths and interests is reflected in the diversity of thesis and dissertation projects of his students - many of which don't even mention Chironomidae in the title. He was also a strong proponent of international collaborations and hosted numerous researchers (both students and faculty) in his lab from countries including Brazil, China, Iceland, and Norway, and he encouraged his undergraduate and graduate students to seek out international research and learning opportunities. For example, he led an environmental science study abroad program in Iceland in 2014, 2016 and 2018, where he brought undergraduate students from the University of Minnesota to Iceland. This experience resulted in one of his graduate students, Corrie Nyquist, who joined the 2018 trip, framing her doctoral research around the impacts of climate change on subarctic midges in Ice-



Figure 8. Len with Petra Kranzfelder, Corrie Nyquist, and some environmental science students near a hot spring (left) and on a glacier (right) in Iceland in 2018. Photographers unknown.

land. Len's international collaborations are clearly apparent in the research in which he was involved in (e.g., Norway, France, Germany, Iceland, Italy, Tasmania, Argentina, New Zealand, South Africa, and Mongolia), the meetings and committees he participated in, and the colleagues with whom he published. Due largely to Len's encouragement, support, and vast network of connections, three of Len's most recent Ph.D. students, Corrie Nyquist (Iceland, 2020-2021), Petra Kranzfelder (Norway, 2014-2015), and Alyssa Anderson (Norway, 2010-2011) received Fulbright Fellowships that allowed them to complete portions of their dissertation research abroad and build their own collaborative networks. Alyssa Anderson states that had it not been for Len's encouragement and strong support for international experiences, she would not have considered the Fulbright program, let alone working an international experience into her graduate program. Now, she views this as the most impactful component of her graduate education, building not only her professional skill set and network, but also enhancing her worldview and collection of friends. Impressively, Len himself was most recently recognized with a prestigious Fulbright Fellow award and was greatly looking forward to performing winter research in Finland for six



Figure 9. Len collecting in Iceland in 2001. Photo by Dean Hansen.



Figure 10. Len sampling midges along a lake shore in western Mongolia in 2005. Photo by Mark Edlund.

months spanning a portion of the 2021-2022 academic year.

One of Len's students and colleagues, Barbara Hayford, had the following to say of working with Len, "When I arrived at the Kansas Biological Survey in 1993, Len was working with Ole Sæther on the early stages of the *Pseudosmittia* revision. Despite this and other research, teaching, service and a full and active family life, he took the time to welcome me to his lab. He provided many opportunities to work on different types of research. During my studies with Len, I worked on an EPA Superfund Cleanup site, a double-blind pesticide study, systematics, and trichomycete/chironomid interactions. He encouraged and facilitated international research, thus I studied chironomids in Mongolia, Tasmania, Panama, Germany, and England. He was unable to participate in the fieldwork in Mongolia in 1995 and encouraged his graduate students to go in his stead. I jumped on the chance, initiating over twenty years of work on Mongolian Chironomidae. I never did do field work with Len in Mongolia although we were both there in 2004 and 2005. In 2018, Will Bouchard and I began analyzing the data resulting from Len's collections of western Mongolia lakes, culminating in our collaboration with Len this past summer of 2021. This collaboration completed a circle of work with Len that began in graduate school, continued throughout my early career, and brought us back together



Figure 11. Len with some of his students and collaborators at lunch during the 2019 Society for Freshwater Science (SFS) Meeting in Salt Lake City (USA: Utah). From left to right: Will Bouchard, Petra Kranzfelder, Corrie Nyquist, Alyssa Anderson, Len Ferrington, Jessica Miller, Lily Fulton, Tracey Anderson, and Barbara Hayford.

studying Mongolia chironomids. I am forever grateful for all he has done for me, for his mentoring, and for the privilege of knowing and working with him.”

When considering his wide range of collaborators and the careers of his students, his impact and contribution to freshwater science has been far reaching not only geographically, but also through time. Many of Len’s past students continued to collaborate with him on research and teaching endeavors long after graduation. One important part of Len’s significant legacy is that he trained and taught many aquatic resource professionals, including researchers and teachers. Many students came to the field of aquatic science because of him. Students might have had a class with him as an undergraduate or came to study with him based on a recommendation from one of Len’s many colleagues. Even if students weren’t aquatic entomologists or biologists when they started working with him, Len’s interest and passion for the field was often kindled in them. Len’s influence and enthusiasm in the classroom, laboratory, and field is also vividly apparent in his students who went on to teach. In the classroom, Len could always be counted on to start a class with a favorite song (usually Bob Marley), YouTube video (typically involving dancing), or a good joke or two (especially on exam days!). These practices are now carried forth by some of his students that are now in the classroom. His legacy includes training teachers and professors at different educational levels

and biologists specializing in public outreach who are educating the public and training students to be the next aquatic biologists. As a result, Len’s training and influence has impacted thousands of students. Len also trained many researchers who are responsible for the protection, conservation, and stewardship of natural resources. It is imperative for the conservation of aquatic resources that there is an experienced and capable community of aquatic science educators and researchers and it is difficult to overestimate Len’s important contribution to the field.

Len is known to many in the world of aquatic ecology and chironomid taxonomy as a scientist, teacher, friend, and colleague. Someone who was a true gentleman with a constant and infectious positive attitude. All who had the honor to know Len recognized how he pursued excellence in life with passion and vigor.

Len was just as encouraging, positive and adventurous with his family as he was with his students. He was known to take his family on elaborate and intricately-planned family trips all around the world, often involving two of his favorite pastimes - bicycling and traveling together. His children note that he was always a present father-figure at sporting events and important milestones growing up, somehow managing the work-life balance. In their adulthood, he was a trusted advisor for their lives’ paths and guided them on their academic, business, and personal endeavors. His encouraging phrase of “just go for it” was one to remember.



Figure 12. Deborah and Len on their wedding day in 1978. Photo by Daniel Carroll.



Figure 13. Len with his children, Len III and Ashley, along a spring in western Kansas in 1984. Photo by Deborah Ferrington.



Figure 14. Len with his growing family in 2019, Ashley, Beau, Len III, Leah, Lindsay, Len, and Deborah. Photographer unknown.



Figure 15. Len playing the mandolin. Photo by Deborah Ferrington.

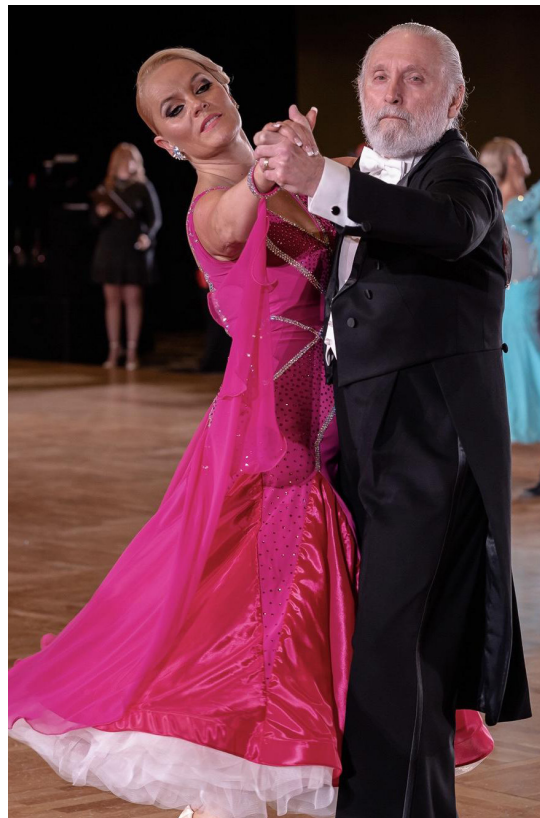


Figure 16. Len with Michelle Hudson during a ballroom dancing competition. Photographer unknown.



Figure 17. Len Ferrington in Iceland, May 2001. Photo by Dean Hansen.

Len was a loving husband of 43 years to Deborah, father to Len III and Ashley, grandfather to Leah. He was fearless in trying and perfecting new activities, including a recent passion for playing the mandolin, and becoming a nationally ranked, award-winning competitive ballroom dancer. Len embodied the persona of a modern Renaissance man, being as comfortable wading in the stream wearing chest waders as he was dancing in a tuxedo. Len was loved greatly by his family, friends, and colleagues and will be dearly missed by them all. His legacy will continue through his family, the contributions he made as a scientist and educator, and the countless lives he touched with his genuine, engaging, humorous, yet gentle personality, and kind heart.

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