



# CHIRONOMUS

## Newsletter on Chironomidae Research

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### CONTENTS

Editorial: CHIRONOMUS - Mitteilungen  
aus der Chironomidenkunde 3

19th International Symposium on  
Chironomidae 5

#### Current Research

Andersen, T. *Mollerietta kaputu* n. sp.  
from the West Usambara Mountains,  
NE Tanzania 8

Kavanaugh et al. Factors affecting  
decomposition rates of chironomid  
(Diptera) pupal exuviae 16

Andersen, T. and Pinho, L. C. A new  
*Thalassosmittia* out of the sea:  
*T. amazonica* n. sp. from the Amazon  
rainforest, Brazil 25

Ferrington Jr., L. C. and Coffman, W. P.  
Differential efficiencies of dip-net  
sampling versus sampling surface-  
floating pupal exuviae in a biodiversity  
survey of Chironomidae 31

Kobayashi, T. A redescription of  
*Zavrelia simantonea* comb. nov. 41

#### Short Communications

Subfossil chironomids from  
Kamchatka 45

The importance of chironomids as  
food for overwintering passerines 48

*Buchonomyia thienemanni* from the  
Czech Republic 51

*Buchonomyia thienemanni* from  
Slovakia 54

Gynandromorphy in fossil Chironomidae  
from Rovno amber 55

The 20th International Symposium on  
Chironomidae 58

News from the editors 60



*Cryptochironomus denticulatus* on oak leaf. Photo by Alan Watson Featherstone.

## ***CHIRONOMUS Newsletter on Chironomidae Research***

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The *CHIRONOMUS Newsletter on Chironomidae Research* is devoted to all aspects of chironomid research and aims to be an updated news bulletin for the Chironomidae research community. The newsletter is published yearly in late autumn, is open access, and can be downloaded free from this website: <http://www.ntnu.no/ojs/index.php/chironomus>. The publisher is the NTNU University Museum at the Norwegian University of Science and Technology in Trondheim, Norway.

Research articles for the *CHIRONOMUS Newsletter* are subject to peer-review. New scientific names are registered in ZooBank (<http://zoobank.org>).

Contributions to *CHIRONOMUS Newsletter on Chironomidae Research* should be submitted online through the online journal system: <http://www.ntnu.no/ojs/index.php/chironomus> following the [author guidelines](#). Submission deadline for contributions to the newsletter is August 1.

Would you like to see your picture on the front page? Please send us your favourite midge photograph or drawing ([torbjorn.ekrem@ntnu.no](mailto:torbjorn.ekrem@ntnu.no)).

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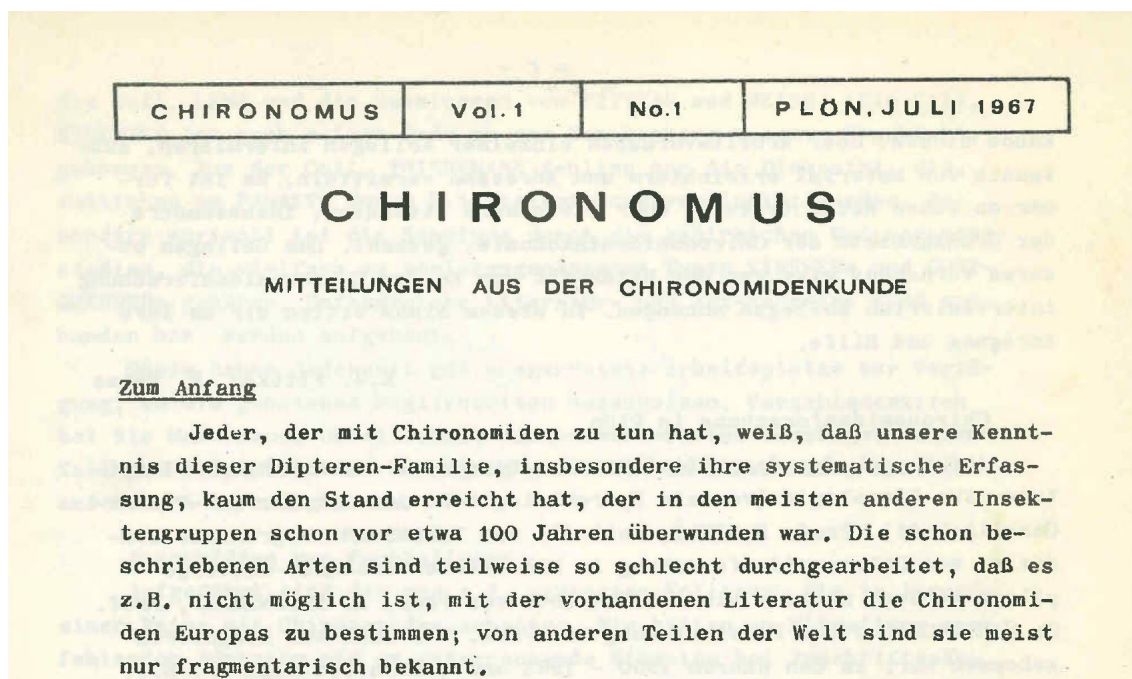
Front page layout: Chironomid in title from photograph by Steve Marshall, Graphic design by Kolbjørn Skarpnæs, NTNU Information Division.

Front page photo: *Cryptochironomus denticulatus* on oak leaf, Scotland.  
Photo by Alan Watson Featherstone

## Editorial

### CHIRONOMUS – Mitteilungen aus der Chironomidenkunde

Such was the title of the first CHIRONOMUS newsletter produced by E.J.Fittkau and F.Reiss in July 1967 - six pages of which nearly four comprised a list of chironomid workers known to the editors arranged alphabetically by nationality. The stated purpose of the newsletter was to enable chironomid workers to publicize their activities, share techniques, to give details of forthcoming symposia and periodically update the directory of those working on Chironomidae. English started to appear in Vol.1 No.7 (1969) and scientific accounts began to be published in Vol 1 No.8 with a paper by P.D.Armitage on 'Chironomid larvae in a lake with a long ice cover period'. Vol. 1 No. 9 (1970) was devoted to an account of the 4<sup>th</sup> International Symposium on Chironomidae held in Ottawa, compiled by D.R.Oliver, complete with a list of participants, program, titles of papers presented, abstracts and a photograph of participants (all of these have been taken over by more recent symposium organisers and the details ceased to be published in the newsletter). In Vol. 1 No.7 Fittkau announces the forthcoming publication of a comprehensive bibliography on Chironomidae compiled by himself and Reiss with the help of O. Hoffrichter. This was the establishment of an ongoing bibliography updated year on year in the newsletter for years until recently by O. Hoffrichter. Also in that edition was a questionnaire: CHIRONOMUS to remain a newsletter or to become a combined newsletter/journal? Readers were requested to give an opinion on such an upgrade and to whether they were prepared to contribute to the costs. It has taken 42 years for that vision to be achieved! After nine years volume 1 comes to an end, No.19 being the final part (April 1976) in which it was announced the transfer of the Chironomid Centre from Plön to Munich on 1<sup>st</sup> May 1976. From 1982 to 1985 the newsletter was edited by Fittkau, Reiss and Jim and Mary Sublette. It ceased publication in 1985.



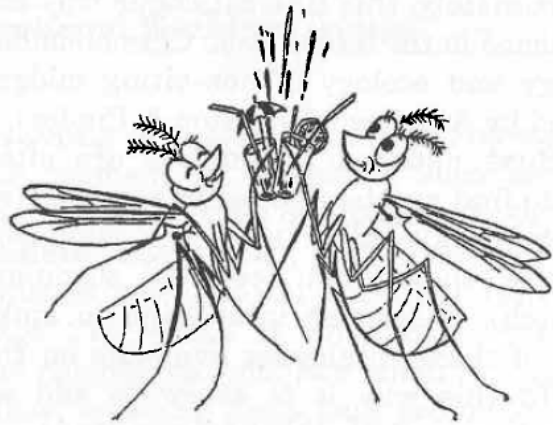
The front page of CHIRONOMUS No. 1

At the Amsterdam Symposium (1991) it was decided to resurrect the newsletter and U. Nolte and R.K. Johnson took on the responsibility. The language changed to English and "Another change in the newsletter's profile is that it excludes contributions with a taste of scientific publication. There are a great number of journals in circulation which offer sufficient opportunities for publishing original scientific data on Chironomidae... but there was a lack of an informal forum for our area of research, and it is exactly this need that provided for the newsletter's revival." So wrote Nolte in her first editorial (No.4, 1992). The page size had increased and so had the number of pages, which averaged 40 during her editorship. From No.6 (1994) the newsletter contained a Current Bibliography section compiled by O. Hoffrichter and this served to keep the number of pages high for years. During Nolte's editorship it was indeed possible to include lit-



tle notes of (hopeful) interest to other chironomid workers: I was able to publish two notes in No.7 (1995), one on a chironomid that was observed on the neck of an open bottle of wine, that then dropped off and staggered about as if drunk ('Six-legged and legless'), the other an account of the construction of a remarkably efficient emergence trap made from a pair of ladies' tights. The pages were embellished with line drawings and cartoons – a magazine for chironomists!

Ulrike Nolte stepped down as editor in 1998 and Ruth Contreras-Lichtenberg and Peter Langton took on the responsibility. Immediately Ulrike's bottom line slipped with the publication of a paper by Burlak, Golygina and Kiknadze on 'Larvae of *Chironomus* can have a different susceptibility to



Tipsy chironomids from CHIRONOMUS No. 7

the entomopathogenic bacterium *Bacillus thuringiensis* subsp. *israelensis* depending on different inversion genotypes': a paper that could have been published in an established entomological journal. That practice has continued to the present day within a section entitled 'Current Research'.

In 2006 Ruth expressed her wish to step down as editor and Torbjørn Ekrem agreed to succeed her. Colour pictures began to grace the cover of the newsletter and the Current Research section expanded. In 2013 Alyssa Anderson was invited on to the editorial board restoring its transatlantic composition. The stage was set for a transformation. Already papers for inclusion in Current Research were being peer reviewed, the newsletter was registered as an open access journal with an ISSN number that is indexed in the Directory of Open Access Journals and Google. The editors felt a change of name was warranted by its new status and at the 19<sup>th</sup> International Symposium in the Czech Republic a vote was taken and practically all the delegates agreed with a name change to CHIRONOMUS Journal of Chironomidae Research: and that will be the title as of No.28 (2015). Chironomists now are blessed with a more complete Bibliography than for most other insect groups, a comprehensive Directory of Chironomid Workers and now a journal on Chironomidae: all metamorphosing from the original newsletter by 'Sepp' Fittkau and Frieder Reiss. There is much that the chironomid community have to be grateful for to these two stalwarts of our preoccupation.

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## 19<sup>TH</sup> INTERNATIONAL SYMPOSIUM ON CHIRONOMIDAE, ČESKÉ BUDĚJOVICE, CZECH REPUBLIC, 17-22 AUGUST 2014

Every three years chironomists from across the world congregate to renew friendships, make new contacts and share their work in a convivial atmosphere: an experience that is compelling and results in repeated attendance symposium on symposium. This year the Symposium was hosted by the Faculty of Science, Charles University of Prague in collaboration with the Biology Centre of the Czech Republic in České Budějovice. About 80 chironomists converged on České Budějovice on Sunday 17<sup>th</sup> August. Though time and fortune take their toll on the ability to attend, there were still four from my first symposium in Dublin (1979): Bruno Rossaro, Huberto Ferrarese, Paddy Ashe and myself. What is encouraging for the health of the study of chironomids is that the number attending successive symposia remains much the same: recruitment appears to be compensating for losses from the community.

Registration opened at 11.00 on the Sunday and closed at 17.00. My rather complex travel arrangements ensured that I arrived after registration had closed. Courtesy of a caretaker, I located the hall of residence, where, whilst booking in, I discovered others who had similarly arrived late. Too late for the tour of the brewery of Budweiser Budvar and “a” welcome drink, we made our way to a restaurant near the town centre. Hardly had we ordered, when in trouped about 20 delegates for whom liquid refreshment hadn’t compensated for

something solid. The swarming of chironomists at specific markers is inadequately researched, but one could start with Paddy’s by now legendary maps!

On the Monday morning delegates were welcomed by Prof. Miloslav Simek, Director of the Biology Centre, Academy of Science of the Czech Republic, Prof. Libor Grubhoffer, Rector of the University of South Bohemia and Prof. Jan Frouz, Director of the Institute of Environment, Charles University, Prague. This was followed by the Thienemann lecture which was delivered by Takashi Okuda on ‘Chironomids as important features in biological sciences’. The capacity of *Polypedilum vanderplanki* to dehydrate and rehydrate repeatedly without destruction is due to the replacement of water by trehalose, conserving the internal structure of cells in so doing and releasing the contents intact on hydration. I watched desiccated larvae of *P. vanderplanki* under a microscope rehydrate and resume activity as an undergraduate over half a century ago and have often wondered how they did it – now I know!

The oral presentations spanned three days with 42 papers delivered in three daily sessions, the sessions separated by lunch in the campus refectory and coffee breaks. The first of the sessions was on ‘Kariology and genetics’ with four presentations, three of them delivered by Veronika Golygina, impressive for the sustained clarity of her speech in a



Lecture hall at the Biology Center of the Czech Academy and South Bohemia University. Photo: Andrey Przhiboro.



Len Ferrington and Andrey Przhiboro. Photo: Vít Syrovátka

language not her own. Then there were sessions on ‘Physiology’ (3 presentations) and ‘Paleological implications (5 – fragments of larval head capsules continue to provide a variety of projects that provide information on the nature of aquatic habitats of former times).

In the lobby outside the lecture theatre 36 posters were displayed; eye catching and informative on a wide variety of Chironomidae related topics. These generated prolonged discussions on an individual basis, that a lecture cannot achieve and are invaluable for cementing contacts and sharing interests.

Tuesday saw sessions on ‘Morphology, taxonomy and systematics’ (7), ‘Pollution and biomonitor-

ring’ (4) and ‘Autecology’ (5) - that included an account of ‘Orthocladiinae acuticauda/Orthocladius species aus Flußsand’, the larva pointed at both ends to facilitate escape into mobile sand; this was accompanied by a video showing one doing just that!). The delegates then boarded coaches to be transported to Trěboň, where we were introduced to the history and technique of carp farming. The emptying of that enormous pond to harvest the fish must be an amazing spectacle! A very quick skim for exuviae, so as not to be left behind produced eight species including the ubiquitous *Glyptotendipes paripes* – the ‘Symposium midge’ (I have souvenir specimens of this species from many of the symposium venues and it was the only species I managed to collect in Tianjin!) We moved on to see the neo-gothic Schwartzberg tomb: an impressive edifice in a tree filled park that was certainly worth the visit. Thence to a restaurant for the symposium banquet – a very enjoyable meal made all the more enjoyable by the company at the table. We were entertained by a very competent traditional jazz clarinet player. Did I really see delegates dancing?!

‘Ecology spatiotemporal distribution’ (4) and ‘Community ecology’ (10) comprised the sessions on the final day, that reported on projects from Japan through Icelandic cold springs to the high mountains of Brazil. Chironomidae please note: chironomists will find you wherever you are!



From the conference trip. Photo: Vít Syrovátka



The final forum included a show of hands for a proposed change of name for the CHIRONOMUS Newsletter on Chironomidae Research to CHIRONOMUS Journal of Chironomidae Research, practically all delegates present voting in favour of the change, a minute's silence for those that had died since the last symposium, an update on the world catalogue by Paddy Ashe and thanks for the admirable arrangements by Jolana Tátosová, Peter Bitusík, Jan Frouz, Josef Matěna and Vít Syrovátka. I reiterate my personal thanks for their orchestration of events, conducted from the floor by Jan Frouz (he has the voice for it!).

It took six buses, five trains, five 'planes and two taxis to get to the Symposium and back. Was it worth the effort? Most decidedly: Yes! A friend of mine would end a meal with 'Well that's lunch; what's for supper?' At first I thought it rather rude

after all the effort that his wife had made to produce the meal, but she understood it as a complement: 'If the next meal is as good as the one I've had, I can't wait!' We'll have to wait: Trento is not for another three years. Bruno and Huberto will be there; Paddy and I will complete the common denominator and look forward to seeing you all again: make the effort!

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Participants at the 19th International Symposium on Chironomidae. Photo: Vít Syrovátka.



## ***MOLLIERIELLA KAPUTU* N. SP. FROM THE WEST USAMBARA MOUNTAINS, NE TANZANIA (DIPTERA: CHIRONOMIDAE)**

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### **Abstract**

The male and female imago of *Mollieriella kaputu* n. sp. are described and figured based on specimens collected in NE Tanzania in 1990. The new species can be separated from *M. calcarella* Sæther and Ekrem by having a slightly narrower, parallel sided anal point and weaker setae on the inferior volsella, longer C extension and distinctly shorter tibiae. The specimens were caught in Malaise traps at about 1500 m altitude in the Mazumbai Forest Reserve in the West Usambara Mountains.

### **Introduction**

The West Usambara Mountains belong to the Eastern Arch, a chain of isolated mountains stretching from the Taita Hills in southern Kenya and the East and West Usambara and Pare mountains in north-eastern Tanzania to the Uluguru and Udzungwa mountains in southern Tanzania. These mountains resulted from uplifting and faulting of the main East African plateau and are much older than the East African volcanoes like Mt. Kilimanjaro and Mt. Meru. They are situated close to the Indian Ocean and warm, wet sea air and easterly winds set up a favorable climate. The mountains are covered with forests of different types depending on altitude and rainfall and contain at least 800 endemic plant species. A thorough description of the vegetation was given by Iversen (1991). There are also many endemic animals, thus the mountains are regarded as an important biodiversity hotspot, i.e. an area with a significant reservoir of biodiversity that is under threat from humans.

In the autumn of 1990 the University Museum of Bergen undertook an expedition to the West Usambara Mountains in Tanzania. The fieldwork, which included extensive use of Malaise traps and sweep nets, was mainly conducted in the Mazumbai Forest Reserve in the eastern part of the mountains. Some of the Malaise traps along the Kaputu Stream were run again in the spring and autumn of 1991.

A number of the taxa found in the material were new to science or previously unrecorded from the African continent (see e.g. Andersen and Sæther

1993, 1994a, 1994b; Andersen and Schnell 2000; Sæther and Andersen 1993, 1995; Sæther and Wang 1993a; Stur and Ekrem 2000). Below I describe one of these and place it in the genus *Mollieriella* Sæther and Ekrem. This monotypic genus is based on *M. calcarella* Sæther and Ekrem, a terrestrial species only known to occur in the Netherlands (Sæther and Ekrem 1999).

### **Material and methods**

The specimens examined were all collected in Malaise traps along the Kaputu Stream and preserved in alcohol. They were later mounted on slides in Canada balsam following the procedure outlined by Sæther (1969). Morphological terminology follows Sæther (1980). Measurements are given as ranges, followed by the mean when four or more specimens have been measured. Coloration descriptions are based on slide mounted specimens.

The holotype and most paratypes are deposited in the Department of Natural History, University Museum of Bergen, Bergen, Norway (ZMBN); some paratypes will be donated to the Zoologische Staatssammlung München, Germany (ZSM).

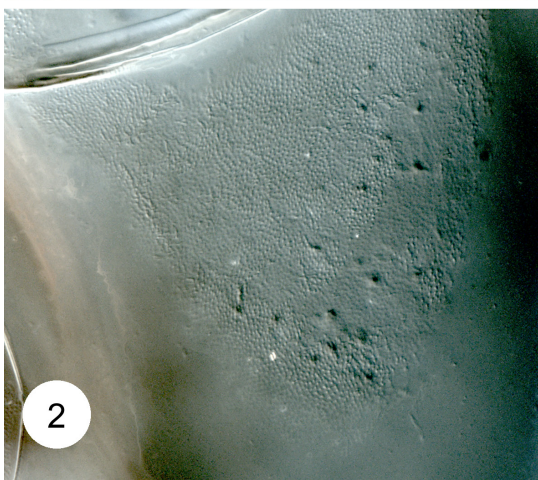
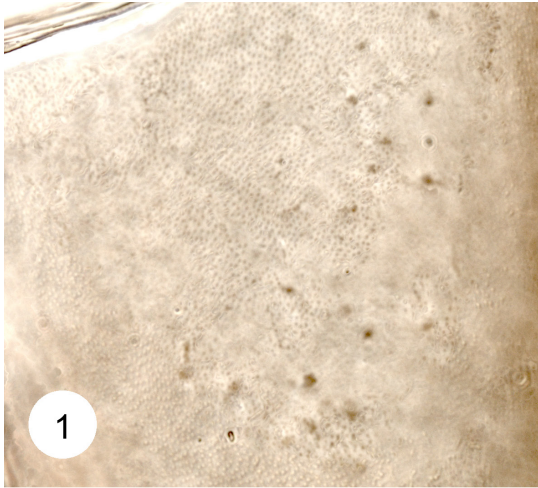
### ***Mollieriella* Sæther and Ekrem**

*Mollieriella* Sæther and Ekrem, 1999: 162.

Type species: *Mollieriella calcarella* Sæther and Ekrem, 1999: 164.

Other included species: *Mollieriella kaputu* n. sp.

The genus is figured and described in detail based on a few specimens of *Mollieriella calcarella* from the Netherlands (Sæther and Ekrem 1999). They stated that the species has tiny preepisternals. However, on closer examination of the type material no preepisternals could be discerned. But the specimens have a number of distinct, small pits on preepisternum (Figs 1–2). In *M. kaputu* n. sp. preepisternum also has a few similar pits and thus, these pits probably do not result from damage. Most probably they have a sensory function or represent the opening of glands. However, examination of fresh material is necessary to establish the true nature of these pits.



Figures 1–2. *Molleriella calcarella* Sæther and Ekrem, 1999, holotype. Details of preepisternum showing the distinct, small pits.

The tibial spurs are described as strongly reduced, thin and weak, with at most a couple of basal hair-like denticles on longer spurs. Examination of the types, however, revealed that all tibiae have a spinose apical scale. In *M. kaputu* n. sp. each leg has a single, long spur and in addition mid and hind tibiae have a single, strong seta at the base of the spur. In *M. calcarella* there appears to be an additional, shorter spur on all legs.

Sæther and Ekrem (1999) suggested two alternative systematic placements for *Molleriella*, a relative isolated position either in the *Heterotrissocladus* group or near *Heterotanytarsus* Spärck. The spinose scales on all tibiae appear to be unique among Orthoclaadiinae and most closely resemble the scales found in *Xiaomyia* Sæther and Wang and *Shangomyia* Sæther and Wang described from Oriental China (Sæther and Wang 1993b). Sæther and Wang (1993b) placed *Xiaomyia* and *Shangomyia* in Chironominae and postulated that they might form the sister group of the tribe Chironomini. However, in a dated molecular phylogeny Cran-

ston et al. (2012) suggested a deeper position as the sister to all other Chironominae, thus justifying tribal or possibly subfamily status.

As there can be no doubt of a placement of *Molleriella* within the subfamily Orthoclaadiinae, the tibial scales and spurs appear to be an autapomorphy in the genus, representing yet another case of convergent rather than phylogenetically informative evolution in Chironomidae. However, a more detailed interpretation and placement must wait until the larvae and pupae are known and/or fresh material for DNA analyses is collected.

### *Molleriella kaputu* new species

(Figs 3–17)

**Type material:** Holotype ♂, Tanzania, Tanga Region, West Usambara Mountains, Mazumbai, Kaputu Stream, loc. 10, 1420 m a.s.l., November 1990, Malaise trap, leg. T. Andersen (ZMB's Tanzania Expedition), (ZMBN). Paratypes: 11 ♂♂ as holotype; 1 ♀, as holotype except loc. 4, 1680 m a.s.l.; 2 ♂♂ as holotype except loc. 7, 1535 m a.s.l., 4–10 February 1991; 3 ♂♂ as previous except 1–8 August 1991 (ZMBN, ZSM).

**Diagnostic characters.** The new species is very similar to *M. calcarella* Sæther and Ekrem in morphological features, but can be distinguished by having slightly more narrow, nearly parallel sided anal point while *M. calcarella* has a wider, slightly spatulate anal point. The new species also has less setae on inferior volsella, the C extension is longer and the tibiae are distinctly shorter.

**Etymology.** Named after the type locality, Kaputu Stream. The species epithet is a noun in apposition without any Latin or Latinized elements.

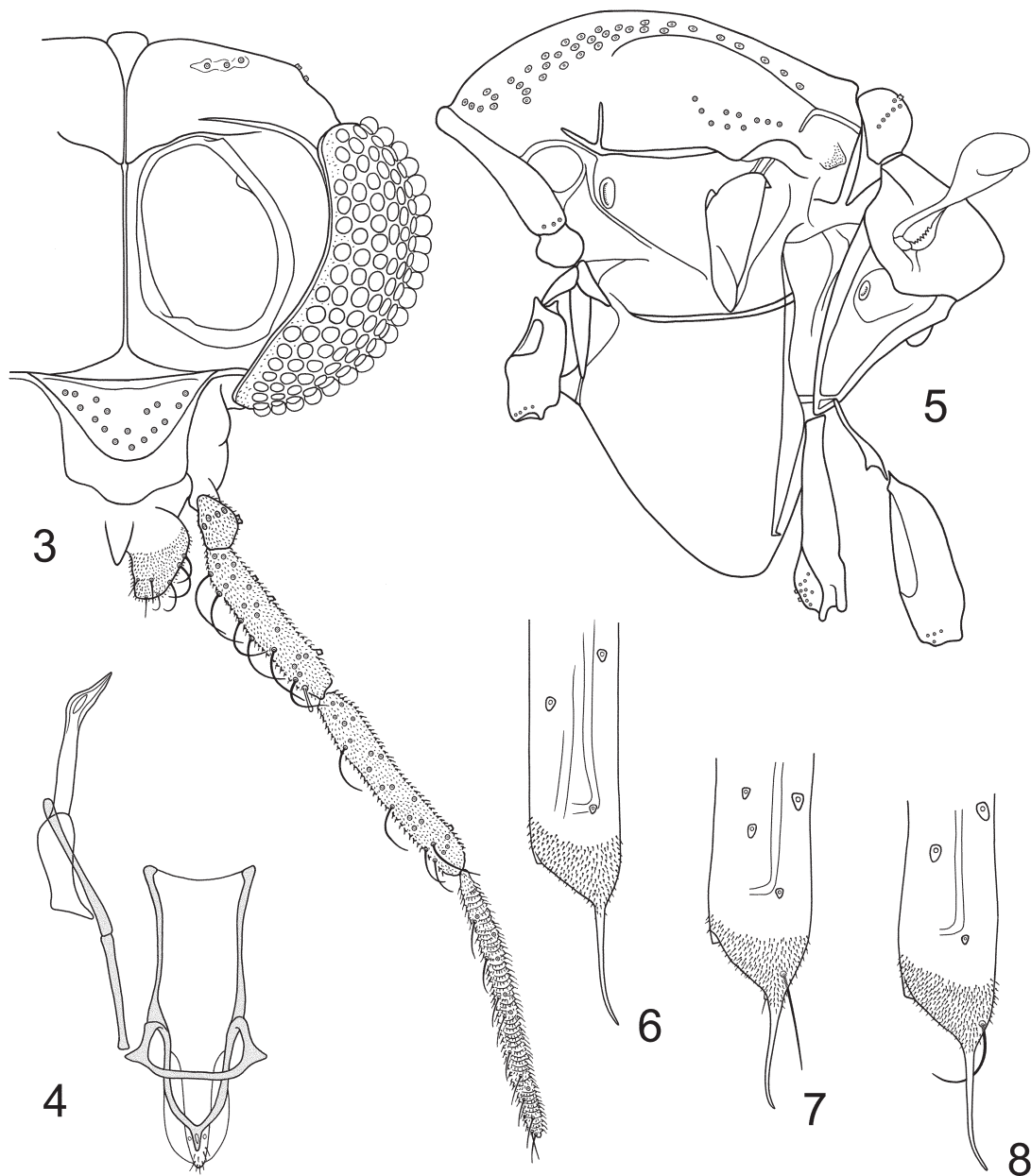
### Description

Male (n = 10, unless stated differently).

Total length 1.70–2.18, 1.90 mm. Wing length 1.01–1.39, 1.15 mm. Total length / wing length 1.57–1.79, 1.65. Wing length / length of fore femur 2.05–2.41, 2.24.

**Coloration.** Head, thorax and abdomen brown; femur and tibiae lighter brown; wings translucent.

**Head** (Fig. 3). AR 0.48 (1). Terminal flagellomere 162–205 (2) µm long. Temporal setae 7–12, 9; consisting of 3–6, 4 inner verticals; 1–3, 2 outer verticals and 2–4, 3 postorbitals. Clypeus with 13–23, 17 setae. Tentorium, stipes and cibarial pump as in Figure 4. Tentorium 98–117, 105 µm long; 18–22, 19 µm wide. Stipes 101–116, 108 µm long. Palpomere lengths (in µm): 22–26, 23; 30–36, 33; 92–104, 98; 108–123, 116 (8); 159 (1). Third



Figures 3–8. *Mollerietta kaputu* n. sp., male. 3, head; 4, tentorium, stipes and cibarial pump; 5, thorax; 6, apex of fore tibia; 7, apex of mid tibia; 8, apex of hind tibia.

palpomere with 1–2 sensilla clavata subapically, longest 11–13  $\mu\text{m}$  long.

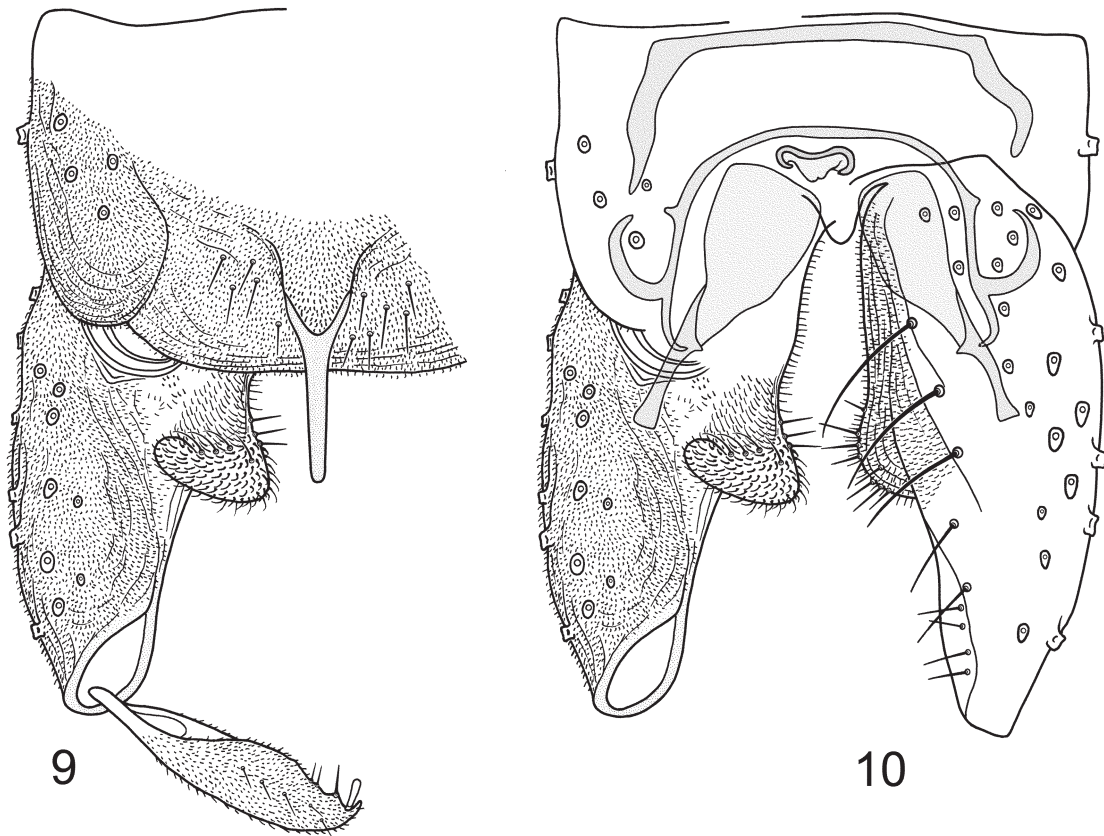
*Thorax* (Fig. 5). Anteprenotum with 2–4, 3 ventrolateral setae. Dorsocentrals 29–45, 35 starting close to anteprenotum; prealars 7–11, 10. Scutellum with 12–16, 14 setae. Preepisternum apparently without minute setae.

*Wing* (Fig. 11). VR 1.27–1.50, 1.37. C extension 104–128, 116  $\mu\text{m}$  long. Brachiolum with 2–3, 3 setae; C extension with about 47–54, 49 non-marginal setae; Sc with about 52–75, 66; R with about

31–42, 36;  $R_1$  with about 22–35, 29;  $R_{4+5}$  with about 33–51, 43; RM with 1–3, 2; M with about 2–5, 3;  $M_{1+2}$  with about 66–85, 74;  $M_{3+4}$  with about 45–56, 48; Cu with about 36–47, 41;  $Cu_1$  with about 25–34, 29; PCu with about 74–97, 85; and An with about 47–54, 49 setae. Wing membrane with about 110 setae in cell m; about 600 in  $r_{4+5}$ ; about 550 in  $m_{1+2}$ ; about 250 in  $m_{3+4}$ ; and about 350 in cu and an combined. Squama with 4–8, 6 setae.

*Legs*. Fore femur 472–568, 508  $\mu\text{m}$  long; fore tibia 268–336, 304  $\mu\text{m}$  long; mid femur 492–589, 528  $\mu\text{m}$  long; mid tibia 376–452, 404  $\mu\text{m}$  long; hind





Figures 9–10. *MollerIELLA kaputu* n. sp., male. 9, hypopygium, dorsal view; 10, hypopygium with anal point and tergite IX removed, dorsal aspect to the left and ventral aspect to the right.

femur 452–516, 484  $\mu\text{m}$  long; hind tibia 436–508, 464  $\mu\text{m}$  long; all tarsi lost. Scale of fore tibia (Fig. 6) 42–54, 45  $\mu\text{m}$  long including 28–35, 30  $\mu\text{m}$  long spur; scale of mid tibia (Fig. 7) 47–56, 50  $\mu\text{m}$  long including 29–36, 33  $\mu\text{m}$  long spur; scale of hind tibia (Fig. 8) 50–55, 52  $\mu\text{m}$  long including 28–36, 32  $\mu\text{m}$  long spur. Width at apex of fore tibia 23–28, 25  $\mu\text{m}$ ; of mid tibia 29–32, 30  $\mu\text{m}$ ; of hind tibia 28–32, 30  $\mu\text{m}$ .

*Hypopygium* (Figs 9–10). Anal point 33–37, 35  $\mu\text{m}$  long; 6–8, 7  $\mu\text{m}$  wide at base; 4–6, 5  $\mu\text{m}$  wide at apex. Tergite IX with altogether 5–11, 8 setae; laterosternite IX with 6–9, 7 setae. Phallosodeme 58–63, 61  $\mu\text{m}$  long; transverse sternapodeme 54–67, 60  $\mu\text{m}$  long. Virga 10–14, 12  $\mu\text{m}$  long; 14–18, 16  $\mu\text{m}$  wide. Gonocoxite 121–144, 130  $\mu\text{m}$  long. Superior volsella absent. Inferior volsella 29–35, 31  $\mu\text{m}$  long; 22–25, 24  $\mu\text{m}$  wide at its widest point. Gonostylus 66–76, 68  $\mu\text{m}$  long; megaseta 8–10, 9  $\mu\text{m}$  long. HR 1.76–1.98, 1.87. HV 2.64–2.96, 2.78.

Female ( $n = 1$ ).

Total length 1.25 mm. Wing length 0.96 mm. Total length / wing length 1.30. Wing length / length of

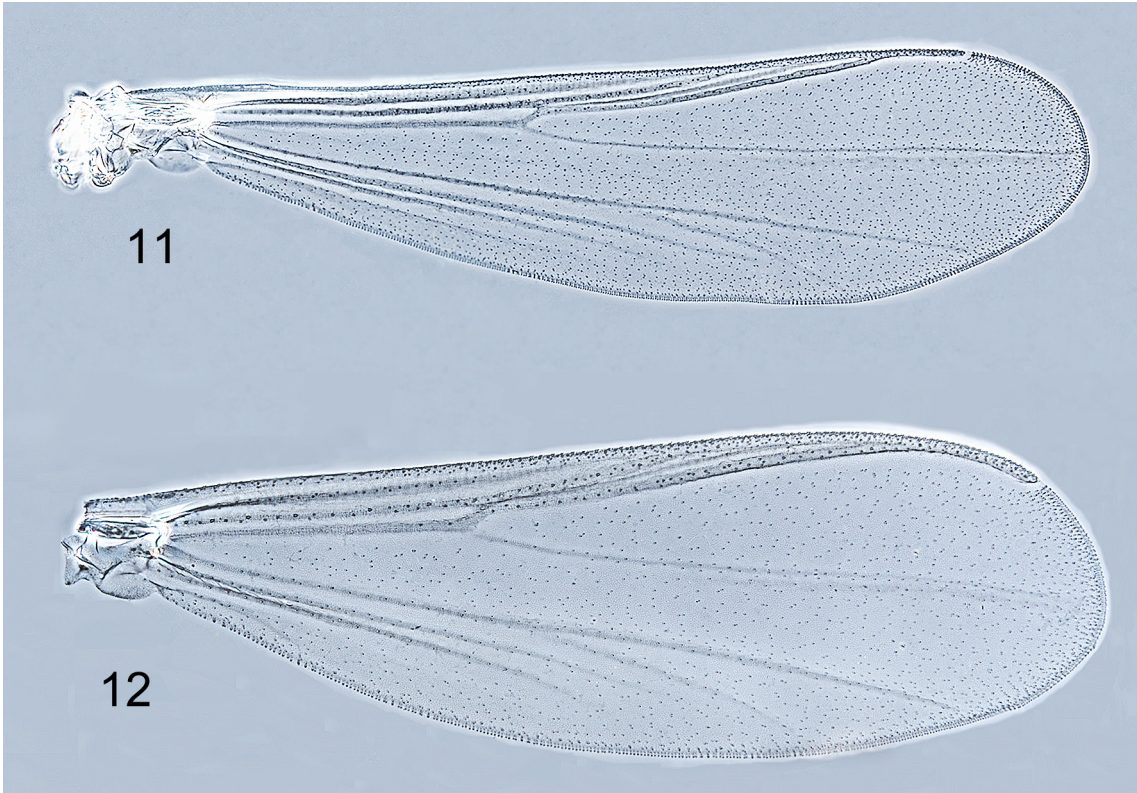
fore femur 2.85.

*Coloration*. Head, thorax and abdomen brown; femur and tibiae lighter brown; wings translucent.

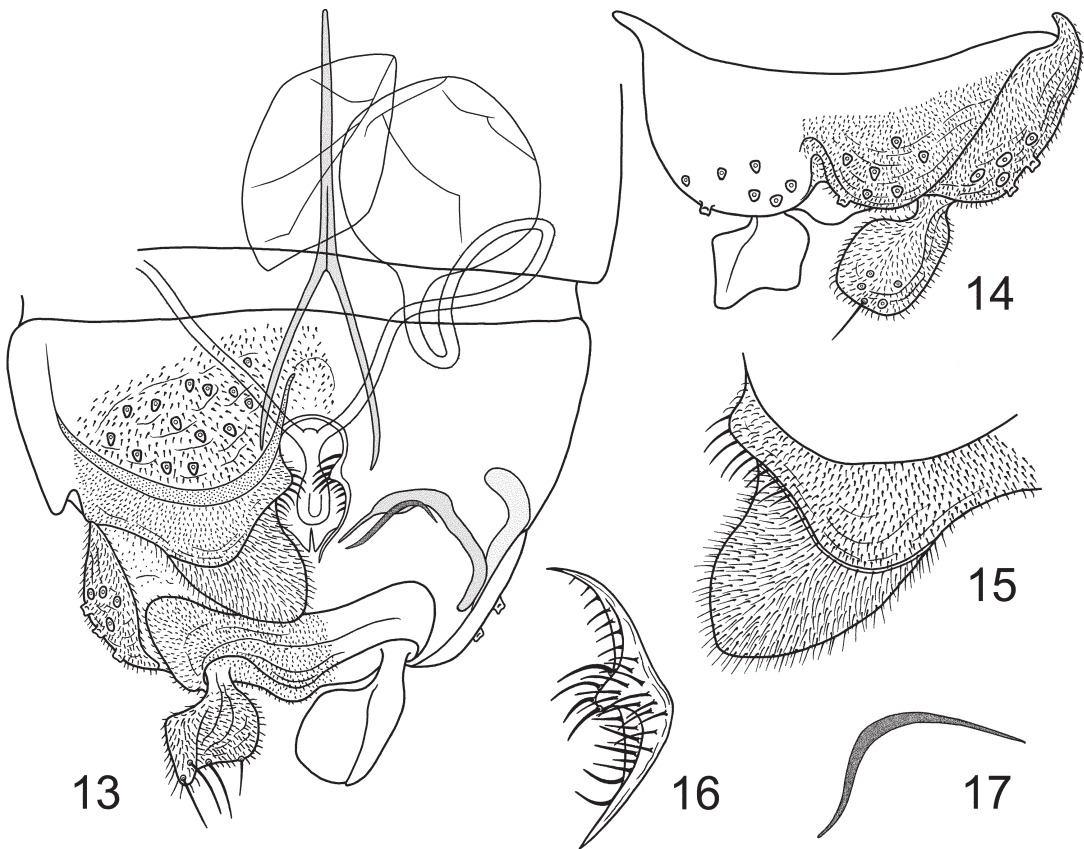
*Head*. Antenna missing. Temporal setae 11, consisting of 5 inner verticals; 2 outer verticals and 4 postorbitals. Clypeus with 21 setae. Tentorium 72  $\mu\text{m}$  long, 8  $\mu\text{m}$  wide. Stipes 90  $\mu\text{m}$  long. Palp segment lengths (in  $\mu\text{m}$ ): 22, 26, 75, 95, fifth palpomere missing. Third palpomere with 2 sensilla clavata subapically, longest 19  $\mu\text{m}$  long.

*Thorax*. Anteprepronotum with 3 ventrolateral setae. Dorsocentrals 47 starting close to anteprepronotum; prealars 9. Scutellum with 14 setae.

*Wing* (Fig. 12). VR 1.37. C extension 173  $\mu\text{m}$  long. Brachiolum with 2 setae; C extension with about 86 non-marginal setae; Sc with about 27; R with about 24;  $R_1$  with about 22;  $R_{4+5}$  with about 41; RM with 2; M with 2;  $M_{1+2}$  with about 56;  $M_{3+4}$  with about 45;  $Cu$  with about 25;  $Cu_1$  with about 23; PCu with about 49; and An with about 29 setae. Wing membrane with about 60 setae in cell m; about 325 in  $r_{4+5}$ ; about 475 in  $m_{1+2}$ ; about 150 in



Figures 11–12. *Mollerietta kaputu* n. sp., 11, wing, male; 12, wing, female.



Figures 13–17. *Mollerietta kaputu* n. sp., female. 13, genitalia, ventral view; 14, genitalia, dorsal view; 15, ventrolateral lobe; 16, dorsomesal lobe; 17, apodeme lobe.



$m_{3+4}$ ; and about 250 in cu and an combined. Squama with 5 setae.

**Legs.** Fore femur 380  $\mu\text{m}$  long; fore tibia 284  $\mu\text{m}$  long; mid femur 420  $\mu\text{m}$  long; mid tibia 344  $\mu\text{m}$  long; hind femur 404  $\mu\text{m}$  long; hind tibia 396  $\mu\text{m}$  long; all tarsi lost. Scale of fore tibia 39  $\mu\text{m}$  long including 26  $\mu\text{m}$  long spur; scale of mid tibia 40  $\mu\text{m}$  long including 26  $\mu\text{m}$  long spur; scale of hind tibia 48  $\mu\text{m}$  long including 30  $\mu\text{m}$  long spur. Width at apex of fore tibia 28  $\mu\text{m}$ ; of mid tibia 30  $\mu\text{m}$ ; of hind tibia 31  $\mu\text{m}$ .

**Genitalia** (Figs 13–17). Gonocoxite IX with 6 setae. Tergite IX with posteromedial deep incision, each side with 7–8 setae. Cercus 47  $\mu\text{m}$  long. Seminal capsules distorted in the single specimen, about 76  $\mu\text{m}$  long, 62  $\mu\text{m}$  wide, with 11  $\mu\text{m}$  long neck. Notum 72  $\mu\text{m}$  long. Ventrolateral lobe bluntly triangular, 52  $\mu\text{m}$  long, 48  $\mu\text{m}$  wide at its widest point, covered with microtrichia. Dorsomesal lobe narrow, 46  $\mu\text{m}$  long, 8  $\mu\text{m}$  wide.

Pupa and larva. Unknown.

#### Remarks

The new species is very similar to *Molleriella calcarella* in most morphological features, and the two species also overlap in most measurements. However, the male *M. calcarella* has a spatulate anal point, while the anal point in *M. kaputu* is slightly narrower and nearly parallel sided. *M.*

*calcarella* apparently also has more and stronger setae on the inferior volsella than *M. kaputu*. In *M. calcarella* the C extension is 79–83  $\mu\text{m}$  long in the male and on average 121  $\mu\text{m}$  long in the female, while in *M. kaputu* it is on average 116  $\mu\text{m}$  long in the male and 173  $\mu\text{m}$  long in the female. The most distinct difference seems however, to be in the length of the tibiae; in *M. calcarella* fore tibia is 430–435  $\mu\text{m}$  long in the male and on average 361  $\mu\text{m}$  in the female, while in *M. kaputu* it is on average 304  $\mu\text{m}$  long in the male, 284  $\mu\text{m}$  long in the female. Using an unconventional leg ratio, length of tibia / length of femur, the fore leg ratio is 0.81 in male *M. calcarella* and 0.54–0.62, 0.58 in male *M. kaputu*, 0.85–0.88, 0.87 in female *M. calcarella* and 0.75 in female *M. kaputu*.

#### Habitat

Two main vegetation types are found in the Mazumbai Forest Reserve: intermediate forest and mountain rainforest. At higher elevations outside the forest reserve dry montane forest occurs. The trees in the intermediate forest can reach a height of 50 m, while those at higher altitudes are reduced in height with increasing altitude.

During our stay at Mazumbai in late autumn 1990 daily maximum temperatures ranged from 17.5 to 23.0°C and the minima from 15.0 to 20.5°C. In the Usambara mountains there are two main rainy seasons, a heavier one from the beginning of March



Figure 18. Kaputu Stream at locality 7 at 1535 m altitude. The Malaise trap was placed across the stream.



to the end of May, and a lighter one in September and October. Mean annual rainfall at Mazumbai (1945–1975) was 1138 mm.

The Kaputu Stream is located on the eastern side of Kwagoroto Hill and is surrounded by nearly undisturbed rainforest. It originates at about 1860 m altitude and runs down to a marshy area at about 1400 m altitude. Four relatively large waterfalls are located along the stream, but in most stretches the water speed is moderate. The water temperatures measured varied between 14.6 and 17.6°C and the pH was 5.9.

The specimens of *M. kaputu* were collected at three of the Malaise trap localities along the Kaputu Stream described in Andersen and Johanson (1993). At locality 4, at 1680 m altitude, the stream was 1–3 m wide and 3–15 cm deep and the current was moderate; the substrate was mostly sand, gravel and stones densely covered with moss. At locality 7 (Fig. 18), at 1535 m altitude, the stream was 2–3 m wide and 5–15 cm deep and the current was rather slow; the substrate was mostly gravel and stones densely covered with moss, with some mud in the backwaters. At locality 10, at 1420 m altitude, the stream was 0.5–2 m wide and 10–20 cm deep and the current was moderate; the substrate was mostly fine sand, mud and some larger stones densely covered with moss.

According to Sæther and Ekrem (1999) *M. cal-carella* is terrestrial; it was caught in “mini-traps” placed on the banks of a small lake about one meter from the water edge. As the material of *M. kaputu* was collected in Malaise traps, this species too might well be terrestrial, having originated in the moss or wet soil along the Kaputu Stream.

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## FACTORS AFFECTING DECOMPOSITION RATES OF CHIRONOMID (DIPTERA) PUPAL EXUVIAE

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### Abstract

Collections of floating chironomid pupal exuviae are used to monitor water quality and assess ecological conditions. Factors controlling exuviae sinking rates are not well known, although they should have an effect on conclusions that can be drawn from collections. The current study was conducted to determine the rate of sinking under controlled laboratory conditions using water from three streams with different nutrient levels. Sinking rates ranged from less than a day to seven days, depending on microbial activity, nutrient concentrations, temperature and turbulence. Results also varied by genus, with pupal exuviae of *Chironomus riparius*, *Diamesa nivoriunda*, *Orthocladius (Euorthocladius) thienemanni* and *Eukiefferiella* sp. used in experiments. Four species of bacteria and eight genera of fungi colonized and metabolized exuviae, with bacteria dominant early and fungi dominant later in the decomposition process. Decomposition was faster in lightly chitinized abdominal conjunctive areas, which resulted in exuviae breaking apart and sinking. Examination of untreated, dewaxed and dewaxed-deproteinized exuviae indicated that untreated exuviae sank faster. Waxes appeared important for colonization and initial microbial metabolization was delayed when waxes were removed. Results confirm the importance of biological degradation of exuviae in determining floatation times. We predict that streams and other waterbodies with high dissolved nutrients will result in rapidly sinking exuviae, while exuviae in low nutrient waterbodies will float longer.

### Introduction

Chitin, an inert and insoluble polysaccharide found in insect cuticle, is among the most abundant naturally occurring organic compounds on Earth (Nation 2008). Pupal exuviae of Chironomidae are primarily chitin-protein complexes with a waxy coating (Anderson 1979, Nation 2008). Chitino-

elastic microbes are common in both aerobic and anaerobic aquatic environments (e.g., Warner and Randles 1977, Bye and Charnley 2008). Therefore, exuviae should be colonized by chitinoclastic bacteria and fungi (Tracy and Valletyne 1968, Dick 1970). Variability in colonization and growth of chitinoclastic bacteria and fungi has potential to change decomposition rates of exuviae.

Collections of chironomid pupal exuviae have been increasingly used to monitor water quality, assess ecological communities, and classify trophic status of waterbodies in both lotic and lentic systems (Coffman 1973, Raunio and Muotka 2005, Raunio et al. 2010, Bouchard and Ferrington 2011). Exuviae are generally easy to locate and efficient to collect, and species occupying many microhabitats can be detected (Wilson and Ruse 2005). Keys in recent decades have made exuviae identification to genus relatively easy (Langton 1991, Ferrington et al. 2008) and species-level keys are available for many genera.

A methodological variable in studies using collections of pupal exuviae is the length of time exuviae remain floating on the surface of the water (Coffman 1973, Wilson and Ruse 2005). A preliminary study on lentic exuviae suggested that sinking generally occurred within 2-10 days, depending on temperature, microbial activity and mechanical disruption (Coffman 1973). However, time necessary for breakdown appeared strongly influenced by the degree of chitinization, so that certain species may be under- or overrepresented in samples depending upon the time interval between population emergence and sampling events.

Although sinking of exuviae has been tentatively linked to microbial activity, to our knowledge, this direct association has not been studied. The present study investigated factors that affect the sinking of exuviae, along with the role of bacteria and fungi in the metabolism and break down of exuviae. Objectives were to: 1) examine factors



that may affect the sinking of exuviae, including temperature, nutrients, microbial activity and mechanical disruption; 2) isolate and identify microorganisms metabolizing chironomid exuviae; 3) document the succession of microorganisms on exuviae; and 4) determine the effect of dewaxing and deproteinization on exuviae sinking.

## Materials and Methods

### *Collection of Exuviae and Water Samples*

Collections occurred between May 1984 and April 1986. Exuviae of *Chironomus riparius* Meigen were collected in spring and summer from Mill Creek, Johnson County, Kansas (T13S, R23E, Sec. 13). Mill Creek received sewage effluent, with a high concentration of nutrients compared to the other study sites. Exuviae of *Diamesa nivoriunda* (Fitch), *Orthocladius* (*Euorthocladius*) *thienemanni* Kieffer and *Eukiefferiella* sp. were collected in winter and spring from Deer Creek, Douglas County, Kansas (T12S, R18E, Sec. 31). Deer Creek was relatively unimpacted, clear, and slow-flowing.

Recently shed pupal exuviae were collected from the stream surface soon after emergence of adults. Exuviae were handpicked using forceps, initially from within a 16'x3' emergence channel constructed of a plywood frame and sides resting on the stream bottom, and a net on the upstream and downstream ends. Once the upstream net was in place, the channel sides allowed an area of stream-bottom substrates to be "enclosed" for a known period of time, providing access to newly shed exuviae. The downstream net was subsequently affixed after allowing enough time for older exuviae to drift through the channel. Later in the season, low water levels required hand picking exuviae without use of the channel. During late-season collections, newly shed exuviae were determined based on lack of water in the cephalothorax, complete and unbroken specimens, and no visible fungal or bacterial growth.

Water samples for experiments and microbial analyses were collected during the same visit from the streams noted above, along with Mud Creek, Douglas County (T12S, R20E, Sec. 7). Mud Creek was turbid due to channelization, fast flowing, lacked riparian vegetation, and received run-off from surrounding agricultural fields. Sterile flasks wrapped in aluminum foil were used to collect water samples for microbial analyses. Flasks were unwrapped just before sampling, plugs were removed and flasks immediately dipped to collect surface water to a depth of ½ inch, then resealed for transport. No more than 60 minutes passed

from collection to plating for microbial analyses. Water samples for chemical analyses and experiments were collected in 1-liter bottles washed in concentrated sulfuric acid and rinsed with distilled water before sampling. Water and exuviae were transported to the laboratory in a cooler at about 10°C. Unless noted below, all experiments were conducted at room temperature (20°C).

### *Experiments*

#### *1) Factors affecting exuviae sinking*

a) *Establishing baseline data:* Exuviae of *Diamesa nivoriunda*, *Orthocladius* (*Euorthocladius*) *thienemanni* and *Eukiefferiella* collected from Deer Creek were held in a single flask containing stream water. The time required for all exuviae to sink was recorded. This experiment was repeated three times.

b) *Nutrients and mechanical shaking:* Ten exuviae each of *D. nivoriunda*, *O. thienemanni* and *Eukiefferiella* collected in Deer Creek were separately transferred to three flasks. A nutrient solution (#1) of 0.4 g (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> and 0.1 g of K<sub>2</sub>H<sub>2</sub>PO<sub>4</sub> was added to 20 mL of Deer Creek water. Two mL of the solution was added to each of two flasks, one kept on a shaker and the other on a table with occasional shaking by hand to simulate the light turbulence of natural conditions. The third flask was also occasionally shaken by hand but had no nutrient solution added. The time required for all exuviae to sink was recorded. This experiment was repeated twice.

c) *Nitrate-nitrogen vs. ammonia-nitrogen:* A second nutrient solution (#2) was prepared using 0.4 g NaNO<sub>3</sub> and 0.1 g K<sub>2</sub>H<sub>2</sub>PO<sub>4</sub> in 20 mL of distilled water. Both nutrient solution concentrations were purposely above ambient stream conditions to ensure any response would be measurable. Ten exuviae of *D. nivoriunda*, *O. thienemanni* and *Eukiefferiella* were transferred to 8 flasks containing 20 mL Deer Creek water. Four flasks had 2 mL of nutrient solution #1 added and four flasks had 2 mL of nutrient solution #2 added. For each treatment, two flasks were kept on a shaker and the other two on a table with occasional hand shaking. The percentage of exuviae sinking in three days was recorded. This experiment was repeated twice.

d) *Temperature, microbial activity and mechanical shaking:* Ten *Chironomus riparius* exuviae were transferred to each of three flasks containing distilled water and untreated stream water. One set of three flasks was kept static with occasional hand shaking and another set of three flasks was kept on a shaker. The experiment was conducted at two

temperatures (22.2°C and 25°C). At 12, 24, 29 and 48 hours, the percentage of exuviae sinking as well as plate counts of bacteria were recorded. This experiment was repeated five times.

e) *Water quality*: Methodology is similar to experiment (d), except that only untreated and aerated water from each stream was used. Flasks were incubated at 29°C. At the time of field collections of *C. riparius* exuviae for this experiment, water chemistry data were collected for nitrate (NO<sub>3</sub>) and ammonia (NH<sub>4</sub>) content using a Hach Ds-EH2 spectrophotometer. This experiment was repeated five times.

f) *Aerobic and anaerobic decomposition*: To assess aerobic decomposition, exuviae of *C. riparius* were heat-fixed on slides, by passing slides over a flame, and then immersed in bottles of aerated Mill Creek water. Anaerobic decomposition used fixed exuviae in Mill Creek water, but bottles were completely filled with no air space, closed airtight, and incubated at 20°C. Anaerobic conditions were expected after two days due to high microbial numbers. For each treatment, two slides were removed daily for 10 days, stained, and observed for progression of decomposition using photomicrographs. To assess anaerobic conditions, the slides were examined after 10 days, then monthly for one year.

g) *Waxes and proteins*: *Chironomus riparius* exuviae were treated to remove waxes, or waxes and proteins. Wax was removed by refluxing with 100 ml of petroleum ether for 30 minutes over a water bath. Half of the dewaxed exuviae were then treated with 100 ml of 10% W/V aqueous solution of NaOH for three hours to achieve deproteinization. Treated exuviae were held in sterile beakers containing aerated Mill Creek water. The time taken for 100% of exuviae to sink was recorded.

## 2) *Succession of microorganisms colonizing exuviae*

Exuviae of *C. riparius* collected from within the wooden channel were held in vials of Mill Creek water in the lab. Exuviae were picked from the water at various intervals from 2-72 hours, washed in sterile water, and placed in tubes containing tryptose broth. Tubes were incubated at room temperature for 36 hours. The growth from tubes was then placed on Dextrose-Tryptone (DT) agar to isolate bacteria and Cook's Rose Bengal (CRB) agar to isolate fungi. DT plates were incubated for another 48 hours to record bacterial growth and CRB plates incubated for 7 days to record fungal growth. The relative growths of bacteria, actinomycetes and fungi from different ages of exuviae

were recorded and curves for succession determined. Relative density values on a scale of 1-4 were assigned for colony growth on plates, with a value of 4 given to the plate with the greatest number of colonies and the rest given qualitatively lower numbers in comparison. Exuviae were also picked at different time intervals (between 2-72 hours) and stained to observe the progression of decomposition. These techniques were also used in experiment (g) above, both in aerobic and anaerobic conditions.

## 3) *Isolation of exuviae-metabolizing bacteria*

Exuviae of *C. riparius* were rinsed to remove passive-adhering bacteria and detritus, and placed on a mineral agar medium devoid of a carbon source. Bacterial growths around the exuviae were isolated after incubation at 20°C for 36 hours. The colonies were pure-cultured on DT agar, and maintained on nutrient agar slants. A 2% chitin agar was prepared using crude crustacean chitin (commercial grade, Sigma Chemicals) and purified using the method described by Skujins et al. (1965). Bacteria were identified using tests described in Buchanan and Gibbons (1974).

## 4) *Isolation and identification of fungi growing on exuviae*

Exuviae from all four taxa were washed twice in sterile water with a 2% solution of streptomycin to inhibit bacterial growth, and then placed on plates with potato-dextrose agar both with and without added antibiotic. A second washing technique used a medium of 1.5% bactoagar. Plates were incubated at 25°C for 5-7 days for most fungi and up to two weeks for slow-growing fungi. Isolated fungi were maintained on pure cultures of potato-dextrose agar and streaked on plates of agar prepared with blended mixtures of exuviae. Two sterilized, blended mixtures were made from water, 1.5% bactoagar and either *C. riparius* from Mill Creek or a mix of the three taxa collected from Deer Creek. Fungi were identified by isolating growths, followed by slide-mounting and staining. Identification keys used were Coker (1923), Barnett and Barry (1972), Fuller (1978) and Alexopoulos and Mins (1979). Water molds isolated from water agar were baited with hemp seeds and transferred to YPSS-Emerson medium to induce spore production to assist with identifications.

## Results

### *Establishing baseline data*

One hundred percent of exuviae of *D. nivoriunda*, *O. thienemanni* and *Eukiefferiella* from Deer Creek sank within seven days in the control group

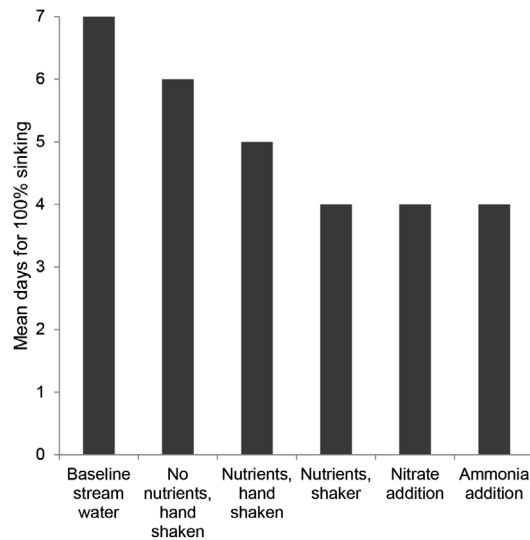


Figure 1. Mean *D. nivoriunda*, *O. thienemanni*, and *Eukiefferiella* exuviae sinking times across treatments, Deer Creek water, 20°C.

(Fig. 1). Without separation into individual flasks, we cannot compare variation of baseline sinking rates for each species.

#### Nutrients and shaking

One hundred percent of *D. nivoriunda*, *O. thienemanni* and *Eukiefferiella* exuviae sank within four days when nutrient solution #1 was added and flasks were kept on a shaker, while those receiving nutrient solution #1 but held stationary sank within five days (Fig. 1). Control flasks with no added nutrients but occasional shaking by hand had 100% sinking in six days. Photomicrographs revealed greater microbial colonization with increased nutrients or aeration via shaking.

#### Nitrate-nitrogen vs. ammonia-nitrogen

The percentage of *Eukiefferiella* that sank varied considerably when comparing Nitrate-N and Ammonia-N shaking treatments, however no difference in sinking rates was observed between these treatments when flasks were only occasionally hand shaken (Fig. 1, Table 1). Neither *D. nivoriunda* nor *O. thienemanni* differed notably in response to nutrient type, but *D. nivoriunda* sank much slower in shaker treatments. In nitrate treatments, it took an average of four days for all exuviae to sink, while 80-100% of exuviae sank in ammonia treatments in the same time.

#### Temperature, microbial activity and mechanical shaking

All *C. riparius* exuviae sank within 48 hours and more than 50% of exuviae sank within 24 hours regardless of treatment (Table 2). All three factors appear to affect the rate of exuviae sinking, with faster sinking generally related to warmer water, presence of naturally-occurring microbes and consistent shaking.

#### Water quality

Proportion of *C. riparius* exuviae sinking is also influenced by water quality and corresponds to nutrients and bacterial numbers (Table 3). A large majority of exuviae sank within 12 hours in Mill Creek water with higher nutrients and bacterial counts, and all sank within 24 hours. Although nutrients were higher in Deer Creek water than Mud Creek, there were no substantive differences between bacterial counts or sinking rates.

Table 1. Average percentage of exuviae sinking in three days under nutrient and shaking treatments, using Deer Creek water at 20°C.

	<i>Eukiefferiella</i> sp.	<i>O. thienemanni</i>	<i>D. nivoriunda</i>
Nitrate-N, shake	90%	30%	10%
Ammonia-N, shake	30%	20%	10%
Nitrate-N, no shake	40%	10%	80%
Ammonia-N, no shake	40%	10%	90%

Table 2. Percentage of *C. riparius* exuviae sinking in distilled water and Mill Creek water, averaged from five replicates.

Time	22.2°C Shaken		25°C Static		25°C Shaken	
	Distilled	Stream	Distilled	Stream	Distilled	Stream
12 hrs	14	37	14	70	50	95
24 hrs	56	80	83	95	80	100
29 hrs	70	100	90	100	90	
48 hrs	100		100		100	

Table 3. Effects of nutrients (nitrate and ammonia) and bacterial numbers on exuviae sinking rate.

	NO <sub>3</sub> (mg/L)	NH <sub>4</sub> (mg/L)	Bacteria (no./mL)	Percent sinking, 12 hours	Percent sinking, 24 hours
Mill Creek	0.488	0.666	2 x 10 <sup>4</sup>	70	100
Deer Creek	0.132	0.244	2 x 10 <sup>2</sup>	30	80
Mud Creek	0.066	0.183	2 x 10 <sup>2</sup>	40	80

*Aerobic and anaerobic decomposition*

Under aerobic decomposition, components of *C. riparius* exuviae were detectable for up to 10 days. After 10 days, no discernable portions of exuviae remained on slides. All exuviae were initially colonized along the pleurites and conjunctives. The intersegmental conjunctives were the first to be metabolized, resulting in segments breaking apart. The cephalothorax was more resistant. Both rod and filamentous microbes dominated the aerobic decomposition process. Anaerobic experiments revealed that exuviae took up to a year to completely decompose. The sequence of colonization and degradation of pleurites and conjunctives was similar to the aerobic process.

*Waxes and proteins*

Dewaxed and deproteinized *C. riparius* exuviae sank faster (5 days for 100% sinking) than dewaxed exuviae (7 days for 100% sinking) in aerated stream water.

*Succession of microorganisms colonizing exuviae*

Qualitative results of succession showed a distinct transition from bacterial to fungal dominance over several days (Fig. 2). Dominance of bacteria appeared stable prior to 31 hours, while fungi were dominant after 49 hours and a distinct transition

from bacterial dominance to fungal dominance occurred in-between.

*Isolation of exuviae-metabolizing bacteria*

Four different bacterial cultures were isolated and maintained on nutrient agar slants (Table 4), indicating that bacteria can utilize exuviae as a sole carbon source. There was no growth on water or mineral agar devoid of any carbon source. Bacteria species names are used tentatively since biochemical characteristics differed slightly from typical species and presumably represent different strains of each species. All four species were gram-negative aerobic rods capable of growth on chitin agar. For descriptions of each species cultured, see Kavanaugh (1988). Photographs of the progression of primarily bacterial decomposition are shown in Fig. 3. Decomposition was initially more pronounced in pleurite and conjunctive regions. Bacteria colonized and metabolized inner parts of the segments later. The cephalothorax was more resistant to metabolization than the abdomen.

*Isolation and identification of fungi growing on exuviae*

Eight genera of fungi were isolated from exuviae, all of which are common aquatic and airborne fungi (Table 4). Microscopic examination of exuviae

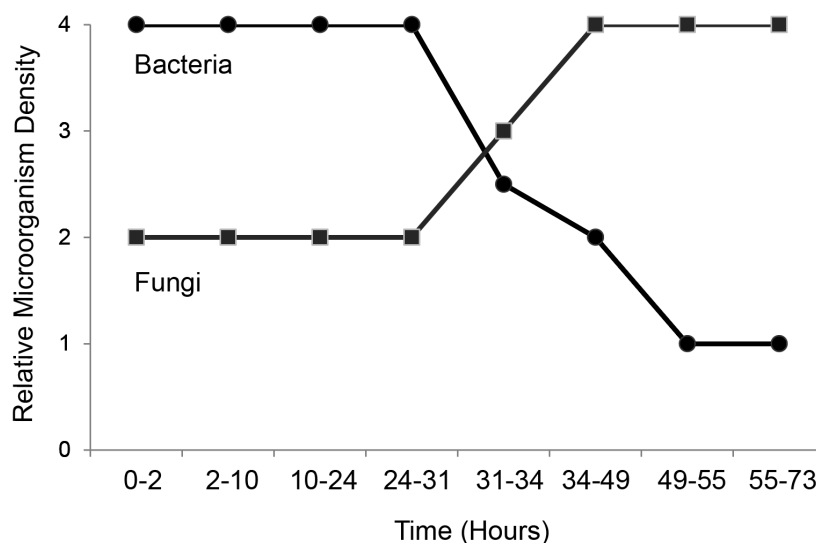


Figure 2. Succession of microbes on exuviae. Qualitatively ranked based on scores for colony growth on plates, with categories ranging from the greatest number of colonies (4) on a plate to the smallest (1).



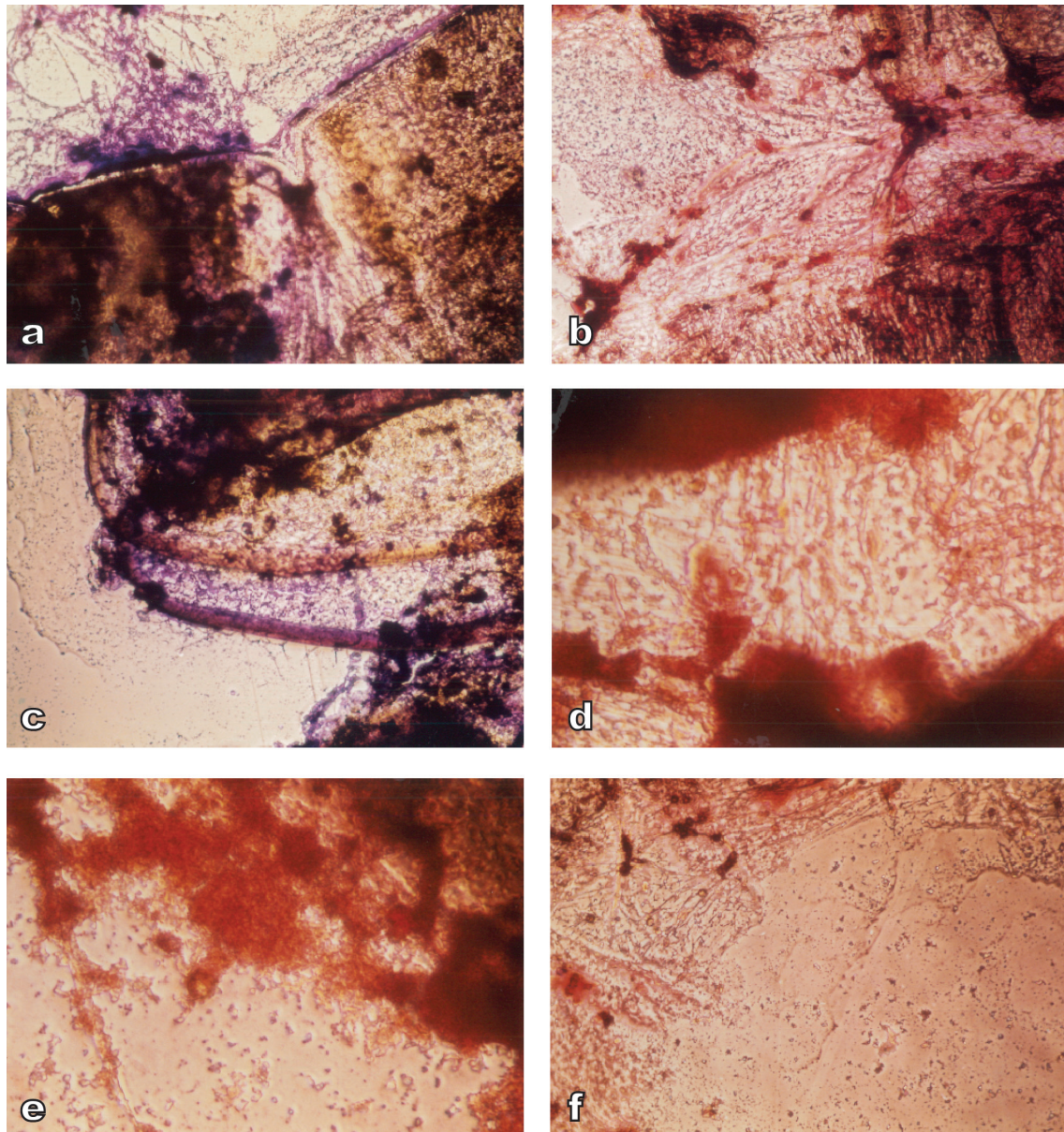


Figure 3. Progress of *C. riparius* exuviae decomposition over time. a) beginning of decomposition, with two abdominal segments and intersegment shown; b) intersegmental region with microbes (400x); c) cephalothorax showing more resistance than abdominal segments to decomposition (100x); d) intersegment decomposed, with bacteria along segment edges (400x); e) large numbers of metabolizing bacteria (400x); f) highly decomposed segment (100x).

showed that fungi were decomposing exuviae, though the antibiotic did not necessarily eliminate all bacterial processes. The cephalothorax was more resistant to decomposition than the abdominal region. During fungal growth, abdominal segments and conjunctive areas were solubilized at about the same rate, although abdominal setae remained undegraded throughout observations.

Water molds were isolated on exuviae of all taxa, but none sporulated and were therefore not identifiable. However, the dominant water mold resembled the genus *Achlya*, with numerous sporangia that were broad in the middle and narrower at the

ends, usually extending from vegetative hyphae. Zoospores were encysted within and released from the sporangium. Vegetative hyphae had numerous gemme or irregular growth. Biflagellate secondary spores were also seen. In "*Achlya*", antheridia and oogonia were not seen.

#### Discussion

The main factor affecting the sinking of exuviae in the laboratory was microbial numbers as indicated by water quality and nutrient concentrations. Additional factors were temperature and mechanical disturbance following initial decomposition. A succession of microorganisms capable of metabo-

Table 4. Bacteria and fungi colonizing chironomid pupal exuviae as a nutrient source.

	<i>Chironomus riparius</i>	<i>O. thienemanni</i> <i>D. nivoriunda</i> <i>Eukiefferiella</i> sp.
Bacteria	<i>Flavobacterium</i> sp. ( <i>aquatile</i> ?)	X
	<i>Pseudomonas</i> sp. ( <i>alcaligenes</i> ?)	X
	<i>Pseudomonas</i> sp. ( <i>facilis</i> ?)	X
	<i>Pseudomonas</i> sp. ( <i>fluorescens</i> ?)	X
Fungi	<i>Alternaria</i>	X
	<i>Aspergillus</i>	X
	<i>Cladosporium</i>	X
	<i>Mucor</i>	
	<i>Pestalotia</i>	
	<i>Phoma</i>	X
	<i>Rhizopus</i>	
	<i>Trichoderma</i>	X

lizing exuviae as a source of carbon were isolated and identified. All exuviae sank within a range of 1-7 days, with rates depending on these factors. In a pristine creek without substantial nutrient additions or turbulence, exuviae collections may be expected to represent emergences from up to seven prior days. In contrast, streams with elevated water temperature, nutrients and agitation create conditions where exuviae may only represent emergences from the previous 12-48 hours.

Rate of sinking for each species was affected by both nutrients and mechanical disruption. *Eukiefferiella* had fast sinking rates in nitrate treatments with constant agitation. *Diamesa nivoriunda* had the opposite response to agitation, with stronger sinking rates in stable conditions. In contrast, *O. thienemanni* had no distinct response to either treatment. These differences illustrate how environmental conditions can influence the availability and abundance of floating exuviae across genera. Although baseline results showed that all species sank within seven days, they were not run concurrently to nutrient and shaking experiments and thus cannot act as a control.

Separate experiments using *C. riparius* also indicated a heightened sinking response to increased temperature, nutrients and agitation. While rates of sinking were faster than the other three genera, *C. riparius* exuviae were exposed *in situ* to higher nutrients and microbes since Mill Creek receives sewage effluent. This water was used in all *C. riparius* experiments, while experiments on Deer Creek genera used water with distinctly lower bacterial counts. No direct comparisons of sinking rates were made under the same nutrient

and microbial conditions for all four genera, but we suspect that *Eukiefferiella*, *Diamesa* and *Orthocladius* would all sink faster in Mill Creek or other enriched waters. Under nutrient-enriched conditions, particularly if turbulence is increasing aeration and helping to weaken exuviae at conjunctives, a maximum of 3-4 days can be expected until most specimens have sunk.

While exuviae can be used effectively to classify waterbodies based on nutrient levels (Ruse 2010), nutrient levels and type (i.e., nitrate vs. ammonia) can conversely influence floating times and thus detectability of taxa. Organic content of water has been shown to be influential to chitinoclasts, with greater abundances in polluted waters (Hood and Meyers 1973), which may influence the colonization rates and subsequent proliferation of bacterial growth on recently shed exuviae. Therefore, sinking rates for taxa are important to consider during study design. Additional experimental testing, using simple methods such as those in the current study, would help determine sinking rates for indicator species of healthy or impaired conditions.

Under laboratory conditions, sinking was more influenced by temperature than mechanical disruption. This indicates that increased microbial activity due to associated temperature is more important than agitation. However, our conclusions may not extrapolate well to natural systems with greater wave action and energy or shear forces. The numbers and activity of chitinoclastic microbes have been shown to be a function of temperature, with a common optimal temperature of 30°C but a range of activity between 0-40°C (Hood and Meyers 1973). As a result, sampling for exuviae should



take into account water temperatures to estimate the timeframe that collections represent. Annual or seasonal data collection with temperature loggers may help reveal how ecological and temperature patterns coincide (Egan 2014).

Coffman (1973) believed that waxes kept exuviae unwettable and resistant to sinking until microbial metabolism removed wax. In the present study, waxes appeared crucial for initial colonization and microbial metabolism because in the absence of waxes, the initiation of metabolism was delayed. Sinking rates were faster for untreated, waxy exuviae than for dewaxed exuviae. In contrast, the faster sinking of dewaxed and deproteinized exuviae, which lead to a disruption of structural integrity, probably mimicked a later stage of decomposition. The progression of decomposition was the same in both aerobic and anaerobic conditions, although it was extended greatly in the absence of oxygen.

Bacteria were dominant early colonizers and began the process of metabolizing exuviae. The conjunctive abdominal regions were solubilized faster, leading to inter-segmental separation. The cephalothorax was resistant and only solubilized after long incubation. The current findings revealed a more truncated pattern of microbial succession of chitinoclasts than observed by Aumen (1980). In his study, bacteria were initial colonizers, fungi dominated over time, yet bacteria regained dominance in the final stages of decomposition. Although the isolated cultures showed growth on chitin media, they might have been involved in metabolizing any of the components of exuviae including chitin. Coffman (1973) suggested that bacteria initially colonize exuviae on the surface of water and only after sinking and contact with substrate did fungal colonization occur.

Microscopic examination revealed fungi utilizing exuviae as a nutrient source and contributing to decomposition. Four fungal genera colonized chironomid exuviae of all species, while four other fungi differed between the Deer Creek chironomids and *C. riparius* from Mill Creek. It is possible that all fungi colonizing exuviae were airborne and settled on floating pupae. As with bacteria, the cephalothorax and abdominal setae were more resistant to decomposition. Fungi belonging to Saprolegniaceae are known to colonize insect exuviae, including Chironomidae (Dick 1970). In living insects, fungal pathogens attack the cuticle of specific hosts using particular enzymes (Bye and Charnley 2008); these fungal enzymes are used in biocontrol efforts and it would be useful to de-

termine if similar enzymes are responsible for the rapid breakdown at abdominal conjunctives.

Variation in genus- or species-level responses of exuviae to sinking is important to consider. Degree of chitinization was noted by Coffman (1973), where lightly chitinized and fragile *Procladius* exuviae had a faster breakdown rate. In contrast, he found *Chironomus plumosus*-type exuviae took one week to break down and *Cryptochironomus* exuviae were still intact after a month. Current results show that decomposition was faster at conjunctives, suggesting that lightly chitinized exuviae should sink faster, particularly with increased physical disturbance. However, in the experience of the authors, while slide mounting exuviae there are some species that break easily at conjunctives and others that tend to break across segments. We believe this indicates that a subjective measurement of chitinization (e.g., dark and heavy sclerotization) may not be a good indicator of likelihood of sinking since weak points, such as the cephalothorax-abdomen junction, could decompose quickly and cause rapid sinking.

## Conclusions

Microbial numbers, temperature, nutrients and mechanical disruption were all found to be influential and interacting factors in the sinking of surface floating pupal exuviae. Microbial metabolism began to break down exuviae at intersegmental conjunctive joints so that mechanical agitation caused them to break apart and sink. Exuviae were readily metabolized by bacteria, actinomycetes, and fungi. Bacteria dominated initial metabolism and fungi dominated later. In waters with considerable organic nutrient content, exuviae can be expected to sink within 1-2 days at 22°C or higher water temperatures. In low-nutrient streams at similar temperatures, some exuviae may float for up to a week. While environmental factors play vital roles in decomposition and sinking of pupal exuviae, standard techniques (e.g., Ferrington et al. 1991, Wilson and Ruse 2005) are likely influenced by generic-level decomposition responses. As a result, researchers using pupal exuviae must consider environmental conditions that influence availability of different species for detection or relative abundances of specimens collected.

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## A NEW *THALASSOSMITTIA* STRENZKE AND REMMERT, 1957 OUT OF THE SEA: *T. AMAZONICA* N. SP. FROM THE AMAZON RAINFOREST, BRAZIL (DIPTERA: CHIRONOMIDAE, ORTHOCLADIINAE)

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### Abstract

The orthoclad *Thalassosmittia amazonica* n. sp. is described based on a male collected in a light trap in the Amazon rainforest. The species is easily separated from its congeners as it has a strongly reduced palp with only a single palpomere.

### Introduction

Most of the 10 known species of *Thalassosmittia* Strenzke *et* Remmert are marine shore dwellers (Tokunaga 1936; Strenzke and Remmert 1957; Morley and Ring 1972; Sæther and Andersen 2011; Andersen *et al.* 2013). However, Wang and Sæther (1993) described *T. montana* Wang *et* Sæther from Xizang (Tibet) in China, where it was taken at 2500 m altitude. The species shows significant differences from the other species of the genus in the wing venation, virga and the phallapodeme. At the same time features of the anal point, gonostylus and the chaetotaxy is characteristic of *Thalassosmittia* and the species keys without difficulty to *Thalassosmittia* in Cranston *et al.* (1989). Wang and Sæther (1993) therefore placed the new species in *Thalassosmittia* and concluded that even if it eventually might be placed in a separate genus it is evidently closely related to *Thalassosmittia*.

Sorting through material collected in Manaus in the Amazon, we came across a species which keys to *Thalassosmittia* in Cranston *et al.* (1989). It has the features of the anal point and gonostylus characteristic of the genus and it groups with *T. montana* in the wing features, like cuneiform shape, R<sub>2+3</sub> running in the middle between R<sub>1</sub> and R<sub>4+5</sub> and absence of setae on R. However, it differs in several other features such as having bare eyes and a reduced palp, a phallapodeme with triangular aedeagal lobe with strongly sclerotized median margin, and a strong virga with lateral lamellae. Even so we place it tentatively in *Thalassosmittia*. As with *T. montana* it might deserve a separate genus, but for the time being we are probably better served with keeping both in the genus *Thalassosmittia* awaiting the discovery of more species.

### Material and Methods

The specimen examined was collected in a light trap in the Reserva Adolpho Ducke in Manaus and preserved in alcohol. It was later mounted in Canada Balsam following the procedure outlined by Sæther (1969). The general morphology follows Sæther (1980).

The holotype is deposited in Museu de Zoologia da Universidade de São Paulo, São Paulo, Brazil (MZSP).

### *Thalassosmittia* Strenzke and Remmert

*Thalassosmittia* Strenzke and Remmert, 1957: 270.

Syn.: *Saundersia* Sublette, 1967: 318. Sæther (1977).

Syn.: *Ikiprimus* Sasa and Suzuki, 1999b: 157. Yamamoto (2004).

**Type species:** *Camptocladus thalassophilus* Bequaert and Goetghebuer, 1914: 373.

**Other included species:** *Thalassosmittia atlantica* (Storå, 1936: 27); *T. christinae* Sæther and Andersen, 2011: 10; *T. clavicornis* (Saunders, 1928: 528); *T. ikijee* (Sasa and Suzuki, 1999b: 157); *T. marina* (Saunders, 1928: 526); *T. montana* Wang and Sæther, 1993: 212; *T. nemalione* (Tokunaga, 1936: 305); *T. pacifica* (Saunders, 1928: 523); *T. tusimoefea* (Sasa and Suzuki, 1999a: 82).

The emended diagnosis is based on the diagnosis in Cranston *et al.* (1989: 243), including the emendations given by Wang and Sæther (1993: 211).

### Emended diagnosis, males

Small species, wing length up to 2.0 mm.

**Antenna.** With 7–8 or 11–13 flagellomeres; antennal groove, when present, beginning at flagellomere 3 or 4; sensilla chaetica variable, present on all flagellomeres in reduced species, perhaps sometimes absent. Antennal plume well developed, variable reduced or virtually absent. Ultimate flagellomere tapering to rounded apex, with-

out subapical, strong setae.

**Head.** Eye round, without dorsomedial extension; bare, pubescent or hairy. Up to 10 uniserial temporals present, including 1–2 inner verticals. Palp with single segment with about 8 sensilla clavata or with 5 segments, often short, with 1–3 sensilla clavata.

**Thorax.** Anteprenotum weakly developed, lobes widely separated by weakly projecting scutum. Few weak, decumbent acrostichals present on anterior to median scutum. Dorsocentrals, prealars and scutellars few, uniserial.

**Wing.** Membrane without setae, finely punctate. Anal lobe absent to well developed. Costa strongly extended.  $R_{2+3}$  either running close to or virtually fused with  $R_{4+5}$ , or running in the middle between  $R_1$  and  $R_{4+5}$ ;  $R_{4+5}$  ending distal to, at same level, or proximal to end of  $M_{3+4}$ ;  $Cu_1$  weakly to moderately sinuous; FCu far distal to RM; postcubitus and anal vein extend to, or slightly beyond, FCu.  $R$ ,  $R_1$  and  $R_{4+5}$  with or without seta. Squama bare.

**Legs.** All legs with 1 strong outer tibial spur; inner spur, if present, very small; comb normal. Sensilla chaetica absent. Pulvilli absent or vestigial; empodium elongate.

**Abdomen.** With few scattered setae.

**Hypopygium.** Anal point broad, apically rounded, covered with short to moderately long microtrichia and setae. Sternapodeme weakly convex, with or without weak, oral projections. Virga small, distinct or pronounced; with or without lateral lamellae. Phallapodeme sometimes heavily sclerotized. Gonocoxite with weakly indicated, rounded, superior volsella; inferior volsella somewhat variably developed, either bilobed or simple, often partly or completely without microtrichia, sometimes with broad spines apically. Gonostylus always with distinct dense microtrichia and setae on inner margin, without crista dorsalis, with normal, weak or without megaseta.

#### Remarks

In the key to the males of the Holarctic Orthoclaudiinae the new species keys without difficulties to couplet 95 - *Thalassosmittia* (part) *thalassophila* Bequaert and Goetghebuer (Cranston et al. 1989); in Sæther et al. (2000) it will key to couplet 175, but not further as the eyes are bare and not pubescent; the genus is not included in the key to the Central American Chironomidae (Spies et al. 2009).

When including the new species in *Thalassosmittia* the generic diagnosis has to be emended to include species with bare eyes and reduced palp.

The new species also has a phallapodeme with triangular aedeagal lobe and strongly sclerotized median margin, and a strong, spine-like virga with distinct lateral lamellae. However, it shares with its congeners the characteristically broad, apically rounded anal point, covered with microtrichia and setae. Further, the gonostylus has brush like setae on the inner margin and the megaseta is weak as in several other species of the genus. The costa is strongly extended, and  $R_{2+3}$  is running and ending closer to  $R_{4+5}$  than to  $R_1$ .

Wang and Sæther (1993) pointed out that *T. montana* might deserve a separate genus. The new species might also deserve a new genus, but it appears to differ too strongly from *T. montana* for the two species to be placed in the same genus. For the time being we are therefore probably better served with keeping both in the genus *Thalassosmittia*.

#### *Thalassosmittia amazonica* new species

(Figs 1–8)

**Type material:** Holotype male: Brazil, Amazonas State, Manaus, Reserva Adolpho Ducke, Igarapé Barro Branco, 02°55'47''S 59°58'22''W, 05–08 February 2010, light trap, leg. L.C. Pinho and H.F. Mendes (MZSP).

**Etymology:** Named after the Amazon region, where the type specimen was collected.

**Diagnostic characters:** The new species can be easily distinguished from its congeners as it has bare eyes and a reduced palp with only a single palpomere.

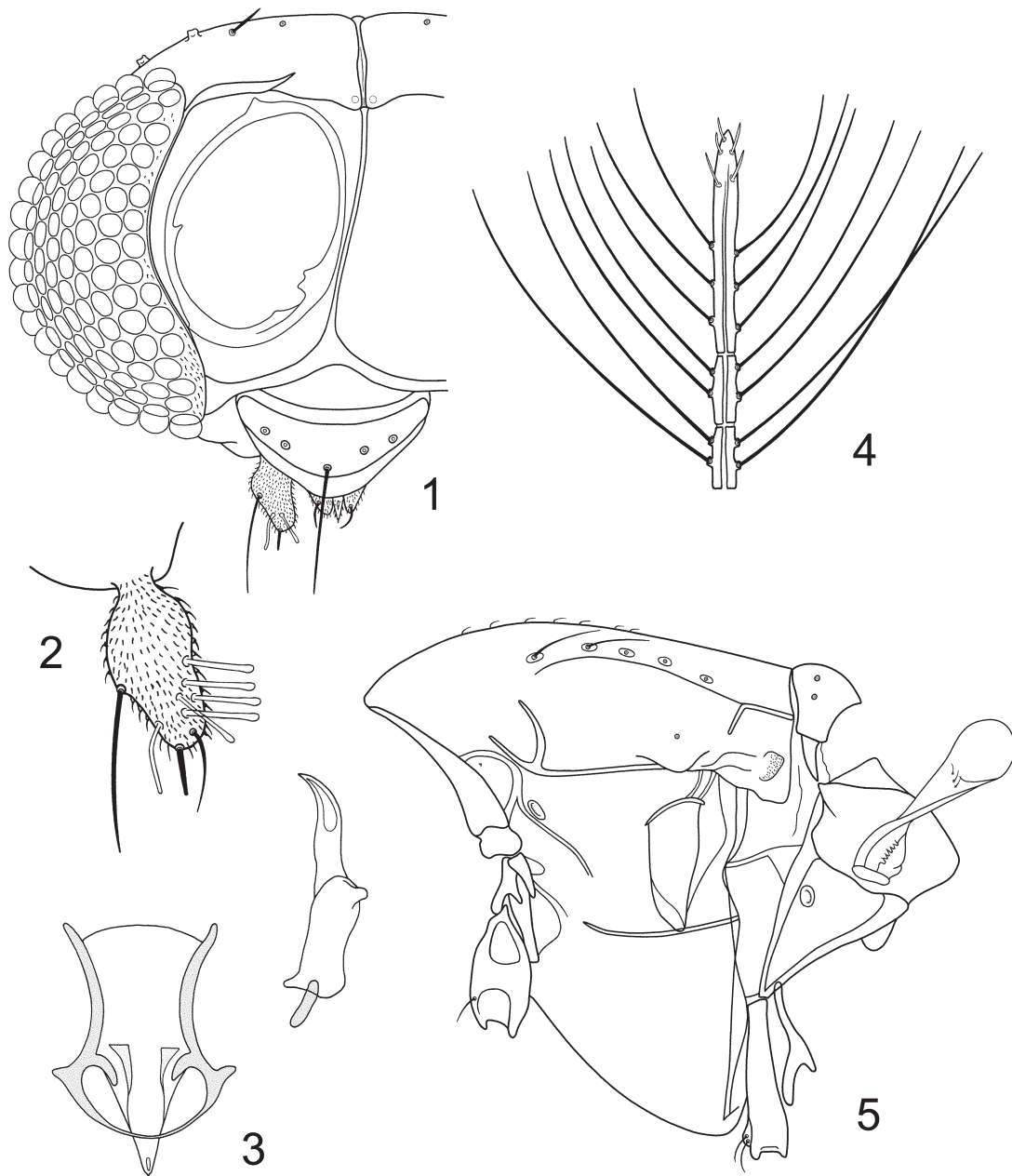
#### Description

Male (n = 1). Total length 1.41 mm. Wing length 858  $\mu$ m. Total length / wing length 1.65. Wing length / length of fore femur 2.76.

**Coloration.** Head, legs and abdomen light brown; thorax light brown with brown vittae; wings translucent.

**Antenna** (Fig. 4). With 13 flagellomeres, fully plumed, AR 0.31. Terminal flagellomere 128  $\mu$ m long, antennal groove starts on flagellomere 4, flagellomeres 2, 3 and terminal with sensilla chaetica.

**Head** (Fig. 1). Temporal setae 4, consisting of 2 inner verticals and 2 outer verticals. Clypeus with 5 setae. Tentorium, stipes and cibarial pump as in Figure 3. Tentorium 66  $\mu$ m long; 14  $\mu$ m wide. Stipes apparently about 15  $\mu$ m long. Palp (Fig. 2) with 1 palpomere, 29  $\mu$ m long, 18  $\mu$ m wide; with about 8 sensilla clavata, longest 12  $\mu$ m long.



Figures 1–5. *Thalassosmittia amazonica* n. sp., male. 1, head; 2, palp, ventral view; 3, tentorium, stipes and cibarial pump; 4, apex of antenna; 5, thorax.

**Thorax** (Fig. 5). Antepronotum without setae. Acrostichals about 7 in mid scutum, dorsocentrals 5; prealar 1. Scutellum with 4 setae.

**Wing** (Fig. 6). VR 1.41. **Wing cuneiform**. **C extension** 105  $\mu\text{m}$  long, narrow, without non-marginal setae. Brachiolum with 1 seta, other veins and membrane bare. Squama bare.

**Legs**. Fore tibia with 29  $\mu\text{m}$  long spur; mid tibia lost; hind tibia with 30  $\mu\text{m}$  and 11  $\mu\text{m}$  long spurs. Width at apex of fore tibia 19  $\mu\text{m}$ ; of hind tibia 22  $\mu\text{m}$ . Hind tibial comb reduced, apparently with 5 setae, longest 18  $\mu\text{m}$  long, shortest 12  $\mu\text{m}$  long.

Sensilla chaetica and pseudospurs absent. Lengths (in  $\mu\text{m}$ ) and proportions of legs as in Table 1.

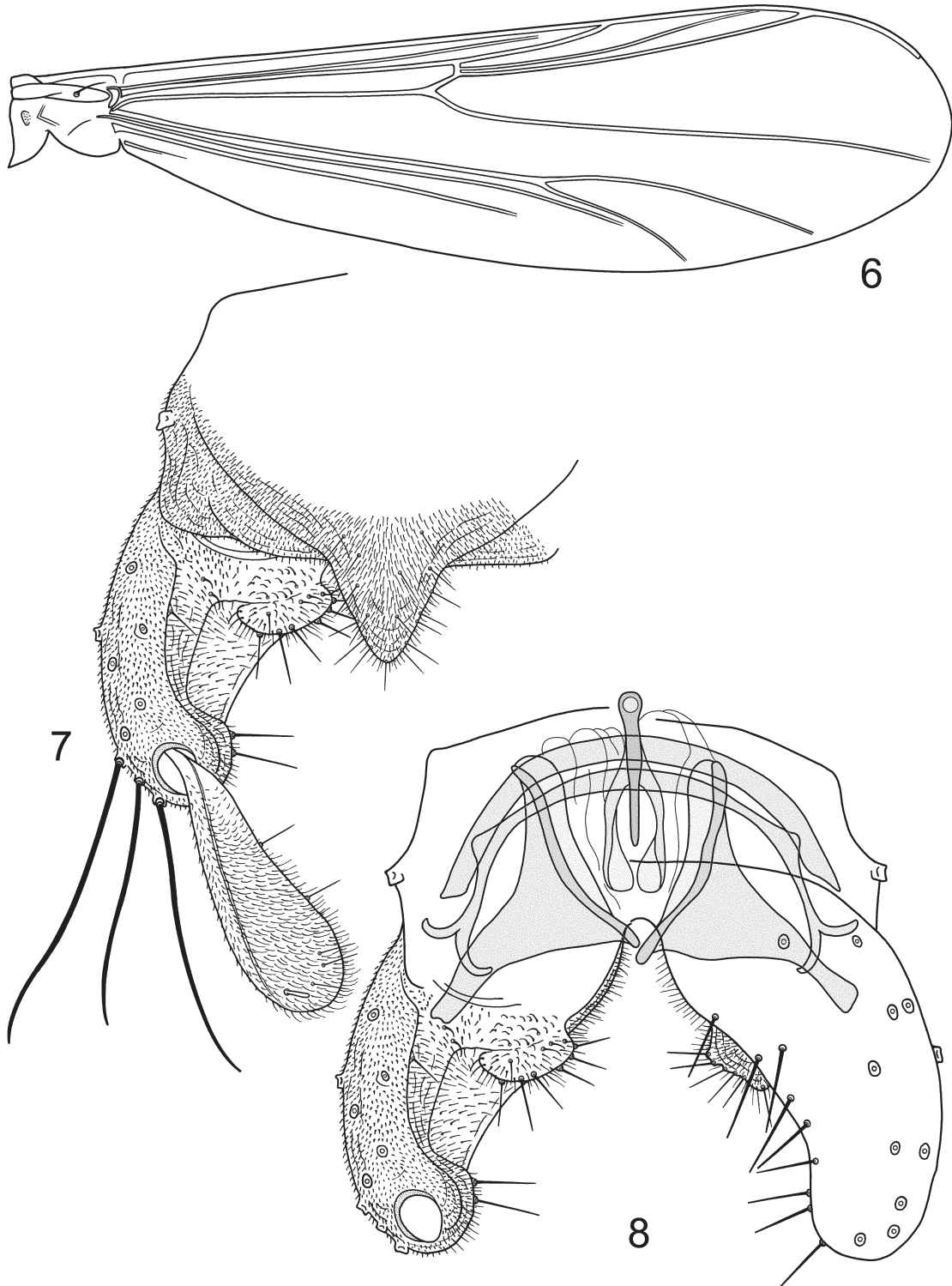
**Abdomen**. Tergite I with 3 setae, tergites II–VII with about 8 setae in transverse band, tergite VIII with 10 comparatively short setae in transverse band.

**Hypopygium** (Figs 7–8). Anal point broadly triangular; 28  $\mu\text{m}$  long, 33  $\mu\text{m}$  wide at base; with about 9 dorsal and 10 marginal, weak setae. Laterosternite IX with 2 setae. Phallapodeme 70  $\mu\text{m}$  long, aedeagal lobe triangular with 55  $\mu\text{m}$  long, strongly sclerotized median margin. Transverse sternap-



Table 1. Lengths (in  $\mu\text{m}$ ) and proportions of legs of *Thalassosmittia amazonica* n. sp., male (n = 1).

	fe	ti	ta <sub>1</sub>	ta <sub>2</sub>	ta <sub>3</sub>	ta <sub>4</sub>	ta <sub>5</sub>	LR	BV	SV	BR
p <sub>1</sub>	320	388	184	116	76	44	32	0.474	3.328	3.848	3.43
p <sub>2</sub>	352	-	-	-	-	-	-	-	-	-	-
p <sub>3</sub>	356	348	208	118	116	36	28	0.598	3.060	3.385	3.75



Figures 6–8. *Thalassosmittia amazonica* n. sp., male. 6, wing; 7, hypopygium, dorsal aspect; 8, hypopygium with anal point and tergite IX removed, dorsal aspect to the left and ventral aspect to the right.

odeme arched with weak oral projections, 80 µm long. Virga consisting of single, strong spine, 44 µm long; with strong, lateral lamellae. Gonocoxite 116 µm long. Inferior volsella subrectangular, 14 µm long. Gonostylus oar-blade shaped, 79 µm long, 23 µm wide at its widest point, densely covered with medially directed, long microtrichia and a few weak setae; megaseta weak, 6 µm long. HR 1.46. HV 1.78.

**Female and immatures.** Unknown.

#### **Distribution and ecology**

The species is only known from the type locality, Reserva Adolpho Ducke, a 10,000 ha reserve in the outskirts of Manaus in the Amazonas State, Brazil. The single male was collected in a light trap situated close to a stream and several temporary pools. The area is covered with primary forest and is relatively flat. During the rainy season numerous small pools are formed scattered on the forest floor.

Reserva Adolpho Ducke is also the type locality for *Dicrotendipes fittkaui* Epler, *Beardius curticaudatus* Pinho, Mendes *et* Andersen, *Litocladius neusae* Mendes, Andersen *et* Hagenlund, and *Saetherocryptus amazonicus* Andersen *et* Pinho (Andersen and Pinho 2014; Epler 1988; Mendes *et al.* 2011; Pinho *et al.* 2013).

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## DIFFERENTIAL EFFICIENCIES OF DIP-NET SAMPLING VERSUS SAMPLING SURFACE-FLOATING PUPAL EXUVIAE IN A BIODIVERSITY SURVEY OF CHIRONOMIDAE

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### Abstract

Relative efficiencies of standard dip-net sampling (SDN) versus collections of surface-floating pupal exuviae (SFPE) were determined for detecting Chironomidae at catchment and site scales and at subfamily/tribe-, genus- and species-levels based on simultaneous, equal-effort sampling on a monthly basis for one year during a biodiversity assessment of Bear Run Nature Reserve. Results showed SFPE was more efficient than SDN at catchment scales for detecting both genera and species. At site scales, SDN sampling was more efficient for assessment of a first-order site. No consistent pattern, except for better efficiency of SFPE to detect Orthoclaadiinae genera, was observed at genus-level for two second-order sites. However, SFPE was consistently more efficient at detecting species of Orthoclaadiinae, Chironomini and Tanytarsini at the second order sites. SFPE was more efficient at detecting both genera and species at two third-order sites. The differential efficiencies of the two methods are concluded to be related to stream order and size, substrate size, flow and water velocity, depth and habitat heterogeneity, and differential ability to discriminate species among pupal exuviae specimens versus larval specimens. Although both approaches are considered necessary for comprehensive biodiversity assessments of Chironomidae, our results suggest that there is an optimal, but different, allocation of sampling effort for detecting Chironomidae across stream orders and at differing spatial and taxonomic scales.

### Introduction

Standardized dip-net (SDN) sampling for bio-assessment of aquatic insects in water quality monitoring programs is a collection method endorsed by the US Environmental Protection Agency (Barbour et al. 1999) and has been widely adopted by state agencies and private corporations. Although widely adopted, sample processing and identification of larval chironomids collected using this method is a time-consuming activity, even when family, subfamily or tribe-level identification are

the measurement end-point, and more efficient methods could result in decreased costs to water quality monitoring programs.

By contrast to SDN sampling, collections of surface-floating pupal exuviae (SFPE) have been shown to be a more time-efficient approach to assessing chironomid composition in a variety of stream settings (Anderson and Ferrington 2012, Bouchard and Ferrington 2011, Ferrington et al. 1991, Sealock and Ferrington 2008). However, no comparisons of the results of simultaneous collections using both methods have been made across stream stretches of differing order in catchments with good water quality and high habitat conditions in the United States, so it is not possible to determine how effective collections of SFPE are in resolving chironomid composition.

For this paper, we have re-analyzed historical data from a biodiversity assessment of aquatic invertebrates of the Bear Run Nature Preserve (BRNR) to calculate the comparative efficiencies of detecting chironomids using both SDN and SFPE methods at sites located on first- through third-order stream stretches. Questions we sought to answer were: what are the comparative efficiencies of detecting chironomid compositions at (1) catchment-scale, (2) stream order scale and (3) at differing taxonomic levels (subfamily/tribe, genus and species levels).

### Materials and Methods

#### *Catchment*

The Bear Run Nature Reserve is located adjacent to PA Route 381, approximately 3 miles south of Mill Run, Stewart Township and Springfield Township in Fayette County, southwestern Pennsylvania. The BRNR is approximately 5,079 acres, most of which has been protected since 1963. Two streams, Bear Run and Lick Run, and their unnamed springs, seeps and small spring runs drain the BRNR.

The plant community types in the reserve range from regenerating old fields and conifer planta-

tions to second-growth deciduous and hemlock forests. Dominant trees include tulip tree, red maple, chestnut oak, sugar maple, and black cherry. American beech is dominant in the upper Bear Run and Lick Run riparian areas. Stands of eastern hemlock occur in the major drainages, especially along Bear Run. Understory forest plants include spicebush, rosebay rhododendron, smooth azalea, witch hazel, and mountain laurel. Wildflowers include moccasin flower, trailing arbutus, painted trillium, Solomon's seal, yellow violet, and sweet violet.

#### *Sample Sites*

Five sample sites were defined on first-order (one, referred to as Site 1), second-order (two, referred to as Sites 2 and 5) and third-order (two, referred to as Sites 3 and 4) stretches of stream. At each site, SDN sampling was done by one of us (LCF) along approximately 10 meters of stream and collections of SFPE were taken (WPC) along approximately 60 meters of stream. On each collection date and at each sample site, the time and effort for each method were similar.

Site 1 (first-order) was located where the stream crosses Rhododendron Trail. The stream varied between 0.5 to approximately 1.2 meters wide and up to 15 cm depth. Stream substrates consisted of rounded boulder, intermixed with sand and gravels. Woody debris was also common. The entire stream site consisted of erosional areas, without well-developed pool or glide habitats and slow water velocity which, in July/August, were further reduced to trickle with barely visible water movement.

Site 2 (second-order) was located downstream of Site 1, where the stream crosses under Ridge Trail. The stream varied between 0.8 to approximately 1.2 meters wide and up to 25 cm depth. Stream substrates consisted of smaller amounts of rounded boulder and sand than Site 1, but greater proportion of gravels. Woody debris was also common. The entire stream site consisted of erosional areas, without well-developed pool or glide habitats. Water velocity was intermediate of sites sampled, and flow was nearly constant across sample dates, even during July/August.

Site 3 (third-order) was located on Bear Run, where the stream crosses under Arbutus Trail. The stream varied between 2.5 to approximately 5.0 meters wide and was often greater than 1.0 meters depth. Stream substrates were highly heterogeneous, and consisted of gravel, cobble and boulder in well-developed riffles, to sand and finer mud in pools. Woody debris was present, but less common

than Sites 1 and 2. Water velocity in riffles was highest of all sites sampled, and flow was nearly constant across sample dates.

Site 4 (third-order) was located on Bear Run, approximately 1.2 kilometers stream-distance downstream of Site 3, where the stream crosses under PA Route 381. The stream varied between approximately 4.0 meters to greater than 5.0 meters wide and up to approximately 0.8 meters depth. Stream substrates were highly heterogeneous, and consisted of gravel, cobble and boulder in well-developed riffles to sand and finer mud in pools. Woody debris was present, but less common than Sites 1, 2 and 3. Water velocity in riffles was less than Site 3 due to less local gradient of slope, and flow was nearly constant across sample dates.

Site 5 (second-order) was located on a tributary of Bear Run that intersects upstream of Site 3 and is upstream of where Hemlock trail crosses this tributary near the south edge of the reserve. This tributary varied between 0.6 to approximately 1.6 meters wide and up to 25 cm depth. Stream substrates consisted of smaller amounts of rounded boulder and sand than Site 1, but greater proportion of gravels similar to substrates at Site 2. Woody debris was also common. The entire stream site consisted of erosional areas, without well-developed pool or glide habitats. Water velocity was intermediate of sites sampled, and flow was nearly constant across sample dates, even during July/August. This site is the only stretch of stream that flowed through unprotected areas of land, approximately 100 meters stream-distance upstream.

#### *Sampling Methods*

Dip-net sampling was performed consistent with the method described in Barber et al. (1999), except that on each sample date some marginal leaf-litter, submerged or partially submerged wood, and larger boulders were inspected and larval specimens from these substrates were hand-picked and added to the sample in the field. SFPE were collected consistent with the method described in Ferrington et al. (1991). This method targets areas where detritus is accumulated by currents and also accumulations of floating foams in which floating pupal exuviae can become entrained. SDN and SFPE samples were both preserved in the field with 70% Ethanol and returned to lab for sorting under 6-12X magnification.

Samples from each site were collected on the same date, approximately monthly from September 1975 through August 1976. At each sample site on each collection date the timed effort spent sampling was similar, but timed effort varied slightly

from winter (less time) to summer. In addition, the SDN samples were strongly oriented to riffle-like conditions and deeper pool habitats were inefficiently sampled.

#### *Specimen Preparation and Identification*

Larval and pupal exuviae specimens were dehydrated in 95% Ethanol then slide-mounted in Euparal for identification using primary literature available at the time of collection. Identifications of larvae and pupal exuviae were performed independently and at the end of the project, our identifications were reconciled by sample site and cumulative lists of species were assembled for each sample site and for the entire catchment. Voucher collections were assembled. The larval vouchers are deposited at the University of Minnesota Insect Collection and the pupal exuviae specimens are deposited at the William P. Coffman lab at the La Selva Biological Station in Costa Rica. Nomenclature for all taxa has been updated to reflect current generic and species definitions, and larval vouchers have been reviewed for quality assurance.

#### **Results**

A total of 7,329 larvae and 8,508 pupal exuviae were collected during this study. Substantially more larvae were collected at Site 1 (1,068 larvae versus 177 pupal exuviae) and Site 5 (3,283 versus 2,153) than pupal exuviae. By contrast, more pupal exuviae were collected at Site 2 (1,993 exuviae versus 964 larvae), Site 3 (1,879 versus 1,044) and Site 4 (2,306 versus 970). Relative to SDN, SFPE samples yielded more specimens as stream order increased.

Results showed SFPE was more efficient than SDN at catchment scales for detecting both genera and species (Table 1). Across all sample sites, 74 genera and 134 species were detected. SDN collections of larvae detected 49 genera (66.2% of total genera) and 77 species (57.5% of total species). By contrast, collections of SFPE detected 72 genera (97.3%) and 128 species (95.5%). Only the genera *Larsia* (1 species, 15 larval specimens) and *Natarsia* (1 species, 1 larval specimen) were not detected as SFPE.

Table 1. Number of taxa (genera, species) detected and the relative detection percentages for larval collections versus collections of SFPE.

	Total taxa	Taxa as larvae	% of total	Taxa as SFPE	% of total
Cumulative project totals (Genera)	74	49	66.2	72	97.3
Cumulative project totals (Species)	134	77	57.5	128	95.5
First order					
Site 1 (Genera)	26	23	88.5	16	61.5
Site 1 (Species)	35	28	80.0	17	48.6
Second order					
Site 2 (Genera)	35	27	77.1	26	74.3
Site 2 (Species)	56	35	62.5	44	78.6
Site 5 (Genera)	52	33	63.5	46	88.5
Site 5 (Species)	89	51	57.3	69	77.5
Third order					
Site 3 (Genera)	56	32	57.1	51	91.1
Site 3 (Species)	93	49	52.7	83	89.2
Site 4 (Genera)	49	31	63.3	48	98.0
Site 4 (Species)	92	51	55.4	83	90.2



Detection efficiencies varied by method at stream order scale (Table 1). At Site 1 (first order stretch of stream), 26 genera and 35 species were detected. SDN collections of larvae detected 23 genera (88.5% of genera detected at this sample site) and 28 species (80% of species detected). Collections of SFPE were less efficient at detecting genera (16, 61.5%) and species (17, 48.6%). The only genera not detected as larvae were *Pseudorthocladus* (1 species, 9 specimens of SFPE), *Mesocricotopus* (1 species, 1 specimen) and *Krenosmittia* (1 species, 2 specimens).

Sample Sites 2 and 5 were located on second order stretches of stream, but had substantially different generic (35 versus 52 genera) and species (56 versus 89 species) richness values. Detection efficiencies did not differ appreciably by method at detecting genera at Sample Site 2 (27 genera detected by SDN of larvae versus 26 as SFPE). However, detection efficiency differed markedly by method at Sample site 5, where 33 genera were detected as larvae by SDN but 46 genera were detected as SFPE. In addition, detection efficiencies differed substantially by method at species-level both within and across the two sample sites. At Sample Site 2, 35 species were detected as larvae by SDN (62.5% of total species) versus 44 detected as SFPE (78.6%). The respective totals for Sample

Site 5 were 51 species (57.3%) detected as larvae by SDN versus 69 (77.5%) detected as SFPE.

Sample Sites 3 and 4 were located on the same third order stretch of stream and had similar generic (56 and 49 genera, respectively) and species richness (93 and 92 species, respectively). Detection efficiencies of larval collections were similar across both sites, with 32 genera (57.1%) detected at Site 3 and 31 genera (63.3%) detected at Site 4. Detection efficiencies of SFPE collections were higher for collections of SFPE across both of the sites compared to larval collections, but were also similar across the two sites, with 51 genera (91.1%) detected at Site 3 and 48 genera (98.0%) detected at Site 4. At the species level, larval collections detected 49 species (52.7%) at Site 3 and 51 species (55.4%) at Site 4. SFPE collections detected 83 species at each site, representing 89.2% and 90.2% of species, respectively, for Site 3 and 4.

The relative efficiencies of the two collection methods varied by taxonomic level (at subfamily/tribe, Tables 2-5, and genus/species, Table 6). Summarized across all sample sites and collection dates, SDN were never more efficient at detecting chironomid genera in the catchment of the Bear Run Nature Reserve than SFPE. Summarized at

Table 2. Number of taxa (genera, species, summed across all sites) detected by subfamily or tribe and the relative detection percentages for larval collections versus collections of SFPE.

	Total taxa	Taxa as larvae	% of total	Taxa as SFPE	% of total
Sample site totals, genera					
Tanypodinae	12	7	58.3	10	83.3
Diamesinae	2	2	100.0	2	100.0
Prodiamesinae	2	1	50.0	2	100.0
Orthoclaadiinae	33	24	72.7	33	100.0
Chironominae					
Chironomini	15	7	46.7	15	100.0
Pseudochironomini	1	0	0.0	1	100.0
Tanytarsini	9	8	88.9	9	100.0
Sample site totals, species					
Tanypodinae	15	9	60.0	13	86.7
Diamesinae	3	3	100.0	2	66.7
Prodiamesinae	2	1	50.0	2	100.0
Orthoclaadiinae	68	41	60.3	67	98.5
Chironominae					
Chironomini	22	10	45.5	22	100.0
Pseudochironomini	1	0	0.0	1	100.0
Tanytarsini	23	13	56.5	21	91.3

the species level, SDN were only more efficient at detecting Diamesinae species (3 versus 2 species) compared to SFPE. By contrast, however, SFPE were much more efficient at detecting genera of Tanypodinae and Prodiamesinae than SDN, and even more efficient at detecting species within the subfamilies Tanypodinae, Prodiamesinae and Orthocladiinae and the tribes Chironomini, Pseudochironomini and Tanytarsini of the subfamily Chironominae.

SDN was always more efficient or equal in efficiency at detecting species within subfamilies or tribes at Site 1 (Table 3); this was also true for detection efficiency at genus-level except for Orthocladiinae, where SFPE was 6.7% more efficient at detecting genera.

For sites on second order stretches of stream, at the genus-level, no consistent trends in efficiencies of either method occurred except for Orthocladiinae, where the SFPE method consistently was more efficient than SDN collections (Table 4). At species-level, however, the SFPE consistently was more efficient for Orthocladiinae, Chironomini and Tanytarsini, but SDN was more efficient for Diamesinae. No consistent pattern was observed for Tanypodinae.

For sites on third order stretches of stream, SFPE

consistently was more efficient at detecting both genera and species of Orthocladiinae, Chironomini and Tanytarsini (Table 5). SDN only consistently outperformed SFPE detecting genera and species of Diamesinae.

Table 6 lists the genera that were most species rich across all sample sites and the species detected by each method. The genera *Eukiefferiella* (9 species), *Tanytarsus* (9 species), *Cricotopus* (8 species) and *Orthocladius* (subgenus *Orthocladius*) (6 species) were the most species richness, and all species of these genera were detected as SFPE. SDN collections of larvae were less efficient at detecting species in these genera (88.9% to 16.7% detected). Fifty of the genera detected at BRNP were each represented by only a single species and 48 of the genera were detected as SFPE (96%) versus only 25 of the genera detected as larvae using SDN sampling (50%).

### Discussion

Comprehensive surveys of Chironomidae biodiversity across stream orders or at catchment scales require substantial sampling effort in seasonal environments such as those that occur at latitudes similar to that of the Bear Run Nature Reserve, and are best achieved using multiple field methods targeting all developing life stages. The results of this

Table 3. Number of taxa (genera, species) detected by subfamily or tribe and the relative detection percentages for larval collections versus collections of SFPE at Site 1, the first order stretch of stream.

	Total taxa	Taxa as larvae	% of total	Taxa as SFPE	% of total
Sample site totals, genera					
Tanypodinae	1	1	100.0	1	100.0
Diamesinae	1	1	100.0	0	0.0
Prodiamesinae	0	0	0.0	0	0.0
Orthocladiinae	15	12	80.0	13	86.7
Chironominae					
Chironomini	4	4	100.0	0	0.0
Pseudochironomini	0	0	0.0	0	0.0
Tanytarsini	5	5	100.0	2	40.0
Sample site totals, species					
Tanypodinae	2	2	100.0	1	50.0
Diamesinae	1	1	100.0	0	0.0
Prodiamesinae	0	0	0.0	0	0.0
Orthocladiinae	20	14	70.0	13	65.0
Chironominae					
Chironomini	5	5	100.0	0	0.0
Pseudochironomini	0	0	0.0	0	0.0
Tanytarsini	7	6	85.7	3	42.9

Table 4: Number of taxa (genera, species) detected by subfamily or tribe and the relative detection percentages for collections of larvae versus SFPE at Sites 2 and 5, the second order stretches of stream.

	Total taxa	Taxa as larvae	% of total	Taxa as SFPE	% of total
SITE 2					
Sample site totals, genera					
Tanypodinae	3	3	100.0	1	33.3
Diamesinae	2	2	100.0	0	0.0
Prodiamesinae	0	0	0.0	0	0.0
Orthocladiinae	16	13	81.3	15	93.7
Chironominae					
Chironomini	7	5	71.4	5	71.4
Pseudochironomini	0	0	0.0	0	0.0
Tanytarsini	5	4	80.0	3	60.0
Sample site totals, species					
Tanypodinae	4	4	100.0	2	50.0
Diamesinae	2	2	100.0	0	0.0
Prodiamesinae	0	0	0.0	0	0.0
Orthocladiinae	33	18	54.5	29	87.9
Chironominae					
Chironomini	9	6	66.7	7	77.8
Pseudochironomini	0	0	0	0	0
Tanytarsini	8	5	62.5	6	75.0
SITE 5					
Sample site totals, genera					
Tanypodinae	7	4	57.1	6	85.7
Diamesinae	1	1	100.0	1	100.0
Prodiamesinae	0	0	0.0	0	0.0
Orthocladiinae	24	18	75.0	23	95.8
Chironominae					
Chironomini	11	6	54.5	10	90.9
Pseudochironomini	1	0	0.0	1	100.0
Tanytarsini	8	5	62.5	7	87.5
Sample site totals, species					
Tanypodinae	9	5	55.6	7	77.8
Diamesinae	2	2	100.0	1	50.0
Prodiamesinae	0	0	0.0	0	0.0
Orthocladiinae	44	28	63.6	36	81.8
Chironominae					
Chironomini	17	9	52.9	12	70.6
Pseudochironomini	1	0	0.0	1	100.0
Tanytarsini	16	8	50.0	12	75.0



Table 5: Number of taxa (genera, species) detected by subfamily or tribe and the relative detection percentages for collections of larvae versus SFPE at Sites 3 and 4, the third order stretches of stream.

	Total taxa	Taxa as larvae	% of total	Taxa as SFPE	% of total
SITE 3					
Sample site totals, genera					
Tanypodinae	6	3	50.0	6	100.0
Diamesinae	2	2	100.0	1	50.0
Prodiamesinae	2	1	50.0	2	100.0
Orthocladiinae	28	15	53.6	27	96.4
Chironominae					
Chironomini	10	5	50.0	7	70.0
Pseudochironomini	0	0	0.0	0	0.0
Tanytarsini	7	6	85.7	7	100.0
Sample site totals, species					
Tanypodinae	8	4	50.0	6	75.0
Diamesinae	2	2	100.0	1	50.0
Prodiamesinae	2	1	50.0	2	100.0
Orthocladiinae	52	26	50.0	46	88.5
Chironominae					
Chironomini	13	7	53.8	12	92.3
Pseudochironomini	0	0	0.0	0	0.0
Tanytarsini	16	9	56.3	16	100.0
SITE 4					
Sample site totals, genera					
Tanypodinae	6	4	66.7	6	100.0
Diamesinae	2	2	100.0	1	50.0
Prodiamesinae	0	0	0.0	0	0.0
Orthocladiinae	25	16	64.0	25	100.0
Chironominae					
Chironomini	7	3	42.9	7	100.0
Pseudochironomini	0	0	0.0	0	0.0
Tanytarsini	10	6	60.0	9	90.0
Sample site totals, species					
Tanypodinae	8	6	75.0	6	75.0
Diamesinae	2	2	100.0	1	50.0
Prodiamesinae	0	0	0.0	0	0.0
Orthocladiinae	51	26	51.0	48	94.1
Chironominae					
Chironomini	11	5	45.5	10	90.9
Pseudochironomini	0	0	0.0	0	0.0
Tanytarsini	20	9	45.0	18	90.0

Table 6. Number of species detected by genus and the relative detection percentages for larval collections versus collections of SFPE.

Genus	Total species	Species as larvae	% of total	Species as SFPE	% of total
<i>Eukiefferiella</i>	9	8	88.9	9	100.0
<i>Tanytarsus</i>	9	3	33.3	9	100.0
<i>Cricotopus</i>	8	6	75.0	8	100.0
<i>Orthocladius</i> ( <i>Orthocladius</i> )	6	1	16.7	6	100.0
<i>Corynoneura</i>	4	2	50.0	4	100.0
<i>Micropsectra</i>	4	2	50.0	4	100.0
<i>Parakiefferiella</i>	4	2	50.0	4	100.0
<i>Polypedilum</i>	4	4	100.0	4	100.0
<i>Conchapelopia</i>	3	3	100.0	3	100.0
<i>Microtendipes</i>	3	1	33.3	3	100.0
<i>Parametriocnemus</i>	3	2	66.7	3	100.0
<i>Thienemanniella</i>	3	2	66.7	3	100.0
<i>Brillia</i>	2	1	50.0	2	100.0
<i>Diamesa</i>	2	2	100.0	1	50.0
<i>Cryptochironomus</i>	2	1	50.0	2	100.0
<i>Krenosmittia</i>	2	1	50.0	2	100.0
<i>Nanocladius</i>	2	1	50.0	2	100.0
<i>Phaenopsectra</i>	2	1	50.0	2	100.0
<i>Procladius</i>	2	1	50.0	2	100.0
<i>Rheocricotopus</i>	2	1	50.0	2	100.0
<i>Rheotanytarsus</i>	2	1	50.0	2	100.0
<i>Stempellina</i>	2	2	100.0	1	50.0
<i>Stempellinella</i>	2	2	100.0	2	100.0
<i>Stilocladius</i>	2	2	100.0	1	50.0
Genera represented by one species per genus	50	25	50.0	48	96.0

project compare favorably to a more intensive field approach used by Coffman (1973) to study composition and phenology in Linesville Creek (134 species versus 143 species). However, the difference in species detected in the two studies suggests that more intensive sampling effort would result in higher species richness at BRNR.

Our field design, which incorporated monthly sampling events, is important in terms of interpreting our results. A concern related to the SFPE method is that it only detects taxa that are emerging at the time of collection, and in highly seasonal environments there is expected to be high temporal variability in species emergence (see Coffman 1989, Coffman and de la Rosa 1998). Consequently, a single annual SDN sample would likely detect more taxa as larvae than as pupal exuviae.

However, this pattern was not seen by Ferrington

et al. (1991) in two organically enriched urban streams in Kansas, where there was strong congruence between larvae detected with SDN samples and pupal exuviae from SFPE samples; on the other hand SFPE was more efficient at detecting species as water quality conditions improved and species richness increased across sites. These results need to be interpreted cautiously, however, because the water quality and habitat conditions of the two streams assessed by Ferrington et al. (1991) were much poorer than exists at BRNR, and the species rich communities at Sites 2-5 may have much greater temporal emergence heterogeneity than the more enrichment-tolerant species of the streams in Kansas.

Given that budgetary limitations influence sample designs, the disparity in efficiencies of the two methods at the catchment-scale suggest that an al-

ternative design consisting of more field sampling events using SFPE could be a more cost-efficient approach to assessing biodiversity than always collecting and processing the more time-consuming and costly SDN samples. However, the patterns of relative efficiency across sample sites with differing stream order suggest that there could be an optimal allocation of field effort employing the two methods among streams of differing orders, size and discharge in a catchment.

The higher efficiency of SDN at Site 1 reflects better coverage of microhabitats in small streams versus larger streams. This also strongly suggests that an equal-effort approach using SDN across streams of differing size will result in decreasing efficiency detecting chironomid taxa in larger streams. By contrast, the low efficiency of SFPE at Site 1 was likely due to difficulty using a pan to dip among large boulders in areas with minimal water depth. In addition, the slower water velocity and reduction to trickle-like flow conditions during summer also decreases the natural accumulating effect on floating pupal exuviae of currents in larger, deeper and faster flowing streams. Use of a drift net or smaller aquarium-size net that would fit into tighter spaces between rocks may have resulted in higher efficiency of collection of SFPE at this site. A drift net, however, would likely have needed to be in place for a 24-hour period or longer to filter a sufficient amount of stream flow, which would have added substantively to field efforts and sampling costs.

At BRNR, SFPE begins to outperform SDN in the transition from second order to third order sample sites characterized by well-developed pool/riffle conditions and widths exceeding about three meters. Our results for sample sites on second order stretches suggest that factors other than just size, flow and depth influence the efficiency of SFPE. These factors could include the size and space between larger boulders and the amount of woody debris extending into the water and creating conditions for back-flow and accumulation of floating detritus and pupal exuviae.

In addition to field sampling design, stream order and habitat conditions, the ability to resolve species is another variable that needs to be considered when comparing the efficiencies of the two methods. All genera detected in this study are well-defined and recognizable in both larval and pupal stages. Consequently, it can be argued that the differences in efficiencies at catchment-scale detection of genera are related to sampling error because of reduced efficiencies of SDN related to stream

size, or difficulties sampling deeper pools, or specialized microhabitats of some larvae. However, at species-levels both sampling error and inability to resolve species-level difference among larvae are both sources of variability that decrease efficiency of SDN. The differential ability to resolve species is especially important in species-rich genera. Examples in this study include the genera *Tanytarsus*, *Cricotopus*, *Orthocladius*, *Corynoneura*, *Micropsectra*, *Parakiefferiella* and *Microtendipes*, where species level identifications of pupal exuviae are more readily achieved compared to identification of larvae.

The SDN sampling in this project was supplemented with targeted hand picking of marginal accumulations of wetted leaves, submerged and partially submerged wood of varying stages of decomposition, and larger boulders. Consequently, we detected larvae of some semi-aquatic species and xylophagous taxa that otherwise might have been undetected with SDN samples from coarser gravel/cobble substrates in riffles. Larvae from the targeted hand-picking efforts were included in the totals for SDN, likely increasing the efficiencies at the higher stream order sites. Despite this targeted effort, SFPE strongly out-performed our efforts aimed at detecting genera and species using larvae and whole pupae. SFPE also was slightly more effective at detecting phoretic taxa compared to the SDN technique.

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## A REDESCRIPTION OF *ZAVRELIA SIMANTONEOA* (SASA, SUZUKI AND SAKAI, 1998) COMB. NOV.

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### Abstract

Examination of the holotype of *Micropsectra simantoneoa* Sasa, Suzuki and Sakai, 1998 revealed that the species should be transferred to the genus *Zavrelia*. The male adult has hairy eyes, antennae with 10 flagellomeres, a costa ending proximal to the tip of  $M_{3+4}$ , and a short and flattened superior volsella. This is the second *Zavrelia* species from Japan, and the 11<sup>th</sup> in the world.

### Introduction

The genus *Zavrelia* was established by Kieffer, Thienemann and Bause in Bause (1913) for *Z. pentatoma* Kieffer and Bause in Bause, 1913 (Ashe and Cranston 1990, Ekrem and Stur 2009). The genus is placed in subtribe *Zavreliina* within tribe *Tanytarsini*, subfamily *Chironominae*, and was recently reviewed by Ekrem and Stur (2009). Ten *Zavrelia* species are known so far: *Z. aristata* Ekrem and Stur, 2009, *Z. bragemia* Guo and Wang, 2007; *Z. casasi* Ekrem and Stur, 2009; *Z. clinovolsella* Guo and Wang, 2004; *Z. elenae* Zorina, 2008; *Z. hudsoni* Ekrem and Stur, 2009; *Z. pentatoma* Kieffer and Bause in Bause, 1913; *Z. pseudopentatoma* Zorina, 2008; *Z. sinica* Ekrem and Stur, 2009, and *Z. tusimatijea* (Sasa and Suzuki, 1999).

Only five records of genus *Zavrelia* have previously been known from Japan, including *Z. tusimatijea* (Sasa and Suzuki 1999) and *Z. kibunensis* (Tokunaga 1938), which was transferred from the genera *Tanytarsus* van der Wulp, 1874 and *Neozavrelia* Goetghebuer, 1941 by Ekrem respectively (2002, 2006). The remaining three belong to unnamed *Zavrelia* species: a larva from Kokubunji Cliff Springs, Tokyo, by Ohno et al. (1999); an adult female from the Shinano River, Ueda, Nagano recorded by Hirabayashi et al. (2001); and a larva from the Takahari River, Okayama by Kitagawa (2003).

During reexamination of *Micropsectra* type specimens described by Sasa, *Micropsectra simantoneoa* Sasa, Suzuki and Sakai, 1998 was found also to fit the diagnosis of *Zavrelia*.

### *Zavrelia simantoneoa* (Sasa, Suzuki and Sakai, 1998) comb. nov.

*Micropsectra simantoneoa* Sasa, Suzuki and Sakai, 1998: 62 (adult male, fig. 15).

Holotype: NSMT-I-Dip.5206 (SC.358-62), adult male labelled as "*Micropsectra simantoneoa*". Collecting data: Shimanto River, Nakamura Town, Shimanto, Kochi; 26.IV.1998, light trap, H. Suzuki.

### Diagnosis

*Zavrelia simantoneoa* (Sasa, Suzuki and Sakai, 1998) can be separated from the other described *Zavrelia* species by the following combination of characters. Superior volsella broad, almost parallel sided and with rounded apex; lamellae of median volsella simple, directed medially; anal point without spinules between crests.

### Redescription

Total length 1.88 mm, wing length 1.08 mm (*cit.* Sasa, Suzuki and Sakai 1998, 62.). Ground colour of scutum, and scutellum yellow; vittae and post-notum brown, abdomen yellowish brown, distal half of femora brownish yellow, other leg portions yellow (*ibid.*).

**Head:** Frontal tubercle absent. Superorbitals 3:3, eye (Fig. 1) hairy, without dorsomedial extension.

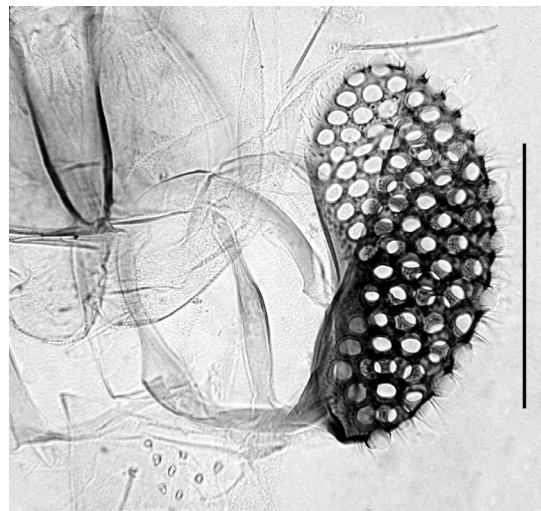
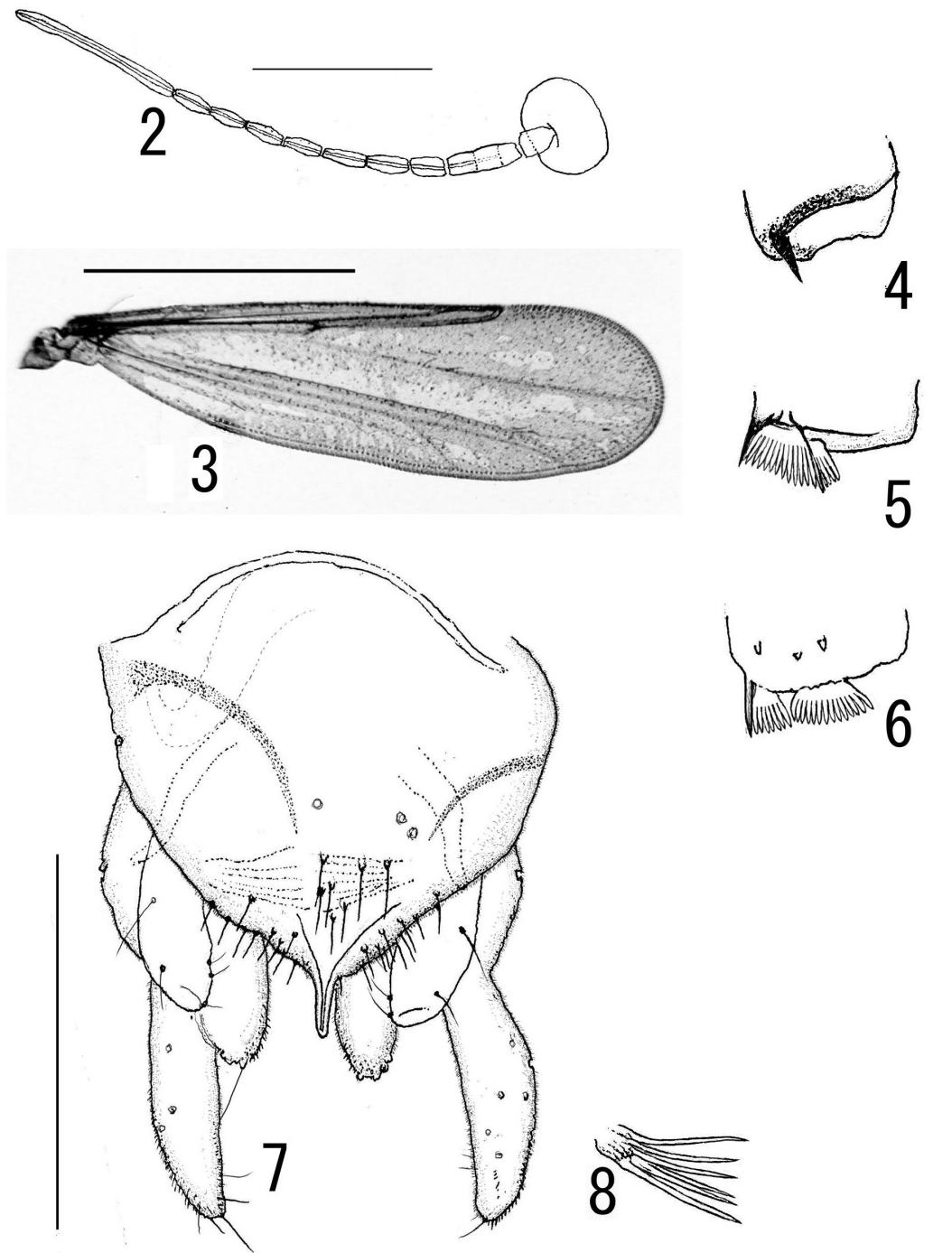


Figure 1. Eye, scale bar = 100µm.



Figures 2-8. 2, antenna, scale bar = 200 $\mu$ m; 3, wing, scale bar = 1,00mm; 4, apex of fore tibia, scale bar = 50 $\mu$ m; 5, apex of mid tibia, scale bar = 50 $\mu$ m; 6, apex of hind tibia, scale bar = 50  $\mu$ m; 7, hypopygium, scale bar = 100 $\mu$ m; 8, median volsella.

Antenna (Fig. 2) 578 $\mu$ m long, with 10 flagellomeres, 2<sup>nd</sup> flagellomere long (75 $\mu$ m) with 2 discernible vestigial joint (incomplete fusion), 3<sup>rd</sup> to 9<sup>th</sup> flagellomeres about 40 $\mu$ m long each one; groove beginning at distal part of 2<sup>nd</sup> flagellomere, AR 0.45. Clypeals 9. Palp long, palpomere lengths in  $\mu$ m I 25, II 28, III 90, IV 93, V 129, palpomere III with sensilla chaetica near apex; tentorium 42 $\mu$ m long

and 5 $\mu$ m wide at most. **Thorax chaetotaxy:** Aps absent. Ac 11 biserial, Dc uniserial 9:9, Pa 1:1, Sct 2. **Wing** (Fig. 3): Wedge-shaped, widest near apex; squama bare, anal lobe weak, with macrotrichia more densely in distal half. Membrane with macrotrichia in all cells except anterior to vein M; all veins with macrotrichia except M and Sc; costal extension absent. R<sub>4+5</sub> ending far proximal to



Table 1. Lengths (in  $\mu\text{m}$ ) and proportions of legs.

	fe	ti	ta <sub>1</sub>	ta <sub>2</sub>	ta <sub>3</sub>	ta <sub>4</sub>	ta <sub>5</sub>	LR
p <sub>1</sub>	410	240	470	225	140	100	60	1.96
p <sub>2</sub>	460	350	215	105	80	60	50	0.61
p <sub>3</sub>	560	-	290	150	-	-	-	-

apex of M<sub>3+4</sub>, R<sub>2+3</sub> obscure, closely along R<sub>4+5</sub>. FCu much distal to RM, VR 1.55, Cu<sub>2</sub> straight and short (150  $\mu\text{m}$ ). **Legs** (Table 1, Figs 4-6): Apex of fore tibia with short spur (10  $\mu\text{m}$  long), tibiae of mid and hind legs with two combs and one short spur at least, sensilla chaetica apparently absent from all tarsomeres. LR1 1.96, LR2 0.61, LR3 unmeasurable ('0.75' in the original description); pulvilli absent. **Hypopygium** (Fig. 7): Laterosternite IX with setae, anal tergite bands separated, 7 median tergite setae placed mainly in dorsomedial area basally of anal point.

Anal point 15  $\mu\text{m}$  long, almost parallel-sided, without spinulae between crests; widest at base and tapering towards round apex, with narrow anal crests; 6 basal and 6 lateral setae. Superior volsella with broad base, setiger rounded apically, with 3 median and 2 lateral setae, without basomedial seta. Median volsella (Fig. 8) 8  $\mu\text{m}$  long, medially directed with 6-7 simple, 22  $\mu\text{m}$  long lamellae. Inferior volsella covered with microtrichiae, with several distal long setae. Gonostylus simple, narrow, inner margin slightly concave.

### Discussion

In the 'Remarks', Sasa et al. (1998) state "this specimen is provisionally classified into the genus *Micropsectra* Kieffer, 1915", and "it is quite unusual as a member of the *Micropsectra-Paratanytarsus* group". As the holotype has hairy eyes, broad and flattened superior volsella, and short, medially directed median volsella with simple lamellae, it certainly does not belong to the genus *Micropsectra* but to *Zavrelia*.

In the key to adult males of *Zavrelia* by Ekrem and Stur (2009), *Z. simantoneoa* cannot advance beyond couplet 3. This couplet separates species with "Setiger of superior volsella with obvious constriction in apical 1/3; anal point bare or with microtrichia in between crests" from species with "Setiger of superior volsella without constriction in apical 1/3; anal point with numerous microtrichia or small spinules". The present specimen has a setiger of superior volsella without constriction in apical 1/3 and a bare anal point in between crests. Thus, it does not fit any other presently keyed *Zavrelia* species and is different from all previously described species.

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## Subfossil chironomids from Kamchatka

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The Kamchatka Peninsula shapes the eastern edge of Siberia and separates the Sea of Okhotsk from the Pacific Ocean (Fig. 1). It is one of the least studied regions in Eurasia. The diverse and variable climatic and ecological conditions in the study region are formed by a complex interplay between geographic position, relief, surrounding seas, the Pacific Ocean, and tectonic activities. The Kamchatka Peninsula belongs to the maritime and sub-oceanic sectors of the boreal zone, characterized by high humidity, relatively low temperatures, short growing seasons, and heavy snowfalls. Monthly mean July temperatures (T July) range between 12 and 16°C, monthly mean January temperatures (T January) between -18 and -20°C (New et al. 2002). Annual precipitation is around 350 mm (Krestov et al. 2008). The climate conditions are ideally suited for tundra in the north and at high elevations as well as widespread forests with dwarf alder (*Alnus fruticosa*), dwarf pine (*Pinus pumila*), and Ermann's birch (*Betula ermanii*) (Krestov et al. 2008).

In 13 investigated lakes from Kamchatka we identified 77 chironomid taxa. Most widely distributed taxa are *Tanytarsus mendax*-type (84.6 % of the lakes; mean 5.1%; max 13.7%), *Procladius* (76.9 % of the lakes; mean 3.2%; max 9.1%), *Psectrocladius sordidellus*-type (69.2 % of the lakes; mean 4.9%; max 16.3%), *Ablabesmyia* (61.5 % of the lakes; mean 4.8%; max 22.0%), *Limnophyes* – *Paralimnophyes* (61.5 % of the lakes; mean 3.7%; max 13.8%), and *Paratanytarsus penicillatus*-type (61.5 % of the lakes; mean 2.2%; max 5.8%). *Chironomus anthracinus*-type (mean 5.8%; max 24.6%), *Sergentia coracina*-type (mean 3.4%; max 14.0%), *Tanytarsus* no spur (mean 3.2%; max 12.3%), *Microtendipes pedellus*-type (mean 2.65%; max 12.3%), *Cladotanytarsus mancusi*-type (mean 1.9%; max 8.9%), *Dicrotendipes nervosus*-type (mean 1.32%; max 4.9%) are found in 7 of 13 lakes (53.8 % of the lakes). *Allopectrocladius*, *Diamesa zernyi/cinerella*-type, *Eukiefferiella fittkau*-type, *Eurycnemus*, *Psectrocladius calcaratus* type, *Pseudodiamesa*, and *Rheocricotopus* were not found in other regions of Siberia (Nazarova et al. 2005, 2008, 2011, 2013a,b).

Earlier surveys have demonstrated similarities between the chironomid fauna of Far East and other parts of Siberia (Karationis et al. 1956; Ogay 1979; Salova 1993; Kiknadze et al. 1996). In total previous investigations (Makarchenko et al. 1999, 2005; Zorina 2001, 2003; 2006a,b; 2013) recorded 74 chironomid species for Kamchatka, which is comparable with the taxonomic richness that was found in our investigation. Most recorded species (60%) are Palaearctic and 40% of all species have Holarctic distribution. We observed a relatively high abundance of head capsules from the subfam-

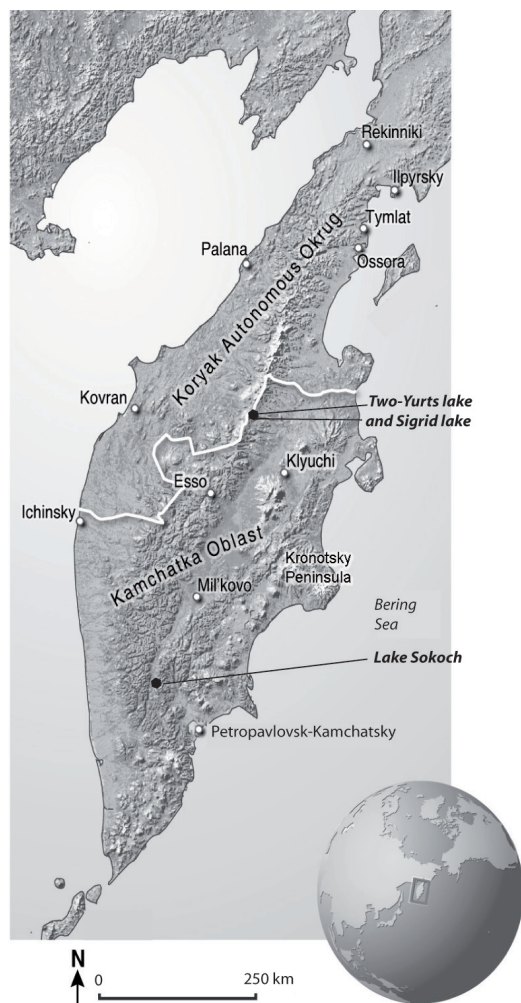


Figure 1. Location of Two-Yurts, Sigrid and Sokoch lakes in Kamchatka.



ily Diamesinae in Kamchatka, which corresponds to investigations of Makarchenko et al. (2005), who recorded 52 Diamesinae taxa in the chironomid fauna of the Russian Far East.

Some of the Tanytarsini specimens had a distinctive mentum with a small single median tooth and minute outer lateral tooth (Fig. 2). The larvae of this morphotype were not found in any modern sediments but were found in 20 sediment layers of the core of Two-Yurts Lake in abundances up to 8.1% (56°49,2'N; 160°06,3'E, Nazarova et al. 2013b), and in 5 sediment layers of the core of the lake Sokoch (53°15.133'N, 157°45.489'E) (Fig. 1). Interestingly this taxon was not found in the lake situated just a few meters away from the Two-Yurts Lake. Taking into account the frequency of the head capsules with this form of the mentum, this morphotype cannot be considered as just a morphological abnormality. None of the known Tanytarsini taxa have a similar mentum, so this taxon was treated as a new morphotype and was named 'Tanytarsini type klein'. The taxon appeared at low to moderate abundances throughout the cores and could not be associated with colder or warmer periods (Nazarova et al. 2013b).

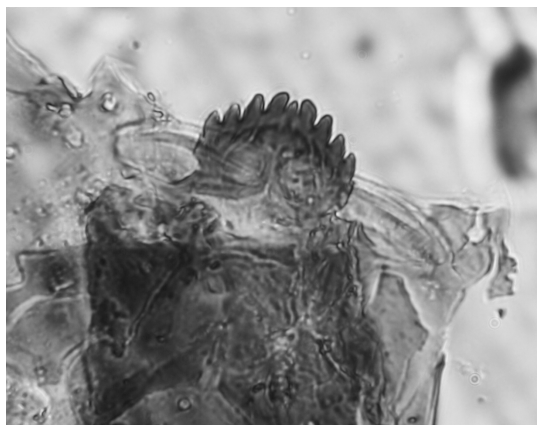


Figure 2. Morphotype *Tanytarsus* type klein from the sediment cores of the Two-Yurts and Sokoch lakes in Kamchatka. Photo: Larisa Nazarova.

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## The importance of emerging chironomids as a food resource for overwintering passerines in an Iberian high altitude lake

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Chironomids form an essential part of the diet of insectivorous birds that feed on emerging aquatic insects in both riparian areas (Lynch et al. 2002, Beck et al. 2013) and other aquatic habitats, providing an important trophic link between the aquatic and the terrestrial environment. High altitude lakes in mountain landscapes are relatively common and valuable habitats that provide vital resources to terrestrial fauna before freezing over in the winter. Chironomids are known to be a major component of the macroinvertebrate fauna of high altitude lakes and are perhaps a particularly important food source during cold weather conditions (Boggero et al. 2006, Füreder et al. 2006, Bouchard et al. 2006).

High altitude lakes are relatively rare on the Iberian Peninsula (Rieradevall and Prat 1999, Hughes et al. 2012). The Picos de Europa mountain range, situated within the Picos de Europa National Park on the northern coast of Spain, is part of the Cantabrian Mountain Range, located in the northwest of Spain. The area, situated in the Eurosiberian biogeographical region, is predominantly calcareous and located within the Atlantic climate domain which has high levels of precipitation (> 1,000 m) in the autumn and winter, mild winters, and cool summers (Moreno et al. 2011). The Covadonga Lakes, a system of two permanent glacial lakes, Enol and La Ercina (1070 m.a.s.l. and 1108 m.a.s.l. respectively) are situated within the National Park in the eastern Cantabrian Mountain Range, in the Asturias principality. Situated in a small catchment (1.5 km<sup>2</sup>), Lake Enol has a surface area of 12.2 hectares and a maximum depth of 22 m. This landscape, which was previously forested, was cleared by man during prehistoric and historic times to produce the present day alpine meadows and pastures that are typical of the area (Montserrat and Fillat 1990).

A collecting campaign of the chironomids of the high altitude lakes of central Portugal and northern Spain was carried out in November 2013. Snow had already fallen and was accumulating at altitudes above 800 m in the Cantabrian Mountains. Collections in Lake La Ercina were not possible as the lake already had a considerable layer of solidifying ice resulting from compacted accumulated snowfall on the lake surface (Fig. 1a). No insect activity was observed at the lakeside. In contrast, Lake Enol, only 500 m from Lake La Ercina, but at lower altitude, had no ice cover at all (Fig. 1b). Collections of exuviae and adults were made on the leeward shore of Lake Enol indicating actively emerging chironomids at the water surface. Exuviae were collected from the water surface using a handnet (250 µm mesh) while adults were taken by hand

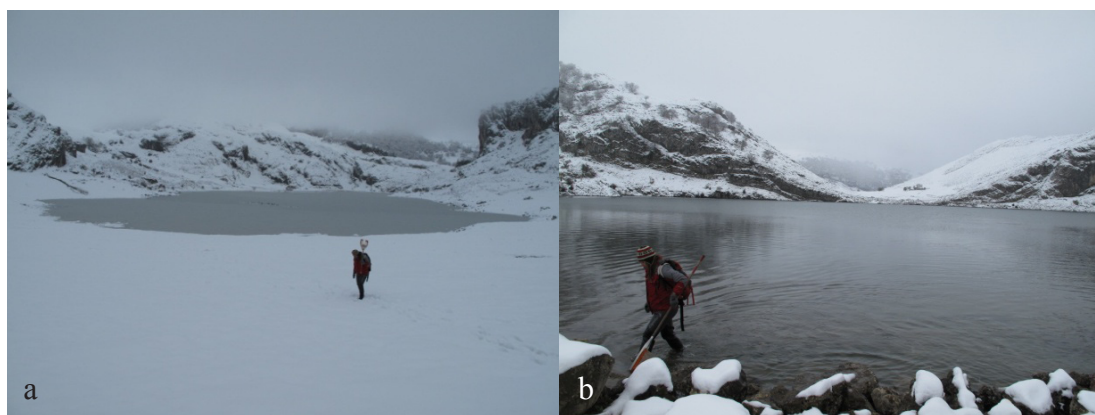


Figure 1. Lakes Ercina and Enol. a, Lake Ercina (1108 m.a.s.l.) in the Picos de Europa National Park, iced over in November 2013; b, Lake Enol (1070 m.a.s.l.) with no ice cover in November 2013. Lake Enol is only 500m distant from Lake Ercina and only ices over for comparatively short periods during the winter.



from the surface of the snow. All collected material was preserved *in situ* in 70% alcohol. Immobile adults were clearly visible resting on snow banks at the lakeside and found up to 100 m away (Fig. 2a, b) from the lake. No other aquatic insects were observed emerging from Lake Enol during our time collecting along the shore (23<sup>rd</sup> November 2013). Weather conditions were largely overcast and the ambient temperature was 3°C, with intermittent periods of snowfall and occasional sunshine. Water temperature was 6.4°C. Exuviae collected from Lake Enol were identified using non-permanent rapid mounts in alcohol. Initial identifications of exuviae collected from Lake Enol resulted in 10 species, including:

*Paratanytarsus bituberculatus* (Edwards, 1929)

*Tanytarsus gibbosiceps* Kieffer, 1922

*Corynoneura edwardsi* Brundin, 1949

*Neozavrelia* Pe1 Langton, 1991 (*fuldensis* Fittkau, 1954 or *luteola* (Goetghebuer and Thienemann, 1941))

*Microtendipes chloris* (Meigen, 1818)

*Polypedilum* (*Pentapedilum*) *sordens* (Wulp, 1874)

*Stictochironomus histrio* (Fabricius, 1794)

*Chironomus plumosus* (L, 1758)

*Metriocnemus obscuripes* (Holmgren, 1869)

*Pseudorthocladius* cf Pe3 Langton, 1991

Some specimens will require additional treatment to elucidate species. Adults have not yet been identified and will be included in a future paper.

While collecting, remarkable field observations were made on the feeding behaviour of three overwintering species of passerines *Motacilla alba* (White Wagtail), *Saxicola rubicola* (Common Stonechat), and *Erithacus rubecula* (European Robin). Individuals of *M. alba*, *S. rubicola* and *E. rubecula* were observed actively feeding on chironomids along the leeward shoreline of Lake Enol. Notably, these species used different feeding strategies. Individuals of *E. rubecula* remained on the lake margins, actively collecting the recently emerged, resting adult chironomids from the snow surface, which rendered them highly visible to predators (Fig 2a, Fig 2b).

In contrast, individuals of *M. alba* and *S. rubicola* were seen initially observing the lake surface while perched on rocks along the shoreline, and then would take off to fly in small circuits over the lake surface, stopping to flutter and hover over the water surface in mid-flight to pluck freshly emerging adult chironomids. *Motacilla alba* is a bird commonly associated with freshwater habitats. In the case of *S. rubicola*, this behaviour is very unusual since this species is associated with predominantly terrestrial feeding habits. We filmed this behaviour while at Lake Enol (<http://www.youtube.com/watch?v=z7y65cCCjmo&feature=youtu.be>).



Figure 2. Recently emerged adult male chironomids (not yet identified) rest completely immobile on the snow surface on the leeward shore of Lake Enol. They are highly visible to predators and easily picked off as a result of their immobility.

Urquhart (2002) describes feeding techniques of *S. rubicola* in terrestrial habitats. The “aerial sally” describes a short, steep aerial ascent and pursuit of flying insect prey, while the “aerial glean” technique involves plucking insect prey items from surrounding vegetation whilst in flight. “Perch to ground sallying” occurs where ground dwelling prey items are observed from an elevated perch and the bird flies to pluck the prey item from the ground, while “flutter pursuit” is where a bird flies to the prey item and flutters after it along the ground or above it in the air. Perch to ground sallying was also reported by Greig-Smith (1983). No scientific papers were found mentioning stonechats feeding over water in the way we observed on Lake Enol following an extensive online literature search (online searches on Google Scholar 02/12/2013 and Scopus 02/12/2013). On the British Birds Rarities Committee website, we found only a short note digitized from the British Birds Magazine dating back to 1977 (Available at: <http://www.britishbirds.co.uk/search?model=pdf&id=5180>) describing a female or immature stonechat taking food from water. We also found wildlife photos of stonechats feeding on invertebrates through ice holes on a frozen lake in the UK on the website of the wildlife photographer Brian Rafferty (Photos available at: <http://brianraffertywildlife-photographer.blogspot.pt/2010/01/stonechats-on-ice-conclusion.html>).

During a typical year, Lake Enol is normally frozen over for only 1-3 weeks (Alfredo Nicieza, University of Oviedo, pers. comm.). Our observations on Lake Enol reveal an apparently rare feeding mode of stonechats over open water that appears to be an adaptation and combination of “perch to ground sallying” and “flutter pursuit”. Observations also emphasise the extreme importance of emerging adult chironomids as a vital food resource for small overwintering insectivore birds in high altitude lakes that do not freeze over or freeze for only short periods of time. This emphasises the strong aquatic-terrestrial trophic link in high altitude ecosystems during the onset of winter when food resources become scarce.

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## First records of *Buchonomyia thienemanni* Fittkau (Diptera: Chironomidae) from the Czech Republic

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*Buchonomyia thienemanni* Fittkau is recorded from the Czech Republic for the first time based on specimens collected in samples from a section of the River Dyje in Podyjí National Park (Moravia) and the Litavka River in the Brdy Mountains (Bohemia).

*Buchonomyia thienemanni* Fittkau was originally described based on a single adult male collected at an altitude of 800 metres in the Wasserkuppe spring region of the River Fulda in Germany (Fittkau 1955). After a long gap of twenty one years a second record was published when adults were found by the River Flesk, S. W. Ireland (Murray 1976). The pupal stage was subsequently discovered and described based on associated material from the River Flesk (Murray and Ashe 1981). The adult stage is more elusive and most records of the species in the western Palaearctic (Europe, North Africa, Iran) are based on the pupal exuviae which can be easily collected in drift samples or in foam samples from rivers.

While attending the 19<sup>th</sup> International Symposium on Chironomidae in České Budějovice (17-22 August 2014) the authors took part in the two day post conference tour. On the 21<sup>st</sup> August the tour group walked for a few hours through a section of the Podyjí National Park. The group eventually reached an elevated area with an excellent view overlooking the Šobes Vineyard and a section of the River Dyje with extensive surrounding natural woodland (Fig. 1). This woodland covers the nearby mountains and extends down to both banks of the river. When the first two authors (PA and JM-B) saw this section of pristine river we immediately suspected that *B. thienemanni* could be found there. We followed the track down from the vineyard to the River Dyje and crossed over the footbridge. On the south bank of the river, about 50 metres below the footbridge, it was possible to directly access the river where the bank was lower. There was quite a lot of river foam which had accumulated behind obstacles such as emergent rocks and tree branches. River foam, forming over several hours or days, may accumulate large numbers of chironomids (pupal exuviae, pharates and drowned adults) and is easily collected with a drift net. One large foam sample was collected just beside the river bank and a more extensive sample was collected further out in shallow water (Fig. 2) which included foam and material washed from aquatic plants and stones. When the large foam sample was being processed for preservation pupal exuviae of *B. thienemanni* were observed and later examination revealed the presence of a total of 98 pupal exuviae and one drowned adult male. The use of drift or hand nets, to collect foam samples, to determine whether or not *B. thienemanni* occurred in the River Dyje proved immediately successful.

Ten days later, on the 31 August 2014, the junior author (DV) was collecting samples from the Litavka



Figure 1. Elevated area overlooking a section of the River Dyje (upstream of sampling site) with extensive natural woodland. Photo: P. Ashe.





Figure 2. Joel Moubayed-Breil sampling, where *B. thienemanni* was found, in the River Dyje about 50 metres downstream of the footbridge. Photo: P. Ashe.



Figure 3. Litavka River, *B. thienemanni* sampling site. Photo: D. Vondrák.



River (Figs 3, 4) which rises in the Brdy Mountains in Bohemia. A hand net was used and pupal exuviae of *B. thienemanni* were found but they were not as numerous in the Litavka sample as those from the River Dyje samples.

Full details of the records are as follows:

**CZECH REPUBLIC: Moravia:** 21 August 2014, circa 1.2 km north of Hnanice, 50 metres downstream of footbridge, south bank of the River Dyje, Podyjí National Park, 48° 48' 39" N, 15° 58' 40" E, leg. P. Ashe & J. Moubayed-Breil; **Bohemia:** 31 August 2014, circa 0.5 km south of road bridge in Lochovice at 314 m a.s.l., next to the soccer field, west bank of the Litavka River, Brdy Mountains, 49° 50' 54" N, 13° 58' 53" E, leg. D. Vondrák.

Voucher specimens from the River Dyje have been deposited in the collections of the Zoologische Staatssammlung, Munich, Germany (3 pupal exuviae and 1 drowned adult male) and the National Museum of Ireland, Dublin, Ireland (4 pupal exuviae)- the remaining material from this site is in the respective collections of the two senior authors (PA and JM-B). Material of *B. thienemanni* from the Litavka River, collected by the junior author (DV), is in the collection of the second author (JM-B).

The locality data detailed above from the River Dyje in Moravia and the Litavka River in Bohemia are the first records of *B. thienemanni* from the Czech Republic.

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Figure 4. Litavka River, 250 metres upstream of sampling site. Photo: D. Vondrák.

## Data on *Buchonomyia thienemanni* Fittkau (Diptera: Chironomidae) from Slovakia

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All known records of *Buchonomyia thienemanni* Fittkau from Slovakia are summarised below which includes two records from the Žilina Region and one record each from the regions of Levice and Sered'. Bitušík (1987) published the first record of the species for Slovakia from the Rajčianka submontane brook. The species was also found during a study on the longitudinal zonation of chironomid assemblages in the River Hron (Bitušík et al. 2006). Specimens from all localities are based on pupal exuviae collected with drift nets. Details on two previously unpublished records are provided.

**SLOVAKIA: Žilina:** 21 July 1979, below the village of Rajecká Lesná at 480 m, Rajčianka submontane brook, 49° 03' 25" N, 18° 37' 21" E, leg. P. Bitušík (Bitušík 1987); 17 July 1994, below the village of Varín at 353 m, Varínka submontane brook, 49° 11' 48" N, 18° 52' 14" E, leg. P. Bitušík; **Levice:** 3 July 2003, close to the village of Jur at 142 m, River Hron, 48° 07' 47" N, 18° 36' 41" E, leg. P. Bitušík (Bitušík et al. 2006); **Sered':** 10 June 2009, above the reservoir of Kráľová at 120 m, River Váh, 48° 15' 40" N, 17° 46' 25" E, leg. S. Ščerbaková.

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## First record of gynandromorphy in fossil Chironomidae (Diptera) from Late Eocene Rovno amber

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Gynandromorphy as an abnormal mixture of sex-specific traits of males and females in an individual is wide spread among the Chironomidae (Martin and Lee 2000). Gynandromorphy could be caused by mutation in the mitosis regulating genes, which creates an abnormal puzzle of “genetically masculine” and “feminine” body parts, or by other factors like Mermithidae (Nematoda) parasites (Martin 1994). Gynandromorphs can be categorized based on morphology and relationships between “male” and “female” parts of the body. Basically they could be divided in 3 groups: a) anteroposterior gynandromorphs – with anterior and posterior parts of the body possessing traits of different sexes; b) lateral – with body possessing different sexes traits on the left and right sides; c) mosaic – with sexual traits creating a sophisticated puzzle where wings and limbs can be attributed to the phenotypes of different sexes (Martin 1994, Rempel 1940). Studies of gynandromorphs are important for understanding Chironomidae sex determination mechanisms and evolution of the group.

Up to now, gynandromorphs have been recorded only in recent Chironomidae. Herein we present the first fossil record of Chironomidae gynandromorphy based on an inclusion in the Late Eocene Rovno amber from Ukraine.

Late Eocene Rovno amber represents a southern coeval analogue of Baltic amber (Baranov et al. 2014). Chironomids are diverse and abundant in Rovno amber, with up to 15 genera from 4 subfamilies recorded from that deposit, three of these genera being known only from Rovno amber (Baranov et al. 2014; Gilka et al. 2013, Zelentsov et al. 2012). The gynandromorph was found during a survey of the I.I. Schmalhausen Institute of Zoology collection of nematoceran Diptera in amber.

### Materials and methods

Rovno amber belongs to the succinites, as does the well-known Baltic amber (Zelentsov et al. 2012). The piece of amber containing the gynandromorphic midge was found in Klesov (Pugach quarry) and obtained from “Ukramber” factory (Rovno). The specimen is moderately well preserved (Fig. 1). However, the wings are folded and thus difficult to examine, and the hypopygium is unavailable for examination, because of an air bubble. The specimen was examined using standard techniques (Baranov et al. 2014). The general terminology follows Sæther (1980). The voucher specimen is housed at the I. I. Schmalhausen Institute of Zoology, National Academy of Science of Ukraine, Kiev (SIZK), Ukraine under catalogue number K-5404.

Photographs were taken at the Paleontological Institute, Russian Academy of Sciences (PIN PAS) in Moscow by Victor Kolyada using a Leica M 165 microscope and Leica DFC 425 camera.

### Description

The specimen is attributed to the subfamily Orthocladiinae (Diptera, Chironomidae) based on the combination of the observable part of the wing, structure, shape of flagellomeres, legs, and especially tibial combs structure (Fig. 1A). Further identification is impossible, due to unavailable details of wings and genitalia. At the left side of scutum a large triangular wound can be seen (Fig. 1A). It could be a marking from an insectivorous biting midge (Diptera, Ceratopogonidae) attack. It has been shown that insectivorous biting midges, like *Eohelea sinuosa* (Meunier, 1904), frequently attacked chironomids in the Rovno amber forest (Perkovsky 2013; Perkovsky and Rasnitsyn 2013).

Wing length is about 800µm. Body length is 835 µm. The specimen possesses evident lateral gynandromorphy, as can be seen from the antennae structure. The left antenna is typically male, with 10 flagellomeres and long bristles. AR = 0,74. In contrast, the right antenna is of typically female structure, consisting of only five flagellomeres and with no long bristles. The pedicellus of the left antenna is much smaller than the right one (Fig. 1B). No signs of mermithids (Nematoda, Mermithidae) or other parasitic worms, have been found on the midge body.

## Discussion

Amber as a fossil container for exceptionally well preserved organisms plays an important role in our understanding of evolution. By studying amber we are gaining more than just a list of new taxa. Often we are able to reconstruct sophisticated connections in the environment of the distant past. Much work has been done on parasitism, mutualistic connection, predation, sexual selection etc. based on amber fossils (Azar 2007). The record of the chironomid gynandromorphy allows us to assume that the sex-determination system of the Chironomidae 40 million years old could have been similar to that of the present (Martin and Lee 2000), because of the similar “typeset errors” like lateral gynandromorphy. We could claim that in this particular case the abnormality was genetically determined, because we have found no signs of Mermithidae or other parasites which could cause such abnormality (Yakovlev pers. comm. 2014). This record once again has proved the importance of amber paleontology for the research in Chironomidae systematics and evolution.

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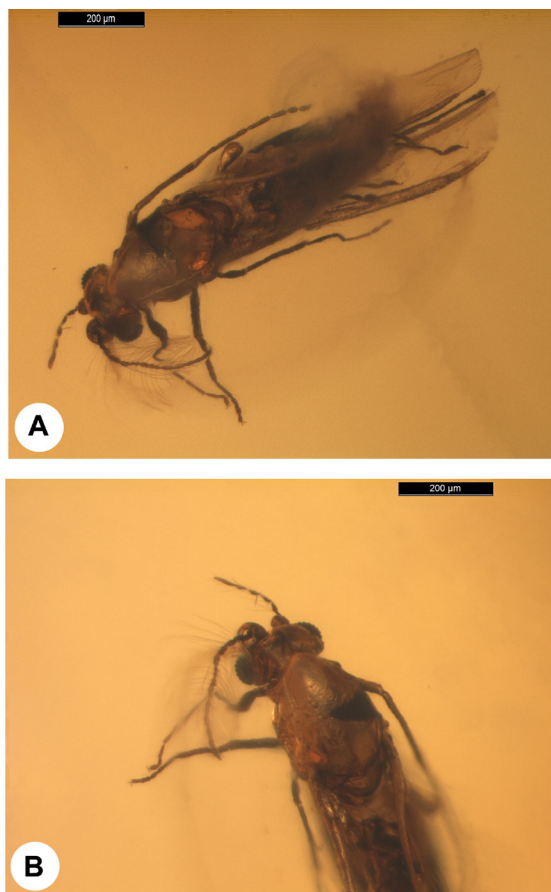


Figure 1. A: Orthoclaadiinae gynandromorph in amber (K-5404) – total view. B: Orthoclaadiinae gynandromorph in amber (K-5404) – head and thorax.

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## The 20<sup>th</sup> International Symposium on Chironomidae

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For the **first time** in its history, the International Symposium on Chironomidae will be organized in Italy. The XX edition of the symposium will be held at the MUSE- Museo delle Scienze in Trento, July 2017.



Town centre, the Cathedral. Photo: Matteo Ianeselli / Wikimedia Commons / CC-BY-3.0& GFDL.

Prince Bishops turned the Buonconsiglio castle into one of the best known castles in the whole of Europe and Trento (historically known in English as Trent) is the capital of the Trentino Province, a mountain region located in the heart of the Alps. The Province covers an area of more than 6,000 km<sup>2</sup>, with a total population of about 0.5 million. Trento lies in the Adige valley just south of the Dolomite Mountains, where the Fersina River and Avisio rivers join the Adige River, the second longest river in Italy. The town centre is more or less a pedestrian area, and walking around the historic centre you can see a number of outdoor frescos on historic buildings.

MUSE- Museo delle Scienze (previously Museo Tridentino di Scienze Naturali) is one of the main cultural centers of the city and was built along the banks of the Adige. It manages, in addition to the main museum located close to the city centre, a network of six museums and local offices at the provincial level and one in Tanzania. MUSE, that traces back its origin in the mid XIX century, is the first museum in Italy that harmoniously blends nature, science and technology, devoted to research, visitor interpretation and education. Since 2013 the museum has been located in a new building whose outline recalls the profile of the surrounding mountains, with a finely balanced contrast between



MUSE and Trento city. Photo: Hufton+Crow, MUSE Archive. MUSE, the building. Photo: Roberto Nova, MUSE Archive.



empty and full spaces that adds charm and prestige to the entire exhibition venue. Built on eco-compatible criteria, MUSE combines characteristics from both traditional natural science museums and modern science centers in a very innovative way.

MUSE has traditionally carried out multidisciplinary research activities, both basic and applied, in the field of the environment with particular attention to the issue of biodiversity and ecology of mountain ecosystems. Participants will have the possibility to visit the exhibition halls of the new museum at any time during the congress. Visits or research in the museum's collections will be also possible (the natural history and archaeological collections of the MUSE account about 300 collections and 5 millions objects, of which the oldest were collected more than two centuries ago).



Noce Bianco glacial stream, Trentino, Italy. Photo: Valeria Lencioni, MUSE Archive.

The scientific program will be developed through a series of sessions during which various issues will be addressed, from systematics to biogeography, ecology and biomonitoring. Mid- and post conference trips will be organized to enjoy alpine freshwaters and chironomid sampling in the Alps, to appreciate artistic heritage of Trento and of the neighboring towns (e.g. Verona, famous in the world for the lyrical season in the Arena, the ancient amphitheatre built by the Romans) and to taste the delicious local food and wines.

The nearest airports are Verona, Venice and Milan. The faster connection with Trento from these airports is by train (about 3 hours from Milan and Venice, one hour from Verona).

MUSE and Trento will welcome you in 2017 to attend the XX International Symposium on Chironomidae. We are proud to host it.

## **News from the editors**

### **Chironomus newsletter changes name to *CHIRONOMUS Journal of Chironomidae Research***

As of the next issue (No. 28, 2015) the newsletter changes name to *CHIRONOMUS Journal of Chironomidae Research*. This change will hopefully make it even more attractive to submit research articles to our journal and further increase the quality and readership of the content. We will continue to publish Short Communications and news items that are not necessarily subjected to peer-review. The journal will remain open access and free of page charges, and will continue to be indexed in the Directory of Open Access Journals and Google.

### **Nomenclatorial acts to be registered in ZooBank**

Taxon names and nomenclatorial acts are listed in the open access registry ZooBank ([www.zoobank.org](http://www.zoobank.org)), to ensure free availability of this information with links to authors and publications. The newsletter and the coming *CHIRONOMUS Journal of Chironomidae Research* has started to register all published nomenclatorial acts from the journal in ZooBank starting from No. 26, 2013.

Alyssa M. Anderson, Torbjørn Ekrem, Peter H. Langton