



*CHIRONOMUS NEWSLETTER OF CHIRONOMID
RESEARCH*

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14th International Symposium on Chironomidae 2000 Rio de Janeiro, Brazil

Dear Colleague,

The organizing committee, consisting of **Dr. Sebastião José de Oliveira, Dr. Maria Conceição Messias** and **Dr. Jorge Nessimian**, invite you to attend the 14th International Symposium on Chironomidae. It is a pleasure to inform you that the Symposium will be part of the scientific activities of the Instituto Oswaldo Cruz/FIOCRUZ centennial. At the last Symposium in Freiburg, Germany it was decided to meet in Rio de Janeiro, Brazil despite the long distances most participants will be travelling. This first Latin America meeting, although faraway for many of you, will offer the opportunity to enjoy tropical nature at reasonable prices.

General information:

The program will run without parallel sessions.

Location: FIOCRUZ Campus, Rio de Janeiro, RJ, Brazil

Date: August 29 - September 2, 2000. (Chosen to harmonize with the XXI International Congress of Entomology to be held at Foz do Iguassu, Brazil - August 20-26, 2000).

Registration fee: Is estimated at US\$ 180 for employed scientists, with reductions for appropriate cases, under consideration. Registration fee includes: welcome cocktail, transfers hotel-symposium-hotel, abstract book, refreshments and lunch.

Post-conference tours preliminary program: All will be by bus, from Rio de Janeiro City. Price of the three tours together US\$ 150/person.

September 3, Tijuca National Park (Rio de Janeiro City).

September 4, Serra dos Órgãos National Park (Teresópolis City)

September 5, Itacuruçá Beach (Mangaratiba City).

The **proceedings of the Symposium** will be published in a special issue of the Memórias do Instituto Oswaldo Cruz.

If you intended to receive the 2nd announcement, please contact:

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We hope to see you in Rio,

Best regards,

Sebastião José de Oliveira

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CURRENT RESEARCH

NEWS FROM RUSSIAN FAR EAST

By **Eugeniy A. Makarchenko**

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I should like to introduce my working group, which consists of four scientists who study systematics and biology of Far Eastern Chironomidae. Three members, **Marina A. Makarchenko**, **Eugeniy A. Makarchenko** and **Oksana V. Zorina** are working in the Laboratory of Freshwater Hydrobiology of the Institute of Biology and Soil Sciences FEB RAS, (Vladivostok). The main purpose of their investigations is to prepare a Key on Chironomidae (males, pupae and larvae) of the Russian Far East. As you know for a long time I studied systematics of Far Eastern Podonominae, Diamesinae and Prodiamesinae. For the other subfamilies only determinations of larvae from hydrobiological samples were made. From last year Marina A. Makarchenko and I began to study in detail taxonomy and systematics of Orthoclaadiinae. Oksana V. Zorina (PhD student) studied Chironominae, Chironomini. We identified 91 species from Kamchatka and 48 species of Orthoclaadiinae from Kurile Islands in 1999. 20 species are recorded for Russia for the first time. For the following species the development from larvae to the adult male was studied: *Orthocladius* (*Orthocladius*) *trigonolabis* EDW., *O.* (*O.*) *obumbratus* JOH.,

Orthocladius (*Eudactylocladius*) *olivaceus* (KIEFF.), *Paracladius conversus* (WALKER) and *Paratrichocladius skirwithensis* EDW. 45 species of Chironomini from the southern part of Primorye Territory (North Korean and Chinese border region) were found, from which three species of *Parachironomus*, *Einfeldia* and *Pagastiella*, appear to be new to science and 27 species are recorded for Russia for the first time.

A fourth colleague, **Tatiana N. Travina** (PhD student) is working in the Kamchatka Fisheries Institute (KamchatNIRO) in (Petropavlovsk - Kamchatsky). She is studying the fauna and biology of chironomids of the Kurilskoe Lake which is situated at the southern part of Kamchatka Peninsula and is the spawning-ground of one of the largest Asian salmon, *Oncorhynchus nerka*. From the Kurilskoe Lake basin 59 species of Chironomidae have been identified. Data on quantity and biomass of chironomid larvae of this lake are available.

EUROPEAN SUBFOSSIL CHIRONOMIDAE WORKING GROUP

By **Steven Brooks¹** and **Maria Rieradevall²**

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2. University of Barcelona SPAIN (email: rdvall@porthos.bio.ub.es)

Since 1996, and under the sponsorship of the EU-funded projects AL:PE 2, MOLAR and CHILL 10,000, participants responsible for Chironomidae midge analysis have had the opportunity to meet and exchange information as well as discuss shared identification difficulties. These meetings have proven to be very useful for harmonisation of taxonomy in studies which cover broad geographic scales. The projects mentioned deal partially or totally with the reconstruction of climatic change during the Holocene, using a wide variety of proxies at sites throughout western Europe.

In April of this year we met in London at the Natural History Museum. The organisers were Steve Brooks (NHM, UK) and Maria Rieradevall (Univ. of Barcelona, Spain) who is staying at the NHM on an EU grant for nine months. Maria is working on chironomids in late-glacial and Holocene sediments from lakes in the Pyrenees and is developing a chironomid-temperature calibration set for the Pyrenees. One of the objectives of her visit is to collaborate with Steve on the production of an identification manual for European subfossil chironomid midge larvae. Although there are some good keys available for the identification of modern midge larvae, some of the critical morphological characters used in these keys are missing from fossil material. This is particularly problematic in Tanypodinae and

Tanytarsini, which are common as fossils in lake sediments and which can provide useful climatic information. Steve and Maria had the opportunity to try out early drafts of some of their keys on the participants of the fifth in the series of European fossil chironomid taxonomy workshops. This year the participants were **Klaus Brodersen** (Copenhagen, Denmark), **Steve Brooks** (London, UK), **Lars Eriksson** (Uppsala, Sweden), **Evastina Grahn** (Abisko, Sweden), **Mari Hakojarvi** (Helsinki, Finland), **Oliver Heiri** (Bern, Switzerland), **Wolfgang Hofmann** (Plön, Germany), **Barbara Milne** (Ormskirk, UK) and **Maria Rieradevall** (Barcelona, Spain).

Previous meetings have been held in London (1996, 1998), Bergen (1997) and Helsinki (1997), and we can add to the above list of participants: **Malcolm Bell** (Leicester, UK), **Ignacio Granados** (Madrid, Spain), **Annika Jonasson** and **Nick Woolley** (Egham, UK), **Anke Halvorsen**, **Øle Sæther**, **Øyvind Schnell**, **Gaute Velle** and **Endre Willassen** (Bergen, Norway), **Heikki Olander** (Helsinki, Finland), **Zoe Ruiz** (Belfast, UK), **Jon Sadler** (Birmingham, UK) and **Ian Walker** (Kelowna, Canada).

The next meeting is planned for April 2000 in Bern (Switzerland). See you there!

A SURVEY OF THE CHIRONOMIDAE (DIPTERA) OF CALAKMUL BIOSPHERE RESERVE, MEXICO

By **A. Contreras-Ramos¹** and **T. Andersen²**

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The chironomid fauna of Mexico is poorly known. At present, only about 60 species of this family have been recorded or described from the country (ANDERSEN et al., in press). Although preliminary, this study has formally started efforts to build a scientific collection of Mexican chironomids. The

results outlined here are part of collecting efforts by Mexico's National University (UNAM) staff in the Calakmul Biosphere Reserve, Campeche State, Mexico, which also focused on estimating the diversity of Odonata and Psocoptera of the reserve. The chironomids are being sorted, processed,

identified, and described as a combined endeavor between UNAM and the University of Bergen. We are also collecting in other areas of Mexico, and gradually we hope to have built up a fairly representative collection of Mexico's fauna. Of course, if estimates of about 1000 species for the Mexican chironomid fauna are close to reality, we still have a long way to go.

Study area, materials, and methods

The Calakmul Biosphere Reserve, with an approximate extension of 723,185 ha, is one of the largest conservation areas in Mexico. It is located on the southeastern corner of Campeche State, being continuous with the Mayan Biosphere Reserve of Guatemala. Dense tropical forests under a strongly seasonal rain regime cover most of the reserve. Rivers are scarce, and when present, they are typically seasonal. Permanent or temporary lentic water bodies, locally called "aguadas", are the main source of water in

the reserve. There were three collecting expeditions to the reserve (28.iv.-10.v.1997, 17-27.ix. 1997, 13-21.ii. 1998), in which 11 sites were visited. Adult chironomids were collected mainly with black and mercury vaporlights and Malaise traps, less frequently with nets. They were sorted in the laboratory and selected specimens were mounted on slides with Canada balsam.

Results and discussion

Several thousand specimens were collected, mostly from lentic habitats. Selectively, a few hundred slides have been prepared. Species identification is difficult as many groups need taxonomic revision. However, we estimate that the Calakmul chironomid fauna consists of at least 100 species, several of which are new to science. At the moment, a total of 42 genera belonging to the subfamilies Tanypodinae, Orthoclaadiinae and Chironomidae, have been recorded from Calakmul Biosphere Reserve (Table 1).

Table 1. Chironomid genera recorded from Calakmul Biosphere Reserve.

<u>Subfamily Tanypodinae</u>	<u>Trib. Chironomini</u>
<i>Ablabesmyia</i> JOHANNSEN, 1905	<i>Apedilum</i> TOWNES, 1945
<i>Clinotanypus</i> KIEFFER, 1913	<i>Axarus</i> ROBACK, 1980
<i>Coelotanypus</i> KIEFFER, 1913	<i>Beardius</i> REISS & SUBLETTE, 1985
<i>Fittkauimyia</i> KARUNAKARAN, 1969	<i>Chironomus</i> MEIGEN, 1803
<i>Labrundinia</i> FITTKAU, 1962	<i>Cladopelma</i> KIEFFER, 1921
<i>Nilotanypus</i> KIEFFER, 1923	<i>Dicrotendipes</i> KIEFFER, 1913
<i>Pentaneura</i> PHILIPPI, 1865	<i>Endochironomus</i> KIEFFER, 1918
<i>Procladius</i> SKUSE, 1889	<i>Glyptotendipes</i> KIEFFER, 1913
<i>Tanypus</i> MEIGEN, 1803	<i>Goeldichironomus</i> FITTKAU, 1965
<u>Subfamily Orthoclaadiinae</u>	<i>Omisus</i> TOWNES, 1945
<i>Antillocladius</i> SÆTHER, 1981	<i>Oukuriella</i> EPLER, 1986
<i>Bryophaenocladus</i> THIENEMANN, 1934	<i>Parachironomus</i> LENZ, 1921
<i>Corynoneura</i> WINNERTZ, 1846	<i>Paratendipes</i> KIEFFER, 1911
<i>Cricotopus</i> VAN DER WULP, 1874	<i>Polypedilum</i> KIEFFER, 1921
<i>Diplosmittia</i> SÆTHER, 1981	<i>Stenochironomus</i> KIEFFER, 1919
<i>Mesosmittia</i> BRUNDIN, 1956	<i>Tribelos</i> TOWNES, 1945
<i>Nanocladus</i> KIEFFER, 1913	<i>Xenochironomus</i> KIEFFER, 1921
<i>Parametriocnemus</i> GOETGHEBUER, 1932	<i>Xestochironomus</i> KIEFFER, 1921
<i>Pseudosmittia</i> EDWARDS, 1932	<u>Trib. Tanytarsini</u>
<i>Thienemanniella</i> KIEFFER, 1911	<i>Rheotanytarsus</i> THIENEMANN & BAUSE in BAUSE, 1913
<u>Subfamily Chironominae</u>	<i>Skutzia</i> REISS, 1985
<u>Trib. Pseudocironomini:</u>	<i>Stempellina</i> THIENEMANN & BAUSE in BAUSE, 1913
<i>Pseudochironomus</i> MALLOCH, 1915	<i>Tanytarsus</i> VAN DER WULP, 1874

It is our goal to continue identifying and describing Calakmul species, as well as gradually continue survey work in different areas of Mexico. A formal taxonomic

collection of Mexican chironomids, as well as training of new specialists in the group, are needed in order to guarantee long-term research on this important insect group.

Acknowledgements

We would like to thank our home institutions for their support regarding field work and research facilities, as well as CONABIO and the MacArthur Foundation for financial support. Thanks also to Javier Alcocer, Peter S. Cranston, John H. Epler, Donald R. Oliver, Friedrich Reiss, Ole A. Sæther, and Martin Spies for help with literature and general support.

References

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"TAKE 25 MG OF INSECT.." - SIMMERING SIGNALS OF PHYLOGENY FROM CHIRONOMID MITOCHONDRIA

by **Endre Willassen**

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Despite the growing popularity of PCR based DNA sequencing in systematics and evolutionary biology, limited use of these techniques have been made in chironomid systematics. It would not take someone like Hieronymus to predict that chironomid phylogenies produced from molecular data will emerge with increasing frequency over the coming years. Assuming that a fair proportion of *Chironomus* readers are not entirely familiar with methods to generate DNA sequence data, I shall briefly outline some basics of laboratory procedures used in my current investigation of Diamesinae species. For more concise recipes and descriptions of necessary equipment I refer to laboratory manuals such as HILLIS et al. (1996) and KARP et al. (1998).

Material

The primary concern for successful work with DNA is that these molecules are easily degraded by several physical and chemical factors (BROWN 1999). The traditional and still best solution to this problem is to freeze fresh samples in liquid nitrogen or dry ice. However, this procedure can be very awkward in field situations and in practice it becomes a quick dash to the lab for someone who wants to investigate DNA from organisms in remote areas. Fortunately, one of the most commonly used fixatives in chironomid collecting is a useful substitute for cryopreservation: ethanol. This means that material which was not intentionally collected for DNA analysis may be used for this purpose. My experience is that specimens that have fairly recently been fixed in alcohol without additives usually give adequate yields for work with fragments that are within the optimal range of direct

sequencing (about 400-800 base pairs). I have amplified DNA from chironomids that have been kept in daylight and room temperature since 1984. However, strange things can happen to DNA under such conditions and samples in alcohol should generally be protected from any sort of radiation, preferably at 4°C. When samples are collected in alcohol, extra measures can be taken to inhibit DNase activity. References to more sophisticated non-cryogenic preservation methods can be found in ZHANG & HEWITT (1998).

Some taxonomists have used fixatives such as Oudemans' solution and Karnoy for insect preservation. These solutions keep tissues softer than with pure alcohol and are certainly good for microscopy. However, the 'softening' component, acetic acid, degrades DNA with aggressive H⁺ ions and gives poor prospects for DNA yield (Koch *et al.* 1998). I can confirm that chironomid DNA is no exception in this case, although chromosome structure does well in acetic acid and ethanol. Neither have I succeeded in attempts to amplify DNA from pinned specimens.

DNA preparation

There are numerous protocols for DNA extraction and isolation. Some are complex and demanding in terms of work. Others are simple and allow for rapid isolation of DNA. The choice of method depends very much on tissue type and the quality and concentration of DNA needed for further processing (PHILLIPS & SIMON 1995; REINEKE et al. 1998). Commonly used methods employ EDTA, TrisHCl, sodium dodecyl sulfate (SDS), and proteinase K for lysis and protein digestion. DNA can subsequently be extracted with high concentration salt and with

ethanol/isopropanol precipitation. Chloroform/phenol extractions may be necessary for more purified extracts.

I have found it convenient to work with the QIAamp Tissue Kit® (Cat. 29304) for DNA isolation and purification. This is a ready-to-use package including spin columns and all necessary reagents (except ethanol). The kit is designed for tissue samples weighing up to 25 mg, and the quote in the headline of this letter is taken from the QIAamp protocol. However, midges don't exactly leave deep footprints on muddy ground, and I would guess that the amounts of tissue I have worked with are sometimes roughly 100 times less than the quantity prescribed by the protocol. To keep body parts with diagnostic features intact for morphology studies, I am mostly using the adult thorax for extraction. I have also used ovary with mature eggs, which makes even the thorax available for conventional slide preparation.

It is common procedure to grind the piece of tissue to be used for extraction in liquid nitrogen. I have skipped the nitrogen and do the grinding in an Eppendorf tube with lysis solution, using a HCl rinsed and autoclaved micro pestle. The lysate is set up for incubation at 70°C for about 15 minutes or more, and afterwards transferred by pipette to the QIAamp spin column where DNA is bound to an incorporated silica gel membrane. Proteins, polysaccharides and other unwanted components in the solution are removed by washing the membrane and spinning the columns in a microcentrifuge. In the final step of this extraction procedure, DNA is eluted from the silica filter and collected in an Eppendorf tube. The eluate is ready to be used directly in polymerase chain reaction (PCR) and can be stored at +4 to -80°C for repeated experiments.

PCR amplification

The critical element for PCR is a pair of oligonucleotide primers with known base sequences. During the annealing phase of a PCR cycle the "forward" and "reverse" primers must attach to complimentary positions in temporarily separated parental strands of the template DNA. The nucleotide stretch to be amplified is bracketed by the forward and reverse primer. Some *a priori* knowledge of the target gene is necessary in order to find an appropriate primer pair. When new taxa are screened, it is a good strategy to start with primers that are

complimentary to conserved sequences in related taxa (see reviews by BROWER & DESALLE 1994; SIMON et al. 1994). Prior knowledge of the annealing positions of the primer pair allows one to predict approximately the length of the stretch to be amplified. When longer stretches are difficult to amplify, the problem can sometimes be solved if there is a suitable primer target internal to one of the originally flanking primers. Particularly from older material, shorter sequences are generally easier to amplify.

PCR can be regarded as a cyclic process with three reaction steps per cycle. Step one takes place in a hot environment (typically 94-96°C). The purpose of heating is to break the bindings between complimentary strands of the DNA template to produce temporary single stranded DNA. This is the *denaturation* or melting phase. In step two, the temperature is lowered to about 50°C to allow primers to bind to matching sites on the single stranded templates. This is the *annealing* phase. At step three, a new strand, complimentary to the template, is synthesized downstream from the primer site. This *extension* of the primer is catalysed by the heat tolerant polymerase *Taq* (*AmpliTaq*® or equivalent synthetic enzyme) which works well at 72°C. An excess of building material for DNA replication is certainly also required. This is contained in the dNTP mix (deoxynucleoside 5'-triphosphates) including dATP, dGTP, dCPT and dTTP. Thus, *Taq*, DNA template, a primer pair, and dNTP are important ingredients in the PCR mixture. These components react in a buffer with a suitable concentration of Mg²⁺ ions. I usually run 25 microlitre reactions.

Temperatures and time intervals for melting, annealing and extension can be programmed on an automatic thermocycler. I am using Perkin-Elmer's GeneAmp® PCR System 2400. Theoretically, one single molecule of template DNA is sufficient for replication with PCR. With 100% efficiency the target sequence would have been copied about 109 fold after 30 cycles. In practice, the yield is closer to 105 fold, depending on several factors such as template length, concentrations, and enzyme half life. The processing speed of *Taq* and the near logarithmic replication of DNA at intervals is certainly the beauty of PCR in a nutshell. A less attractive side of this powerful kinetics is that PCR is susceptible to

contamination with alien DNA. Precautions must therefore be taken at all times to avoid unintentional amplifications, and all PCR sessions must be run with a negative control which contains all ingredients of the reaction except template DNA. Another potential snag is that *Taq* replication is lacking a proofreading mechanism. (Some other polymerases have that property.) Although this is generally not considered as a very big problem, it may result in chimeric PCR products.

Electrophoresis of PCR products

When the thermocycling programme is terminated, the next step is to test for PCR product and to determine the length, purity, and quantity of possible DNA yield. This is usually done by electrophoresis on a 1% agarose gel submerged in TAE buffer. Small quantities of PCR samples are mixed with a dye that helps to visualise the loading of the sample into a gel slot, and also to indicate the progress of the electrophoresis. At some stage, the gel must be stained with ethidiumbromide to detect any presence of DNA after electrophoresis. Ethidiumbromide binds easily to DNA and makes it fluorescent in UV light.

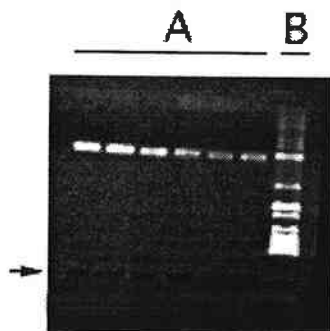


Figure 1. Agarose gel with PCR products (A) and standard ladder (B). Arrow pointing to loading slots.

The length of bands in the PCR product can be determined by loading one of the slots in the gel with a standard. The standard is a mix of DNA fragments of known lengths and quantities. During electrophoresis, these fragments will be separated out to a ladder of bands (Figure 1) which can be used as references to determine the length of bands in the PCR products. With a trained eye, one can also approximately estimate the quantity of PCR product simply by comparing emission intensities with the standard under

UV light. Quantification can otherwise be done with a spectrophotometer.

Illumination of the agarose gel is perhaps the most exciting moment in the lab, because when one is working with rare specimens, hopes are broken with fruitless PCR. What we want to see are sharp clear bands, one in each lane with loaded PCR product (Figure 1). Bands in the negative control would indicate DNA contamination of a chemical or some piece of equipment. It would indeed be an unpleasant observation. Multiple bands in the PCR product attest to unspecific primer binding. No bands at all means that there is a wealth of options for repeated trial and error experiments. The specificity of PCR is strongly affected by three factors: The concentration of *Taq*, the annealing temperature, and the concentration of Mg^{2+} . Optimisation of these factors may be a tedious procedure, so opportunity knocks for rejoicing or frustration in UV light.

Sequencing the PCR product

There are several routes to obtain information about the sequence of nucleotides. Direct sequencing of the PCR product is the fastest way. By means of the fluorescent ddNTP chain termination method, the preparations are also amazingly simple. Because thermocycling is also used in the sequence reaction, the procedure is somewhat similar to amplification. However, there are three important differences: First, instead of crude DNA, purified PCR product is now used as template. Second, forward and reverse replications of strands are run as separate reactions. This is achieved by adding only one primer of the original pair to the reaction mix. Still, both primers should be used, but now in separate reactions. Third, but not least important, is an additional set of ingredients to the PCR cocktail: 2'-3' dideoxynucleosides (ddNTP). Each of the four ddNTPs (ddCTP, ddGTP, etc.) may be accepted as substitutes for their respective dNTP as precursors in the growing DNA strand. However, each time a ddNTP is incorporated instead of a dNTP the strand will be terminated. This is because the phosphodiester bond necessary to extend the chain further cannot be formed. The result of thermocycling under these conditions is a nested set of single stranded nucleotide fragments. What they all have in common is the primer sequence in the 5' end. Each ddNTP is uniquely labelled with a fluorescent dye. The shortest fragment produced thus

carries a ddNTP that signifies the base identity in the first position after the primer. The second shortest fragment has one unlabelled base in between the primer sequence and the labelled base. This fragment thus carries information about the second base after the primer, and so on. Accordingly, a complete nested set may comprise several hundred fragment lengths differing by increments of single bases, and each carrying the identity of a particular base position in the 3' end dNTP derivative.

In our laboratory we are using ABI PRISM® BigDye for cycle sequencing. This

kit has all necessary chemical components for the reaction described above. After precipitation of the sequencing product with absolute EtOH and NaOAc and successive rinses with 70%EtOH, the fragments in the nested set can be separated by electrophoresis in an automated sequencing machine. The machine has a detector unit with CCD camera. Special software interprets the fluorescent signals and transforms them into electronic chromatograms. Most of my sequences have been run on an ABI PRISM® 377XL DNA Sequencer. Results are usually collected in the form of chromatogram files.

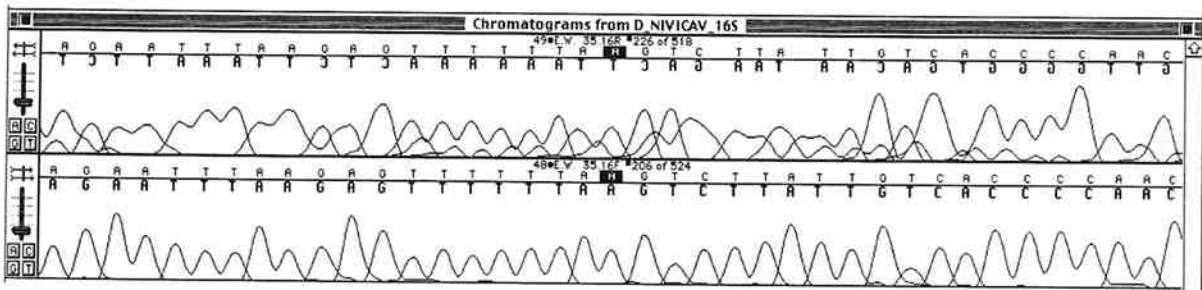


Figure 2. Chromatograms of reverse (top) and forward (bottom) sequences assembled for inspection. The forward sequence has clear signals whilst the reverse shows some less acute peaks and potential signal noise in secondary peaks.

Treatment of raw sequence data

Computer software is needed to inspect and edit chromatogram files. I prefer Sequencher from GeneCodes®, not so much for its rip-off price, but rather because it has a wide spectre of functions that can save a great deal of work, including some painstaking reading of four letter texts. The first thing to do is to assemble the chromatogram files from the forward and reverse reactions for comparison (Figure 2). This is usually made very quickly with one of Sequencher's alignment algorithms. The result is a so called contig into which other sequences can also be imported. Sequences can be messy over short stretches, sometimes due to impurities including traces of salt, ethanol and excess dNTP. Unfortunately, such impurities may also sometimes disperse from neighbour lanes in the sequencing gel and produce faulty base calls in a clean sequencing product. Ambiguous base calls in one sequence may be edited and corrected if the complimentary sequence has unambiguous signals in those positions. But there is certainly a limit to how much of a mess one can accept before a sequence must be discarded and reproduced. After editing clearly erroneous base calls and

trimming untidy ends, a consensus sequence can be made from the forward and reverse sequences. If appropriate, the consensus sequence can contain N's (or other more specific characters) symbolizing unresolved base calls.

When an entirely new sequence has been produced, it may be a good idea to compare it with data accessible from GENBANK. Particularly if the sequence has been generated with "universal" primers, a BLAST search over Internet may exclude the possibility that an alien fragment of DNA was unintentionally sequenced. Interested readers might like to try a basic BLASTn search with one of the 30 bp sequences in Figure 3A:

GTAATAAATAATAAAAAGTTTATTTAATTT.
(<http://www.ncbi.nlm.nih.gov/BLAST/>)

Sequence alignment

Using BLAST is an application of an alignment algorithm to compare pairwise sequence similarities. The purpose of aligning multiple sequences is to establish positional identities between bases and nucleotide regions. When used to infer phylogeny, an alignment is thus an implicit statement of homology (in a general sense). This is why alignment is perhaps one of the bigger

epistemological challenges in molecular systematics, and also the nodal point of diverging philosophies. Alignments can be made manually, and literature indicates that this is a method preferred by many investigators. However, "alignment by eye" becomes increasingly difficult with more disparate sequences and the expression probably often boils down to *a posteriori* editing and adjustments in computer aligned output. Several alignment programmes including W.Wheeler's "MALIGN" and the popular "ClustalW" are available over Internet. Many net sites also provide services for interactive work with alignment programmes.

Will DNA sequencing make a revolution in chironomid systematics?

Many aspects of molecular evolution clearly suggest that molecular data are not a wizard's formula to direct reconstructions of species phylogenies. From the perspective of someone who is trained more with nets than pipettes, it seems that molecular systematics is coming to terms with less assured perceptions of molecular providence in all areas of inference. The prospects for new insights in chironomid systematics with sequence data are probably best when it comes to questions of relationships between 'phenetically gapped' taxa. In many such cases, morphological synapomorphies are either disputed or hard to find at all. In due time, it is thus a possibility that molecular data may come to challenge particularly some of the deeper branches in current hypotheses of chironomid phylogenies. At the present stage of my investigation I am not in a position to overthrow any previous ideas of relationships. More to the contrary, - much of my data seem to fit with current hypotheses of species groups in *Diamesa*. For example, through a peephole to some of my preliminary findings (Figure 3) an earlier suggestion (WILLASSEN 1985) appears to be supported: *Diamesa nivicaavernicola* HANSEN, 1976, could be the sister species to

the *D.davisi* complex. More complete sequence sampling and rigid analyses are necessary to ascertain this conclusion. Sequencing will certainly shed new light on chironomid systematics and will be a valuable supplement to morphology studies.

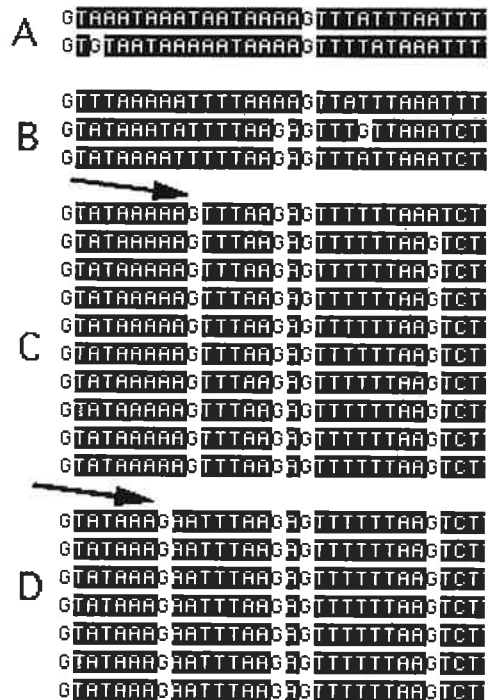


Figure 3. Alignment of partial sequences from Simuliidae and Culicidae (A), non-*Diamesa* chironomids (B), *Diamesa* (C,D), *D.nivicaavernicola* and *D.davisi*-group (D). Arrows pointing to two possibly synapomorphic base substitutions

Epilogue: Diamesinae wanted!

I take this opportunity to respectfully ask field workers for material of Diamesinae species. Adult or pupal material in alcohol is preferred, but larvae may also be of interest. Contributions will certainly be acknowledged in published results emerging from examination of the material. Please write to Museum of Zoology, Muséplass 3, N-5007 Bergen, Norway, or contact me by e-mail: Andre.Willassen@zmb.uib.no

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The following contribution is an abstract of a paper which is going to be published in *Regulated Rivers*:

THE EFFECT OF WATER INTAKE ON MACROINVERTEBRATE COMMUNITIES IN ALPINE STREAMS (TIROL, AUSTRIA)

By **M. Margreiter-Kownacka¹, A. Kownacki² & H. Kraus³**

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2. Institute of Freshwater Biology, Academy of Sciences, Ul. Slawkowska 17. PL-31016, Krakow. Poland

3. Herzog – Eugen-Str. 44, A-6020 Innsbruck

The aim of this study was to determine the effect of water intake, connected with a power site, on macroinvertebrate communities in alpine streams. The study was carried out in the Stubai Alps in four high mountain streams: Fischbach, Längentaler Bach, without technical constructions, and Horlachbach, Gleirschbach with water intakes for the power site in Kühtai. Water intakes cause a considerable decrease in the water flow below.

The stream Fischbach has an invertebrate community characteristic of glacial streams. Chironomidae of the genus *Diamesa* dominate these streams, mayflies appear more numerous only in the middle and lower reaches. The stream Längentaler Bach is a typical mountain stream, flowing from springs. Here mayflies dominated (*Baetis alpinus*, *Rhithrogena* spp.) and only in its upper reach were Chironomidae of the genus *Diamesa* found more often.

A great difference was observed between the Horlachbach and Gleirschbach (glacial type streams) when the macroinvertebrate fauna communities were compared above and below a water intake. In the Horlachbach stream, in spite of seasonal differences, at site G2, above the water intake, the density was always higher, than that at site H3, below the water intake. However, the percentage share of higher taxonomic groups was very similar at both sites. Chironomidae constituted about 65% at both sites. Another situation was observed in the Gleirschbach stream. The density of macroinvertebrate fauna was always lower at site G2, above the water intake, and higher at site G3, below the water intake. At site G2, the Chironomidae never constituted greater than 60%, and mayflies as well as stoneflies were numerous. However, at site G3, the Chironomidae share was always higher than 80%. It is difficult now to give an explanation for this phenomenon.

KEY WORDS: alpine streams, water intake, macroinvertebrate community.

THESES

**DOCTOR OF SCIENCES THESIS CHIRONOMIDS OF THE SUBFAMILY DIAMESINAE
(DIPTERA, CHIRONOMIDAE) OF NORTHERN HEMISPHERE. SYSTEMATICS,
BIOLOGY AND BIOGEOGRAPHY (1998) (IN RUSSIAN)**

by Eugenyi A. Makarchenko

Institute of Biology and Soil Sciences Far East Branch Russian Academy of Sciences, 690022 Vladivostok, Russia

The revision of the Diamesinae of the Northern Hemisphere is completed. As a result 153 recent and 1 fossil species from 14 genera are recognised. 29 species and 3 genera (*Arctodiamesa*, *Kaluginia*, *Sasayusurika*) new to science are described. 12 species are registered for the first time in Eurasia, 31 records are new for the territory of Russia and former USSR republics. The names of 18 species are shown to be synonyms, 26 species are considered to be nomina dubia. The classification of the subfamily Diamesinae of the Northern Hemisphere considering all three stages of metamorphosis is given. Pupal and larval morphology of 56 species from 10 genera on

basis of reared material from larvae up to imago are given. New diagnostic characters of adult males, pupae and larvae, are given and used to make the original complex diagnoses of subfamily, genera and species, to prepare a key. Some processes of life history (hatching of imago, swarming, laying of eggs, life of larvae and pupae and others) are described in detail. Original distribution-maps of the investigated species are given. Natural habitats are analysed and classified. Hypothesis of the two centres (Alps and Baikal Lake basin) of origin and distribution of the recent Diamesinae is presented. Text - 518 pages, Figures - 285, References - 268.

XINHUA WANG DEFENDS HIS PHD-THESIS ON CHINESE CHIRONOMIDS

By Trond Andersen

Museum of Zoology, University of Bergen, Muséplass 3, N-5007 Bergen, Norway. (e-mail: trond.andersen@zmb.uib.no)

On May 12th Associate Professor Xinhua Wang, Nankai University, Tianjing, China, defended his thesis: «A Biosystematic Study on Chironomidae from China (Diptera)» for the Norwegian Doctor of Philosophy degree. Dr. Patrick Ashe, Ireland, was first opponent, Dr. Geir E. E. Søli, Oslo was second opponent while Dr. Kjell Arne Johanson, Bergen, served as third member of the committee. The defense was held in the old Natural History Museum building at the University of Bergen.

The thesis includes 16 papers most of which are co-authored by O. A. Sæther and other staff members at the Zoological Museum in Bergen, and consists a summary of Wang's biosystematic studies on chironomids from China over the last 10 years. Most of the papers are descriptions, revisions and phylogenies of different genera

with one or more representatives in China. A check-list of Chinese chironomids is also included, listing 472 species in 137 genera, of which 86 species are endemic to China. Altogether 5 new subspecies, 29 new species and 4 new genera are described in the papers.

In the first paper, distribution patterns and vicariance events of the Chinese chironomids are summarized and discussed. Five major fauna elements are discerned: 1) Cosmopolitan species, 2) Holarctic species, 3) West Asian - High Asian elements, 4) Gondwanian elements, and 5) the East Asian - South Asian endemics. Different biogeographical methods show many links between the East Asian and Nearctic fauna, which is explained by distribution across the Bering Strait during the last Ice Age when East Asia and North America were connected. The Chironomidae fauna in South

China show strong affinities to the fauna found in Australia and Africa indicating that the southern parts of China also might have formed part of Gondwanaland.

The two obligatory lectures given on May 11th were titled: «Biodiversity of China - Present situation and protection», and «Are Nematocera and Brachycera sistergroups? - Towards a better understanding of the higher level phylogeny of Diptera».

Xinhua Wang was born in Neimonggul in China in 1952. He took his M.Sc. exam in 1975 at the Nankai University in Tianjin, where he has been working, first as assistant professor, and from 1993 as associate professor. He visited the Museum of Zoology, Bergen, from August 1991 to June 1992 and again from June 1998 to June 1999.

Xinhua Wang has published the following scientific papers:

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* see also the Current Bibliography

DYNAMICS OF METAL ADAPTATION IN RIVERINE CHIRONOMIDS

Ph.D Thesis of Dick Groenendijk
University of Amsterdam (1999, in English)

Dick has researched the effect of zinc contamination on *Chironomus riparius* populations in the River Dommel close to the Dutch-Belgian border. His thesis has eight chapters consisting mainly of coauthored papers published, in press or submitted for publication:

1. General Introduction, 2. Seasonal dynamics and larval drift of *Chironomus riparius* (Diptera) in a metal-contaminated lowland river, 3. Fluctuating asymmetry and mentum gaps in populations of the midge *Chironomus riparius* (Diptera: Chironomidae) from a metal-contaminated lowland river, 4. Efficient shedding of accumulated metals during metamorphosis in metal-

adapted populations of the midge *Chironomus riparius*, 5. Fluctuating life-history parameters indicating temporal variability in metal adaptation in riverine Chironomids, 6. A method for crossbreeding strains of chironomid midges (Diptera: Chironomidae) and its application to ecotoxicological studies, 7. Loss of metal adaptation in *Chironomus riparius* (Diptera: Chironomidae) by simulating gene flow in adapted field populations, 8. Concluding remarks.

It is helpful to have these papers bound together in this way. I found it a very interesting read!

PHL

SHORT COMMUNICATIONS

ANNOUNCEMENT OF A NEW PUBLICATION:

A CHIRONOMID TAXA CODING SYSTEM FOR USE IN ECOLOGICAL AND PALAEOECOLOGICAL DATABASES

by Øyvind A. Schnell, Maria Rieradevall, Ignacio Granados, and Oddvar Hanssen

With the increasing interest in chironomids as palaeoindicators, large amounts of

data on both subfossil and contemporary community composition have been and are

being collected. Our main aim in proposing this standardised coding system is to facilitate between-site comparison for a large number of sites sampled in previous and future projects, to make easier the interchange of databases to compare community results over a broad regional scale or among different projects. Such a system will make it easier to interpret the results of statistical analyses, and also make communication of the results simpler.

This first version of the coding system includes about 960 species from all over Europe, with emphasis on the western part of the region. We have built a coding grammar of 8 characters at maximum, which makes the code suitable for most of the available statistical programs and databases. The proposed grammar allows to name the specimens at different levels depending

on the taxonomic resolution available: Species level, Species-group level, uncertain species, Genus level, uncertain genus, provisional species; name and Tribe and subfamily levels.

Diatomists established a coding system some years ago, and this has achieved a great level of acceptance. Therefore we hope that such a system will prove useful also for chironomid studies, and that the present system, and its future improvements, will be used by chironomid researchers.

The paper is published by NIVA (Norway) as a Report. Copies are available upon request to the first author at: Department of Zoology, University of Bergen. Allégt. 41, N-5020 Bergen, Norway.

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University of Barcelona (Spain).
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Pedes spurii

by Peter H. Langton

In my keys to the pupal exuviae of British Chironomidae (1984) and of West Palaearctic Chironomidae (1991) I incorporated novel terms to improve precision of description. On the whole these have been studiously ignored! Moreover, one structure is consistently misunderstood and misused: pedes spurii A. A **pes spurius** (false foot) is a general term used to cover any swelling, armed or not, on the abdomen of the pupa, believed to aid locomotion. Different types of pedes spurii (ZAVREL 1926, 1942, referred to in HIRVENJOJA 1973) are not homologous in origin, **pedes spurii A** occur postero-laterally on the sternite, whereas **pedes spurii B** occur posteriorly on the pleuron.

The term **pes spurius B** causes no problems, but the use of **pes spurius A** is frequently inaccurate. SÆTHER's 1980 definition of this structure is correct: pedes spurii A occur on the caudolateral corners of the sternite. My interpretation of this definition is confirmed by his use of the term in his earlier (e.g. presence on *Hydrobaenus hudsoni* 1977) and later papers (e.g. presence in *Heterotrissocladius boltoni* 1992); these are Orthoclaadiinae which do not possess the whorl of spines on the parasternite frequent in the Chironominae that I call the **vortex**.

The paratergite is defined by SÆTHER (1980) as the lateral sclerite of abdominal segments: in pupae it and the parasternite lie

lateral to the longitudinal adhesion marks. The vortex is a development of the posterior part of the parasternite armament, though in *Rheotanytarsus* it can be displaced anteriorly to lie mid-laterally. Confusion can arise in some Orthoclaadiinae (e.g. *Cricotopus* spp.) where the enlarged posterior spinules of the parasternite are displaced inwards to form a combined structure with **pes spurius A**, but the direction in which the spines point delimits the extent of each component.

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On the length of names

Most of us, I suppose, who are involved in submitting papers for publication will have come across the nameless referees who go beyond objectively reviewing the manuscript by incorporating their response to the editor some pet foible of their own. For example, "Five syllable specific epithets are unacceptably long". As a comfort to those who have suffered thus, I append a table of the number of syllables in published genera

and species taken from ASHE & CRANSTON 1990. It includes all generic names and specific epithets, including synonyms and nomina dubia from the beginning of the list to the end of the Diamesinae, and from the beginning of the Chironominae until I became bored by the exercise!

	number of syllables							
	1	2	3	4	5	6	7	8
genera	0	0	6	14	24	20	4	1
species	1	56	223	344	119	31	4	2

69 genera are included in the table and 780 species (not included are *Demicryptochironomus* LENZ, *quatuordecimpunctatus* GOETGHEBUER nor *er* SÆTHER!) It is obvious that traditionally systematic chironomists enjoy polysyllabic names.....and why not?!

ASHE, P. & CRANSTON, P.S. 1990, Family Chironomidae in SOÓS, A. & PAPP, L. (eds.), Catalogue of Palaearctic Diptera Vol. 2. Elsevier.

PHL

NEWS FROM INDIA

Works initiated at the Department of Zoology, Arunachal University (India)

Dr. A. Mazumdar and **Mr. B. K. Bhuyan** have initiated studies of the chironomid communities of Arunachal Pradesh. This state is situated at the far eastern tip of India and major portion of it is under virgin forest cover. The state has been recognized as one of the major 'hot spot' areas of biodiversity of this subcontinent. The group is eager to collaborate with other international and national workers specially on the following aspects - biodiversity and biosystematics of this "hot spot".

Dr. A. Mazumdar
Department of Zoology, Arunachal University
Rono Hills, Itanagar
Arunachal
Pradesh 791 111, India.

Research activities at the University of Pune

A tropical species of midge, *Chironomus ramosus* has been successfully reared in the laboratory. A novel mass rearing technique has been standardised and in course of time an inbred line has been derived. This inbred line of *C. ramosus* is used for cytological characterization of chromosomes. A laboratory population of *C. ramosus* is cultured for cell and developmental studies, stress response and in the study of puffing phenomena. A few behavioural parameters of the species have been investigated.

Dr. B. B. Nath
Department of Zoology, University of Pune
Pune 411 007, India

**Research works in progress in
Presidency College, Calcutta**

Chironomus striatipennis KIEFFER is a widespread species in freshwater aquatic bodies of West Bengal. Egg masses were collected from different habitats and were subjected to rearing in the laboratory in prescribed culture medium. Larvae of different stages were then processed to obtain polytene chromosome preparations. A sequence of polytene chromosome formation was recorded for this species. In the salivary gland cells, this species exhibited four polytene chromosomes with distinct organisation.

Chromosomes I, II and III have been noted to be metacentric and the IV is acrocentric. There are at least seven distinct steps of differentiation of polytene chromosome in this species which can be detected morphologically. The present finding suggests the formation of polytene chromosomes from the paired metaphase homologues in the salivary gland cells.

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**Prof. A. K. Dutta Gupta and his co-
workers**

have initiated works in Molecular genetics of chironomids adapted to polluted water in and around Calcutta on a research project sponsored by Department of Science & Technology, Govt. of West Bengal.

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New workers (see also PERSONALIA)

Ms. Khajja has been working on the Systematics and Biology of Chironomid midges of Jammu and Kashmir, hitherto unexplored for chironomid -studies.

OBITUARY NOTICE

**In Memory of Friedrich Reiss
(24 December 1937 - 17 August 1999)**



On 17 August 1999, Friedrich ("Frieder") Reiss – dear friend, colleague and advisor to many in our profession – died, in his 62nd year, from a recurrence of heart problems. Although the latter had been cause for concern for some years, this sudden end to Frieder's central

presence in our circle has come as an unexpected shock. He had hoped to still solve many taxonomic puzzles in the three years until his retirement, and surely would have continued to do so afterwards.

Frieder is survived by Annemarie Reiss, his wife for 37 years, and their son Peter. On their behalf we would like to express our sincere gratitude for the kind thoughts of all who have responded with condolence letters and emails to our earlier notices about Frieder's passing.

In Friedrich Reiss, chironomid research has lost a scientist who had for decades set the highest standards in this field of aquatic entomology. He was fortunate in being able to concentrate exclusively on the midges ever since the completion of his academic education. He took full advantage of this opportunity, not only for himself, but also with his constant efforts to make his achievements available to colleagues and aspiring biologists becoming interested in taxonomy and the Chironomidae. All who have known him deeply appreciate not only Frieder's knowledge in our specialty, but equally so his wide-ranging interests in the natural sciences, and intellectual openness in general. From discussions with him, on any subject, one always walked away with something gained.

Born 24 December 1937 in Stuttgart, Frieder grew up in Schorndorf in Baden-Württemberg state, where he completed school in 1957 with the Abitur, the high school diploma and general qualification for college. In the same year still he began taking courses in biology, chemistry, and geography at Stuttgart University. In 1965 he transferred to the University at nearby Hohenheim, from which he graduated in 1966 with both a doctorate degree and the scientific part of a high school teaching certificate.

Following his interests in zoology/entomology and aquatic ecology – the latter developed during limnological lectures by Prof. J. Grim – he had turned to Prof. O. Pflugfelder of Hohenheim University to find an appropriate topic for his doctoral thesis. Under this guidance, Frieder was able to secure a grant from the German Academic Society's aquatic research program for a dissertation entitled "Ecological and systematic studies on the Chironomidae (Diptera) of Lake Constance. A contribution to the chironomid lake fauna of the northern prealpine area" (see No. 8 of the bibliography below). This was carried out beginning in 1961, at the laboratories of the Lake Constance public water works in Sipplingen, whose director was Prof. Grim.

During his time as a doctoral candidate, Frieder managed several times to visit and use the extensive chironomid resource collection

established by Thienemann and curated by Fittkau at the Max-Planck-Institute for Limnology in Plön. These contacts led to a post-doctoral research grant to Frieder from the Max Planck Society to continue his chironomid studies at Plön, and in 1967 to his employment there as a research associate. His duties then were to help develop the chironomid center in continuation of the tradition of Thienemann and his school. The goals were to advance the taxonomy and diagnostics of Chironomidae through revisionary work, the international coordination of information and documentation, and not least through creating opportunities for basic and more in-depth studies by outside colleagues some of whom were then still working under rather isolated conditions. Frieder took an integral part in producing the new "Chironomus. Newsletter of chironomid research" as well as the first bibliography of all publications on chironomids, incorporating 7000 titles at the time. He also took care of the colleagues visiting the Plön collections of specimens and literature. It was back in those days, too, when the idea was first conceived to gather the knowledge of competent researchers worldwide for the development of definitive generic concepts in the Chironomidae, and of keys to the genera of the Holarctic fauna.

When it became apparent that a taxonomic research focus like the chironomid center would not have a lasting future within an ecology-oriented institution, Frieder did not hesitate to accompany the first author to Munich, in 1976, to continue chironomid science in a museum environment at the Zoologische Staatssammlung (ZSM).

Thanks to the courtesy of the directorate at Plön, both the Thienemann collections and the library could be transferred to ZSM. In Munich, Frieder became responsible for the Diptera Section, and in 1998 was appointed head of the entire Department of Entomology.

His enthusiasm and drive for chironomid research were not in the least affected by the originally comparatively poor work conditions at ZSM, then insufficiently housed in the north wing of the Nymphenburg castle. However, it was not until ZSM had moved to its new facility on Münchhausenstrasse that Frieder was given the means to make the ever growing chironomid collections completely accessible, and to provide comfortable work conditions to visiting guests.

Next to his personal scientific projects Frieder

gave high priority also to the continuous taxonomic analysis, incorporation, and availability to others of the already present and newly incoming collection materials. We all owe it to his diligent, persistent efforts in these regards, and to his thorough, determined style of work, that the chironomid collections at ZSM have reached the value they represent today.

Friedrich Reiss has been among the most influential people in the development of chironomid science from the beginning of his dissertation work. As one of the youngest participants of the first international symposium on Chironomidae at Plön in 1964, he was able early on to establish contacts, soon developing into friendship, with most colleagues who were active then. One quick reward for his achievements was the invitation from Prof. Lars Brundin to accompany him on a three-month expedition in 1969/70 to southern Chile and Patagonia. With his critical analysis of the species from Lake Constance Frieder had started out by gaining comprehensive knowledge of the central European fauna. Later, he focussed especially on the taxonomy and systematics of the Chironomini and Tanytarsini, and on issues of chironomid faunistics and biogeography in general.

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The chironomid center at Plön was part of the institute's Department for Tropical Ecology. In this context, Frieder was given the opportunity to work out of Manaus, Brazil, from 1971 to 1972 to apply and compare his lake-ecological field experience to central Amazon habitats, and to gain further knowledge of the South American biota. After the move to Munich, the Neotropics continued to be one of his major research interests, next to European material and the previously poorly known local southern German fauna. Of special value among the results of Frieder's work, apart from his revisions and new descriptions of many genera and species, is his part in the compilation of the first comprehensive catalog of Neotropical Chironomidae. In 1997, Frieder was honored by the invitation to the second Brazilian chironomid congress at São Carlos, where for several days he conducted a course on taxonomy. Having enjoyed this trip without problems he gained new trust in his health, and renewed motivation to help develop chironomid research in Brazil.

Friedrich Reiss was a great gift to our science. His untimely death fills us all with deep sorrow. We will sorely miss his competence, advice and friendship.

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A CALL FOR PAPERS

Special Issue of SPIXIANA

in Memory of Dr. Friedrich Reiss

We are glad to be able to announce to you that one of the regular issues of the journal SPIXIANA in the year 2000 is to become a posthumous tribute to Dr Friedrich Reiss. You are, thus, cordially invited to send any contribution adequate for this occasion – original scientific manuscripts, but possibly also more personal remembrances – either to the editor-in-chief of SPIXIANA:

Zoologische Staatssammlung, Dr. Martin Baehr, Münchhausenstr. 21, D-81247 München, Germany; (Fax: +49 89 8107 300; Email: kld1122@mail.lrz-muenchen.de) or to me or another member of the Munich chironomid group you know.

In order to be able to produce this commemorative issue within the shortest possible time, Dr. Baehr has suggested a first deadline for submissions of 31 December 1999. Given the circumstances, we are hoping you will excuse such tight scheduling. If you would like to contribute, but this announcement reaches you after that date, please, contact us as soon as you can.

Since the volume of the work will be limited to not much more than 96 pages, we may not be able to consider individual papers which exceed a certain length. To make the tasks of the editors easier, please, consult a recent SPIXIANA issue or article for matters of formatting, style, etc., and type your contribution accordingly. Manuscripts should be submitted in electronic form – on disk or as

email attachments – formatted in Word or WordPerfect versions for IBM-compatible computers. We are looking forward to hearing from you, with manuscripts or any suggestions, to help us make this a work to duly honor the friend and colleague we have already begun to miss, but will always remember.

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LAST MINUTE NEWS

Overlooked Historic Descriptions

by M. Spies

Several 19th century publications by F. M. van der Wulp have been rediscovered, which contain valid descriptions of taxa previously thought to have been established elsewhere. In detail:

A) 1859: 4 papers, including triple descriptions each for six new species. Determining the respective effective publication dates (using Barendrecht, G. & Kruseman, G., jr. 1957. - *Tijdschr. Ent.* 100: 1-4) according to the Code for Zoological Nomenclature yields the following **altered page numbers in species citations**: *Chironomus dilatatus* van der Wulp, 1859: **9** (?syn. *Psectrocladius obvius* (Walker, 1856)); *Chironomus ambiguus* van der Wulp, 1859: **6** (nom. dub., Chironominae); *Chironomus* "unicolor van der Wulp", 1859: **5** (homonym repl. by *monochromus* van der Wulp, 1874: 129; now in *Parachironomus*); *Chironomus blandus* van der Wulp, 1859: **5** (syn. *Polypedilum convictum* (Walker, 1856)); *Chironomus sylvaticus* van der Wulp, 1859: **6** (now in *Tanytarsus*); *Chironomus marmoratus* van der Wulp, 1859: **9** (type sp. of *Zavreliella*).

B) 1874: genus descriptions for *Camptocladius*, *Cricotopus*, *Eurycnemus* (incl. type species designation), *Metriocnemus*, *Orthocladius*, and *Tanytarsus*; each published twice, effectively simultaneously (Barendrecht & Kruseman 1957, see above). The page number for all genera in van der Wulp (1874a) is LXX.

So far, it appears that identification and nomenclature are not affected by these multiple publications. However, readers are cautioned to verify this when working on any of the taxa involved.

Sincere thanks to Paul L. Th. Beuk (Amsterdam, NL), who contributed a lot of the above data.

Martin Spies (Munich, Germany)

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MEETING

BRAZIL

3RD BRAZILIAN MEETING ON TAXONOMY AND ECOLOGY OF CHIRONOMIDAE

The 3rd Brazilian Meeting on Taxonomy and Ecology of Chironomidae will be held on November 4 and 5, 1999, at the Instituto Oswaldo Cruz, Rio de Janeiro, Brazil.

It will be supported by the Instituto Oswaldo Cruz and Fundação de Amparo à Pesquisa do Estado do Rio de Janeiro (FAPERJ).

Contributions are likely to be published in a special issue in the journal *Entomología y Vectores*. For further information please contact:

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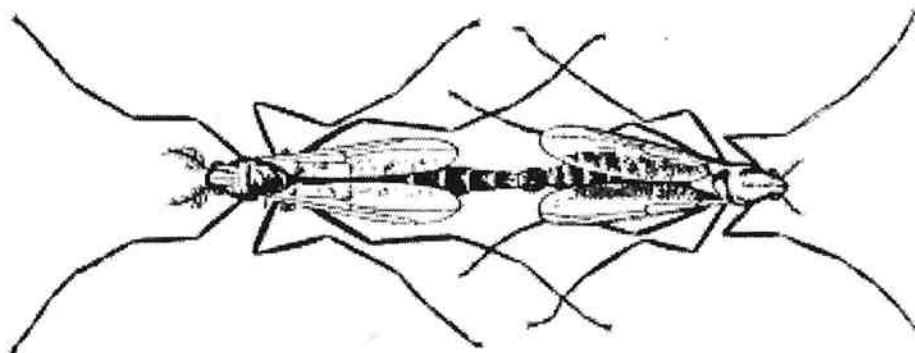
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