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Stenochironomus sp. from La Selva Research Station, Costa Rica. Photo: Carlos de la Rosa.

CHIRONOMUS Journal of Chironomidae Research

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

Front page photo: *Stenochironomus* sp. from Costa Rica. Reared in the laboratory on field-collected wood from a small unnamed stream at the La Selva Research Station. Photo: Carlos de la Rosa.

Editorial

Online early – only online

This issue (Number 28) of the CHIRONOMUS Journal of Chironomidae Research includes three research papers and six short communications. The number of contributions is lower than what we have had in a while, and we have therefore considered which actions to take to make the journal a more attractive publication channel for the Chironomidae research community. In a world in which the number of online, open access journals is rapidly increasing and the turn-around time between manuscript submission and publication is rapidly decreasing, the competition between small journals is tough. A periodical like CHIRONOMUS, in which a submission in January cannot be expected to be published before December, is of course less appealing than one with frequent publication dates.

The CHIRONOMUS Journal for Chironomidae Research has taken a number of steps over the last few years to make the publication more widely available and appealing to the community. Current Research articles are peer-reviewed by expert reviewers and publication complies with the International Code of Zoological Nomenclature (ICZN-Code); the journal's recent name change is meant to reflect the enhanced rigor, making CHIRONOMUS a more attractive venue for publication. Additionally, publications are freely accessible online and are deposited in the Directory of Open Access Journals, and there are no page-charge fees for publication. But, until now, we have been stuck to one issue per year in which all included papers have the same publication date. This has been necessary to avoid discrepancies between publication time for online and paper versions of the same article, as paper versions have been required to comply with the ICZN-Code.

The requirement for printed copies has now changed. As of January 1, 2016, CHIRONOMUS will only be electronically available and its publications separately downloadable through our website (http://www.ntnu.no/ojs/index.php/chironomus). This is possible since CHIRONOMUS contributions now also are stored in the permanent and approved electronic depository system PKP-LOCKSS enabled through the Open Journal System that we use. Together with ZooBank (www.zoobank.org) registrations of all new scientific names, this meets the requirement by the ICZN-Code for publication of new taxa.

There are several immediate advantages of online-only publication. Printing costs are reduced to zero, but more importantly, we can now evaluate, accept and publish papers and short communications continuously as they are submitted to the journal. Thus, the turn-around time for a paper submitted to CHIRONOMUS will now only depend on the speed of the editorial board members, reviewers and authors, not the Gregorian calendar. All papers will still belong to a yearly issue and are citeable as we are used to. Along with publication of current research and short communications, the journal will still remain an important venue for relaying announcements and other significant news items relevant to the community of Chironomidae researchers; these items will be published annually in December. The CHIRONOMUS Journal aims to be a valuable resource for the Chironomidae research community and we hope the newest changes will make our journal a more attractive place to publish research results, news and short communications.

With best wishes for 2016.

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

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A Note on Sampling Chironomids for RNA-Based Studies of Natural Populations that Retains Critical Morphological Vouchers

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Abstract

The rapid uptake of transcriptomic approaches in freshwater ecology has seen a wealth of data produced concerning the ways in which organisms interact with their environment on a molecular level. Typically, such studies focus either at the community level and so don't require species identifications, or on laboratory strains of known species identity or natural populations of large, easily identifiable taxa. For chironomids, impediments still exist for applying these technologies to natural populations because they are small-bodied and often require time-consuming secondary sorting of stream material and morphological voucher preparation to confirm species diagnosis. These procedures limit the ability to maintain RNA quantity and quality in such organisms because RNA degrades rapidly and gene expression can be altered rapidly in organisms; thereby limiting the inclusion of such taxa in transcriptomic studies. Here, we demonstrate that these limitations can be overcome and outline an optimised protocol for collecting, sorting and preserving chironomid larvae that enables retention of both morphological vouchers and RNA for subsequent transcriptomics purposes. By ensuring that sorting and voucher preparation are completed within <4 hours after collection and that samples are kept cold at all times, we successfully retained both RNA and morphological vouchers from all specimens. Although not prescriptive in specific methodology, we anticipate that this paper will assist in promoting transcriptomic investigations of the sublethal impact on chironomid gene expression of changes to aquatic environments.

Introduction

Understanding the way in which genes and organisms interact with the environment is central to many areas of fundamental and applied biology. Recent advances in DNA and RNA sequencing technologies have driven an explosion of interest in functional studies of such interactions (Pauls et al. 2014, Hughes et al. 2014). Of increasing importance is the way in which these interactions occur in response to anthropogenic change to ecosystems (e.g., Hoffman and Willi 2008, Marchand et al. 2013). In freshwater systems, emerging research is demonstrating the utility of genomic (DNA-based) and transcriptomic (RNA-based) approaches to assessing sublethal impacts of ecosystem degradation on natural populations (Pujolar et al. 2012). In particular, transcriptomics can be used to explore differential expression patterns among populations or species that experience different stressors (both in a lab-based ecotoxicological setting and in natural habitats) and make associations between genes and environmental factors (e.g., Altshuler et al. 2011, Piña and Barata, 2011, Wang et al. 2012). Such research, aimed initially at fundamental biological questions, may allow development of functional molecular proxies in key species to detect and track sublethal adaptive shifts that occur in response to changes to ecosystems – the so-called 'ecotoxicogenomic' approach (Snape et al. 2004) – and will extend and complement current aquatic biomonitoring practices (e.g., Hoffmann and Willi, 2008, Kim et al. 2011, Connon et al. 2012, Tsai and Sung, 2013).

Field sampling of many aquatic macroinvertebrate groups, including chironomids, necessarily involves collection of immature stages (larvae), and thus molecular studies at the species level can be limited by difficulties in making accurate taxonomic identifications. This is due to both the small size of immatures for many groups and lack of diagnostic morphological characters visible under low magnification. For such taxa, accurate diagnoses are impossible without preparation of morphological voucher specimens, which often involves slide-mounting body parts in tissue-clearing fluids and visualisation under high magnification. This represents a significant problem when both molecular and morphological vouchers need to be retained. For example, in DNA-based studies of chironomids, preservation of bulk samples in high purity ethanol and secondary sorting using room temperature ethanol is commonplace. Once tissues

are preserved in ethanol, DNA is stable and excision and slide-mounting of taxonomically critical head capsules as morphological vouchers can be conducted without time constraint. Storage of the remainder of the larval body in high strength ethanol for use in molecular protocols, with unique specimen codes that connect morphological and molecular vouchers 1:1, has been highly successful (e.g., Krosch et al. 2011, 2012). Recent techniques developed for use with pupal exuviae (Krosch and Cranston, 2012), and adopted for use with whole pupae and adults (Krosch and Cranston, 2013, Krosch et al. 2015), utilise whole individuals for non-destructive DNA extractions and retain cuticle intact for voucher preparation post-extraction. The use of DNA barcoding to identify species can alleviate some of these difficulties and remove the need for retention of morphological vouchers in some well-known groups; however, this approach is still limited in some groups where connections between barcodes and morphological taxonomy are lacking. Thus, RNA-based research concerning natural populations of larval chironomids presents new problems for sample storage, voucher preparation and species identification for these invertebrate groups.

RNA degrades much more rapidly than DNA, so storage of specimens in ethanol may not be sufficient to maintain RNA integrity even in the short-medium term (hours-days). Although preservatives exist that are more appropriate for RNA (e.g., RNA*later*®, liquid nitrogen), they are not broadly suitable for initial preservation of chironomids. This is because collecting methods for chironomid larvae are necessarily time-intensive, normally involving collection of a bulk sample (possibly size-sorted using sieves) that contains detritus and non-target taxa, followed by secondary sorting for target taxa under low magnification, voucher preparation of particular body parts from target specimens and confirmation of diagnosis under higher magnification. For studies focused on a single taxon, rather than a whole community, this secondary sorting and species identification to exclude non-targets is essential. Furthermore, post-extraction species diagnosis – either via barcoding (by co-extracting DNA or using bioinformatic approaches on resulting transcriptome data) or retention of cuticle for morphology as described above – may not be feasible for RNA studies of chironomids because RNA extraction techniques are often destructive (e.g., involving crushing of tissues in liquid nitrogen) and multiple specimens may need to be pooled in a single extraction to obtain sufficient RNA quantity for subsequent uses (e.g., cDNA library preparation for high-throughput sequencing).

Taken together, RNA-based studies of natural populations of chironomids clearly require a defined protocol for storage, sorting and vouchering of specimens that both maintains RNA integrity suitable for subsequent processes and retains gene expression profiles as close to natural as possible. Here, we describe such a protocol that extends from stream site to RNA extraction. This protocol was developed and optimised for three Australian species of the chironomid genus *Cricotopus* Wulp (Chironomidae: Orthocladiinae) as part of a broader assessment of differential expression between streams of varying human impact. Development of protocols that facilitate use of RNA-based techniques on natural freshwater macroinvertebrate populations is expected to open up a wealth of novel research areas.

Materials and Methods

Sample collection and transport

Australian species of *Cricotopus* inhabit diverse freshwater ecosystems and some species tolerate ecosystem degradation (Drayson et al. 2015, Krosch et al. 2015). Multiple collections of *Cricotopus* larvae were made from two locations in southeast Queensland, Australia, throughout 2014 and early 2015 (Table 1). Three species were recorded (*C. draysoni* Cranston & Krosch, *C. albitarsis* Drayson, Cranston & Krosch and *C. parbicinctus* Drayson, Cranston & Krosch); the presence and abundance of each varied through the year, with each species generally present at both sites during the same time periods. These species were chosen based on a parallel project on *Cricotopus* systematics led by the senior author that confirmed species diagnoses for all three taxa by associating DNA barcode data with morphological vouchers and found no evidence for cryptic lineages within any of the three species at either location (Drayson et al. 2015, Krosch et al. 2015). Collections involved kick-sampling in riffle sections using a 0.9mm x 0.3mm funnel-tapered polyester sweep net for 30-45 minutes depending on availability of suitable microhabitat. When moving through the stream to subsample in different sections, care was taken to ensure net bag remained submerged and thus not expose specimens to open air which may potentially affect gene expression. Total net samples were strained firstly through a coarse grade (~1mm) then a fine grade (~0.2mm) sieve to remove coarse particulate organic matter and larger invertebrates whilst retaining chironomid larvae (with detritus and non-target organisms). Bulk

samples were transferred to 100mL plastic bottles and immediately fixed with cold absolute analytical reagent (AR) grade ethanol (transported to the sample site on ice). This both euthanases organisms and captures gene expression as close as possible to the point of removal and is a critical step for differential expression studies.

Secondary sorting was conducted as soon as possible but always less than two hours after collection, under low magnification, in a small sorting tray (113mm x 86mm x 18mm) to separate out putative target chironomid larvae: the rest of the bulk sample was held in a 4°C refrigerator and sorting trays were successively filled, sorted and non-target material discarded. Generally, secondary sorting took 2-3 hours. All target specimens were transferred from sorting trays immediately into RNA*later*® (Ambion, Life Technologies, USA) in a glass well on ice. Once in RNA*later*®, RNA fragments are significantly more protected from degradation than in ethanol, but cold storage remains crucial.

Morphological voucher preparation

Morphological vouchers of larval head capsules were prepared immediately on completion of secondary sorting. This involved dissection of the head capsule from the body, using fine-tipped forceps on clean microscope slides (never dissecting two larvae on the same part of a slide), before placement of individual dissected heads in single drops of Hoyer's mountant (van der Meer, 1977) on microscope slides. Immediately as each head was dissected and placed in mountant, larval bodies were transferred to individual RNase-free tubes containing 0.2mL RNAlater® on ice and labelled with codes that related individually and uniquely to each vouchered larval head. Head capsules in mountant were incubated at room temperature for 5 mins to allow mountant solution to rehydrate the head capsule, before compression under a 12mm diameter circular cover slip. Once voucher preparation was complete (which, for a single specimen, takes only 6-7 minutes including incubation), all tubes were transferred to a -20°C freezer until transport on ice to the Molecular Genetics Research Facility (MGRF) at the Queensland University of Technology (QUT).

RNA extraction

On arrival at the MGRF, larval bodies were transferred to a -80°C freezer until use in RNA extraction, while slide vouchers were examined and species identifications recorded against each unique voucher code. Larval body samples of confirmed target species were then selected from the frozen collection. Initial RNA extraction trials (using three and five pooled individuals of *C. draysoni* or *C. albitarsis* or 12 individuals of *Rheocricotopus sp.*) were conducted to determine if and how many individual larvae needed to be pooled into a single extraction to return sufficient RNA. All extractions were conducted in a dedicated RNA fume hood (except for the tissue lysis step) and used dedicated pipettes, sample racks, centrifuge, and filter tips. Prior to commencing extractions all surfaces were cleaned first with 70% AR grade ethanol (diluted with RNase-free water) then with RNase*Zap* (Life Technologies) to remove any remaining RNase contamination. Extractions were conducted following a standard guanidine isothiocyanate-phenol-chloroform protocol (Simms et al. 1993), with some modifications. Briefly, selected larval bodies were thawed on ice and transferred to fresh RNase-free 1.5 mL microcentrifuge tubes containing 1 mL TRIsure™ (Bioline, Australia) or TRIzol® (Life Technologies, USA) and a single 5 mm stainless steel bead (Qiagen, Australia). Tubes were sealed with Parafilm® (Sigma Aldrich, USA) and shaken in a TissueLyser II (Qiagen) at 30Hz for 3 mins. Samples were transferred to new RNase-free tubes and stainless steel beads discarded, as tubes that retain beads can burst during the first centrifugation step (14000rpm/20000g for 15 mins). The RNA phase was separated using chloroform and RNA pellets were precipitated with isopropanol and cleaned with 70% ethanol. RNA was resuspended in 50 µL DEPC-treated water (Bioline) and all samples were visualised by agarose gel electrophoresis (50-100 mL 2% w/v agarose gels with 1-2 µL added GelRed, imaged under UV transillumination) to assess extraction success and detect signatures of degradation and DNA/protein contamination. RNA quality and DNA/protein contamination was also assessed, along with quantification of total RNA, using an Agilent 2100 Bioanalyzer (Agilent Technologies, USA) and the RNA 6000 Nano kit for total RNA following manufacturer's guidelines. Total RNA was then sent to the Australian Genomics Research Facility (AGRF, Melbourne) for cDNA library preparation and high-throughput sequencing on an Illumina Hiseq2500 using either 100bp paired end or 50bp single end read chemistry as part of a broader parallel study (unpublished data).

Results

The described technique, which was designed specifically to alleviate difficulties in collecting wild chironomid midges for transcriptomic studies, has been used successfully for a total of 40 separate RNA extractions (Table 1). The number of extractions actually represents the sum of 192 individ-

ual chironomid larvae because RNA extractions were conducted on 2-6 individual larvae pooled as a single sample. Moreover, over the course of this research, ten field collections we conducted which resulted in a total of >400 putative target specimens that were preserved and vouchered using the described protocol and include both non-target species and additional unused specimens. It is expected that these samples, currently stored in a -80C freezer, remain valuable for future studies. The protocol, excluding RNA extraction, can be completed by a single person in less than a day: field collection of 30-45 minutes (excluding travel time), secondary sorting of 2-3 hours, voucher preparation 1-2 hours (depending on the number of putative target taxa collected). All morphological vouchers of larval head capsules showed that diagnostic characters (e.g., menta, mandibles, pigmentation) were retained intact, allowing identification using existing species-level keys (designed by the

Table 1. Summary of *Cricotopus* samples used for RNA extraction and resulting template concentration. * indicates samples that formed part of the initial trial extractions. [1]Superscript numbers indicate samples that appear in the agarose gel in Figure 1a and the lane number they were loaded in. ^ indicates the sample for which the exemplar Bioanalyzer plot is provided in Figure 1b.

Site	Species	No. pooled specimens	Concentration (ng/uL)
Cedar Creek	*C. albitarsis*	3	105*
	C. draysoni	5	126*
	C. draysoni	5	341*
	C. draysoni	6	50
	C. draysoni	5	422
	C. draysoni	5	183
	C. draysoni	5	275
	C. draysoni	5	32
	C. draysoni	4	190
	C. draysoni	4	51
	C. draysoni	3	8
	C. draysoni	4	446[8]
	C. draysoni	4	268[9]
	C. draysoni	6	542[10]
	C. draysoni	6	556[11]
	C. draysoni	6	480[12]
	C. parbicinctus	6	62
	C. parbicinctus	6	120
	C. parbicinctus	6	450
	C. parbicinctus	6	57
	C. parbicinctus	6	289
North Pine River	*C. albitarsis*	2	1132
	C. draysoni	5	113
	C. draysoni	5	111
	C. draysoni	5	157
	C. draysoni	5	147
	C. draysoni	5	391
	C. draysoni	5	749
	C. draysoni	5	98
	C. draysoni	5	410
	C. draysoni	5	143
	C. draysoni	4	30
	C. draysoni	4	34
	C. draysoni	6	295[3]^
	C. draysoni	5	130[4]
	C. draysoni	5	260[5]
	C. draysoni	2	58[6]
	C. draysoni	3	227[7]
	C. parbicinctus	5	482
	C. parbicinctus	5	262

senior author, published in Drayson et al. 2015). Identifiability of RNA*later*-preserved specimens did not differ from other *Cricotopus* specimens collected into ethanol/isopropanol for parallel projects (Drayson et al. 2015, Krosch et al. 2015). This demonstrates that storage of larvae in RNA*later*®, at least in the short-medium term, does not cause significant and irredeemable impact on the head capsule cuticle, provided they are incubated for a short period in mountant prior to application of and compression with the coverslip.

All attempted RNA extractions were in some way successful, and pooling of different numbers of individuals did not correlate with resulting RNA concentration. Initial trials suggested that three or five individuals pooled would produce >100 ng/uL RNA (Table 1), whereas 12 individuals, albeit from a different genus, resulted in a relatively lower yield (50 ng/µL). Of the remaining samples, extractions from two pooled individuals gave a range of 58-1132ng/uL, whereas six pooled individuals gave 50-556ng/uL. In this particular case, a total RNA concentration of >100ng/uL was advised (AGRF sample submission guidelines), thus most extractions were conducted on five or six pooled individuals. Exceptions to this occurred where sample sizes were limited for a given collection and biological replicates (for differential expression analyses) were preferred over increasing RNA yield.

Regardless of concentration, resulting total RNA was consistently of high quality (Fig. 1). We provide only exemplars of RNA extraction quality control results for brevity and because all 40 samples are essentially similar, but all results can be made available on request. Agarose gel electrophoresis (Fig. 1a) showed that all samples possessed a strong 18S/28S rRNA band, minimal short (~100 bp) RNA fragments and no high molecular weight gDNA band. Likewise, the exemplar Bioanalyzer plot (Fig. 1b) shows no evidence of DNA/protein contamination (which would otherwise be indicated by the presence of very long fragments) or of RNA degradation (short fragments). Unfortunately, RNA Integrity Numbers (RIN – Schroeder et al. 2006), a standard metric

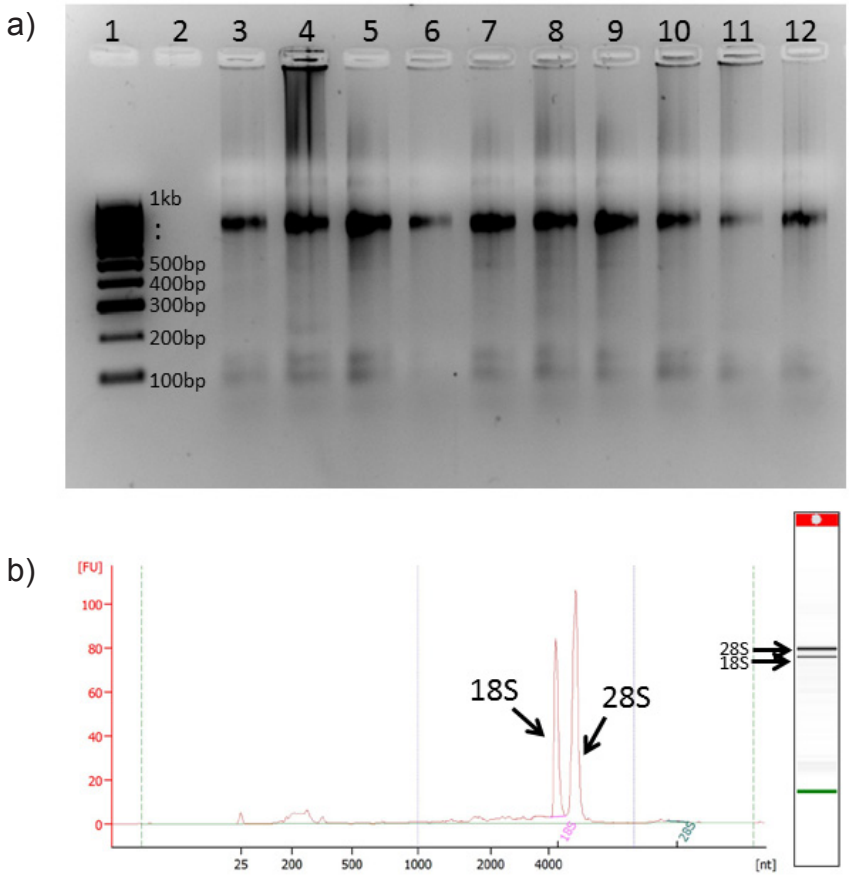

Figure 1. Exemplar RNA extraction QA/QC assessments from the 42 extractions conducted. a) ten extractions analysed using 2% w/v agarose gel electrophoresis: lane 1 contains 1 µL Hyperladder IV (Bioline), lane 2 was intentionally blank, samples are from lanes 3-12; b) exemplar Agilent 2100 Bioanalyzer plot of fragment lengths (x axis) against fluorescence units (y axis). The software automatically attempts to identify the 18S and 28S peaks (labelled below the peak trace in smaller font on an angle) to calculate a RIN.

of RNA quality, could not be calculated reliably for these samples because their peak traces, as interpreted by the Bioanalyzer, were atypical. This most likely related to the software misplacing the 28S peak (e.g., Fig. 1b), possibly driven by differences between the RIN model's expected length of the 28S fragment (based on model eukaryotes) and that observed in *Cricotopus*. Although this can be emended manually, it was not critical to subsequent sample processing. Nevertheless, in general all samples appeared to fit the expected peak trace for samples of RIN 8-10, indicating RNA of high integrity (Schroeder et al. 2006).

Discussion

The expansion of possible RNA-based studies concerning freshwater ecosystems to incorporate small-bodied immature insects that are difficult to identify under low magnification surely will enhance the field by improving our understanding of how ecosystem change impacts taxa. Currently, many studies rely either on laboratory-based ecotoxicogenomic studies of monoculture lab strains (e.g., Li et al. 2009, Planello et al. 2010, David et al. 2012) or on natural populations of larger-bodied, more easily identified species (e.g., Pujolar et al. 2012, Schulteis et al. 2014). A major impediment for research on small aquatic macroinvertebrates like chironomids is the necessity to maintain RNA integrity, 'true' gene expression profiles and morphological vouchers, with sample processing time and storage conditions the most critical factors. The greatest benefit of the described technique, therefore, is to complement and extend field-based ecotoxicogenomic research to incorporate a greater diversity of aquatic macroinvertebrates and explore fully the varied responses of such taxa to ecosystem change.

The key improvements or changes to existing sampling protocols that are critical for maintaining RNA when sampling chironomids can be summarised as follows:

1. When moving through the stream to subsample in different sections, ensure net bag remains submerged and thus does not expose specimens to open air which may potentially affect gene expression.
2. Minimise the time between collection and secondary sorting, and transport samples as cold as possible between collection site and laboratory. It is possible secondary sorting could be conducted at the collection site using a car-powered refrigerator/freezer and portable microscopes. Secondary sorting is best completed within hours of collection to avoid RNA degradation.
3. During secondary sorting and voucher preparation keep sorted target specimens cold, preferably on ice. Ensure vouchering protocol minimises cross-contamination risk.

Although this paper describes an optimised protocol that is likely, with minor adjustment, to have wider applicability across many macroinvertebrate groups and sampling scenarios, this paper does not intend to be prescriptive. There are several alternative options for various steps of the procedure that deserve exploration. Firstly, preservation of bulk samples at the collecting site could conceivably involve transferring the whole sample into RNA*later*® or liquid nitrogen (LN). However, using RNA*later*® for storage of the bulk sample would be expensive and increase the risk of retaining contaminant (non-target) RNA. LN is perhaps most ideal for snap-freezing and storing specimens for RNA work; however, LN possesses considerable health and safety risks when transported into the field (e.g., requiring separation from the main cab of a vehicle, appropriate signage). This can make LN impractical for remote study sites (although it is easy to refill LN dewers 'on the road'). Moreover, subsequent thawing of samples for secondary sorting may still degrade RNA. On the other hand, samples could conceivably be kept alive using water from the site and small aquarium pumps and aerators. Although this method would retain RNA intact while specimens were alive, this may be highly inappropriate for differential expression studies as expression levels may change for some environment-associated genes. Whether this alternative is suitable or not would depend on the system under investigation. For example, heavy-metal enriched stream water will still be high in heavy metals in a sample container and so expression of genes associated with those conditions probably will not change; however, hypoxic water may change in dissolved oxygen content under aeration and thus gene expression associated with hypoxia may change).

Secondly, once bulk stream samples have been secondarily sorted and putative target specimens selected out into cold RNA*later*®, specimens could be transferred to a single vial of RNA*later*® for longer-term storage or transport before morphological vouchers are prepared. However, this risks potential contamination of specimens resulting from storage in multi-species vials, RNA integrity may be reduced by multiple freeze-thaw cycles, and cuticle becomes brittle from long-term storage in RNA*later*®, potentially limiting diagnostic qualities of morphological vouchers. Finally, many alternative methods exist for isolating RNA from tissue specimens, including

a variety of commercially available kits designed around silica matrix spin columns. The method described here inherently relies on precise removal of the aqueous layer to minimise DNA and protein contamination, whilst maximising RNA retention. Although we demonstrate this method can achieve this effectively, spin columns are a more simple option for less experienced lab users (albeit more expensive per reaction). Moreover, extraction kits that are optimised for small amounts of starting tissue may alleviate the need to pool specimens in single extractions and would be strongly recommended for species for which comprehensive systematic knowledge is lacking.

The protocols associated with sampling and preservation to minimise contamination whilst retaining RNA integrity, along with vouchering and specimen tracking are of great importance for many macroinvertebrate groups. Not only do unique vouchers allow specimens to be identified prior to molecular work, specimens can be revisited and perhaps obscure or overlooked morphology validated that may explain unexpected molecular patterns. Furthermore, many taxonomic groups are yet to be comprehensively catalogued in existing DNA barcode libraries (e.g., GenBank, BOLD) and thus molecular species identification remains tenuous for such taxa. Taken together, we consider the described approach optimal to minimise such difficulties and facilitate inclusion of additional taxonomic groups in RNA-based research.

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A Preliminary Survey of the Non-biting Midges (Diptera: Chironomidae) of the Dominican Republic

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Abstract

Chironomidae (Diptera) are among the most diverse and widespread aquatic insects, with roughly 5,500 described species. However, prior to the present work, no species of Chironomidae had been documented from the island of Hispaniola. Collections of non-biting midges, with emphasis on the lotic fauna, were made in the Dominican Republic during July of 2015. In total, 578 specimens belonging to 27 genera and at least 44 species within the subfamilies Chironominae (20 taxa), Orthocladiinae (16 taxa) and Tanypodinae (8 taxa) were found. The subfamilies Chironominae and Orthocladiinae predominated. *Polypedilum* was the most widespread and diverse genus of Chironominae. *Metriocnemus* were collected in bromeliad tanks. The chironomid fauna in Dominican Republic includes multiple genera with worldwide distributions, including Holarctic and Neotropical components.

Introduction

Non-biting midges, belonging to the family Chironomidae (Diptera), are the most widely distributed free-living holometabolous insects (Ferrington 2008). The immature stages of most species occur in freshwater, but numerous terrestrial or marine species are known (Sæther and Ekrem 2003). The adult life stage of chironomids is short, and most of the lifespan is spent as a larva. The great species and habitat diversity makes this family not only a valuable indicator species for lentic and lotic aquatic ecosystems, but also a most interesting group for phylogenetic and biogeographical analyses (Silva and Ekrem 2015). However, in order to obtain the most biologically informative data, it is crucial to determine taxa to species, since within a single genus, species may respond in a different way to environmental changes (Lenat and Resh 2001). Usually, the lack of descriptions and keys to a local fauna prevents species identifications, or workers choose to overlook the Chironomidae in favor of groups (e.g. Ephemeroptera, Plecoptera, Trichoptera) that are more restricted in number and diversity (Spies et al. 2009).

Approximately 900 chironomid species are recognized from the Neotropical region (M. Spies, personal communication). This number has been increasing in recent years thanks to intense taxonomic work being done, particularly in Brazil (e.g. Andersen and Pinho 2014, Andersen et al. 2015, Oliveira et al. 2013, Silva and Wiedenbrug 2015, Silva et al. 2014a,b, Siri and Donato 2015, Trivinho-Strixino et al. 2013, 2015). Regarding Central America and the Caribbean, the chironomid fauna remains poorly known. No list of Chironomidae in Dominican Republic has been published so far and our knowledge consists basically of fossil records (Grund 2004, 2006, Perez-Gelabert 2008). Recent fauna have been sparsely mentioned by some authors. Margalef (1986), investigating the limnology of Lake Enriquillo in Dominican Republic, documented some chironomid larvae, identified only to family. Moreover, Perez-Gelabert (2008) compiled a checklist of arthropods of Hispaniola, which included chironomid species in amber and only one recent species, *Chironomus redeuns* Walker, considered a nomen dubium by Spies and Reiss (1996). In this context, the goal of our study was to provide data on the Chironomidae of the Dominican Republic, in order to contribute to the overall knowledge of Caribbean fauna.

Material and Methods

Study area

Hispaniola (Fig. 1) is the second largest island (76,480 km^2) in the archipelago of the Greater Antilles, Caribbean Region. It is centrally located in the Caribbean basin just south of the Tropic of Cancer at 17º40' and 19º56' North latitude and 68º20' and 72º01' West longitude. This natural geographic unit is shared by two countries with different languages and cultures, Haiti on the western one third (27,750 km^2) and the Dominican Repub-

lic on the eastern two thirds (48,730 km^2) (Perez-Gelabert 2008). The Dominican shores are washed by the Caribbean to the south and the Atlantic Ocean to the north. It has a varied terrain comprising rainforest, savannah and highlands, including Pico Duarte which, at about 3100 m elevation, is the Caribbean's tallest mountain. Climate in the Dominican Republic is tropical maritime, ranging from 18 to 32°C, with relatively high humidity. Mean annual precipitation along the southeastern coast around Santo Domingo is 1,400 mm with a distinct wet season from May to October. The island has some large saline or hypersaline inland lakes. There are several smaller islands and cays that are part of the Dominican territory. A complex topography and variety of local weather patterns generate numerous microhabitats, which support a rich flora and fauna with significant numbers of endemic species (Perez-Gelabert 2008).

Collection and identification

Collections were made at 13 localities in July 2015 (Table 1, Fig. 1). Most were lotic environments, ranging from small springs to large rivers, at elevations from 20 to 2,250 m above sea level. The main emphasis was on adult sampling, collected with a sweep net near aquatic systems. Dipnets (20 cm dia, 250 µm mesh) were used to collect immatures at some localities. Samples were also taken by aspiration of the water and organisms from the central tank of bromeliads. All samples were field-preserved using 96% ethanol. In the laboratory, after initial sorting and identification, representatives of every taxon in each sample were slide mounted in Euparal for species identification following the procedure outlined by Pinder (1983, 1986, 1989). Voucher specimens will be deposited in the Museum of Comparative Zoology at Harvard University.

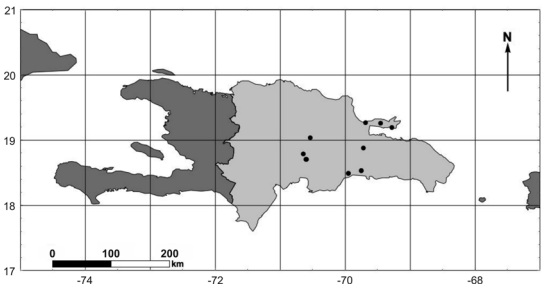

Figure 1. Map of the island of Hispaniola showing collecting localities in Dominican Republic (shown in light grey shading). Sampling sites are denoted as black dots. Some sites overlap due to their proximity and scale of map. See table 1 for more details on sampling localities.

Table 1. Localities, habitat type and geographic coordinate of chironomid collections in the Dominican Republic, July 2015.

Code	Province	Habitat type	Latitude	Longitude
A	Santo Domingo	River, heavy organic pollution	18°31'52.2"N	69°45'12.7"W
B	Samaná	River, with rocky bottom	19°16'06.2"N	69°41'15.7"W
C	La Vega	Pool, surrounded by grass	18°42'15.8"N	70°36'14.3"W
D	Santo Domingo	Lake, without surrounding vegetation	18°29'42.3"N	69°57'15.9"W
E	La Vega	Stream, with sparse riparian vegetation	18°42'23.1"N	70°36'12.7"W
F	La Vega	River, with moderate canopy cover	19°02'10.0"N	70°32'35.2"W
G	Santo Domingo	River, heavy organic pollution	18°31'51.1"N	69°45'12.8"W
H	Samaná	River, with dense canopy cover	19°15'48.5"N	69°27'29.9"W
I	Santo Domingo	River, heavy organic pollution	18°29'41.5"N	69°56'59.6"W
J	Monte Plata	Stream, with dense riparian vegetation	18°52'46.6"N	69°43'20.7"W
K	Samaná	Stream, with moderate canopy cover	19°11'32.9"N	69°17'04.5"W
L	La Vega	River, surrounded by grass	18°47'32.4"N	70°38'46.9"W
M	La Vega	Bromeliad tanks	18°42'15.9"N	70°35'56.0"W

Results

In total, 578 representatives of three subfamilies were collected (Table 2). Twenty-seven genera have been identified, containing at least 44 species. At least two additional orthoclad species could not be assigned to any described genera. Chironominae and Orthocladiinae predominated in all samples. Tanypodinae were often sampled, but rarely in large numbers. Chironomini was represented by at least 10 genera, but species diversity was low. Except for *Polypedilum*, no genus was represented by more than two species. The most widespread Chironomini were *Polypedilum* (*Asheum*) *beckae* (Sublette) and other *Polypedilum* spp. *Polypedilum* is one of the largest chironomid genera containing about 440 described species (Saether et al. 2010). Larvae of *Polypedilum* occur in virtually all kinds of still and flowing waters. A few species are also found in bromeliad tanks (Epler et al. 2013). Other commonly encountered Chironominae were *Goeldichironomus* spp. With the exception of *Chironomus* sp., *Paralauterborniella nigrohalteralis* and *Stenochironomus* sp. 1, collected in two localities, the remaining species were rare and found in only one locality. Two species of *Stenochironomus* and one of *Xestochironomus* were found, the larvae of these genera are often found mining decayed leaves or wood in freshwater habitats, which suggests woody debris as an available food item in those streams. The tribe Pseudochironomini was represented by *Manoa pahayokeensis* Jacobsen & Perry and *Pseudochironomus* sp., while *Tanytarsus* was the only genus belonging to Tanytarsini.

Ten genera of Orthocladiinae have been identified. Except for *Cricotopus*, most of these were collected from single localities. *Compterosmittia croizati* Mendes, Andersen & Saether and a related, undescribed species were collected in three localities in Dominican Republic. Furthermore, numerous larvae of *Metriocnemus* were collected in bromeliad tanks at the base of leaves, petals or bracts. *Metriocnemus* is a cosmopolitan midge with seven endemic species from the Neotropics and commonly found in pitcher plants, hollow trees and phytotelmata (Siri and Donato 2014). Despite much searching near the bromeliad phytotelmata where larvae were found, no adults of *Metriocnemus* were collected. Finally, several specimens belonging to *Diplosmittia* were collected at the National Botanical Garden (Santo Domingo) in a highly organic, polluted river. The examination of this not readily identifiable material, suggests that this species does not belong to any of the currently recognized *Diplosmittia* species. The new species can be separated from other *Diplosmittia* by the narrow and elongate anal point. The description of the new species will be done in a later manuscript.

Tanypodinae was the least abundant subfamily sampled in the Dominican Republic. Pentaneurini was represented by at least four genera with very low species diversity. *Labrundinia* was represented by three morphospecies, while *Pentaneura* by two. There appear to be numerous undescribed species of *Labrundinia* from the Neotropical region (Roback 1987). In a worldwide revision of the genus Silva et al. (2014a) described four species of *Labrundinia* from Central America. However none of the Dominican species could be ascribed to any of the previously described species. Only immatures of *Thienemannimyia* were collected. Although we assigned these specimens to *Thienemannimyia*, an identification of *Thienemannimyia* group (Silva and Ekrem 2015) may be more appropriate since the immatures of this complex are difficult to separate at the generic level (Cranston and Epler 2013). Only one pupal exuviae belonging to *Procladius* (*Holotanypus*) was collected.

Table 2. Chironomid taxa collected in the Dominican Republic, July 2015. A = adult, L = larva, P = pupal exuviae. Localities according to the table 1.

Chironomidae taxa	Life Stage	Locality
Chironominae		
Chironomini		
Chironomus sp.	A, L	A, H
Einfeldia sp.	A	H
Goeldichironomus sp. 1	A	G, H
Goeldichironomus sp. 2	A	A, G
Microchironomus sp.	A	D

Chironomidae taxa	Life Stage	Locality
Parachironomus yanomani Spies, Fittkau & Reiss	A	D
Paralauterborniella nigrohalteralis (Malloch)	A	C, H
Polypedilum (*Asheum*) *beckae* (Sublette)	A	A, D, G, H
Polypedilum sp. 1	A	B, E, J, K, L
Polypedilum sp. 2	A	B, F, H, K
Polypedilum sp. 3	A	H, J
Polypedilum sp. 4	A	H
Polypedilum sp. 5	A	H
Stenochironomus sp. 1	A	H, J
Stenochironomus sp. 2	A	H
Xestochironomus sp.	A	H
Pseudochironomini		
Manoa pahayokeensis Jacobsen & Perry	A	J
Pseudochironomus sp.	A, P	L
Tanytarsini		
Tanytarsus excavatus group	P	C, L
Tanytarsus sp.	A	H
Orthocladiinae		
Antillocladius sp.	A	F
Compterosmittia croizati Mendes, Andersen & Saether	A	H, I
Compterosmittia sp. 1	A	D
Cricotopus sp. 1	A	B, C, D, H
Cricotopus sp. 2	A	B, H, J
Diplosmittia sp.	A	I
Lipurometriocnemus glabalus Sæther	A	C
Lipurometriocnemus sp.	A	F
Metriocnemus sp.	L	M
Orthocladiinae sp. 1	A	H
Orthocladiinae sp. 2	A	J
Orthocladius sp.	A	D
Parametriocnemus sp.	A	L
Psectrocladius sp. 1	L, P	L

Chironomidae taxa	Life Stage	Locality
Psectrocladius sp. 2	L	C
Pseudosmittia sp.	A	D
Tanypodinae		
Pentaneurini		
Labrundinia sp. 1	A	H
Labrundinia sp. 2	A	H, J
Labrundinia sp. 3	A	H
Monopelopia sp.	A	A, K, L
Pentaneura sp. 1	A	J
Pentaneura sp. 2	A	F, H
Thienemannimyia sp.	A, P	E
Procladiini		
Procladius (*Holotanypus*) sp. 1	P	C

Discussion

At the generic level the chironomid fauna in Dominican Republic includes multiple genera with worldwide distributions with Holarctic and Neotropical components. The Neotropical component is typically an extension of the warm adapted fauna of lowland South America (Watson and Heyn 1992). At the level of species it is possible to infer that *Compterosmittia croizati*, known from Brazil and Venezuela (Mendes et al. 2004) and *Parachironomus yanomami* described from Brazil (Spies et al. 1994) would represent the Neotropical component. Within this component, genera such as *Diplosmittia, Goeldichironomus*, *Labrundinia*, *Lipurometriocnemus*, *Pentaneura* and *Polypedilum* (*Asheum*) possess a Pan-American distribution, having secondarily dispersed into the southern Nearctic via Central America or the Caribbean (Reiss and Sublette 1985). According to Jacobsen and Perry (2002) *Manoa pahayokeensis* Jacobsen & Perry would belong to the fauna with primarily Neotropical or pantropical distribution. Other genera such as *Microchironomus* and *Orthocladius* are most widespread in the Holarctic region. *Paralauterborniella*, monotypic for *P. nigrohalteralis* (Malloch), has almost a worldwide distribution, only unrecorded for the Australian region (Sæther and Spies 2013). Spies and Reiss (1996) catalogued information and references for all chironomid taxa known from the entire Neotropical Region. However, no species was documented from the Dominican Republic or Haiti. Our study recorded 44 chironomid species in the Dominican Republic.

Assuming the limited duration and extension of our study, this species richness documented here is probably lower than the true richness of chironomid fauna in the Dominican Republic. Comparing our results with Ferrington et al. (1993), who sampled in a mountain stream during one year in the neighbouring island of Puerto Rico, we could expect that some species of the following genera are still found in streams of the Dominican Republic: *Ablabesmyia*, *Corynoneura*, *Djalmabatista*, *Larsia*, *Paratendipes*, *Rheotanytarsus*, *Skutzia* and *Thienemanniella.* Additional orthoclad genera were found on Saint Vincent by Saether (1981): *Bryophaenocladius*, *Eurycnemus*, *Onconeura*, *Paraphaenocladius* and *Smittia*. A study performed by Anderson et al. (2014) on San Salvador Island (Bahamas) identified only 12 chironomid species. The authors suggested that low diversity might occur on individual islands, with a much larger collective community in the Bahamas as a whole. According to Bass (2003), other important factors influencing the diversity of freshwater macroinvertebrate communities on Caribbean islands probably include climate and island dispersal capabilities or limitations of specific populations.

Although our results document a relatively small chironomid community in the Dominican Republic, we believe that collections in different periods

(including both the rainy and dry seasons) and broadening the variety of sampling habitats and geographic area will reveal much greater diversity than currently detected. While recent years have seen increased activity concerning the chironomid fauna in the Neotropical region, the knowledge of the diversity and taxonomy as well as biogeography and phylogeny, especially in the Central America and Caribbean region, remains fragmentary. Thus, additional inventories are required to discover and analyse possible areas of endemism in the Greater Antilles archipelago. The present study contributes to the knowledge of chironomid fauna in the Dominican Republic and will hopefully motivate further studies in the area.

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Chironomidae Types in the Reference Collection of the Laboratory of Ecology of Aquatic Insects, São Carlos, Brazil

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Abstract

The Chironomidae (Insecta: Diptera) type collection at the Laboratory of Ecology of Aquatic Insects (LEIA - UFSCar) is reviewed. It comprises 103 primary types, as well as 95 paratypes, mostly resulting from research by S. Trivinho-Strixino and G. Strixino. Notes updating the taxonomic status are provided for some species.

Introduction

The type collection of Chironomidae at the Laboratory of Ecology of Aquatic Insects (LEIA - UFSCar) comprises numerous slide mounts, as well as large amounts of adult and immature specimens preserved in 70% ethanol. It is an important chironomid collection, especially regarding the Neotropical region. Moreover, it possesses valuable yet unstudied material mainly from south-east Brazil. It is historically relevant, but not well known. The largest part of this material results from research activities of S. Trivinho-Strixino and G. Strixino, who during more than three decades at the LEIA - UFSCar described numerous species.

The annotated checklist presented below (Table 1) was generated during a research visit of the present first author to LEIA - UFSCar in March 2015, which was partly financed by a Putnam expedition grant from the Museum of Comparative Zoology at Harvard University.

Results

The collection at the LEIA - UFSCar includes holotypes for 103 species of Chironomidae, as well as 95 paratypes, all slide mounted, with a total of 109 species represented. Most of the types belong to the subfamilies Chironominae and Tanypodinae, but there are also types of Orthocladiinae, Podonominae and Telmatogetoninae. In the annotated checklist presented below (Table 1), all species names and references were checked with the corresponding literature. The notes on the species' taxonomic status are based on Systema Dipterorum (Pape and Thompson 2013), as well as on published papers, keys (e.g. Cranston and Epler 2013, Spies and Reiss 1996, Trivinho-Strixino and Sanseverino 2003, Sanseverino et al. 2010) and/or on information from collection labels.

Table 1. Type specimens of Chironomidae deposited in the collection of the Laboratory of Ecology of Aquatic Insects (LEIA - UFSCar). Data in the "Type" and "Sex/Stage" columns refer to what is housed at UFSCar only, and that for some species this is less than the full material listed in the respective original publication. Abbreviations: H = holotype, P = paratype, f = female, l = larva, m = male, p = pupa.

Type number	Original genus	Original species	Author/ Reference	Type	Sex/ Stage	Note
			Chironominae			
A1/01-07	*Aedokritus*	*coffeatus*	Trivinho-Strixino, 1997: 13	H, P	m, f, p, l	
M2/01-09	*Beardius*	*cristhinae*	Trivinho-Strixino and Siqueira, 2007: 281	H, P	m, f, p	
M2/37-45	*Beardius*	*phytophilus*	Trivinho-Strixino and Strixino, 2000b: 245	H, P	m, l	
M2/27-33	*Beardius*	*roquei*	Trivinho-Strixino and Siqueira, 2007: 282	H, P	m, p	

Type number	Original genus	Original species	Author/ Reference	Type	Sex/Life stage	Note
M2/19-24	*Beardius*	*xylophilus*	Trivinho-Strixino and Strixino, 2000b: 246	H, P	m, l	
C1/01	*Caladomyia*	*angelae*	Trivinho-Strixino, 2012: 11	H	m	
C2/01, C2/03, C2/06, C2/08	*Caladomyia*	*bruneola*	Trivinho-Strixino, 2012: 13	H, P	m, f, p, l	
C1/02-03	*Caladomyia*	*canine*	Trivinho-Strixino, 2012: 16	H, P	m, p	
C1/06-07, C1/09-11	*Caladomyia*	*capaopreto*	Trivinho-Strixino, 2012: 18	H, P	m, p, l	
C1/04-05	*Caladomyia*	*carolae*	Trivinho-Strixino, 2012: 21	H, P	m	
C2/12-19	*Caladomyia*	*carolensis*	Trivinho-Strixino, 2012: 22	H, P	m, f, p, l	
C1/45-48	*Caladomyia*	*comunis*	Trivinho-Strixino, 2012: 26	H, P	m, p, l	
C1/13	*Caladomyia*	*curumim*	Trivinho-Strixino, 2012: 29	H	m, p, l	
C3/04-15	*Caladomyia*	*friederi*	Trivinho-Strixino and Strixino, 2000a: 168	H, P	m, f, p, l	
C1/15-18	*Caladomyia*	*jaragua*	Trivinho-Strixino, 2012: 31	H	m, p, l	
C2/28-29, C2/32-33, C2/35	*Caladomyia*	*kapilei*	Trivinho-Strixino, 2012: 33	H, P	m, p, l	
C1/20-25	*Caladomyia*	*yara*	Trivinho-Strixino, 2012: 36	H, P	m, f, p, l	
B1/01-13	*Chironomus*	*amissum*	Correia, Trivinho-Strixino and Michailova, 2013: 134	H, P	m, p, l	1
M2/30-32	*Chironomus*	*antonioi*	Correia and Trivinho-Strixino, 2007: 57	H, P	m, p, l	
M1/42-44	*Chironomus*	*detriticola*	Correia and Trivinho-Strixino, 2007: 54	H, P	m, p, l	
M1/48	*Chironomus*	*fittkaui*	Correia and Trivinho-Strixino, 2007: 65	H	m, p, l	
F1/15-26	*Chironomus*	*inquinatus*	Correia, Trivinho-Strixino and Michailova 2006: 58	H, P	m, f, p, l	
B2/11-14	*Chironomus*	*oliveirai*	Correia and Trivinho-Strixino, 2007: 63	H, P	m, p, l	
B2/20-26	*Chironomus*	*phytophilus*	Correia and Trivinho-Strixino, 2007: 61	H, P	m, p, l	

Type number	Original genus	Original species	Author/ Reference	Type	Sex/Life stage	Note
F1/01-15	*Chironomus*	*reissi*	Correia, Trivinho-Strixino and Michailova 2005: 30	H, P	m, f, p, l	
B2/01-06	*Chironomus*	*sancticaroli*	Trivinho-Strixino and Strixino, 1981: 333	H, P	m, f	2
M1/01-04	*Cryptochironomus*	*brasiliensis*	Silva, Trivinho-Strixino and Oliveira, 2010: 19	H, P	m, p, l	
M1/08-12	*Cryptochironomus*	*mantiqueira*	Silva, Trivinho-Strixino and Oliveira, 2010: 23	H, P	m, p, l	
M1/15-21	*Cryptochironomus*	*reshchikovi*	Silva, Trivinho-Strixino and Oliveira, 2010: 27	H, P	m, p, l	
E3/07-11	*Endotribelos*	*bicolor*	Trivinho-Strixino and Pepinelli, 2015: 3	H, P	m, f, p, l	
E1/15-17	*Endotribelos*	*calophylli*	Roque and Trivinho-Strixino, 2008: 193	H, P	m, p, l	
E2/01-02	*Endotribelos*	*euterpe*	Roque and Trivinho-Strixino, 2008: 196	H, P	m, f, p, l	
E3/01-03	*Endotribelos*	*ficus*	Roque and Trivinho-Strixino, 2008: 201	H, P	m, p, l	
E3/39-43	*Endotribelos*	*fulvidus*	Trivinho-Strixino and Pepinelli, 2015: 11	H, P	m, f, p, l	
E2/37-42	*Endotribelos*	*jaragua*	Trivinho-Strixino and Pepinelli, 2015: 17	H, P	m, f, p, l	
E3-25	*Endotribelos*	*jiboia*	Trivinho-Strixino and Pepinelli, 2015: 24	H	m, p, l	
E2/20-27	*Endotribelos*	*semibruneus*	Trivinho-Strixino and Pepinelli, 2015: 24	H, P	m, f, p, l	
E3/34-42	*Endotribelos*	*sublettei*	Trivinho-Strixino and Pepinelli, 2015: 29	H, P	m, f, p, l	
E1/01-05	*Endotribelos*	*talaumae*	Roque and Trivinho-Strixino, 2008: 203	H, P	m, f, p, l	
L1/01-09	*Goeldichironomus*	*luridus*	Trivinho-Strixino and Strixino, 2005: 441	H, P	m, f, p, l	
L1/40-41	*Goeldichironomus*	*maculatus*	Trivinho-Strixino and Strixino, 1991b: 593	H, P	m, f	
L1/30-36	*Goeldichironomus*	*neopictus*	Trivinho-Strixino and Strixino, 1998: 271	H, P	m, f, p, l	
L1/14-20	*Goeldichironomus*	*petiolicola*	Trivinho-Strixino and Strixino, 2005: 442	H, P	m, f, p, l	
L2/01	*Nimbocera*	*paulensis*	Trivinho-Strixino and Strixino, 1991a: 175	H, P	l	3
M1/36	*Nimbocera*	*rhabdomantis*	Trivinho-Strixino and Strixino, 1991a: 173	H, P	l	4

Type number	Original genus	Original species	Author/ Reference	Type	Sex/Life stage	Note
L1/47-48	*Oukuriella*	*jatai*	Trivinho-Strixino and Messias 2005: 285	H, P	m, f, p, l	
A1/25-32	*Parachironomus*	*lupus*	Trivinho-Strixino, Silva and Roque, 2010: 2	H, P	m, p, l	
A3/16-25	*Paratanytarsus*	*corbii*	Trivinho-Strixino, 2010: 60	H, P	m, f, p, l	
A3/14-15	*Paratanytarsus*	*silentii*	Trivinho-Strixino, 2010: 65	H, P	m	
A2/25-30	*Pelomus*	*psammophilus*	Trivinho-Strixino and Strixino, 2008: 224	H, P	m, f, p, l	
A2/01-05	*Pelomus*	*sophiae*	Trivinho-Strixino and Silva 2011: 274	H, P	m, p, l	
O2/48-50	*Riethia*	*manauara*	Neubern, Trivinho-Strixino and Silva, 2011: 596	H, P	m, p, l	
H1/23-25	*Tanytarsus*	*alfredoi*	Sanseverino and Trivinho-Strixino, 2010: 78	H, P	m, p, l	
H2/01-20	*Tanytarsus*	*caipira*	Trivinho-Strixino and Strixino, 2007: 62	H, P	m, f, p, l	
H2/43-48	*Tanytarsus*	*corumba*	Trivinho-Strixino, Wiedenbrug and Silva, 2015: 92	H, P	m	
H1/29-30	*Tanytarsus*	*fittkaui*	Sanseverino and Trivinho-Strixino, 2010: 75	H, P	m, p, l	
H1/33-43	*Tanytarsus*	*giovannii*	Sanseverino and Trivinho-Strixino, 2010: 71	H, P	m, p, l	
H3/33-37	*Tanytarsus*	*hirsutus*	Trivinho-Strixino, Wiedenbrug and Silva, 2015: 93	H, P	m, p	
H3/01-13	*Tanytarsus*	*impar*	Trivinho-Strixino and Strixino, 2004: 160	H, P	m, f, p, l	
H3/40-43	*Tanytarsus*	*jatai*	Trivinho-Strixino, Wiedenbrug and Silva, 2015: 95	H, P	m	
H1/46-49	*Tanytarsus*	*lenyae*	Sanseverino and Trivinho-Strixino, 2010: 68	H, P	m, p	
H2/29-36	*Tanytarsus*	*longitubuli*	Trivinho-Strixino, Wiedenbrug and Silva, 2015: 96	H, P	m, p, l	
H3/19-27	*Tanytarsus*	*magnus*	Trivinho-Strixino and Strixino, 2004: 155	H, P	m, f, p, l	
H1/01-21	*Tanytarsus*	*obiriciae*	Trivinho-Strixino and Sonoda 2006: 2	H, P	m, f, p, l	

Type number	Original genus	Original species	Author/ Reference	Type	Sex/Life stage	Note
H3/30-31	*Tanytarsus*	*pseudocurvicristatus*	Trivinho-Strixino, Wiedenbrug and Silva, 2015: 98	H, P	m, p, l	
A3/01-05	*Tapajos*	*cristinae*	Trivinho-Strixino, Silva and Oliveira, 2013: 396	H, P	m	
M3/01-15	*Xenochironomus*	*ceciliae*	Roque and Trivinho-Strixino, 2005: 232	H, P	m, f, p, l	
		Orthocladiinae				
N3/01	*Gravatamberus*	*nidularium*	Mendes and Andersen, 2008: 49	P	m, p, l	
N3/02	*Physoneura*	*paulseni*	Stur and Andersen, 2000: 132	P	m	
		Podonominae				
N1/13-14	*Podonomus*	*pepinellii*	Roque and Trivinho-Strixino, 2004: 2	H	p	
		Tanypodinae				
P1/25	*Ablabesmyia*	*arquata*	Neubern, 2013: 9	H	m	
P3/02-06	*Ablabesmyia*	*cauame*	Neubern, 2013: 13	H, P	m	
P1/12-20	*Ablabesmyia*	*commata*	Neubern, 2013: 21	H, P	m	
O1/01-21	*Ablabesmyia*	*communiba*	Neubern, 2013: 24	H, P	m, p, l	
P2/12-14	*Ablabesmyia*	*cordeiroi*	Neubern, 2013: 30	H, P	m	
O2/37-38	*Ablabesmyia*	*depaulai*	Neubern, 2013: 33	H	m, p, l	
P2/40-42	*Ablabesmyia*	*diversa*	Neubern, 2013: 39	H, P	m	
O2/01-05	*Ablabesmyia*	*ducke*	Neubern, 2013: 41	H, P	m, p, l	
O2/32-33	*Ablabesmyia*	*fazzari*	Neubern, 2013: 47	H, P	m, p, l	
P1/45-46	*Ablabesmyia*	*fusariae*	Neubern, 2013: 53	H, P	m, p, l	
P1/36-39	*Ablabesmyia*	*gessnerae*	Neubern, 2013: 59	H, P	m, p, l	
P2/33-37	*Ablabesmyia*	*gigas*	Neubern, 2013: 64	H, P	m	
O2/29	*Ablabesmyia*	*jaquirana*	Neubern, 2013: 71	H	m	

Type number	Original genus	Original species	Author/ Reference	Type	Sex/Life stage	Note
P2/18	*Ablabesmyia*	*manauara*	Neubern, 2013: 80	H	m	
P2/20-22	*Ablabesmyia*	*martha*	Neubern, 2013: 83	H, P	m	
P3/09-10	*Ablabesmyia*	*novema*	Neubern, 2013: 89	H, P	m, p, l	
O3/12-16	*Ablabesmyia*	*oliveirai*	Oliveira and Fonseca-Gessner, 2006: 740	H, P	m, p, l	
P1/29-33	*Ablabesmyia*	*parannulata*	Neubern, 2013: 95	H, P	m	
O2/39-40	*Ablabesmyia*	*parareissi*	Neubern, 2013: 99	H, P	m	
O2/20-27	*Ablabesmyia*	*pinhoi*	Neubern, 2013: 102	H, P	m, p	
P2/30	*Ablabesmyia*	*rafaeli*	Neubern, 2013: 106	H	m	
P1/42	*Ablabesmyia*	*separata*	Neubern, 2013: 109	H	m	
P2/25-27	*Ablabesmyia*	*strixinoae*	Neubern, 2013: 113	H, P	m, p, l	
P1/21-23	*Ablabesmyia*	*suiamissu*	Neubern, 2013: 118	H, P	m	
O1/41-42	*Clinotanypus*	*caritus*	Oliveira, Silva and Trivinho-Strixino, 2014a: 319	P	m, p, l	
O1/31-32	*Clinotanypus*	*gymnos*	Oliveira, Silva and Trivinho-Strixino, 2014a: 325	H, P	m, p, l	
O1/44-45	*Clinotanypus*	*setosus*	Oliveira, Silva and Trivinho-Strixino, 2014a: 330	H, P	m, p, l	
O1/34-39	*Clinotanypus*	*striatus*	Oliveira, Silva and Trivinho-Strixino, 2014a: 336	H, P	m, p, l	
B3/11-14	*Guassutanypus*	*oliveirai*	Roque and Trivinho-Strixino, 2003: 161	H, P	m, f, p, l	5
O3/04-10	*Hudsonimyia*	*araxa*	Silva, Wiedenbrug, Oliveira, Trivinho-Strixino and Pepinelli, 2012: 1629	P	m, f, p, l	
O3/01	*Hudsonimyia*	*caissara*	Silva, Wiedenbrug, Oliveira, Trivinho-Strixino and Pepinelli, 2012: 1623	P	m, p, l	
O2/42-44	*Larsia*	*gelhausi*	Oliveira and Silva, 2011: 30	H, P	m, p, l	

Type number	Original genus	Original species	Author/ Reference	Type	Sex/Life stage	Note
O2/12-14	*Larsia*	*hamadae*	Oliveira and Silva, 2011: 35	H	m, p, l	
B3/07-08	*Larsia*	*labartheae*	Serpa-Filho, 2005: 295	P	m	
O3/25-32	*Metapelopia*	*corbii*	Silva, Oliveira and Trivinho-Strixino, 2014: 86	H, P	m, f, p, l	
O2/07-10	*Monopelopia*	*paranaensis*	Oliveira, Mendes and Silva, 2010: 54	H, P	m, f, p, l	
O1/24-27	*Parapentaneura*	*brunnescens*	Oliveira, Silva and Trivinho-Strixino, 2014b: 27	H, P	m, p, l	
O1/29	*Parapentaneura*	*flavescens*	Oliveira, Silva and Trivinho-Strixino, 2014b: 31	H	m, p, l	
Telmatogetoninae						
P1/01-09	*Thalassomya*	*gutae*	Oliveira, Silva and Trivinho-Strixino, 2013: 590	H, P	m, p, l	

Notes in Table 1:

1. In *Chironomus amissum* Correia, Trivinho-Strixino and Michailova, 2013, the original spelling of the species epithet, "*amissum*", meaning loss, has to be emended. Correia *et al*. (2012: 134) gave an etymological derivation from a Latin noun "*amissum*", meaning loss, but that Latin noun is "*amissus*", with masculine rather than neuter gender and ending.

2. *Chironomus sancticaroli* Trivinho-Strixino and Strixino, 1981 has been treated as a junior synonym of *C. xanthus* Rempel, 1939 by some authors since Spies and Reiss (1996).

3. *Nimbocera paulensis* Trivinho-Strixino and Strixino, 1991 has been treated as a junior synonym of *Caladomyia ortoni* Säwedal, 1981 since Trivinho-Strixino and Strixino (2003).

4. *Nimbocera rhabdomantis* Trivinho-Strixino and Strixino, 1991 has been carried as *Tanytarsus rhabdomantis* (Trivinho-Strixino and Strixino, 1991) since Trivinho-Strixino and Sanseverino (2003).

5. *Guassutanypus oliveirai* Roque and Trivinho-Strixino, 2003 has been carried as *Alotanypus oliveirai* (Roque and Trivinho-Strixino, 2003) since Cranston and Epler (2013).

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Chironomids: A Personal Journey

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The year was 1977. I was an undergraduate student at the newly created Biology Department at the Simón Bolívar University in Venezuela. We were a group of about 30 students, mostly transfers from other careers (I had been in Engineering for 3 years when I transferred to Biology), who had waited patiently for the bureaucratic process of creating a new department in our university. A team of new faculty members, some very well known scientists, some barely older than we were, worked with the students to create the new curriculum, outfit the laboratories, and develop research projects where the students could participate as research assistants. It was a wonderful time filled with creativity, endless energy, hard work, and fantastic scholarship and idealism. Some of us students drifted from lab to lab, learning techniques, going on field trips with our professors, and learning the ropes of this new career we had chosen: to be a professional biologist.

This particular summer I was working in the aquatic biology lab, run by the Academic Coordinator of the department, Dr. Roger Carrillo. Roger had recently returned from his Ph.D. degree in aquatic ecology at the University of Pittsburgh, under Dr. William Coffman. Roger invited Bill to visit him in Venezuela, go collecting in a few places, and give a talk at the department. Bill gave a presentation on his research on Chironomidae, in English of course, which most of us understood only a little. In spite of the language barrier, I was fascinated by his work and hoped to have a chance to meet him later at the lab and show him my samples. I had been collecting midges in Lake Valencia, an endorheic lake in north-central Venezuela. I collected a wide range of aquatic insects and struggled with the lack of field guides to identify the creatures. Particularly vexing was the collection of Chironomidae. I had many larvae that I could tell were probably different species, but I had no way to get them further identified. The only books available to me were old copies of Pennak (1953) and Edmondson (1959) and the recently-published Spanish version of Needham and Needham (1978), all of which had proven useful to identify things to order or family, but not beyond.

Bill came to the lab that afternoon and I showed him a dish filled with chironomid larvae. He looked through the microscope and started sorting the larvae into three groups. Pointing with his forceps to one group he said, (and Roger translated to me): "*This group are probably new species. I can recognize the genera, but they are likely new. This other group*," he said while pointing at the second little pile of larvae, "*I can't recognize the genera. I'm sure they are totally new*." And finally, pointing to the third and smaller pile, he added excitedly, "*And this last group. Well, these are unique and bizarre. I can't even put them to subfamily!"*

Figure 1. Carlos de la Rosa collecting chironomid pupal exuviae in Costa Rica, 1989.

At that moment, something clicked in my mind. The demonstration and seeing his enthusiasm, his passion for the midges, left me speechless. My thought was "*This is exactly what I want to do with my life.*" I wanted to be able to do what he had just done, look into a dish of creatures and know what they were and what they were not and what to do about them. From that day on, all my energy became focused in getting accepted at the University of Pittsburgh to study under Coffman. For the next fourteen years or so I spent most of my time studying midges to get my Ph.D. and later collecting in Costa Rica and Florida in collaboration with Bill (Fig. 1).

Now, move forward 38 years to today. My career has taken many turns, some of them a bit odd but all interesting and productive. But all throughout these decades, midges have always been part of my life. I have not worked steadily in taxonomy or ecology of the family, and my publications on them or that include the family are very few (de la Rosa, 1985, 1997, 1992, 1996, 1997a,b, 2002, 2013, 2014; de la Rosa and Nastase, 1987; Coffman, Yurasits and de la Rosa, 1988; de la Rosa and Barbee, 1993, 1995; Epler and de la Rosa, 1995; Nastase, de la Rosa and Newell, 1995; Coffman, de la Rosa, Cummins and Wilzbach, 1992; de la Rosa, Koebel and Cummins, 1998; Coffman and de la Rosa, 1998). However, regardless of the job I had at any given time, I continued to collect midges when I could, particularly tens of thousands of specimens of pupal exuviae, in Venezuela, Pennsylvania, Florida and Costa Rica, all of which ended up in Bill's extraordinary collection of over 100,000 specimens. Bill even made a trip to Costa Rica and we collected in 26 different streams, rivers and lakes around the country (Fig. 2). This collection is now being maintained, curated and expanded at the La Selva Research Station of the Organization for Tropical Studies in Costa Rica, thanks to the generosity and expediency of a number of people that allowed us to rescue the collection and save it from a possible loss after Bill's death in 2012 (de la Rosa, 2012; Levin, 2013). Through all those years, I worried over the fate of all this extraordinary material, mounted and prepared with exquisite care, cataloged, illustrated and identified by Bill but never published. The collection and catalogs hold over 450 undescribed pupal exuviae from Venezuela, Trinidad and Costa Rica, most of which will end up being new species to science (Fig. 3). Who is going to be able to work on this material and how are we going to make it work for science and society?

Figure 2. William Coffman collecting pupal exuviae in Costa Rica, February 1997.

There is a complex set of circumstances and challenges that have made this an important part of my past and current work. I have always felt that we should describe all of Bill's pupal exuviae types as proper species, using the pupal exuviae as the taxonomically significant life stage. My arguments have been: (1) they are easily collected, preserved, mounted and identified, characteristics that make them accessible to biologists in developing countries; (2) most aquatic biologists would welcome the opportunity to use exuviae as a survey tool for examining their communities, especially in the tropics where relatively little is known about this group, but only if they could identify them; (3) the collection of pupal exuviae causes minimal to no impact to the aquatic communities, in contrast to benthic sampling. This is an important consideration in fragile ecosystems and in protected areas; (4) the immediate problems with synonymy would be relatively small compared with the enormous value of having the fauna described and named as pupae. To date we have about 90 chironomid species recorded for Costa Rica (Epler, personal communication),

most of which have only been described as adults. Additionally, a new study in a small area of north-central Costa Rica has produced over 100 new species and records for the country (Epler, personal communication). Bill estimated there were at least 1,000 species in Costa Rica, although that number could be still higher. The possible synonymy issues could be resolved over time; and (5) the availability of keys and descriptions for pupal exuviae would galvanize the necessary work of obtaining associations through rearing larvae to adults. As you can imagine, this proposal never received much support, either from Bill or from the other chironomid taxonomists I spoke to about it. The most common response I received from my taxonomy colleagues was "*Get associated material! It is the only way.*" So the dilemma lingered.

In the last two years (2014-2015) I have been working with two extraordinary parataxonomists (see Janzen et al., 1993 for the definition of what a parataxonomist does), several volunteers, and a few generous scientists in obtaining additional pupal exuviae material from Costa Rica (Fig. 4), focusing on areas that had not been collected during my previous tenure in this country from 1987 to 1997. We have accumulated so far several hundred samples from 78 separate freshwater ecosystems, including bi-weekly year series from 8 streams and rivers. More importantly, we have obtained over 700 associations of larvae-pupae-adult or pupae-adult (plus a few larvae-pupae that failed to emerge), and we get more every day (Figs. 5 and 6). With this new material, the Coffman collection is likely to become (if it is not already) the most comprehensive and complete chironomid collection from a Central American country, and likely from Latin America.

New technologies, such as DNA barcoding applied to pupal exuviae and chironomids in general (see Ekrem and Stur, 2007; Ekrem et al., 2010; Stur and Ekrem, 2011; Anderson et al, 2013; and Kranzfelder et al., 2015, as examples) also promise to increase exponentially the number of correctly associated material without having to rear them from larvae, as well as open a huge opportunity to correctly identify species using DNA from pupal exuviae (Krosch and Cranston, 2012). Additionally, the use of chironomid pupal exuviae sampling in tropical environments has received a strong boost from recently published papers (Kranzfelder et al., 2015b; Kranzfelder and Ferrington, 2015; and the very interesting Kranzfelder et. al., 2015a which uses video to present their techniques). These and other papers forthcoming in Spanish will facilitate access to proper techniques, protocols, applications and, eventually, keys to the many genera and species that live in this huge bioregion to an underrepresented but eager audience of Latin American scientists and students.

Figure 3. Pupal exuviae of undescribed *Apsectrotanypus*.

Figure 4. Socorro Avila, parataxonomist, collecting larvae and pupal exuviae in the Sarapiquí River, April 2015.

I feel that we are on the threshold of a tropical chironomid taxonomy revolution, one where a new generation of aquatic biologists and taxonomists-in-training, together with parataxonomists and the international chironomid expert community, can take a giant leap forward in placing chironomids in their proper place in studies of aquatic ecosystems, especially in the Neotropics where many aquatic ecosystems are under severe threat. The interest in effective, minimally destructive, and useful monitoring techniques is rapidly increasing, as Latin America faces the challenge of hasty development and its accompanying destruction of aquatic and riparian ecosystems. Rivers and streams are too often seen as resources to be exploited for drinking water, irrigation, transportation, generation of electricity, aquaculture, and even waste disposal. Most studies of the environmental impact of industry never consider the rivers as true ecosystems, filled with unique species assemblages and communities, fragile and in need of protection and management.

Figure 5. Chironomid rearing setup at the Coffman Laboratory of Aquatic Entomology, at the La Selva Biological Station, Costa Rica.

And here is where the international chironomid community can play an important role. Supporting the local inventories, the training of Latin American technicians (e.g., Ekrem et al., 2013), biologists and taxonomists, collaborating in projects, and helping secure funds for processing and describing the materials collected, will be key for building a genuine capacity in developing countries like Costa Rica to pursue sustainability, ecosystem viability and conservation goals. I hope you join the effort.


Figure 6. Association of larvae, pupae and adult of undescribed *Corynoneura*.

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Preliminary data on the chironomid fauna of the Middle Volga region within the Republic of Tatarstan (Russia) based on hydrobiological monitoring studies

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The chironomid fauna of Middle Volga within the Republic of Tatarstan, Russia, has never been a subject of a special taxonomic investigation and only larval stages of chironomids have been studied from hydrobiological samples. Taxonomic chironomid lists are known only for some water bodies of the region: Zainsk water reservoir (55°18′N 52°01′E, Nazarova, 1999), Cheboksar reservoir (56°09′N 47°14′E, Nazarova et al., 2004), as well as several small rivers (Yakovlev 2003; Torsuev et al. 2005; Kondrateva, Nazarova, 2011; Mingazova 2012).

Here we provide a new list of chironomid taxa from the middle section of the River Volga (within the Republic of Tatarstan, Fig.1) and its tributaries (Mesha, Kazanka, Svijaga, Steppe Zai, Vjatka, Big Cheremshan, Tojma, Avlashka, Jurashka, and small inundated reservoir of Kazan, Fig. 1). Chironomids were investigated from hydrobiological collections of zoobenthos completed by the Meteorological service of Russia (FPBI "Management of hydrometeorology and environmental monitoring of the Republic of Tatarstan"). Samples were collected between 1991 and 2014 using standard hydrobiological methods (Abakumov 1983). On large rivers (Volga, Vjatka, Big Cheremshan) samples were collected using a Petersen dredge. Small rivers were sampled using a rod dredge and hydrobiological net. The comprehensive list of chironomids collected includes 131 species from 5 subfamilies. Names of the genera and species are listed according to the Fauna Europaea catalogue: http://www.faunaeur.org/index.php.

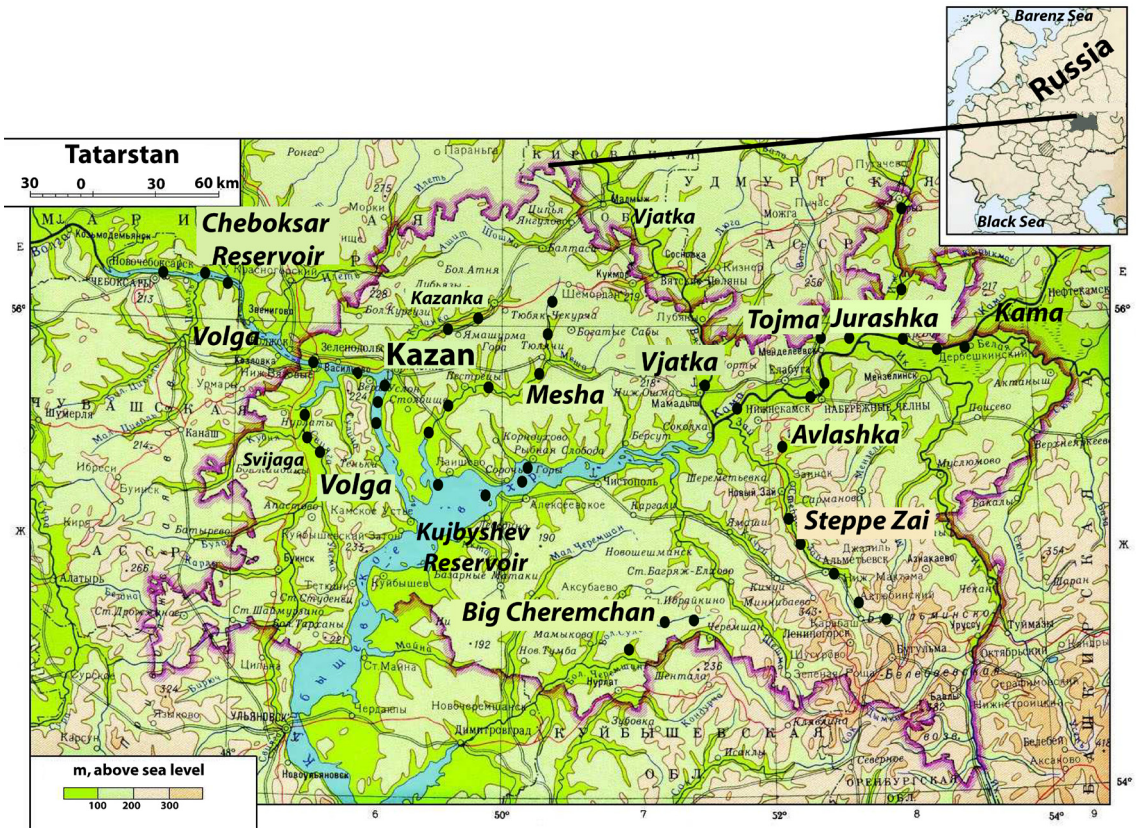

Figure 1. Map of the region. Sampling sites are given in black circles.

Table 1. List of chironomid taxa collected from the middle stream of the River Volga (within the Republic of Tatarstan, Fig. 1) and its tributaries. Abbreviations: M - Mesha, K - Kazanka, S -Svijaga, SZ - Steppe Zai, Vj - Vjatka, BC - Big Cheremshan, T - Tojma, A - Avlashka, J - Jurashka, V – Volga, VS – small inundated reservoir of Kazan.

Chironomidae collected	Rivers										
	M	K	S	SZ	Vj	BC	T	A	J	V	VS
Tanypodinae											
Ablabesmyia (*Ablabesmyia*) *monilis*	+	+	+	+							
A. (*Ablab.*) *phatta*		+									
Ablabesmia sp.	+	+	+								
Apsectrotanypus trifascipennis	+			+				+			
Arctopelopia sp.				+					+		
Clinotanypus pinguis											+
C. nervosus											+
Coelotanypus concinnus											+
Conchapelopia melanops											+
Conchapelopia sp.	+										
Guttipelopia guttipennis		+									
Macropelopia nebulosa			+								
Monopelopia tenuicalcar											+
Psectrotanypus varius											+
Procladius (*Holotanypus*) *choreus*	+				+						+
P. (*Holotanypus*) *ferrugineus*	+	+	+	+	+	+	+			+	+
Procladius (*Holotanypus*) sp.			+								+
Procladius (Psilotanypus) imicola	+										
Rheopelopia ornata				+							
Rheopelopia sp.				+		+				+	
Telopelopia okoboji				+							
Tanypus (*Tanypus*) *kraatzi*											+
Diamesinae											
Diamesa steinboecki					+						
Potthastia longimanus								+		+	
P. gaedii				+							+
Protanypus caudatus				+							
Protanypus sp.											
Pseudodiamesa (*Pseudodiamesa*) *nivosa*				+							
Prodiamesinae											
Monodiamesa bathyphila	+	+		+		+					
Odontomesa fulva				+				+			
Prodiamesa olivacea	+	+	+	+		+	+	+		+	+
Prodiamesa sp.											
Orthocladiinae											
Brillia bifida				+							
Chaetocladius sp.				+							
Corynoneura scutellata				+							
Corynoneura carriana		+									
Cricotopus (*Cricotopus*) *algarum*		+	+								
C. (*Cricotopus*) *bicinctus*				+	+			+		+	
C. (*Cricotopus*) *cylindraceus*				+	+					+	
C. (*Isocladius*) *sylvestris*	+	+		+	+		+	+		+	+
C. (*Cricotopus*) *trifascia*				+	+		+			+	+
Eukiefferiella coerulescens									+		
Eukiefferiella claripennis				+							
Eukiefferiella sp.		+									+
Euryhapsis subviridis											+
Orthocladius (*Orthocladius*) *clarki*				+				+		+	+
Orthocladius (*Pogonocladius*) *consobrinus*				+							

Chironomidae collected	Rivers										
	M	K	S	SZ	Vj	BC	T	A	J	V	VS
Paratrissocladius excerptus				+				+			
Pseudorthocladius (Pseudorthocladius) curtistylus										+	
Psectrocladius (*Allopsectrocladius*) *obvius*				+				+			
P. (*Allopsectrocladius*) gr. *dilatatus*	+										
P. (*Psectrocladius*) *bisetus*											+
P. (*Psectrocladius*) *pancratovae*				+							
P. (Psectrocladius) psilopterus	+		+								
P. (*Psectrocladius*) *sordidellus*				+						+	+
P. (*Psectrocladius*) *simulans*				+							
P. (*PPsectrocladius*) *sokolovae*				+							
Psectrocladius sp.				+			+	+			
Rheocricotopus robacki				+							
Rheocricotopus sp.			+								
Chironominae											
Tanytarsini											
Cladotanytarsus (*Cladotanytarsus*) *mancus*	+	+		+	+	+	+	+		+	+
Micropsectra sp.		+									
Paratanytarsus austriacus									+		+
Paratanytarsus sp.	+	+	+								
Rheotanytarsus curtistylus				+			+				
Rheotanytarsus sp.		+									
Stempellinella edwardsi											+
Stempellinella minor Edwards				+						+	
Stempellina subglabripennis										+	
Tanytarsus bathophilus				+	+						+
Tanytarsus pallidicornis				+							+
Tanytarsus excavatus				+	+	+		+			
Tanytarsus sp.											+
Chironomini											
Beckidia zabolotzkyi	+										
Chironomus (*Chironomus*) balatonicus	+	+									+
C. (*Chironomus*) *plumosus*	+	+	+	+	+						+
C. (*Chironomus*) *pallidivittatus*	+	+									
C. (*Chironomus*) *riparius*	+										
Chironomus (*Chironomus*) sp.	+	+	+	+	+	+	+	+		+	+
Cladopelma lateralis											
Cladopelma viridulum	+		+								
Cryptochironomus (*Cryptochironomus*) *psittacinus*					+						
C. (*Cryptochironomus*) *defectus*	+	+	+	+						+	+
C. (*Cryptochironomus*) *obreptans*				+						+	+
C. (*Cryptochironomus*) *albofasciatus*		+									
C. (*Cryptochironomus*) *psittacinus*		+									
C. (*Cryptochironomus*) *ussouriensis*	+										
Cryptotendipes holsatus				+							
Cryptotendipes nigronitens	+										
Cryptotendipes sp.	+	+	+								
Dicrotendipes modestus				+		+				+	+
Dicrotendipes nervosus				+			+			+	
Dicrotendipes notatus	+	+	+		+					+	
Demicryptochironomus (*Demicryptochironomus*) *vulneratus*							+			+	
Einfeldia pagana											+

Chironomidae collected	Rivers										
	M	K	S	SZ	Vj	BC	T	A	J	V	VS
Endochironomus albipennis	+	+	+	+		+	+			+	+
E. tendens						+					+
Endochironomus sp.			+								
Glyptotendipes (*Glyptotendipes*) *glaucus*	+	+	+	+	+	+				+	+
G. (*Glyptotendipes*) *cauliginellus*	+	+	+				+			+	+
G. (*Glyptotendipes*) *barbipes*										+	
G. (*Glyptotendipes*) *paripes*	+	+	+							+	
Glyptotendipes sp.	+	+	+							+	
Harnischia curtilamellata				+							
Harnischia sp.				+						+	
Lipiniella araenicola	+										
Lipiniella moderata				+							
Microchironomus tener	+	+	+							+	
Microtendipes pedellus				+			+				+
Parachironomus gracilior	+										
Parachironomus varus											
Parachironomus pararostratus		+									
Paracladopelma camptolabis	+			+							
Paralauterborniella nigrohalteralis					+						
Paratendipes albimanus	+	+	+	+			+	+			
Paratendipes nudisquama				+							
Paratendipes sp.	+										
Polypedilum (*Tripodura*) *bicrenatum*			+	+			+			+	+
P. (*Uresipedilum*) *convictum*	+	+	+	+	+		+			+	
P. (*Pentapedilum*) *exsectum*		+		+							
P. (*Polypedilum*) *nubeculosum*	+	+	+	+	+		+			+	+
P. (*Polypedilum*) *nubifer*										+	
P. (*Tripodura*) *scalaenum*	+	+	+	+	+				+	+	
P. (*Pentapedilum*) *sordens*											
Polypedilum (*Tripodura*) *tetracrenatum*	+										
Polypedilum sp.	+		+								
Stictochironomus crassiforceps				+							
S. rosenschoeldi				+							+
Stictochironomus sp.	+				+						+
Synendotendipes dispar	+	+	+								
S. impar	+	+	+	+	+					+	+

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Identification of *Chironomus* (*Chironomus*) *melanescens* Keyl, 1962 in North America

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Chironomus melanescens was originally described by Keyl (1962) on the basis of the morphology of polytene chromosome arms A, E, and F in populations from Germany. Keyl ascribed the name to Strenzke, but although Strenzke collected and reared the specimens, he died before he was able to describe them. Keyl also had used the name in a 1961 paper but there was no information that could be considered a species description (i.e. it was a nomen nudum). The type localities in Keyl (1962) are given as Duemer Lake and a pool south of Clauthal-Zellerfeld. Wülker et al. (1981) quoted a personal communication from Keyl in which he nominated the type specimen as slide S1149 B2 (misread as S1149 82), and giving the locality as Harz, Acker, which is a loose reference to Clauthal-Zellerfield. The latter paper gave a more full description of the chromosomes as well as some information about the adult male and the larva of material from Germany and Switzerland, although specifically aimed at distinguishing *C. melanescens* from the closely related *C. holomelas* Keyl, 1962 and *C. saxatilis* Wülker et al., 1981. Kiknadze et al. (1991) illustrated the larva and redescribed the cytology from Russian populations.

Larvae from Ontario and Wisconsin, labelled as 'Species e' in Martin (2015) were found to belong to the pseudothummi-cytocomplex, which is uncommon in North America. Analysis of the mitochondrial cytochrome c oxidase subunit 1 (COI) barcode sequence from two larvae indicated that they differed by only 2.6-3.4% from the European *C. melanescens* sequence of Guryev et al. (2001) (GenBank accession number AF 192204). The conspecificity of the specimens from the two regions was confirmed by a comparison of the available morphological data and the banding patterns of the polytene chromosomes.

The purpose of this note is to provide information on North American specimens, compared to Palearctic descriptions, which are in German or Russian, so they will be more accessible for North American workers. Terminology generally follows Sæther (1980), larval characters essentially as Proulx et al. (2013). VMR is the ratio of the anterior marginal band of the ventromentum to the distance to the base of the striae (X/Y in Fig. 3d).

Description

Adult male: Some adults were also collected and some are in the collection of J.E. Sublette in the museum of the University of Minnesota, for which limited data was obtained (e.g. hypopygium, Fig. 1). However, one reared male from Wisconsin was available for study and details are listed here with comparison to characters of Palearctic specimens (in brackets) where these are available from Wülker et al. (1981).

Wing length 3.74 mm (3.42-4.66), wing width 0.99 mm; AR 3.7 (3.20-4.58); LR 1.52 (1.42-1.58); FeI/TiI 1.05 (1.00-1.11); BR 2.0-2.2 (1.5-3.0).

Length/width of frontal tubercles 22 x 10 µm; lengths of palpomeres (µm) 50:53:230:255:355. Clypeal setae 43. thoracic setae: at least 13 acrostichals; 22 dorsolaterals; 5-6 prealars; 1 supra-alar; scutellars in approximately three rows, posterior row with 19 setae, other two rows less clearly defined and comprising 25 setae.Three sensilla campaniforia on brachiolum of wing, 26 setae in squamal fringe.

Leg proportions (in µm) and ratios:

	Fe	Ti	Ta1	Ta2	Ta3
PI	1400	1330	2025	995	835
PII	1455	1405	880	500	365
PIII	1710	1745	1335	735	580
	Ta4	**Ta5**	**LR**	**Fe/Ti**	**BR**
PI	755	380	1.52	1.05	2.0-2.2
PII	255	175	0.63	1.04	-
PIII	335	195	0.76	0.76	-

Abdominal tergites with brown bands across the anterior part, darker along the midline, becoming more extended on the more posterior segments. Nine (4-16) setae in center of 9th tergite. Hypopygium (Fig. 1a) as that of European specimens of *C. melanescens* in being similar to *C. riparius* Meigen, 1804, with a superior volsella of the S-type (Strenzke 1959). Setae of inferior volsella simple. Gonostylus relatively gradually tapered from about two thirds along its length.

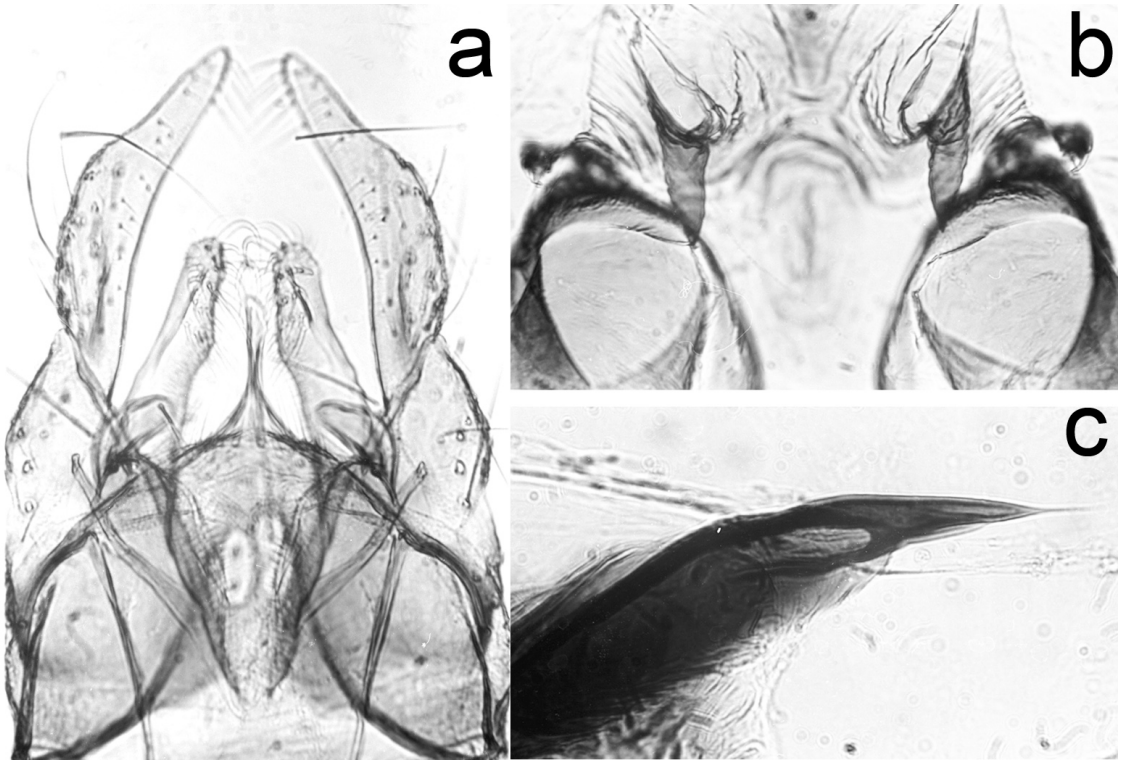

Figure 1. Reared male from Trout Lake, Vilas Co., Wisconsin. a) male hypopygium; b) pupal cephalic tubercles; c) spur of segment VIII.

Pupa: Typical of the genus, light brown in color. Length about 8.3 mm, posterior margin of wing case 3.5 mm. Cephalic tubercles (Fig. 1b) about 55 µm in length, slightly longer than their basal diameter.

Basal ring of respiratory organ about 56 x 48 µm, prealar tubercle weakly developed, about 50 µm in length.

About 51 recurved hooks on posterior margin of tergite II, central hooks with a small spine dorsally; hook row approximately half of width of the segment. Light shagreen pattern particularly near the centerline towards the rear of the segments, small adhesion marks on segments I-III; obvious pedes spurii B on segment III and large pedes spurii A on segment IV; posterolateral spur of segment VIII with 1 - 2 spines (Fig. 1c). Hair fringe on each side of the anal lobe with about 75 filamentous setae.

Larva: Medium sized (length, female about 13.0 - 16.5 mm, male 10.3 mm), bathophilus-type with ventral tubules of equal length (ant. 0.8 - 2.75 mm; post. 0.8 - 2.73 mm); anal tubules (Fig. 2) long, about 6 times longer than wide.

Gular region pale to slightly dark on posterior third, frontoclypeus pale to slightly darkened. Mentum (Fig. 3c) with pointed teeth; 4th laterals hardly reduced (type I); c1 tooth long and narrow with c2 teeth well separated (type III). Ventromental plate (Fig. 3d) with about 37 - 43 not very obvious striae; VMR about 0.35-0.41 of distance to base of striae. Pecten epipharyngis (Fig. 3a) with about 13 - 16 moderately broad sharp teeth, although larvae from the Clarence Creek population had the pectin epipharyngis and its teeth somewhat deformed. Premandible (Fig. 3b) with teeth about equally long, unless outer more worn, inner tooth about 1.6-2.3 times the width of outer tooth. Antenna (Fig. 3e) with relatively long, narrow basal segment, about 4

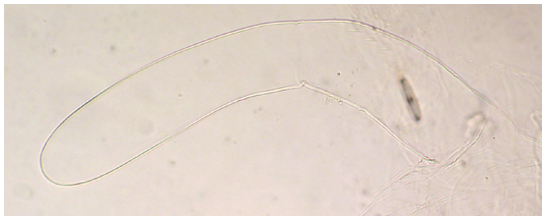

Figure 2. Anal tubule of North American larva.

times as long as wide, with ring organ between a third and half way up from base; AR about 1.88 - 2.3; ratio of segments (in µm) about 183:43:13:15:9. Mandible (Fig. 3f) with 3rd inner tooth only slightly darkened and partly to nearly completely separated (type II-IIIB), and with about 11 - 14 furrows on the outer surface at the base.

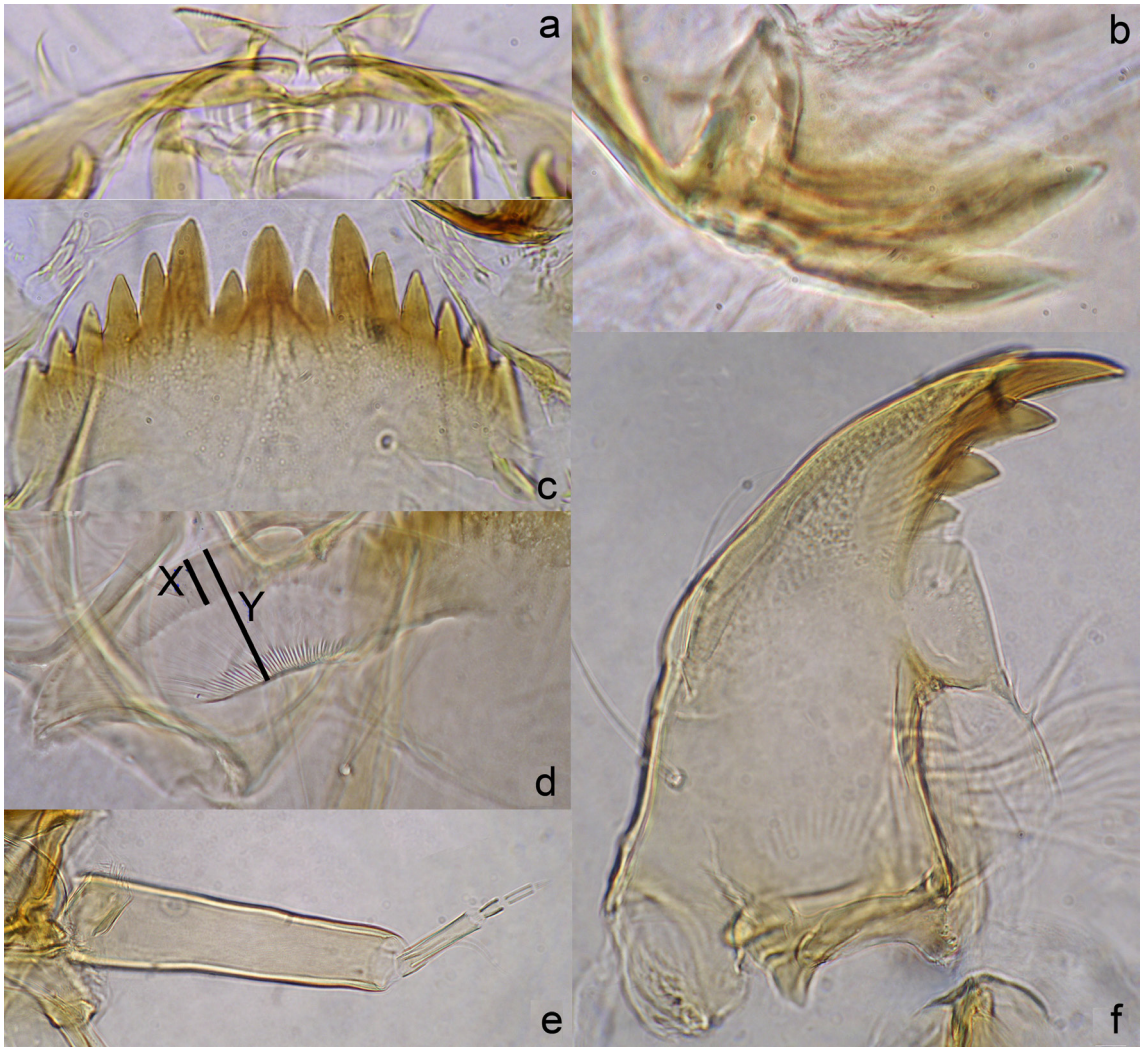

Figure 3. Larval mouth parts of North American C. melanescens. a) pecten epipharyngis; b) premandible; c) mentum; d) ventromentum; e) antenna; f) mandible.

Ventral tubules of Wisconsin specimens were much longer than those from Ontario.

These larval characters are similar to those shown in the excellent figures in Kiknadze et al. (1991) and compatible with the few characters given by Wülker et al. (1981)

Cytology: Four polytene chromosomes with pseudothummi-cytocomplex arm combination AE, BF, CD, G (Fig. 4). Sequences are as in Palearctic populations except in arm B, which is inverted compared with the Palearctic sequence. Polymorphism for simple paracentric inversions is recorded for arms A, C and G, the inversions also being present in the Palearctic. Arm G is generally paired unless heterozygous, with a sub terminal nucleolus and 2 Balbiani rings which vary in position depending on the sequence. No nucleoli occur in the other arms.

DNA Barcodes: A COI barcode sequence of Palearctic *C. melanescens* was published by Guryev et al. (2001) (GenBank accession number AF192204), and at least partial sequences have been obtained from two North American populations (Clarence Creek, Carleton Co., Ontario, Canada (45.50° N, 75.22° W); Arboretum, Madison, Dane Co., Wisconsin (43.08° N, 89.42° W) using the same primers as Guryev et al. (2001).

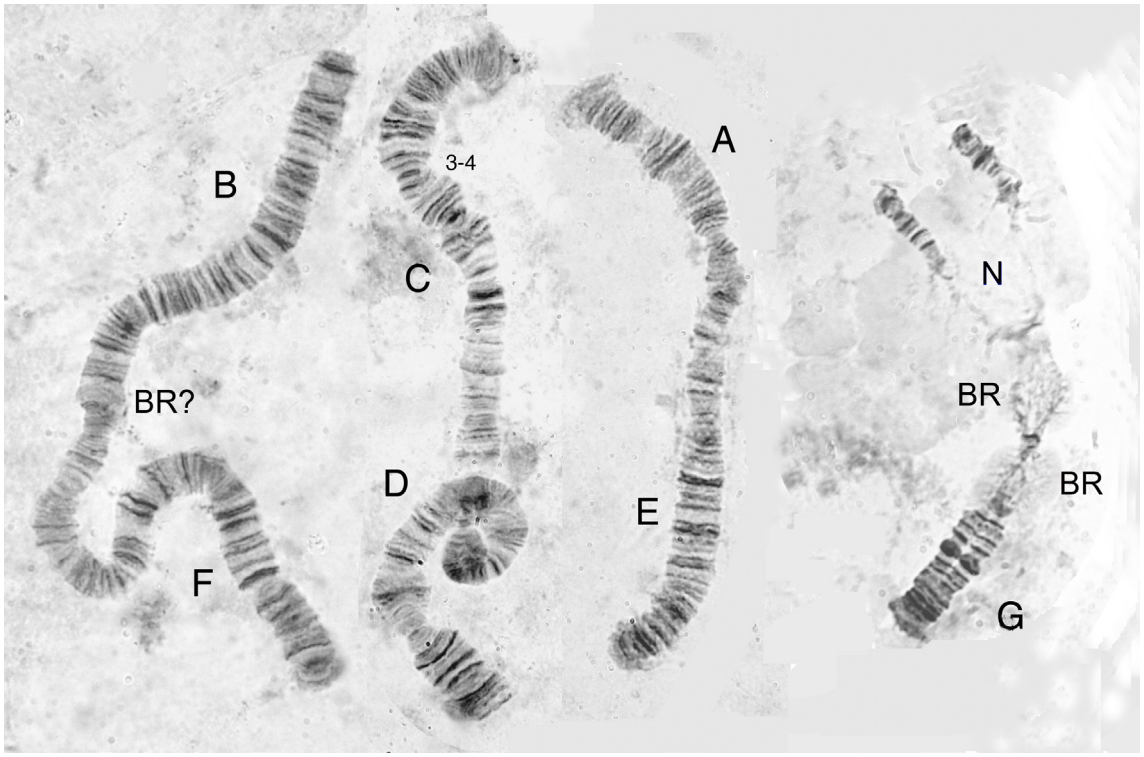

Figure 4. Polytene chromosomes of *C. melanescens*. Chromosome arms on Keyl (1962) system (A-G); nucleolus (N); Balbiani rings (BR).

A further 19 sequences from Ontario and one from Nova Scotia, from GenBank and the BOLD database, have been included in the comparison of uncorrected pairwise genetic distance. The 21 Nearctic sequences represented 7 haplotypes which varied by 0.2-1.3%. The distance between the Palearctic sequence and those from the Nearctic range from about 2.6-3.7%. Since the morphology and cytology confirm that all refer to a single species, the COI divergence is regarded as intraspecific variation. Speculation about the significance of the Palearctic/Nearctic difference is best left until data on variation across the Palearctic are available – at present even the locality of the existing specimen is uncertain, although it likely to be from Russia.

Conclusions

The morphology and polytene chromosome patterns confirm that the North American taxon provisionally called "species e" is conspecific with *C. melanescens.* Emphasis in this and previous studies of Palearctic material has been on the larvae. It seems likely that they can be identified by the combination of a medium sized bathophilus type larva with anal tubules over 6x longer than wide; pale or slightly darkened gula and frontoclypeus; a mentum of type I with the central trifid tooth of type III; and a mandible with the third inner tooth partially to completely separated, but only slightly darkened. Other characters may also be useful, but they can be variable. It also cannot be guaranteed that there are no other currently insufficiently studied species whose larvae share this combination of characters.

Acknowledgements

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First records of *Lasiodiamesa gracilis* (Kieffer, 1924), *Parochlus kiefferi* (Garrett, 1925) and several other Chironomidae from the Czech Republic and Slovakia

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The species composition of the Chironomidae fauna in the Czech Republic is still poorly known despite a long tradition in Chironomidae research, already established by Prof. Jan Zavřel in the beginning of the 20th century. The major reasons are a low number of researchers dealing with Chironomidae in the Czech Republic and their traditional focus on larvae, which usually cannot be identified to species. As a result, only a small portion of the chironomid fauna living in the Czech Republic has been discovered so far and many chironomid species are first to be recorded in the future. This concerns both (common) species that are already known from Slovakia and other neighbouring countries, and species living in poorly studied habitats, such as montane springs or bogs.

Times are getting better. Recently, Ivan Skála (2011, 2012, 2013, 2014) contributed to the list of species known from the Czech Republic and the last Chironomidae Symposium revealed even a new subfamily (Ashe et al. 2014).

The first author of this paper came across some new records as well, and this is the first time an annotated list of these species from the Czech Republic is provided.

Podonominae

In Europe, the representatives of the subfamily Podonominae are found mostly in Scandinavia, but they are also known from several other countries across Europe including all countries neighbouring the Czech Republic (Bitušík and Brabec 2009, Sæther and Spies 2015). The Czech Republic has been an exception. Two species of Podonominae are now recorded from the Czech Republic for the first time.

Lasiodiamesa gracilis (Kieffer 1924)

During the first-author's (VS) visits to his grandmother living in Škrdlovice he regularly collected pupal exuviae from a peat pool within a mineral poor *Sphagnum* fen, Radostínské rašeliniště National Nature Reserve (Fig. 1). This small reserve is located in the Žďárské Vrchy Protected Landscape Area within the Bohemian-Moravian Highlands. VS found a single pupal exuviae of *Lasiodiamesa* on 14 May 2011 and identified it as *L. gracilis*. Despite the fact that the identification was confirmed by P. Langton at the Chironomid Symposium in Trondheim a few months later, VS felt uncertainty about it because of subtle characters that discriminate this species from *L. sphagnicola* (Kieffer 1925).

During one of his next visits, 15 March 2015 VS succeeded to collect tens of larvae of *Lasiodiamesa* from the same locality and reared two male and several female adults. Adult males bear much better characters than pupae and the two males could be easily identified as *L. gracilis* using the key by Brundin (1966).

The habitat where the species was found corresponds well with the known restriction of the genus to *Sphagnum* bogs in the Middle Europe, where it is considered a glacial relict, as stated by Brundin (1966). Radostínské rašeliniště *Sphagnum* bog is the only place in the Bohemian-Moravian Highlands where pools are developed, while the closest *Sphagnum* bogs with pools may be found as far away as in Sudetenland. Indeed, the closest known locality is the type locality just across the Czech/Poland border in Poland some 90 km to the north from the Radostínské rašeliniště (Brundin 1966, Sæther and Spies 2015). Other populations may be expected in Sudetenland, however VS has been unsuccessful there so far. This might be caused by the rarity of the species as stated by Verberk et al. (2003), but also by unsuitable time of the author's sampling – in late spring and summer the species might be already emerged and can not be found in water anymore. Other known localities in Europe are in The Netherlands (Verberk et al. 2003) and in Scandinavia (Brundin 1966, Sæther and Spies 2015).

Figure 1. Radostínské rašeliniště National Nature Reserve, *Lasiodiamesa gracilis* sampling site (site nr. *S15* in the List). Photograph taken during sampling 22 March 2015.

The chironomid assemblage at Radostínské rašeliniště was dominated by *Monopelopia tenuicalcar* (Kieffer 1918) and *Corynoneura* spp. in early spring, in late spring also by *Psectrocladius bisetus* Goetghebuer 1942 and *Psectrocladius oligosetus* Wülker 1956. Throughout the year *Macropelopia adaucta* Kieffer 1916, *Procladius choreus* (Meigen 1804), *Ablabesmyia phatta* (Egger 1864) and *Telmatopelopia nemorum* (Goetghebuer 1921) were found. Full record information is summarized in Table 1.

Parochlus kiefferi (Garrett 1925)

On a cross-country skiing trip on 20 February 2015 in the Krkonoše Mountains National Park (Giant Mts.) VS collected a tuft of decomposing vegetation with mosses from a helocrene spring located just next to a ski-track (Fig. 2). This spring is usually covered with snow in the winter, but due to the combination of lack of snowfall during the previous days, sunny weather and slope exposition to the south, it was uncommonly snow-free at that time. The inspection of the collected material back in the laboratory revealed one larva of *Parochlus*.

The identification of *Parochlus* larvae into species is not possible, but only a single species *P. kiefferi* is known from the northern hemisphere. Thus, the collected specimen most certainly belongs to this species.

P. kiefferi seems to be cold-stenothermic, living mostly in cold springs and upper courses of spring-fed streams (Brundin 1966). It was also reported from large streams with strong current in North America (Brundin 1966) or from moss in a pond fed by ice-melt water in Italian Alps (Lencioni et al. 2007).

It is possible that the species emerges just after the snow melts and is no longer present at the locality, as was observed by Wülker (1958) in Feldberg springs. Such phenology would prevent the species from being recorded by conventional sampling, which is usually carried out in the vegetation season. It is worth noting, however, that in some regions the species emerges practically throughout the whole snow-free period: from the beginning of June to the beginning of October in Swedish mountains or from the end of April to the beginning of November in temperate Canada (Brundin 1966). In the Italian Alps, Lencioni et al. (2007) recorded a pupal exuviae in the middle of September. Please see Table 1 for summarized information.

Other species new to the Czech Republic

Traditionally, the area of the Czech Republic is divided into Bohemia (the western part) and Moravia (the eastern part). New records are therefore designated for these parts separately. Apart from the two Podonominae species we recorded further 111 chironomid species that are new to either part of Czech Republic, to Czech Republic as a whole, or exceptionally to Slovakia. All these records are summarized in Table 1.

As the species were recorded from 65 localities (Appendix 1), we use abbreviations to refer to them in the table. Most localities may be classified either as helocrene springs (abbreviation starts with *H* followed by a number), flowing waters (*F*) or standing waters (*S*). Two highly artificial localities were kept separately as other (*O*). The details about the localities are given in appendix 1.

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Figure 2. A spring close to Nová Klínovka chalet (in the background) in the eastern part of a meadow called Klínové Boudy, The Krkonoše Mountains National Park. *Parochlus kiefferi* sampling site (site nr. *H11* in the List). Photographed during sampling 20 February 2015.

Table 1. The list of species new to Bohemia (B), Moravia (M), Czech Republic (CZ) or Slovakia (SK). Subfamilies/tribes are listed taxonomically, species within subfamilies/tribes are in alphabetical order. The column **New to** describes the area for which a species is reported for the first time. Taxa new to Bohemia or Moravia indicated with B or M have already been reported from the other part of the Czech Republic. Species new to the Czech Republic are indicated with region in parentheses. The column **Record details** includes date of collection, locality abbreviation (*in italic*): number of larvae (L), pupae (P), pupal exuviae (PE), imagines (I). All material was identifed by the first author, except where noted. Localities are listed in the following habitat order: Helocrene spring (H), flowing water (F), standing water (S), other (O). See appendix 1 for list of localities.

Species	New to	Record details
PODONOMINAE		
Lasiodiamesa gracilis	CZ(B)	**B**: 14 May 2011, *S15*: 1PE, det. P. Langton; 15 Mar 2015, *S15*: 2 reared I♂
Parochlus kiefferi	CZ(B)	**B**: 22 Feb 2015, *H11*: 1L
TANYPODINAE-Procladiini		
Procladius crassinervis	CZ(B)	**B**: 29 May 2015, *S1*: 5PE; 17 Oct 2013, *S7*: 1PE, leg. D. Němejcová.
TANYPODINAE-Pentaneurini		
Ablabesmyia longistyla	CZ(B,M)	B: 24 Apr 2007, *F28*:C6428: 1PE, leg. M. Straka, det. P. Langton. M: 26 Sep 2006, *F1*: 2PE, det. P. Langton.
Ablabesmyia phatta	CZ(B)	**B**: 16 May 2007, *S4*: 4PE+2L; 20 May 2007, *S15*: 1PE.
Conchapelopia hittmairorum	CZ(M)	**M**: 15 Sep 2006, *F1*: 2PE; 26 Sep 2006, *F1*: 1PE, all det. P. Langton.
Conchapelopia viator	M	**M**: 15 Sep 2006, *F1*: 1PE, det. P. Langton.

Species	New to	Record details
Hayesomyia tripunctata	M	**M**: 23 Aug 2005, *F1*: 1PE, det. P. Langton.
Thienemannimyia pseudocarnea	B	**B**: 24 Apr 2007, *F28*: 4PE, leg. M. Straka.
Zavrelimyia barbatipes	B	**B**: 21 Aug 2006, *F21*: 1PE, det. P. Langton.
DIAMESINAE-Tribus Diamesini		
Diamesa permacra	CZ(M)	**M**: 25 Sep 2006, *H7*: 1PE, leg. J. Bojková, det. P. Langton.
Potthastia longimana	B	**B**: 24 Apr 2007, *F28*: 2PE, leg. M. Straka.
PRODIAMESINAE		
Odontomesa fulva	B	**B**: 24 Apr 2007, *F28*: 1PE, leg. M. Straka.
ORTHOCLADIINAE		
Chaetocladius melaleucus	CZ(B,M)	**B**: 24 Feb 2008, *F22*: 3PE, det. P. Langton. **M**: 25 Sep 2006, *H5*: 1PE, leg. J. Bojková.
Chaetocladius perennis	CZ(B,M)	**B**: 15 Mar 2008, *F7*: 2PE. **M**: 25 Feb 2007, *F8*: 2PE, det. P. Langton.
Corynoneura celtica	B	**B**: 14 May 2007, *F26*: 1PE.
Corynoneura gratias	B	**B**: 24 Sep 2007, *S16*: 3PE, det. P. Langton.
Corynoneura lacustris	CZ(B,M)	**B**: 29 Apr 2008, *F13*: 1PE; 24 Sep 2007, *F20*: 6PE, det. P. Langton; 09 Apr 2007, F30: 3PE, det. P. Langton. **M**: 19 Nov 2006, *F15*: 1PE, det. P. Langton; 29 Aug 2006, *F27*: 1PE, det. P. Langton.
Corynoneura lobata	CZ(B)	**B**: 29 Apr 2008, *F13*: 7PE; 24 Sep 2007, *F19*: 7PE; 09 Apr 2007, *F22*: 6PE, det. P. Langton.
Corynoneura scutellata	M	**M**: 30 Aug 2006, *S9*: 4PE; 27 Aug 2008, *S20*: 10PE, leg. P. Komzák & S. Větříček; 28 May 2008, *S20*: 3PE, leg. P. Komzák & S. Větříček.
Cricotopus albiforceps	B	**B**: 29 Apr 2008, *F13*: 4PE; 24 Apr 2007, *F28*: 1PE, leg. M. Straka, all det. P. Langton.
Cricotopus festivellus	CZ(B)	**B**: 23 Sep 2007, *S19*: 6PE, det. P. Langton.
Cricotopus tremulus	CZ(B,M)	**B**: 24 Sep 2007, *F20*: 1PE, det. P. Langton. **M**: 29 Apr 2007, *F5*: 4PE; 04 Oct 2002, *F25*: 1PE.
Eukiefferiella coerulescens	B	**B**: 24 Sep 2007, *F19*: 5PE; 14 May 2007, *F26*: 4PE, det. P. Langton; 23 Sep 2007, *F29*: 6PE.
Eukiefferiella devonica	M	**M**: 31 Mar 2007, *F2*: 8PE; 31 Mar 2007, *F3*: 4PE; 31 Mar 2007, *F4*: 7PE; 31 Mar 2007, *F12*: 6PE; 03 Oct 2002, *F25*: 3PE.
Eukiefferiella dittmari	CZ(B,M)	**B**: 23 Sep 2007, *F29*: 1PE, det. P. Langton. **M**: 31 Mar 2007, *F2*: 1PE.
Eukiefferiella fittkaui	CZ(M)	**M**: 18 May 2011, *F9*: 1PE; 20 Apr 2011, *F9*: 3PE.
Eukiefferiella fuldensis	B	**B**: 20 Apr 2007, *F11*: 10PE, det. P. Langton; 24 Sep 2007, *F19*: 1PE; 24 Sep 2007, *F20*: 1PE; 14 May 2007, *F26*: 5PE.
Eukiefferiella tirolensis	CZ(M)	**M**: 31 Mar 2007, *F12*: 1PE, det. P. Langton; 03 Oct 2002, *F25*: 1PE; 29 Oct 2008, *F25*: 1PE.
Heleniella ornaticollis	B	**B**: 20 Apr 2007, *F11*: 2PE, det. P. Langton; 14 May 2007, *F26*: 1PE.
Heterotanytarsus apicalis	M	**M**: 09 May 2006, *H7*: 1P; 25 Sep 2006, *H7*: 6P+25L, all leg. J. Bojková.

Species	New to	Record details
Hydrobaenus lugubris	M	**M**: 19 Nov 2006, *S8*: 1PE, det. P. Langton.
Krenosmittia boreoalpina	CZ(B,M)	**B**: 14 May 2007, *F26*: 3PE. **M**: 18 May 2011, *F9*: 2PE.
Limnophyes edwardsi	CZ(B,M)	**B**: 09 Apr 2007, *F22*: 1PE, det. P. Langton. **M**: 31 Mar 2007, *F3*: 1PE, det. P. Langton.
Limnophyes punctipennis	CZ(M)	**M**: 21 Apr 2008, *S12*: 1PE, leg. P. Komzák & S. Větříček; 24 Sep 2015, *O1*: 1 reared I ♀ (parthenogenetic) + 2 P, leg. K. Benesch.
Limnophyes spinigus	CZ(B)	**B**: 23 Sep 2007, *F29*: 1PE, det. P. Langton.
Nanocladius balticus	CZ(M)	**M**: 22 Jul 2008, *S20*: 1PE; 25 Jun 2008, *S20*: 1PE; 27 Aug 2008, *S20*: 3PE, all leg. P. Komzák & S. Větříček.
Nanocladius parvulus	CZ(B)	**B**: 14 May 2007, *F26*: 1PE.
Orthocladius ashei	B	**B**: 24 Apr 2007, *F28*: 1PE, leg. M. Straka.
Orthocladius glabripennis	M	**M**: 31 Mar 2007, *F2*: 2PE, det. P. Langton; 31 Mar 2007, *F3*: 1PE; 31 Mar 2007, *F4*: 2PE; 10 Apr 2012, *F24*: 1 reared pharate imago; 21 Apr 2008, *S10*: 1PE, leg. P. Komzák & S. Větříček; 21 Apr 2008, *S11*: 1PE, leg. P. Komzák & S. Větříček; 21 Apr 2008, *S12*: 2PE, leg. P. Komzák & S. Větříček.
Orthocladius lignicola	CZ(B,M)	**B**: 09 Apr 2007, *F30*: 1PE. **M**: 29 Apr 2007, *H4*: 1L; 26 Apr 2012, *F10*: 6L; 18 May 2011, *F9*: 1PE; 20 Apr 2011, *F9*: 8PE.
Orthocladius oblidens	B	**B**: 29 Apr 2008, *F13*: 1PE.
Orthocladius pedestris	B	**B**: 20 Apr 2007, *F11*: 5PE, det. P. Langton; 29 Apr 2008, *F13*: 1PE.
Orthocladius saxosus	B	**B**: 20 Apr 2007, *F11*: 5PE; 24 Sep 2007, *F19*: 4PE.
Paracladius conversus	CZ(B)	**B**: 17 Oct 2013, *S7*: 17PE; 24 Jul 2013, *S7*: 1PE; 29 Aug 2013, *S7*: 1PE, all leg. D. Němejcová.
Parakiefferiella bathophila	CZ(B,M)	**B**: 29 May 2015, *S1*: 6PE; 30 May 2015, *S6*: 1PE, leg. D. Němejcová; 28 May 2015, *S13*: 10PE. M: 03 Oct 2002, *F25*: 1PE, det. P. Langton; 29 Aug 2006, *F27*: 1PE, det. P. Langton.
Psectrocladius limbatellus	B	**B**: 23 Sep 2007, *S19*: 2PE.
Psectrocladius octomaculatus	CZ(B)	**B**: 28 May 2015, *S13*: 1PE.
Psectrocladius oxyura	CZ(B,M)	**B**: 30 May 2015, *S6*: 2PE; 17 Oct 2013, *S7*: 3PE; 24 Jul 2013, *S7*: 1PE, all leg. D. Němejcová. **M**: 28 Aug 2008, *S10*: 1PE; 22 Apr 2008, *S20*: 6PE; 22 Jul 2008, *S20*: 2PE; 25 Jun 2008, *S20*: 2PE; 27 Aug 2008, *S20*: 2PE; 28 May 2008, *S20*: 14PE, all leg. P. Komzák & S. Větříček.
Psectrocladius platypus	CZ(B)	**B**: 24 Sep 2007, *S16*: 11PE; 12 Oct 2008, *S17*: 1PE.
Psectrocladius schlienzi	CZ(B)	**B**: 23 Sep 2007, *S19*: 4PE.
Rheocricotopus atripes	CZ(M)	**M**: 11 May 2006, *H1*: 1PE, leg. J. Bojková, det. P. Langton.
Rheocricotopus effusus	B	**B**: 24 Sep 2007, *F19*: 5PE, det. P. Langton; 14 May 2007, *F26*: 1PE.
Thienemanniella vittata	B	**B**: 29 Apr 2008, *F13*: 3PE; 24 Sep 2007, *F20*: 1PE.

Species	New to	Record details
CHIRONOMINAE-Chironomini		
Chironomus acidophilus	CZ(B)	**B**: 23 Apr 2007, *S21*: 1PE, det. P. Langton.
Chironomus anthracinus	CZ(B)	**B**: 16 May 2007, *S4*: 2PE, det. P. Langton.
Chironomus entis	M	**M**: 02 May 2005, *F1*: 1PE, det. P. Langton.
Chironomus lacunarius	CZ(B)	**B**: 16 May 2007, *S4*: 2PE; 23 Apr 2007, *S21*: 1PE, all det. P. Langton.
Chironomus lugubris	CZ(B)	**B**: 20 Aug 2006, *S18*: 1PE, det, P. Langton.
Chironomus luridus	M	**M**: 02 Sep 2006, *O2*: 4PE, leg. J. Sychra, det. P. Langton.
Chironomus montuosus	CZ(B)	**B**: 16 May 2007, *S4*: 6PE, det. P. Langton.
Cladopelma virescens	B	**B**: 30 Aug 2013, *S3*: 2PE, leg. D. Němejcová.
Cladopelma viridulum	CZ(B)	**B**: 30 Aug 2013, *S3*: 2PE, leg. D. Němejcová.
Cryptochironomus obreptans	B	**B**: 29 Aug 2013, *S7*: 1PE, leg. D. Němejcová.
Cryptochironomus supplicans	CZ(M)	**M**: 22 Jul 2008, *S20*: 3PE; 25 Jun 2008, *S20*: 1PE, all leg. P. Komzák & S. Větříček.
Cryptotendipes holsatus	CZ(M)	**M**: 30 Aug 2006, *S9*: 5PE.
Cryptotendipes pseudotener	M	**M**: 31 Jul 2012, *F14*: 1 reared P.
Cryptotendipes usmaensis	CZ(B,M)	**B**: 24 Jul 2013, *S3*: 2PE; 24 Jul 2013, *S7*: 9PE, leg. D. Němejcová; 29 Aug 2013, *S7*: 1PE, leg. D. Němejcová. **M**: 24 Jun 2008, *S11*: 1PE, leg. P. Komzák & S. Větříček; 28 Aug 2008, *S11*: 1PE, leg. P. Komzák & S. Větříček.
Dicrotendipes pulsus	CZ(M)	**M**: 22 Jul 2008, *S20*: 70PE, leg. P. Komzák & S. Větříček; 25 Jun 2008, *S20*: 39PE, leg. P. Komzák & S. Větříček; 27 Aug 2008, *S20*: 25PE, leg. P. Komzák & S. Větříček.
Glyptotendipes paripes	M	**M**: 24 Jun 2008, *S10*: 6PE; 28 Jul 2008, *S10*: 1PE; 29 May 2008, *S10*: 1PE; 24 Jun 2008, *S11*: 25PE; 28 Jul 2008, *S11*: 4PE; 24 Jun 2008, *S12*: 4PE; 28 Aug 2008, *S12*: 1PE; 28 Jul 2008, *S12*: 14PE; 29 May 2008, *S12*: 3PE, all leg. P. Komzák & S. Větříček.
Harnischia curtilamellata	CZ(B,M)	**B**: 24 Jul 2013, *S3*: 1PE; 24 Jul 2013, *S7*: 3PE, leg. D. Němejcová; 29 Aug 2013, *S7*: 1PE, leg. D. Němejcová. **M**: 24 Jun 2008, *S11*: 1PE, leg. P. Komzák & S. Větříček.
Kiefferulus tendipediformis	B	**B**: 30 Aug 2013, *S3*: 2PE, leg. D. Němejcová.
Microchironomus tener	B	**B**: 23 Jul 2013, *S5*: 1PE; 29 Aug 2013, *S5*: 1PE, all leg. D. Němejcová.
Microtendipes confinis	CZ(B)	**B**: 24 Apr 2007, *F28*: 1PE, leg. M. Straka, det. P. Langton.
Microtendipes diffinis	B	**B**: 29 Apr 2008, *F13*: 3PE, det. P. Langton.
Paracladopelma nigritula	CZ(B)	**B**: 24 Jul 2013, *S7*: 37PE, leg. D. Němejcová.
Parachironomus parilis	B	**B**: 23 Sep 2007, *S19*: 1PE.
Parachironomus vitiosus	CZ(M)	**M**: 28 May 2008, *S20*: 2PE, leg. P. Komzák & S. Větříček.
Paralauterborniella nigrohalteralis	CZ(M)	**M**: 25 Jul 2012, *F17*: 1PE.

Species	New to	Record details
Paratendipes nudisquama	CZ(M)	**M**: 10 Jul 2006, *H7*: 3L; 29 Apr 2007, *H9*: 31L, all leg. J Bojková.
Phaenopsectra flavipes	B	**B**: 29 May 2015, *S1*: 20PE; 29 May 2015, *S2*: 3PE; 30 Aug 2013, *S3*: 3PE, leg. D. Němejcová; 28 May 2015, *S13*: 20PE; 30 May 2015, *S14*: 9PE.
Polypedilum albicorne	CZ(B,M)	**B**: 24 Sep 2007, *F20*: 1PE, det. P. Langton; 29 May 2015, *S2*: 1PE; 28 May 2015, *S13*: 8PE. **M**: 03 Oct 2002, *F25*: 1PE; 29 Aug 2006, *F27*: 1PE.
Polypedilum nubens	CZ(M)	**M**: 25 Sep 2008, *S12*: 1PE; 22 Jul 2008, *S20*: 2PE; 27 Aug 2008, *S20*: 16PE, all leg. P. Komzák & S. Větříček.
Polypedilum sordens	CZ(B,M)	**B**: 24 Jul 2013, *S3*: 1PE; 23 Jul 2013, *S5*: 2PE, leg. D. Němejcová. **M**: 25 Sep 2008, *S11*: 5PE, leg. P. Komzák & S. Větříček; 28 Jul 2008, *S12*: 2PE, leg. P. Komzák & S. Větříček.
Polypedilum tritum	CZ(M)	**M**: 25 Jun 2008, *S20*: 1PE, leg. P. Komzák & S. Větříček.
Robackia demeijerei	CZ(M)	**M**: 25 Jul 2012, *F17*: 1L.
Stenochironomus gibbus	CZ(B)	**B**: 24 Jul 2013, *S7*: 1PE, leg. D. Němejcová.
Tribelos intextum	CZ(B)	**B**: 29 May 2015, *S1*: 14PE.
Tribelos intextus	CZ(B)	**B**: 24 Jul 2013, *S3*: 1PE.
Xenochironomus xenolabis	M	**M**: 24 Jun 2008, *S10*: 1PE; 28 Aug 2008, *S10*: 2PE; 29 May 2008, *S10*: 2PE, all leg. P. Komzák & S. Větříček.
CHIRONOMINAE-Tanytarsini		
Cladotanytarsus lepidocalcar	CZ(M)	**M**: 30 Aug 2006, *S9*: 1PE, det. P. Langton; 28 Jul 2008, *S10*: 4PE, leg. P. Komzák & S. Větříček; 25 Sep 2008, *S11*: 7PE, leg. P. Komzák & S. Větříček; 28 Aug 2008, *S11*: 9PE, leg. P. Komzák & S. Větříček; 28 Aug 2008, *S12*: 3PE, leg. P. Komzák & S. Větříček; 28 Jul 2008, *S12*: 2PE, leg. P. Komzák & S. Větříček; 22 Jul 2008, *S20*: 59PE, leg. P. Komzák & S. Větříček; 25 Jun 2008, *S20*: 31PE, leg. P. Komzák & S. Větříček; 27 Aug 2008, *S20*: 104PE, leg. P. Komzák & S. Větříček; 28 May 2008, *S20*: 8PE, leg. P. Komzák & S. Větříček.
Lithotanytarsus emarginatus	CZ(M), SK	**M**: 20 Apr 2011, *F9*: 4L**.** **SK**: 13 May 2012, *F6*: 5L; 22 Apr 2013, *F31*: 2L.
Micropsectra apposita	CZ(M)	**M**: 31 Mar 2007, *F4*: 4PE, det. P. Langton.
Micropsectra atrofasciata	CZ(B,M)	**B**: 24 Sep 2007, *F19*: 5PE; 24 Sep 2007, *F20*: 12PE; 09 Apr 2007, F22: 4PE, det. P. Langton. **M**: 31 Mar 2007, *F2*: 4PE, det. P. Langton; 31 Mar 2007, *F4*: 7PE; 14 Oct 2007, *F23*: 2PE; 29 Oct 2008, *F25*: 4PE.
Micropsectra bidentata	B	**B**: 29 Apr 2008, *F13*: 1PE; 24 Sep 2007, *F19*: 3PE.
Micropsectra junci	B	**B**: 24 Sep 2007, *F20*: 1PE, det. P. Langton.
Micropsectra lindrothi	CZ(B,M)	**B**: 09 Apr 2007, *F22*: 1PE. **M**: 30 Aug 2006, *S9*: 1PE, all det. P. Langton.

Species	New to	Record details
Micropsectra longicrista	CZ(M)	**M**: 04 May 2009, *H6*: 2I♂.
Micropsectra pallidula	CZ(M), SK	**M**: 14 Sep 2011, *H8*: 1P. **SK**: 13 Sep 2011, *H2*: 1P.
Neostempellina thienemanni	CZ(M)	**M**: 29 Apr 2007, *H4*: 1P+5L; 29 Apr 2007, *H10*: 1P+3L, all leg. J. Bojková.
Paratanytarsus bituberculatus	B	**B**: 24 Jul 2013, *S7*: 1PE, leg. D. Němejcová.
Paratanytarsus penicillatus	CZ(B)	**B**: 23 Apr 2007, *S21*: 1PE, det. P. Langton.
Rheotanytarsus muscicola	M	**M**: 02 May 2005, *F1*: 1PE, det. P. Langton; 03 Oct 2002, F*25*: 1PE.
Stempellina almi	CZ(B,M)	**B**: 24 Jul 2013, *S7*: 7PE, leg. D. Němejcová. **M**: 27 Aug 2008, *S20*: 1PE, leg. P. Komzák & S. Větříček.
Stempellinella edwardsi	CZ(B,M)	**B**: 29 Apr 2008, *F13*: 18PE; 24 Apr 2007, *F28*: 3PE, leg. M. Straka. **M**: 22 Jul 2008, *S20*: 3PE, leg. P. Komzák & S. Větříček; 25 Jun 2008, *S20*: 2PE, leg. P. Komzák & S. Větříček; 27 Aug 2008, *S20*: 46PE, leg. P. Komzák & S. Větříček.
Stempellinella flavidula	CZ(M)	**M**: 29 Apr 2007, *H4*: 8PE, leg. J. Bojková; 18 May 2011, *F9*: 2PE; 31 Aug 2006, *F18*: 1PE, det. P. Langton.
Tanytarsus bathophilus	CZ(B,M)	**B**: 24 Jul 2013, *S7*: 21PE, leg. D. Němejcová. **M**: 22 Jul 2008, *S20*: 41PE; 25 Jun 2008, *S20*: 12PE; 27 Aug 2008, *S20*: 4PE; 28 May 2008, *S20*: 8PE, all leg. P. Komzák & S. Větříček.
Tanytarsus curticornis	CZ(M)	**M**: 02 May 2005, *F1*: 2PE, det. P. Langton; 28 Aug 2008, *S11*: 1PE, leg. P. Komzák & S. Větříček.
Tanytarsus debilis	CZ(B)	**B**: 30 May 2015, *S14*: 1PE.
Tanytarsus ejuncidus	M	**M**: 24 Jun 2008, *S10*: 2PE; 28 Aug 2008, *S11*: 2PE; 22 Jul 2008, *S20*: 2PE; 27 Aug 2008, *S20*: 14PE, all leg. P. Komzák & S. Větříček.
Tanytarsus eminulus	M	**M**: 19 Nov 2006, *F15*: 2PE, det. P. Langton.
Tanytarsus longitarsis	CZ(B)	**B**: 29 Apr 2008, *F13*: 29PE, det. P. Langton.
Tanytarsus medius	CZ(B,M)	**B**: 24 Jul 2013, *S7*: 1PE, leg. D. Němejcová. **M**: 29 May 2008, *S10*: 9PE, leg. P. Komzák & S. Větříček.
Tanytarsus mendax	M	**M**: 30 Aug 2006, *S9*: 1PE, det. P. Langton; 24 Jun 2008, *S10*: 1PE, leg. P. Komzák & S. Větříček.
Tanytarsus telmaticus	CZ(M)	**M**: 19 Nov 2006, *S8*: 1PE, det. P. Langton.

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Appendix 1. Collection sites with details on their location and protection status.

Helocrene springs

H1 – a helocrene in Bukovec nature reserve, 2 km east of Bukovec, 49°32’57.658”N, 18°51’29.019”E, Moravia, CZ.

H2 – a helocrene in the floodplain of river Hron, south bank, approx. 2 km upstream Červená Skala, 48°49’35.645’’N, 20°9’47.527’’E, Slovakia.

H3 – a helocrene on the south margin of Horní Lomná 49°31’6.288”, 18°37’48.932”, CZ, Moravia.

H4 – helocrene Kalábová, nature monument, 1.5 km north of Březová, 48°56’23.788”N, 17°44’38.353”E, Moravia, CZ.

H5 – helocrene, Kyčmol nature monument, 2 km southwest of Horní Lomná, 49°30’46.607”N, 18°37’24.557”E, Moravia, CZ.

H6 – a helocrene approx. 1 km southeast of Lopeník 48°56’33.653”N, 17°47’52.779”E, CZ, Moravia.

H7 – a helocrene in Obidová nature monument, 400 m southwest of Visalaje 49°31’1.171”N, 18°31’26.765”E, Moravia, CZ.

H8 – helocrene V Krátkých, nature monument, approx. 2 km southwest of Vápenice, 48°57’24.443”N, 17°49’28.765”E, Moravia, CZ.

H9 – helocrene Chmelinec, nature monument, directly south of Vyškovec, 48°56’25.587”N, 17°51’19.175”E, Moravia, CZ.

H10 – a helocrene in Hutě nature reserve, approx. 2 km northeast of Žítková 48°59’25.389’’N, 17°54’19.785’’E, Moravia, CZ.

H11 – a helocrene spring above the road about 100 m east of Nová Klínovka chalet (formerly Tesla) in the eastern part of a meadow called Klínové Boudy. About 4 km southeast of Špindlerův Mlýn, The Krkonoše Mountains National Park, 50°42’30.812”N, 15°39’35.048”E, about 1230 m a.s.l., Bohemia, CZ. Fig. 2.

Flowing waters

F1 – Bečva river, 1.5 km east of Černotín, 49°32’3.041’’N, 17°47’45.601’’E, Moravia, CZ.

F2 – Bobrůvka (brook), 700 m upstream of Olešínky, 49°29’9.02”N, 16°8’53.64”E, Moravia, CZ.

F3 – Bobrůvka (brook), 800 m southeast of Podolí, 49°28’52.57”N, 16°5’28.96”E, Moravia, CZ.

F4 – Bobrůvka (brook) 1 km north of Řečice, 49°31’25.29’’N, 16°3’49.59’’E, Moravia, CZ.

F5 – Bobrůvka (brook) 400 m upstream of Strážek, 49°26’42.52”N, 16°11’21.45”E, Moravia, CZ.

F6 – Čierný potok (brook), 1.5 km west od Súľov, 49°9’44.064’’N, 18°34’4.584’’E, Slovakia.

F7 – Doubrava (brook) 1.5 km southwest of Radostín, 49°38’36.075”N, 15°51’33.729”E, Bohemia, CZ.

F8 – Fryšávka (brook) 500 m upstream of Jimramov, 49°38’12.293”N, 16°12’59.322”E, Moravia, CZ.

F9 – a brook flowing from south and touching Chmelinec nature monument at its eastern margin, 160 m east of Vyškovec 48°56’26.357”N, 17°51’19.527”E, Moravia, CZ.

F10 – Jasénka brook, 3 km upstream (northeast) of Horní Jasénka, 49°22’36.487”N, 18°1’26.930”E, Moravia, CZ.

F11 – Křemelná (brook), 3 km downstream of Prášily, 49°8’7.17”N, 13°23’7.76”E, Bohemia, CZ.

F12 – Bobrůvka (brook), 1 km east of Mirošov, above the bridge, 49°27’56.47”N, 16°10’25.28”E, Moravia, CZ.

F13 – Lužnice (river), Horní Lužnice nature reserve, approx. 2 km upstream of Halámky, 48°50’7.093”N, 14°55’41.59”E, Bohemia, CZ.

F14 – Morava (river) near Babice, 49°7’6.704”N, 17°29’30.097”E, Moravia, CZ.

F15 – Morava (river) just at the confluence with Dyje (river), 12 km south of Lanžhot, 48°37’2.400”N, 16°56’26.470”E, Moravia, CZ.

F16 – Morava (river), near Spytihněv 49°8’23.085”N, 17°30’14.110”E, Moravia, CZ.

F17 – Morava (river), Osypané Břehy nature monument, 3 km northwest of Strážnice, 48°55’17.694”N, 17°16’44.529”E, Moravia, CZ.

F18 – Radějovka (brook), below the Kejda fish-pond, 5 km upstream of Radějov, 48°52’3.635”N, 17°24’25.935”E, Moravia, CZ.

F19 – Roklanský potok (brook), Bohemian Forest NP, Modrava 1 km upstream of Rybárna, 3.5 km upstream of Modrava, 49°1’59.253”N, 13°27’37.472”E, Bohemia, CZ.

F20 – Řezná (brook), Bohemian Forest NP, 1.5 km upstream of Železná Ruda, 49°8’47.614’’N, 13°15’3.633’’E, Bohemia, CZ.

F21 – a small brook on the northern margin of Senotín, 49°4’11.328”N, 15°8’35.034”E, Bohemia, CZ.

F22 – Sklenský potok (brook), Světnovské údolí nature monument, 1,5 km northeast of Světnov, 49°37’47.07”N, 15°58’12.7”E, Bohemia, CZ.

F23 – Svratka (brook), approx. 1.5 km north of Cikháj, 49°39’25.453”N, 15°58’19.544”E, Moravia, CZ.

F24 – Svratka (river) 0.5 km downstresm of Tišnov, 49°20’10.935”N, 16°25’14.907”E, Moravia, CZ.

F25 – Svratka (river) 0.5 km downstream of Unčín, 49°36’48.839”N, 16°14’4.124”E, Moravia, CZ.

F26 – Úhlava (river), just upstream of the Zadní Hamry bridge, approx. 2 km upstream of Hamry, 49°12’13.931”N, 13°11’0.702”E, Bohemia, CZ.

F27 – Velička (brook), just upstream the downstream bridge in Vápenky, 48°52’29.032”N, 17°37’56.017”E, Moravia, CZ.

F28 – Vltava (river), upstream the bridge in Hluboká, 49°2’58.664”N, 14°26’46.393”E, Bohemia, CZ.

F29 – Vltava (river), Bohemian Forest NP, just downstream the bridge 1 km west of Pěkná, 48°51’7.639”N, 13°55’13.344”E, Bohemia, CZ.

F30 – Vortovský potok (brook), just downstream a bridge, 0.5 km upstream (south) Zlámanec fish-pond, 1.5 km upstream of Vortová, 49°41’52.39”N, 15°56’19.49”E, Bohemia, CZ.

F31 – a brook flowing from the Strážov hill to the southwest, approx 50 m upstream a bridge, 1.8 southeast of Zliechov, 48°56’46.68”N, 18°27’22.11”E, Slovakia.

Standing waters

S1 – Černé lake, Bohemian Forest NP, approx. 6 km northwest of Železná Ruda, 49°10’53.643’’N, 13°11’8.634’’E, Bohemia, CZ.

S2 – Čertovo lake, Bohemian Forest NP, approx. 4 km northwest of Železná Ruda, 49°9’54.959’’N, 13°12’0.216’’E, Bohemia, CZ.

S3 – Dukla lake, coal dump, 1 km northwest of Újezdeček, 50°39’9.261’’N, 13°46’40.673’’E, Bohemia, CZ.

S4 – peat pool, Chalupská slať raised bog nature monument, Bohemian Forest NP, approx. 1 km north of Borová Lada, 48°59’53.44”N, 13°39’33.65”E, Bohemia, CZ.

S5 – Kateřina lake, coal dump, 0.5 km south of Soběchleby, 50°39’51.5”N, 13°53’21.9”E, Bohemia, CZ.

S6 – Laka lake, Bohemian Forest NP, 4 km south of Nová Hůrka, 49°6’36.771”N, 13°19’37.522”E, Bohemia, CZ.

S7 – Lake ČSM, coal dump, 0.5 km southwest of Dubí-Pozorka, 50°39’18.5’’N, 13°46’54.9’’E, Bohemia, CZ.

S8 – an oxbow in the floodplain area of the confluence of Morava and Dyje rivers, approx. 10 km south of Lanžhot, 48°37’44.426”N, 16°56’53.685”E, Moravia, CZ.

S9 – the downstream fish pond in Lopeník, 48°56’53.604”N, 17°46’37.796”E, Moravia, CZ.

S10 – Nové Mlýny - lower reservoir, just northeast of Pavlov 48°53’2.104”N, 16°41’11.455”E, Moravia, CZ.

S11 – Nové Mlýny - middle reservoir, just northwest of Dolní Věstonice 48°53’28.868”N, 16°38’18.291”E, Moravia, CZ.

S12 – Nové Mlýny - upper reservoir, south of Pasohlávky, 48°53’58.676”N, 16°34’53.342”E, Moravia, CZ.

S13 – Plešné lake, Bohemian Forest NP, approx 4 km south of Jelení, 48°46’39.051”N, 13°52’4.220”E, Bohemia, CZ.

S14 – Prášilské lake, Bohemian Forest NP, approx 3.5 km southeast of Prášily, 49°4’31.638”N, 13°24’4.509”E, Bohemia, CZ.

S15 – a peat pool in Radostínské rašeliniště, mineral poor Sphagnum fen national nature reserve, 1 km northeast of Radostín, 49°39’23.37”N, 15°53’14.82”E, Bohemia, CZ. Fig. 1.

S16 – a peat pool in Modravské slatě raised bog nature monument, Bohemian Forest NP, approx. 5.5 km west of Modrava, 49°1’26.588”N, 13°25’0.427”E, Bohemia, CZ.

S17 – a peat pool in Rolavská vrchoviště raised bog national nature reserve, approx. 5 km north of Přebuz 50°24’32.387”N, 12°36’33.625”E, Bohemia, CZ.

S18 – a pool on a small brook on the northern margin of Senotín, 49°4’11.328”N, 15°8’35.034”E, Bohemia, CZ.

S19 – an oxbow in the floodplain of Vltava (river), Bohemian Forest NP, north of the road from Pěkná to Pěkná railway station, 1.3 km west of Pěkná, 48°51’6.654”N, 13°54’56.386”E, Bohemia, CZ.

S20 – Vranov reservoir, 0.5 km north of Vranov nad Dyjí, 48°54’21.579”N, 15°49’4.072”E, Moravia, CZ.

S21 – a pool, Bohemian Forest NP, approx 700 m northeast of Pamferova Huť and 3 km northeast of Železná Ruda, 49°9’15.772’’N, 13°15’46.462’’E, Bohemia, CZ.

Other

O1 – tap water from Hrušovany nad Jevišovkou, 48°49’47.671”N 16°24’9.747”E, Moravia, CZ.

O2 – barrel with rain water, Rapotice, 49°11’32.361”N, 16°15’14.252”E, Moravia, CZ.

Lost and found in Ireland; how a data label resulted in a postal delivery to *Metriocnemus* (*Inermipupa*) *carmencitabertarum* (Orthocladiinae)

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Langton and Cobo (1997) erected the subgenus *Inermipupa* within *Metriocnemus* (Orthocladiinae) and described all life history stages of *Metriocnemus* (*Inermipupa*) *carmencitabertarum* from specimens obtained in Spain and Portugal in 1989. The pupa had been known prior to the description of the new species and was included in Langton (1991) as "Orthocladiinae gen.? sp.? Pe3". The first record outside the Iberian Peninsula was from a collection of the distinct pupal exuviae in October 1998 on the Azorean island of Terceira (Murray et al. 2004). Since then and more recently the species has been recorded from The Netherlands (Kuper and Moller-Pillot 2012), England (Langton 2012) and from numerous pupal exuviae and adults collected in Ireland in March 2012 (Murray 2012). Subsequently on 17 October 2012 I collected some adults and pupal exuviae from a rain-filled barrel in the yard at my home near Ashbourne, Co. Meath and placed them in alcohol in a SARSTEDT 2ml plastic vial to send to P. H. Langton (PHL) in Northern Ireland who had requested some specimens for his personal collection. With an appropriate data label in the vial (Fig. 1) detailing species name, date and site of collection etc., it was packed and posted to PHL in Coleraine, Co. Derry, Northern Ireland. However, when the package was delivered the vial was missing - it had been separated from the package. When PHL reported this loss on 22 October a second vial with more specimens was prepared and posted. This time delivery was successful.

But that was not the end of the affair. Five months after the loss of the vial from the first mailing, in March 2013 my postman showed me a "Royal Mail" envelope and, pointing to the address label, asked if could be for me. It was - the envelope contained the original vial that was posted to PHL in October 2012. The specimens were undamaged and intact. The back of the envelope (Fig. 2) was stamped "*Found opened or damaged and officially secured*" and initialled "N.I.M.C." (Northern Ire-

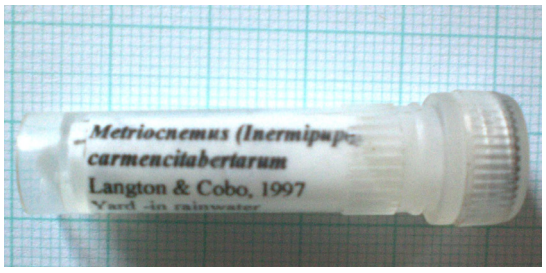

Figure1. The internally labelled vial that was lost in the post

Figure 2. Back of envelope from the Northern Ireland Royal Mail Centre (N.I.M.C.) also showing the Irish "An Post" seal.

land Mail Centre). The vial had obviously been found in the Northern Ireland Mail Centre where, on examination, an astute official read and copied relevant data information from the label in the vial on to the envelope in an attempt to return it through the postal system to the original sender.

The hand written address on the envelope (Fig. 3) read:

Metriocnemus carmencitabertarum
Langton & Cobo 1997,
yard – in rainwater,
Meadesbrooke, Ashbourne,
Co Meathe IGR

To which the Northern Ireland official added "R.O.I" (Republic of Ireland)

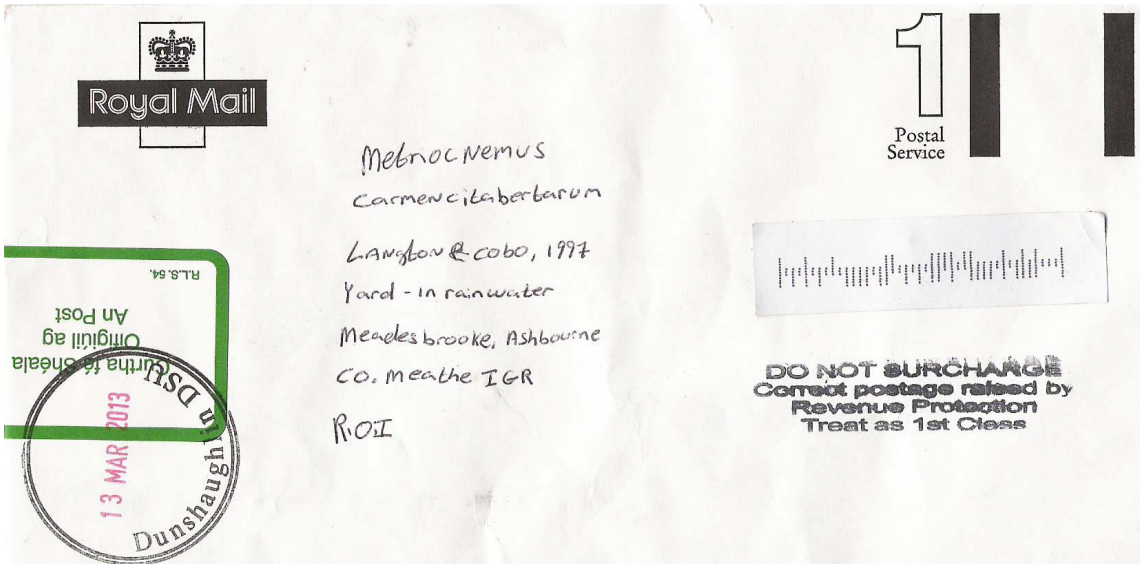

Figure 3. Front of the Royal Mail envelope that contained the returned vial addressed to "*Metriocnemus carmencitabertarum*" and showing the An Post date stamp 13 March 2013.

On its return journey the envelope containing the original vial had also been opened, examined and resealed by an official of An Post, the Irish Postal Service, at the Dunshaughlin sorting office since it bore a label "Officially Sealed by An Post and was date stamped 13 March 2013.

Credit is due to the official in the Northern Ireland Mail Centre for his/her initiative in copying the label information in the alcohol-filled vial and to the officials in the postal service in the Republic of Ireland and to my postman who recognised the possibility that the peculiar name and address for such an unusually named individual as "*Metriocnemus carmencitabertarum*" must have been linked with the entomologist who lived at "Meadesbrook" !

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In memoriam

Maria Rieradevall (23.02.1960 - 15.10.2015)

Our dear colleague Maria Rieradevall passed away on October 15th, 2015. A scientific obituary will be published in the second issue of the journal Limnetica (2015) and made available through the web page of her research group: http://www.ub.edu/fem/. Messages of condolences can be posted at: http://mariarieradevall.name/

In the obituary, Bonada et al. write: "Maria was a very versatile researcher who helped to advance the knowledge of Iberian limnology. She studied a wide variety of ecosystems, from rivers to lakes to coastal lagoons, focusing on macroinvertebrates (specially Chironomidae) as model organisms. Her contributions on these topics have been acknowledged by many national and international researchers"

Torbjørn Ekrem

Maria Rieradevall in London, 2008. Photo Narcís Prat.

Nikolai Alexandrovich Shobanov (21.06.1958 - 05.11.2015)

Our dear colleague Nikolai Shobanov passed away on November 5th, 2015. Nikolai graduated from Ivanovsk State University in Ivanovo, Russia, in 1980. From April 1981 to November 2015 he worked at the Institute of Biology of Inland Waters of the Russian Academy of Sciences in Borok (near the Rybinsk reservoir). He was a disciple of the famous Russian chironomidologist, Dr. A. I. Shilova, and defended in 2000 his doctoral thesis on the "Genus *Chironomus* Meigen (Diptera, Chironomidae): systematics, biology, evolution".

Eugenyi Makarchenko

Nikolai A. Shobanov in St. Petersburg, 2004. Photo Eugenyi Makarchenko.

Hiroshi Hashimoto (? – 26.02.2015)

Our dear colleague Dr. Hiroshi Hashimoto from (Professor emeritus of Shizuoka University) passed away on 26th February this year. He was the second chironomidologist in Japan and succeeded Dr. M. Tokunaga.

Tadashi Kobayashi