Zootaxa 3741 (4): 401–458
www.mapress.com/zootaxa/

Article

ISSN 1175-5326 (print edition)
ZOOTAXA
ISSN 1175-5334 (online edition)

http://dx.doi.org/10.11646/zootaxa.3741.4.1
http://zoobank.org/urn:lsid:zoobank.org:pub:0D2276A9-5295-4622-BCE8-A7D996A30367

Using various lines of evidence to identify *Chironomus* species (Diptera: Chironomidae) in eastern Canadian lakes

ISABELLE PROULX[1], JON MARTIN[2], MELISSA CAREW[3] & LANDIS HARE[1,4]

[1] *Institut national de la recherche scientifique – Centre Eau Terre Environnement, 490 rue de la Couronne, Quebec City, Quebec, G1K 9A9, Canada. E-mails: isabelle_proulx@hotmail.com & landis@ete.inrs.ca*

[2] *Department of Genetics, University of Melbourne, Melbourne, Victoria 3010, Australia. E-mail: j.martin@unimelb.edu.au*

[3] *Centre for Aquatic Pollution Identification and Management, Bio21 Institute, University of Melbourne, Melbourne, Victoria 3010, Australia. E-mail: mecarew@unimelb.edu.ca*

[4] *Corresponding author*

Table of contents

Abstract

Chironomus Meigen (Diptera, Chironomidae) larvae are usually the largest sediment-burrowing chironomids, and as such often constitute a major part of the freshwater infaunal biomass. However, use of this genus in ecological, environmental and paleoecological studies is hampered by the fact that *Chironomus* larvae are difficult to identify to species because the larvae of many species are morphologically similar. We used a combination of morphological, cytological and genetic techniques to distinguish *Chironomus* larvae collected from 31 water bodies located in eastern Canada, producing 17 distinguishable groupings. These groups of larvae were ultimately identified as belonging to 14 known species (*C. anthraci-*

Accepted by B. Rossaro: 25 Oct. 2013; published: 29 Nov. 2013

nus, *C. bifurcatus*, *C. cucini*, *C. decorus*-group sp. 2, *C. dilutus*, *C. entis*, *C. frommeri*, *C. harpi*, *C. maturus*, *C.* nr. *atroviridis* (sp. 2i), *C. ochreatus*, *C. plumosus*, *C. staegeri* and *C. 'tigris'*) and three other species that remain unidentified (*C.* sp. NAI-III). No single approach served to delimit and identify larvae of all 17 *Chironomus* species that we collected. Although we expected that morphological criteria alone would be insufficient, our results suggest that DNA barcoding, using either the mitochondrial *cox1* or the nuclear *gb2β* gene, was also inadequate for separating some *Chironomus* species. Thus we suggest that multiple approaches will often be needed to correctly identify *Chironomus* larvae to species.

Key words: *Chironomus*, morphology, cytology, DNA barcoding, *cox1*, *gb2β*, Canada

Introduction

The insect genus *Chironomus* Meigen (Diptera, Chironomidae) is found in fresh waters on all continents except Antarctica. It includes several hundred species, now classified into three subgenera (*Chaetolabis*, *Chironomus*, *Lobochironomus*) (the subgenus *Camptochironomus* is no longer recognized—see Sæther (2012)), as well as other species that are yet to be described (Ryser *et al.* 1985; Ashe & Cranston 1990; Martin 2013). In lakes from the tropics (Hare & Carter 1986), to the temperate (Jónasson 1972), to the Arctic (Butler 1982), *Chironomus* larvae are usually the largest sediment-burrowing chironomid and often represent a major part of the infaunal biomass. Thus *Chironomus* larvae can be an important source of food for fish and are widely used in ecological (Jónasson 1972), environmental (Martin *et al.* 2008) and paleoecological (Brooks *et al.* 2007) studies of fresh waters. If we are to understand their roles in aquatic ecosystems, it is important to be able to correctly identify *Chironomus* species.

The identification of *Chironomus* larvae to species can be problematic because there are few conspicuous morphological differences among many *Chironomus* species (Lindeberg & Wiederholm 1979). As a result, larvae are often referred to simply as *Chironomus* spp. (Nyman *et al.* 2005) or at best are grouped into types according to the presence and form of their abdominal tubules (Shobanov *et al.* 1996) or the shape of their mouth parts (Brooks *et al.* 2007). Such groupings can limit the use of *Chironomus* larvae in ecological, environmental and paleoecological studies because behavioural and ecological differences among species are often important. For example, cadmium concentrations in sympatric *Chironomus* species can vary by an order of magnitude because of differences in their feeding habits and consequent contaminant exposure (Martin *et al.* 2008; Proulx & Hare 2008, 2013). Pooling such species would clearly limit their use as contaminant biomonitors. If we cannot correctly identify *Chironomus* larvae to species, then it is difficult to use them to infer environmental impacts.

In early studies, features of the head capsule and abdominal tubules were used to identify *Chironomus* larvae to species (Johannsen 1937). Subsequently, *Chironomus* species were also separated on the basis of the structure of polytene chromosomes located in their salivary glands (Keyl 1962; Martin 1979; Wülker *et al.* 1989). In the last decade or so, genetic techniques have been used to supplement these earlier taxonomic methods. For example, the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) approach has been used to produce species-specific DNA profiles that can differentiate among *Chironomus* species (Carew *et al.* 2003; Sharley *et al.* 2004). This technique involves first amplifying specific genes or regions with PCR, and then digesting the resulting PCR amplicons with restriction endonucleases. Restriction endonucleases cut PCR amplicons differentially based on nucleotide differences in their DNA sequence, thereby generating a species-specific RFLP or DNA profile. The DNA profiles are visualised by gel electrophoresis as DNA fragments of different lengths. Although this method is inexpensive and useful for screening large numbers of individuals, it only examines a subset of the variation present in PCR amplicons (Pfrender *et al.* 2010).

Another genetic technique used to separate and identify species is DNA sequencing (also known as Sanger sequencing) of PCR amplicons. This technique, referred to as DNA barcoding when used for identifying species, is more exact than PCR-RFLP as it detects all nucleotide differences. The standard gene used for DNA barcoding is the 3' end of the mitochondrial *cytochrome oxidase subunit I* (*cox1*; Hebert *et al.* 2003). Advantages of using the *cox1* gene are that universal primers are able to amplify this gene from many animal groups (Folmer *et al.* 1994) and sequence variations in *cox1* can be used to discriminate among many closely-related species (Hebert *et al.* 2004a). In insects, DNA barcoding using the *cox1* gene has been used to identify species from a range of groups including the Collembola (Hogg & Hebert 2004), the Ephemeroptera (Ball *et al.* 2005; Elderkin *et al.* 2012), the Coleoptera (Davis *et al.* 2011) and the Chironomidae (Carew *et al.* 2007; Ekrem *et al.* 2007; Pfenninger *et al.* 2007; Sinclair & Gresens 2008; Ekrem *et al.* 2010; Carew *et al.* 2011; Stur & Ekrem 2011). Although *cox1* sequences can

References

Andersen, F.S. (1949) On the subgenus *Chironomus*. Studies on the systematics and biology of Chironomidae III. *Videnskabelige Meddelser fra Dansk Naturhistorisk Forening*, III, 1–66.

Ashe, P. & Cranston, P.S. (1990) Family Chironomidae. *In:* Soos, A. & Papp, L. (Eds.), *Catalogue of Palearctic Diptera. Vol. 2. Psychodidae - Chironomidae*. Elsevier, Amsterdam, pp. 113–355.

Ball, S.L., Hebert, P.D.N., Burian, S.K. & Webb, J.M. (2005) Biological identifications of mayflies (Ephemeroptera) using DNA barcodes. *Journal of the North American Benthological Society*, 24, 508–524. http://dx.doi.org/10.1899/04-142.1

Beermann, W. (1955) Cytologische Analyse eines *Camptochironomus*-Artbastards. I. Kreuzungsergebnisse und die Evolution des Karyotypus. *Chromosoma*, 7, 198–259. http://dx.doi.org/10.1007/bf00329725

Brooks, S.J., Langdon, P.G. & Heiri, O. (2007) *The identification and use of Palearctic Chironomidae larvae in palaeoecology*. QRA Technical Guide No. 10, Quaternary Research Association, London, 276 pp.

Butler, M.G. (1982) Production dynamics of some artic *Chironomus* larvae. *Limnology and Oceanography*, 27, 728–736. http://dx.doi.org/10.4319/lo.1982.27.4.0728

Butler, M.G., Kiknadze, I.I., Cooper, J.K. & Siirin, M.T. (1995) Cytologically identified *Chironomus* species from lakes in North Dakota and Minnesota, USA. *In:* Cranston, P.S. (Ed.), *Chironomids, from Gene to Ecosystems - Proceedings of the 12th International Symposium on Chironomidae (Canberra, January 23–26, 1994)*. CSIRO, Canberra, pp. 31–37.

Butler, M.G., Kiknadze, I.I., Golygina, V.V., Martin, J., Istomina, A.G., Wülker, W.F., Sublette, J.E. & Sublette, M.F. (1999) Cytogenetic differentiation between Palearctic and Nearctic populations of *Chironomus plumosus* L. (Diptera, Chironomidae). *Genome*, 42, 797–815. http://dx.doi.org/10.1139/g99-014

Carew, M.E., Marshall, S.E. & Hoffmann, A.A. (2011) A combination of molecular and morphological approaches resolves species in the taxonomically difficult genus *Procladius* Skuse (Diptera: Chironomidae) despite high intra-specific morphological variation. *Bulletin of Entomological Research*, 101, 505–519. http://dx.doi.org/10.1017/s000748531100006x

Carew, M.E., Pettigrove, V., Cox, R.L. & Hoffmann, A.A. (2007) DNA identification of urban Tanytarsini chironomids (Diptera:Chironomidae). *Journal of the North American Benthological Society*, 26, 587–600. http://dx.doi.org/10.1899/06-120.1

Carew, M.E., Pettigrove, V. & Hoffmann, A.A. (2003) Identifying chironomids (Diptera: Chironomidae) for biological monitoring with PCR-RFLP. *Bulletin of Entomological Research*, 93, 483–490. http://dx.doi.org/10.1079/ber2003268

Carew, M.E., Pettigrove, V. & Hoffmann, A.A. (2005) The utility of DNA markers in classical taxonomy: Using cytochrome oxidase I markers to differentiate Australian *Cladopelma* (Diptera: Chironomidae) midges. *Annals of the Entomological Society of America*, 98, 587–594.

City of Greater Sudbury (2013) Individual Lake Information. Available from: http://www.greatersudbury.ca/living/lakes-facts/local-lake-descriptions/ (accessed 17 September 2013)

Davis, G.A., Havill, N.P., Adelman, Z.N., Caccone, A., Kok, L.T. & Salom, S.M. (2011) DNA barcodes and molecular diagnostics to distinguish an introduced and native *Laricobius* (Coleoptera: Derodontidae) species in eastern North America. *Biological Control*, 58, 53–59. http://dx.doi.org/10.1016/j.biocontrol.2011.03.016

DeSalle, R., Egan, M.G. & Siddall, M. (2005) The unholy trinity: taxonomy, species delimitation and DNA barcoding. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 360, 1905–1916. http://dx.doi.org/10.1098/rstb.2005.1722

Ekrem, T., Stur, E. & Hebert, P.D.N. (2010) Females do count: Documenting Chironomidae (Diptera) species diversity using DNA barcoding. *Organisms Diversity and Evolution*, 10, 397–408. http://dx.doi.org/10.1007/s13127-010-0034-y

Ekrem, T., Willassen, E. & Stur, E. (2007) A comprehensive DNA sequence library is essential for identification with DNA barcodes. *Molecular Phylogenetics and Evolution*, 43, 530–542. http://dx.doi.org/10.1016/j.ympev.2006.11.021

Elderkin, C.L., Corkum, L.D., Bustos, C., Cunningham, E.L. & Berg, D.J. (2012) DNA barcoding to confirm morphological traits and determine relative abundance of burrowing mayfly species in western Lake Erie. *Journal of Great Lakes Research*, 38, 180–186. http://dx.doi.org/10.1016/j.jglr.2011.11.010

Epler, J.H. (2001) *Identification manual for the larval Chironomidae (Diptera) of North and South Carolina. A guide to the taxonomy of the midges of the southeastern United States, including Florida*. Special Publication SJ2001-SP13 North Carolina Department of Environmental and Natural Resources, Raleigh, NC, and St. John's River Water Management District, Palatka, FL, 526 pp.

Folmer, O., Black, M., Hoeh, W., Lutz, R. & Vrijenhoek, R. (1994) DNA primers for amplification of mitochondrial cytochrome *c* oxidase subunit I from diverse metazoan invertebrates. *Molecular Marine Biology and Biotechnology*, 3, 294–299.

Fortin, C., Couillard, Y., Vigneault, B. & Campbell, P.G.C. (2010) Determination of free Cd, Cu and Zn concentrations in lake waters by in situ diffusion followed by column equilibration ion-exchange. *Aquatic Geochemistry*, 16, 151–172. http://dx.doi.org/10.1007/s10498-009-9074-3

Goeldi, E.A. (1905) Os mosquito no Pará. Reunião de quatro trabalhos sobre os Mosquitos indigenas, principalmente as especies que molestam o homem. *Memorias do Meseu Goeldi (Museu Paraense) de Historia Natural e Ethnographia*, 4, 134–137.
http://dx.doi.org/10.5962/bhl.title.65997

Gunderina, L.I. (2012) Species-specific PCR primers for identification of the sibling species *Chironomus plumosus* (Linnaeus, 1758) and *Chironomus balatonicus* (Devai, Wuelker et Scholl, 1983) (Chironomidae, Diptera). *Fauna Norvegica*, 31, 151–157.
http://dx.doi.org/10.5324/fn.v31i0.1381

Gunderina, L.I. & Katokhin, A.V. (2011) Variation and divergence of the rDNA ITS-1 region in species of the genus *Chironomus* (Diptera: Chironomidae). *In:* Wang, X. & Liu, W. (Eds.), *Contemporary Chironomid Studies - Proceedings of the 17th International Symposium on Chironomidae (July 6-9, 2009 Nankai University, China)*. Nankai University Press, Tianjin, pp. 22–35.

Gunderina, L.I., Kiknadze, I.I., Istomina, A.G. & Butler, M. (2009) Geographic differentiation of genomic DNA of *Chironomus plumosus* (Diptera, Chironomidae) in natural Holarctic populations. *Russian Journal of Genetics*, 45, 54–62. http://dx.doi.org/10.1134/s1022795409010086

Guryev, V., Makarevitch, I., Blinov, A. & Martin, J. (2001) Phylogeny of the genus *Chironomus* (Diptera) inferred from DNA sequences of mitochondrial *Cytochrome b* and *Cytochrome oxidase I*. *Molecular Phylogenetics and Evolution*, 19, 9–21. http://dx.doi.org/10.1006/mpev.2001.0898

Guryev, V.P. & Blinov, A.G. (2002) Phylogenetic relationships among Holarctic populations of *Chironomus entis* and *Chironomus plumosus* in view of possible horisontal transfer of mitochondrial genes. *Russian Journal of Genetics*, 38, 239–243.

Hall, T.A. (1999) BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symposium Series*, 41, 95–98.

Hankeln, T., Friedl, H., Ebersberger, I., Martin, J. & Schmidt, E.R. (1997) A variable intron distribution in globin genes of *Chironomus*: Evidence for recent intron gain. *Gene*, 205, 151–160. http://dx.doi.org/10.1016/S0378-1119(97)00518-0

Hare, L. & Carter, J.C.H. (1976) The distribution of *Chironomus* (*s.s.*) ?*cucini* (*salinarius* group) larvae (Diptera: Chironomidae) in Parry Sound, Georgian Bay, with particular reference to structural deformities. *Canadian Journal of Zoology*, 54, 2129–2134.
http://dx.doi.org/10.1139/z76-246

Hare, L. & Carter, J.C.H. (1986) The benthos of a natural West African lake, with emphasis on the diel migrations and lunar and seasonal periodicities of the *Chaoborus* populations (Diptera, Chaoboridae). *Freshwater Biology*, 16, 759–780. http://dx.doi.org/10.1111/j.1365-2427.1986.tb01016.x

Harnisch, O. (1942) Die sogenannten "Blutkiemen" der Larven der Gattung *Chironomus* Mg. *Biologia Generalis*, 16, 593–609.

Hebert, P.D.N., Cywinska, A., Ball, S.L. & DeWaard, J.R. (2003) Biological identifications through DNA barcodes. *Proceedings of the Royal Society B: Biological Sciences*, 270, 313–321. http://dx.doi.org/10.1098/rspb.2002.2218

Hebert, P.D.N., Penton, E.H., Burns, J.M., Janzen, D.H. & Hallwachs, W. (2004a) Ten species in one: DNA barcoding reveals cryptic species in the neotropical skipper butterfly *Astraptes fulgerator*. *Proceedings of the National Academy of Sciences of the United States of America*, 101, 14812–14817. http://dx.doi.org/10.1073/pnas.0406166101

Hebert, P.D.N., Stoeckle, M.Y., Zemlak, T.S. & Francis, C.M. (2004b) Identification of birds through DNA barcodes. *PLoS Biology*, 2, 1657-1663.
http://dx.doi.org/10.1371/journal.pbio.0020312

Hogg, I.D. & Hebert, P.D.N. (2004) Biological identification of springtails (Hexapoda: Collembola) from the Canadian Arctic, using mitochondrial DNA barcodes. *Canadian Journal of Zoology*, 82, 749–754. http://dx.doi.org/10.1139/z04-041

Ineichen, H., Meyer, B. & Lezzi, M. (1983) Determination of the developmental stage of living fourth instar larvae of *Chironomus tentans*. *Developmental Biology*, 98, 278–286.

Johannsen, O.A. (1908) New North American Chironomidae. *New York State Museum Bulletin*, 124, 264–285.

Johannsen, O.A. (1937) Part IV. Chironomidae: Subfamily Chironominae. *In:* Johannsen, O.A. (Ed.), *Aquatic Diptera - Eggs, Larvae, and Pupae of Aquatic Flies.*Memoirs of the Cornell University Agricultural Experiment Station, 210, 1-52.

Jónasson, P.M. (1972) Ecology and production of the profundal benthos in relation to phytoplankton in Lake Esrom. *Oikos Supplement*, 14, 1–148.

Kao, W.Y., Trewitt, P.M. & Bergtrom, G. (1994) Intron-containing globin genes in the insect *Chironomus thummi*. *Journal of Molecular Evolution*, 38, 241–249.
http://dx.doi.org/10.1007/bf00176086

Keyl, H.G. (1960) Die cytologische Diagnostik der Chironomiden. II. Diiagnosen der Geschwisterarten *Chironomus acidophilus* n. sp. und *Ch. uliginosus* n. sp. *Archiv für Hydrobiologie*, 57, 187–195.

Keyl, H.G. (1962) Chromosomenevolution bei *Chironomus* II. Chromosomenumbauten und phylogenetische Beziehungen der Arten. *Chromosoma*, 13, 464–514.
http://dx.doi.org/10.1007/bf00327342

Kiknadze, I.I., Aimanova, K.G., Butler, M. & Cooper, K. (1993) The pattern of the reduction of the chromosome number in the chironomid evolution. *Tsitologiya*, 35, 96–104. [in Russian]

Kiknadze, I.I., Butler, M.G., Golygina, V.V., Martin, J., Wulker, W.F., Sublette, J.E. & Sublette, M.F. (2000a) Intercontinental karyotypic differentiation of *Chironomus entis* Shobanov, a Holarctic member of the *C. plumosus* group (Diptera, Chironomidae). *Genome*, 43, 857–873.
http://dx.doi.org/10.1139/g00-054

Kiknadze, I.I., Butler, M.G., Golygina, V.V., Wuelker, W.F., Martin, J., Sublette, J.E. & Sublette, M.F. (2000b) Macrogeographic patterns of banding sequences in Holarctic *Chironomus entis* Shobanov. *In:* Hoffrichter, O. (Ed.), *Late 20th Century Research on Chironomidae: an Anthology from the 13th International Symposium on Chironomidae*. Shaker Verlag, Aachen, pp. 621.

Kiknadze, I.I., Butler, M.G., Gunderina, L.I., Istomina, A.G., Gusev, V.D. & Nemytikova, L.A. (2010) Chromosomal evolution of Nearctic and Palearctic *Chironomus* species (Diptera: Chironomidae). *In:* Ferrington, L.C. (Ed.), *Proceedings of the XV International Symposium on Chironomidae*, Chironomidae Research Group, University of Minnesota, Saint Paul, Minnesota, pp. 203–221.

Kiknadze, I.I., Golygina, V.V., Istomina, A.G. & Gunderina, L.I. (2004) Pattern of chromosomal polymorphism during population and species divergence in *Chironomus* (Diptera, Chironomidae) (In Russian, English summary). *Sibirskiy Ekologicheskiy Zhurnal*, 11, 635–651.

Kiknadze, I.I., Shilova, A.I., Kerkis, I.E., Shobanov, N.A., Zelenkov, N.I., Grebenchov, L.P., Istomina, A.G. & Prasolov, B.A. (1991) *Karyotype and Morphology of Larvae of the Tribe Chironomini* ATLAS, Novosibirsk, 114 pp.

Kiknadze, I.I., Wuelker, W.F., Istomina, A.G. & Andreeva, E.N. (2005) Banding sequence pool of *Chironomus anthracinus* Zett. (Diptera, Chironomidae) in Palearctic and Nearctic. *Euroasian Entomological Journal*, 4, 13–27.

Kimura, M. (1980) A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. *Journal of Molecular Evolution*, 16, 111–120.
http://dx.doi.org/10.1007/bf01731581

Lindeberg, B. & Wiederholm, T. (1979) Notes on the taxonomy of European species of *Chironomus* (Diptera Chironomidae). *Entomologica Scandinavica Supplements*, 10, 99–116.

Linnaeus, C. (1758) *Systema naturae per regna tria naturae. Vol. 1. 10th ed.* Laurentii Salvii, Stockholm, 824 pp.

Lundbeck, W. (1898) Diptera groenlandica. *Videnskabelige Meddelelser fra den Naturhistorisk Forening i Kjøbenhavn*, 1898, 236–314.

Makarevich, I.F., Berezikov, E.V., Guryev, V.P. & Blinov, A.G. (2000) Molecular phylogeny of the *Chironomus* genus deduced from nucleotide sequences of two nuclear genes, *ssp160* and the Globin 2b gene. *Molecular Biology*, 34, 606–612.
http://dx.doi.org/10.1007/bf02759569

Malloch, J.R. (1915) The Chironomidae, or midges, of Illinois, with particular reference to the species occuring in the Illinois River. *Bulletin of the Illinois State Laboratory of Natural History*, 10, 275–543.

Malloch, J.R. (1934) III. Chironomidae, Sciaridae, Phoridae, Syrphidae, Oestridae, Piophilidae, Helomyzidae, Calliphoridae and Tachinidae. *In:* Holland, W.J. (Ed.), *The Exploration of Southampton Island, Hudson Bay, by G.M. Sutton Supposed by J.B. Semple 1929–1930*. Memoirs of the Carnegie Museum, pp. 13–32.

Martin, J. (1979) Chromosomes as tools in taxonomy and phylogeny of Chironomidae (Diptera). *Entomologica Scandinavica Supplements*, 10, 67–74.

Martin, J. (2011) From bands to base pairs: Problems in the identification of species using the example of *Chironomus oppositus* Walker (Honorary Thienemann Lecture). *In:* X. Wang & W. Liu (Eds.), *Contemporary Chironomid Studies - Proceedings of the 17th International Symposium on Chironomidae (July 6–9, 2009 Nankai University, China)*. Nankai University Press, Tianjin, pp. 126–143.

Martin, J. (2013) North American species of the genus *Chironomus*. Available from: http://genetics.unimelb.edu.au/Martin/NACytfiles/NAChiron.html (accessed 17 September 2013)

Martin, J., Andreeva, E.N., Kiknadze, I.I. & Wülker, W.F. (2006) Polytene chromosomes and phylogenetic relationships of *Chironomus atrella* (Diptera: Chironomidae) in North America. *Genome*, 49, 1384–1392.
http://dx.doi.org/10.1139/g06-095

Martin, J., Guryev, V. & Blinov, A. (2002) Population variability in *Chironomus* (*Camptochironomus*) species (Diptera, Nematocera) with a Holarctic distribution: Evidence of mitochondrial gene flow. *Insect Molecular Biology*, 11, 387–397.
http://dx.doi.org/10.1046/j.1365-2583.2002.00348.x

Martin, J., Sublette, J.E. & Caldwell, B.A. (2010) Description of *Chironomus quinnitukqut*, n. sp., closely related to the *C. decorus* group in North America, with characterization of an additional larval form from halobiontic habitats. *Zootaxa*, 2716, 29–41 & 2743, 2768.

Martin, J. & Wülker, W. (1971) Inversion polymorphism in *Chironomus staegeri* Lundbeck. *Canadian Journal of Genetics and Cytology*, 13, 306–321.

Martin, J., Wülker, W. & Sublette, J.E. (1974) Evolutionary cytology of the genus *Chironomus* (Diptara: Chironomidae). *Studies in Natural Sciences (Portales, N.M.)*, 1, 1–12.

Martin, S., Proulx, I. & Hare, L. (2008) Explaining metal concentrations in sympatric *Chironomus* species. *Limnology and Oceanography*, 53, 411–419.
http://dx.doi.org/10.4319/lo.2008.53.2.0411

Meier, R., Shiyang, K., Vaidya, G. & Ng, P.K. (2006) DNA barcoding and taxonomy in Diptera: a tale of high intraspecific variability and low identification success. *Systematic Biology*, 55, 715–728.
http://dx.doi.org/10.1080/10635150600969864

Ministère du Développement durable de l'Environnement et des Parcs (2012) Le réseau de surveillance volontaire des lacs. Available from: http://www.mddep.gouv.qc.ca/eau/rsvl (accessed 20 August 2012)

Nei, M. & Kumar, S. (2000) *Molecular Evolution and Phylogenetics*. Oxford University Press, New York, 352 pp.

Nyman, M., Korhola, A. & Brooks, S.J. (2005) The distribution and diversity of Chironomidae (Insecta: Diptera) in western Finnish Lapland, with special emphasis on shallow lakes. *Global Ecology and Biogeography*, 14, 137–153.
http://dx.doi.org/10.1111/j.1466-822x.2005.00148.x

Oliver, D.R., Dillon, M.E. & Cranston, P.S. (1990) *A catalog of Nearctic Chironomidae. Vol. 1857/B*. Research Branch Agriculture Canada Publication, 89 pp.

Pfenninger, M., Nowak, C., Kley, C., Steinke, D. & Streit, B. (2007) Utility of DNA taxonomy and barcoding for the inference of larval community structure in morphologically cryptic *Chironomus* (Diptera) species. *Molecular Ecology*, 16, 1957–1968.
http://dx.doi.org/10.1111/j.1365-294x.2006.03136.x

Pfrender, M.E., Ferrington, L.C. Jr., Hawkins, C.P., Hartzell, P.L., Bagley, M., Jackson, S., Courtney, G.W., Larsen, D.P., Creutzburg, B.R., Lévesque, C.A., Epler, J.H., Morse, J.C., Fend, S., Petersen, M.J., Ruiter, D., Schindel, D. & Whiting, M. (2010) Assessing macroinvertebrate biodiversity in freshwater ecosystems: Advances and challenges in DNA-based approaches. *Quarterly Review of Biology*, 85, 319–340.
http://dx.doi.org/10.1086/655118

Ponton, D.E. & Hare, L. (2009) Assessment of nickel contamination in lakes using the phantom midge *Chaoborus* as a biomonitor. *Environmental Science and Technology*, 43, 6529–6534.
http://dx.doi.org/10.1021/es900920b

Proulx, I. & Hare, L. (2008) Why bother to identify animals used for contaminant monitoring? *Integrated Environmental Assessment and Management.* 4, 125–126.
http://dx.doi.org/10.1002/ieam.5630040115

Proulx, I. & Hare, L. (2013) Differences in feeding behaviours among *Chironomus* species revealed by measurements of sulphur stable isotopes and cadmium in larvae. *Freshwater Biology*, in press.

Ryser, H.M., Wuelker, W. & Scholl, A. (1985) Revision der Gattung *Chironomus* Meigen (Diptera) X: *Lobochironomus* n. subgen. (*C. montuosus* n. sp., *C. storai* Goetgh., *C. mendax* Stora). *Revue Suisse de Zoologie*, 92, 385–404.

Sæther, O.A. (1975) Nearctic chironomids as indicators of lake typology. *Verhandlungen der Internationale Vereinigung für Theoretische und Angewandte Limnologie*, 19, 3127–3133.

Sæther, O.A. (2012) The *Chironomus* group (Diptera: Chironomidae) in Lake Winnipeg, Canada. *Zootaxa*, 3275, 1–19.

Saitou, N. & Nei, M. (1987) The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Molecular Biology and Evolution*, 4, 406–425.

Sharley, D.J., Pettigrove, V. & Parsons, Y.M. (2004) Molecular identification of *Chironomus* spp. (Diptera) for biomonitoring of aquatic ecosystems. *Australian Journal of Entomology*, 43, 359–365.
http://dx.doi.org/10.1111/j.1440-6055.2004.00417.x

Shobanov, N.A. (1989) The morphological differentiation of *Chironomus* species of *plumosus* group (Diptera, Chironomidae). Larvae. *Acta Biologica Debrecina, Supplementum Oecologica Hungarica*, 2, 335–344.

Shobanov, N.A. (2002) Evolution of the genus *Chironomus* (Diptera, Chironomidae). 1. Ancestral form and major lines of phylogenesis. *Entomological Review*, 82, 487–493.

Shobanov, N.A., Kiknadze, I.I. & Butler, M.G. (1999) Palearctic and Nearctic *Chironomus* (*Camptochironomus*) *tentans* (Fabricius) are different species (Diptera: Chironomidae). *Insect Systematics and Evolution*, 30, 311–322.
http://dx.doi.org/10.1163/187631200X00147

Shobanov, N.A., Shilova, A.I. & Belyanina, S.I. (1996) Extent and content of the genus *Chironomus* Meig. (Diptera, Chironomidae): review of world fauna. *In:* Shobanov, N.A. & Zinchenko, T.D. (Eds.), *Ecology, Evolution and Systematics of Chironomids*, *Inst. Biol. Inland Waters & Inst. Ecol. Volga Basin*. Russian Academy of Sciences, Tolyatti, Boruk, Russia, pp. 44–96. [in Russian]

Sinclair, C.S. & Gresens, S.E. (2008) Discrimination of *Cricotopus* species (Diptera: Chironomidae) by DNA barcoding. *Bulletin of Entomological Research*, 98, 555–563.
http://dx.doi.org/10.1017/s0007485308005865

Stur, E. & Ekrem, T. (2011) Exploring unknown life stages of Arctic Tanytarsini (Diptera: Chironomidae) with DNA barcoding. *Zootaxa*, 2743, 27–39.

Sublette, J.E. & Sublette, M.F. (1971) A review of the genus *Chironomus* (Diptera, Chironomidae) B. Description of the immature stages and adults of the *Chironomus staegeri* group. *Studies in Natural Sciences (Portales, N.M.)*, 1 (1), 6–21.

Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M. & Kumar, S. (2011) MEGA5: Molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Molecular Biology and Evolution*, 28, 2731–2739.
http://dx.doi.org/10.1093/molbev/msr121

Thienemannn, A. (1941) Lappländische Chironomiden und ihre Wohngewasser. (Ergebnisse von Untersuchungen im Abiskogebeit in Schwedisch-Lappland). *Archiv für Hydrobiologie Supplement*, 17, 1–253.

Thompson, J.D., Higgins, D.G. & Gibson, T.J. (1994) CLUSTAL W: Improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Research*, 22, 4673–4680.
http://dx.doi.org/10.1093/nar/22.22.4673

Townes, H.K. Jr. (1945) The Nearctic species of Tendipedini [Diptera, Tendipedidae (= Chironomidae)]. *American Midland Naturalist*, 34, 1–206.
http://dx.doi.org/10.2307/2421112

Vallenduuk, H.J. & Moller Pillot, H.K.M. (1997) Key to the larvae of *Chironomus* in Western Europe. *In: RIZA Rapport 97.053,* pp. 1–13 + appendix.

Webb, C.J. & Scholl, A. (1985) Identification of larvae of European species of *Chironomus* Meigen (Diptera: Chironomidae) by morphological characters. *Systematic Entomology*, 10, 353–372.
http://dx.doi.org/10.1111/j.1365-3113.1985.tb00143.x

Webb, C.J., Scholl, A. & Ryser, H.M. (1985) Comparative morphology of the larval ventromental plates of European species of *Chironomus* Meigen (Diptera: Chironomidae). *Systematic Entomology*, 10, 373–385.
http://dx.doi.org/10.1111/j.1365-3113.1985.tb00144.x

Webb, D.W. (1969) New species of chironomids from Costello Lake, Ontario (Diptera: Chironomidae). *Journal of the Kansas Entomological Society*, 42, 91–108.

Wiederholm, T. (1979) Morphology of *Chironomus macani* Freeman, with notes on the taxonomic status of subg. *Chaetolabis* Town. (Diptera: Chironomidae). *Entomologica Scandinavica Supplements*, 10, 145–150.

Wuelker, W. (2010) The role of chromosomes in chironomid systematics, ecology and phylogeny. *In:* Ferrington, L.C. (Ed.), *Proceedings of the XV International Symposium on Chironomidae*. Chironomidae Research Group, University of Minnesota, Saint Paul, Minnesota, pp. 1–13.

Wuelker, W., Martin, J., Kiknadze, I.I., Sublette, J.E. & Michiels, S. (2009) *Chironomus blaylocki* sp. n. and *C. bifurcatus* sp. n., North American species near the base of the decorus-group (Diptera: Chironomidae). *Zootaxa*, 2023, 28–46.

Wülker, W., Dévai, G. & Dévai, I. (1989) Computer assisted studies of chromosomes evolution in the genus *Chironomus* (Dipt.). Comparative and integrated analysis of chromosome arms A, E and F. *Acta biologica debrecina, supplement oecologica Hungarica*, 2, 373–387.

Wülker, W. & Götz, P. (1968) Die Verwendung der Imaginalscheiben zur Bestimmung des Entwicklungszustandes von *Chironomus*-Larven (Dipt.). *Zeitschrift für Morphologie und Okologie der Tiere*, 62, 363–388.
http://dx.doi.org/10.1007/bf00401562

Wülker, W. & Martin, J. (1974) A review of the genus *Chironomus* (Diptera, Chironomidae) VI. Cytology of the maturus-complex. *Studies in Natural Sciences (Portales, N.M.)*, 1 (9), 1–21.

Wülker, W., Sublette, J.E. & Martin, J. (1991) *Chironomus utahensis* Malloch and *Chironomus harpi* new species and their karyosystematic relationships to other species in the *decorus*-group of *Chironomus*. *Spixiana*, 14, 71–94.

Wülker, W.F. & Butler, M.G. (1983) Karyosystematics and morphology of Northern *Chironomus* (Diptera: Chironomidae): Freshwater species with larvae of the *salinarius*-type. *Entomologica Scandinavica*, 14, 121–136.

Wülker, W.F. & Martin, J. (1971) A review of the genus *Chironomus* (Diptera, Chironomidae) C. Karyosystematics of the *Chironomus staegeri* group. *Studies in Natural Sciences (Portales, N.M.)*, 1 (1), 22–30.

Wülker, W.F., Sublette, J.E., Sublette, M.F. & Martin, J. (1971) A review of the genus *Chironomus* (Diptera, Chironomidae) I. The *staegeri* group. *Studies in Natural Sciences (Portales, N.M.)*, 1 (1), 1–89.

Zetterstedt, J.W. (1860) *Diptera Scandinaviae disposita et descripta. Tomus decimus quartus seu ultimus, continens addenda, corrigena & emendada tomis prioribus, una cum indice alphabetico navarum specierum hujus & praecedentis tomi, atque generico omnium tomorum*. *Lundae*, 14, 6497–6512.