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

Jon Martin

Genetics, Genomics & Development, School of Biosciences, University of Melbourne VIC 3010, Australia. E-mail: j.martin@unimelb.edu.au

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.

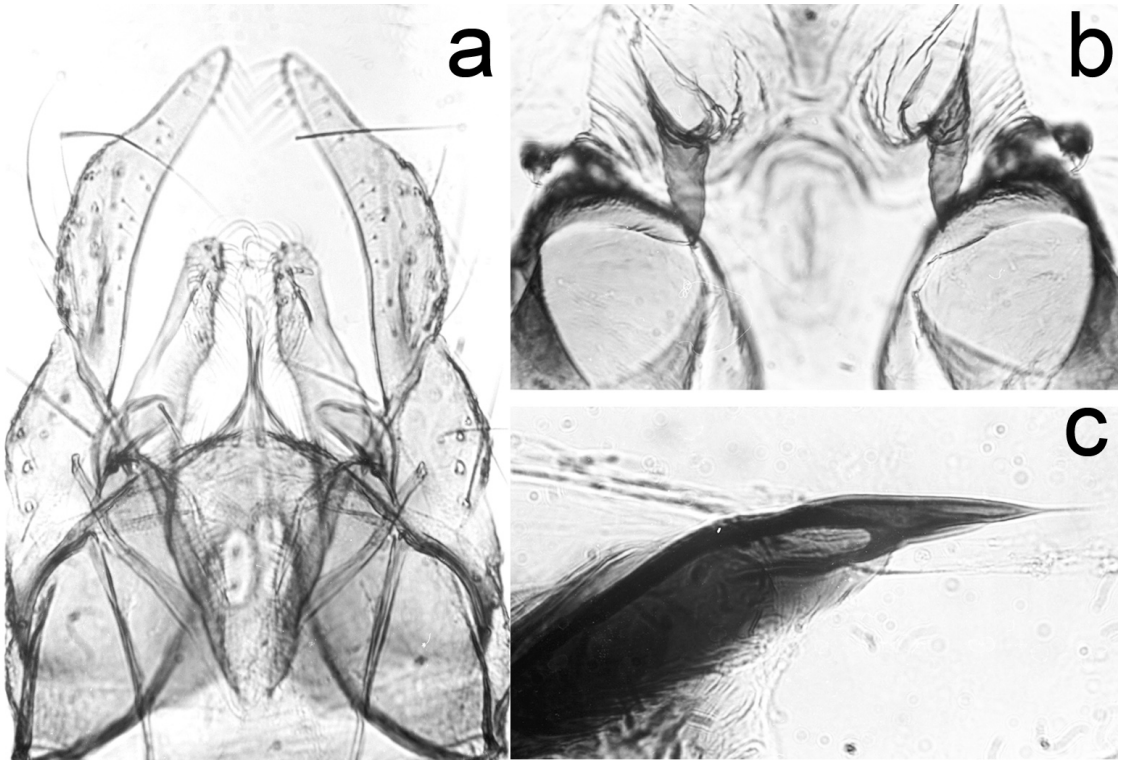

Figure 1. Reared male from Trout Lake, Vilas Co., Wisconsin. a) male hypopygium; b) pupal cephalic tubercles; c) spur of segment VIII.

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

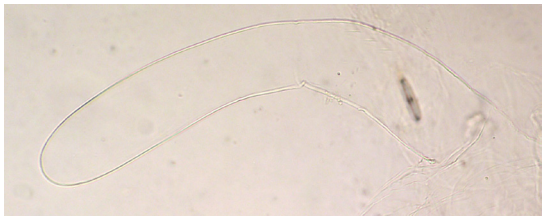

Figure 2. Anal tubule of North American larva.

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.

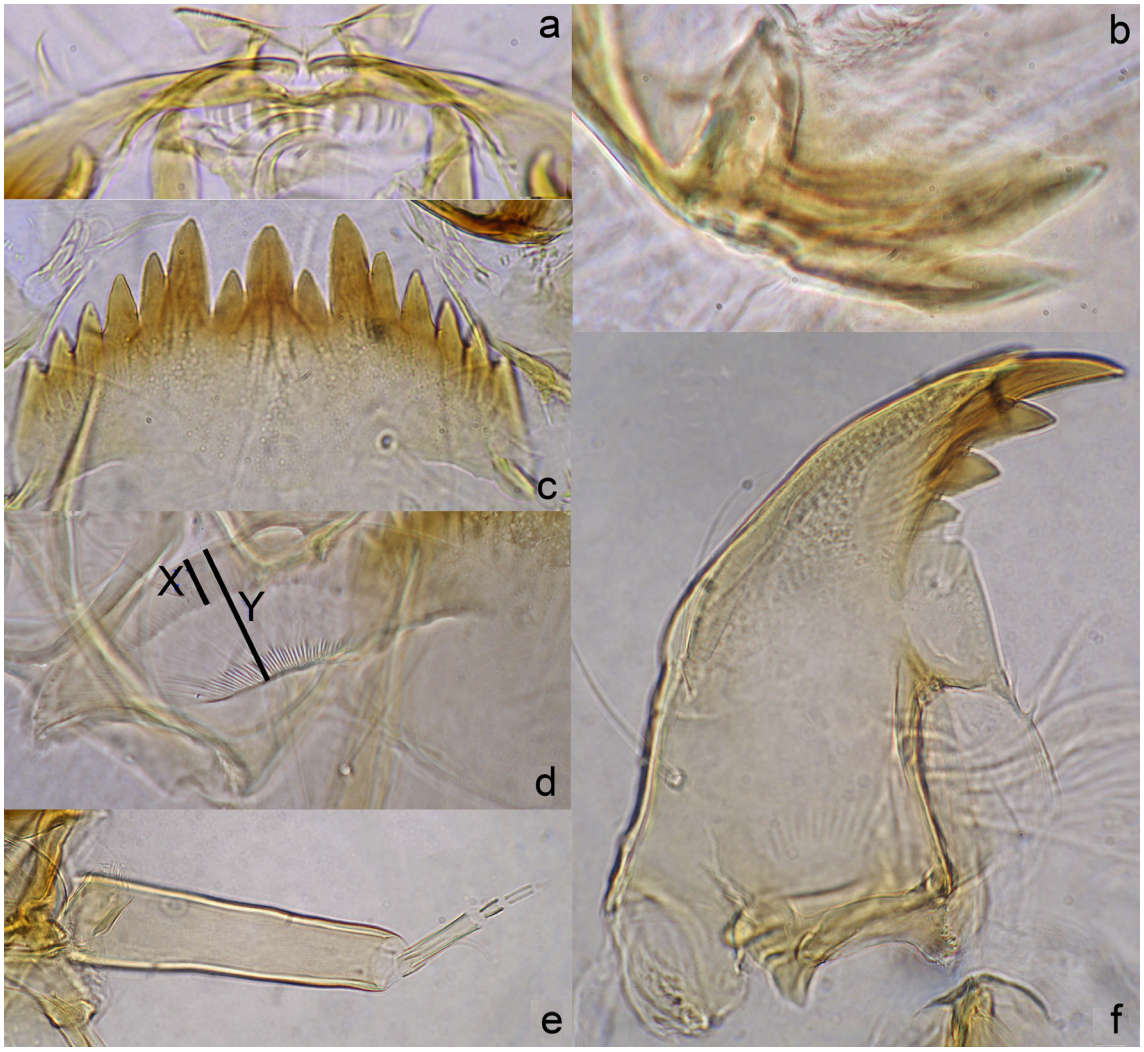

Figure 3. Larval mouth parts of North American C. melanescens. a) pecten epipharyngis; b) premandible; c) mentum; d) ventromentum; e) antenna; f) mandible.

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).

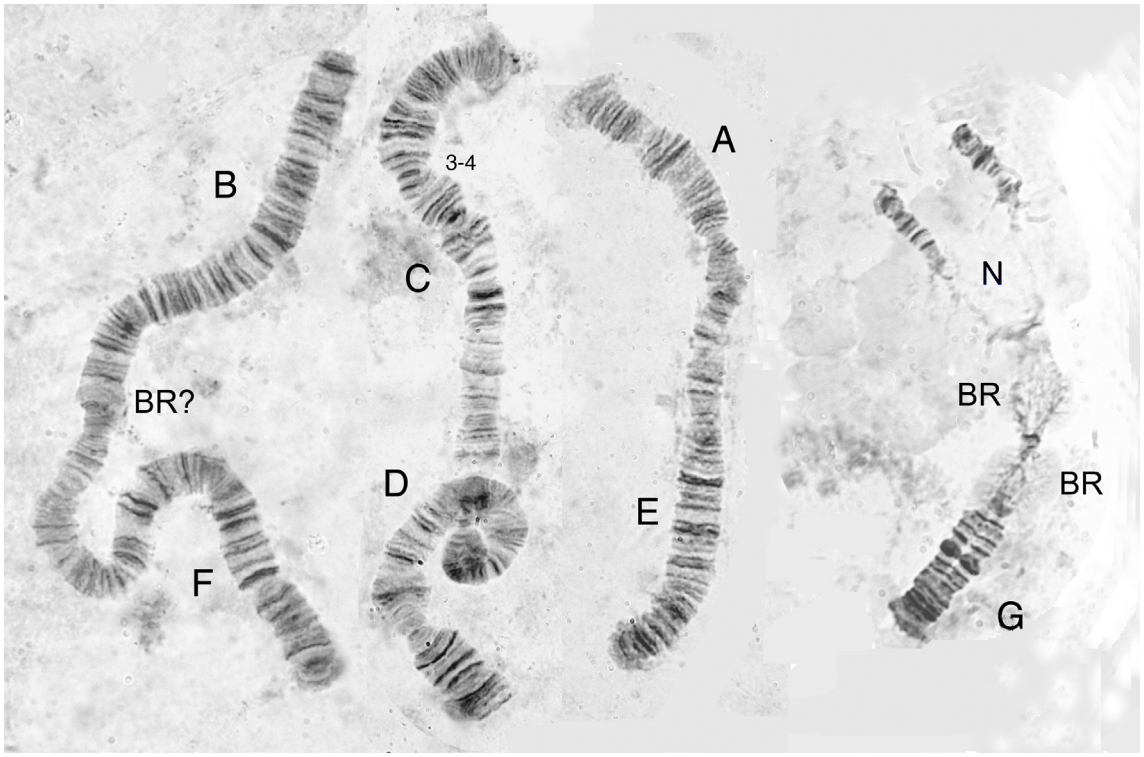

Figure 4. Polytene chromosomes of *C. melanescens*. Chromosome arms on Keyl (1962) system (A-G); nucleolus (N); Balbiani rings (BR).

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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References

Guryev V., Makarevitch, I., Blinov, A. and 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/10.11646/zootaxa.3741.4.1)

Keyl, H.-G. 1961. Chromosomenevolution bei *Chironomus* I. Struktur-abwandlungen an Speicheldrüsen-Chromsomen. - *Chromosoma* 12: 26-47. (http//dx.doi.org 10.1007/BF00328912)

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., Shilova, A.I., Kerkis I.E., Shobanov, N. A., Zelenkov, N.I., Grebenchov, L.P., Istomina, A.G., and Prasolov, B.A. 1991. *Karyotype and morphology of larvae of the tribe Chironomini* (In Russian). ATLAS, Novosibirsk, 114pp.

Martin, J. 2015. North American *Chironomus* species. Available from http://www.genetics.unimelb.edu.au/Martin/NACytfiles/NAChiron.html (accessed 27 October 2015).

Proulx, I., Martin, J. Carew, M. and Hare, L. 2013. Using various lines of evidence to identify *Chironomus* species in eastern Canadian lakes. - *Zootaxa* 3741: 401-458.

Sæther, O.A. 1980. Glossary of chironomid morphology terminology (Diptera: Chironomidae). - *Entomologica scandinavica, Supplement* 14: 1-51.

Strenzke, K. (1959) Revision der Gattung *Chironomus* Meig. I. Die Imagines von 15 nord-deutschen Arten und Unterarten. - *Archiv für Hydrobiologie* 56: 1-42.

Wülker, W., Ryser, H.M. and Scholl, A. 1981. Revision der Gattung *Chironomus* Meigen (Dipt.) VI. *C. holomelas* Keyl, *C. saxatilis* n. sp., *C. melanescens* Keyl. - *Revue suisse de Zoologie* 88: 903-924.