NORTH AMERICAN SPECIES OF THE GENUS CHIRONOMUS

as recognized by morphology and larval cytology (includes *CHAETOLABIS*, *LOBOCHIRONOMUS* and *EINFELDIA* (sens. lat.) and species included in *Tendipes* by Townes (1945) but now placed in other genera)

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Analysis of the karyotype of the polytene chromosomes in the fourth instar larvae has indicated the presence of a much larger *Chironomus* fauna than indicated in the revision of Townes (1945) or the Nearctic catalog of Oliver, Dillon & Cranston (1990). Some of these species have been described subsequently, but many remain undescribed.

As well, since more information is becoming available, Greenland has been included as it is essentially part of the continent of North America.

This list gives some information on the morphology, karyotype, mt*COI* sequence and distribution of about 118 species that have been recognized, but it should be noted that even this list is not exhaustive as available material includes a number of specimens that do not easily fit this expanded list. The use of DNA sequences, as in the Barcode of Life Database (BOLD) (Ratnasingham & Hebert, 2007), has supported the reality of many of these proposed species. Where available, the BOLD Bin in which this sequence is recorded is listed in the species description.

In many cases the assigned names result from unpublished studies with the late Jim Sublette, and without the assistance of Jim, and the late Wolfgang Wülker, this list would not have reached even the present degree of development. Many other people have assisted with material or in many other ways, and to them I am most grateful.

Part 1

Species of the thummi-cytocomplex and close relatives with unknown cytology.

Other cytocomplexes are in Part 2.

The References and known geographic distribution of the species are in Part 2.

General information for both parts:

In the locality listings a couple of species are listed as "Hudson Bay Territory" in the absence of better information on the site of collection. Hudson Bay Territory (also known as Prince Rupert's Land) existed from the 17th to 19th centuries and included the northern parts of Quebec and Ontario, all of Manitoba, and parts of Saskatchewan, Alberta, Nunavut Territory, Minnesota and North Dakota.



Map of Prince Rupert's Land (from Wikipedia)

The initial samples of many of the provisional species were collected during my tenure of a Canadian National Research Council Post-Doctoral Fellowship at the then Entomological Research Institute, Ottawa, Canada in 1966-67. Consequently, most of the adults or rearings of these species are in the collection of the Canadian National Insect Collection at the Biosystematics Research Institute. Subsequent material was supported by research funds from my friend and colleague James E. Sublette, some on a New Mexico Energy Institute Grant. The rearings from this material are mostly in the Sublette Collection in the Museum of the University of Minnesota. Further funding came from The University of Melbourne, during periods of study leave, and from a grant to Professor Stephen M. Case at the University of Mississippi. Numerous other people have assisted with material and advice, notably Professor Dr. Wolfgang F. Wülker, Dr. Malcolm G. Butler, Professor Iya. I. Kiknadze and her group at Novosibirsk, Professor Peter S. Cranston and Dr. Martin Spies. I express my thanks to all those who have so generously assisted with the work and ideas included in the compilation.

The broad diagnosis of *Chironomus* used here reflects the fact that at the time this study was begun, the nature of *Einfeldia* was quite unclear, and that there was a broad overlap of characters with those of *Chironomus*. While many aspects have been clarified, it is still not agreed that *Benthalia*, as used here, should be recognized as distinct from *Chironomus*. The subgenus *Camptochironomus* is not recognized here, in line with the decision of Townes (1945, p.116). The subgenus was defined only by the enlarged genitalia of the male, but that is a common response to the adoption of mating on the substrate, and cytological and morphological studies indicate that this has occurred independently in unrelated species. All species listed as *Tendipes (Tendipes)* by Townes (1945) are noted, including a species of *Goeldichironomus* or *Kiefferulus* in the U.S.A., but they were not included in the subgenus *Tendipes* by Townes, or have not been included in *Chironomus* (s.s.) at any stage).

In general, the morphological terminology used in this document follows Sæther (1980), Webb & Scholl (1985) and Vallenduuk & Moller Pillot (1997).

Abbreviations:

- ASA distance between antennal bases
- AT Anal tubules
- BOLD Barcoding of Life Database (http://www.boldsystems.org/views/login_interim.php)
- BR Balbiani Ring
- BV length of (fe + ti + ta1) / length ta2-5
- COI Cytochrome oxidase subunit I
- CT pupal cephalic tubercles
- FC Frontoclypeus (more correct for *Chironomus* than Frontoclypeal apotome)
- FT adult frontal tubercles
- GC Gonocoxite IX
- GS Gonostylus
- H setae Humeral setae. These occur only in the females of *Chironomus* species and are usually included with the dorsocentrals, but the arrangement can be a useful character.
- HR ratio of length to width of pupal respiratory base
- IPD Inter-plate distance i.e. distance between the Ventromental Plates
- IVo Inferior volsella
- MD male determining (gene)
- Mdt-Mat distance from tip of apical tooth of mandible to tip of the dorsal tooth
- Mt Mitochondrial
- MW width of Mentum
- N Nucleolus (i.e. the sac produced by an active NOR)
- NOR Nucleolar Organizing Region (i.e. the chromosomal locus capable of producing a nucleolus)
- PE Pecten Epipharyngis
- PMa Pecten Mandibularis
- Po Post occipital margin
- PreM Premandible
- PsA Pedes spurii A
- PsB Pedes spurii B
- S4A distance between S4 setae
- SCf Sensilla campaniformia
- SCh Sensilla Chaetica
- SSV length of (fe + ti) /length of ta1
- SVo Superior Volsella
- TIX Tergite IX of adult male
- TLt (or PLT) Lateral tubules
- VHL Ventral Head Length of larva
- VM Ventromental plates
- VMR ratio of the width of the marginal region of ventromentum (usually seen as a granular band under light microscopy) to the distance from the anterior margin to the base of the striae (see figure below)
- VPA (also IPD) distance between the inner margins of the two VM.
- VR Venarum ratio. There are 2 variants of this measure, one used mainly in the United Kingdom, the other in Europe, North America. The value used here is that given in Sæther (1981): length of M-vein to length of crossvein.
- VT Ventral tubules.
- ¿ presence at locality not confirmed.

In the adult descriptions reference is made to the types of SVo shape as recognized by Strenzke (1959). This is a helpful initial classification, but experience has shown that the

types are not discrete but are part of a continuum. The three categories as described by Strenzke are:

S-type: The SVo is shoe shaped, i.e. it is drawn out distal-medially into a broad, rounded lobe (Fig. a-c, below) (Strenzke's figure suggests the most distal point will be at the toe of the shoe),

D-type: The SVo is ribbon-like: distally it may have a weakly thickened shoulder (Fig. d, below) (most distal point is not at the internal margin) or bent in a shallow sickle-shape (Fig. e-f, below).

E-type: The SVo has the form of an elephant's tusk; distally it is sharply graded to a point, or with an expanded knob (Fig. g-i, below) (line from base to most distal point goes outside the limits of the SVo).



Abb. 4. Grundformen der Claspette des Chironomus-Hypopygs (♂), a-c S-Typ (a halophilus, b thummi thummi, c luridus), d-f D-Typ (♂, c dorsalis, f striatus), g-i E-Typ (g cingulatus, h salinarius, i annularius).

In the following descriptions, reference is made to the larval type. The scheme used here is the revision of older classifications as proposed by Proulx *et al.* (2013), which recognizes 9 categories. The categories are:

salinarius - lacking posterolateral (TLt) and ventral tubules (VT)

Lacking TLt:

halophilus - anterior VT very short or absent, posterior VT short, less than the width of the segment.

bathophilus – moderate to long, essentially straight VT, greater than the width of the segment.

fluviatilis - VT slightly curved and coming to a point at ends. (often hard to distinguish from bathophilus-type, particularly in some fixed material)

thummi – long, anterior VT with 'elbows', posterior VT coiled

Possessing TLt:

reductus – lacking ventral tubules.

semireductus – short straight or slightly curved VT, less than the width of the segment. melanotus – moderate to long, essentially straight VT.

plumosus - long, anterior VT with 'elbows', posterior VT coiled.



 halophiles type (total length 12 cm; the ford; 27.1V.1942); seen from the left, slightly from the ventral side.

9. salinarius type (total length 15 cm; the flord; 27.1V.1942); seen from the left.

from Andersen 1949

Reference is also made to the mentum and mandible types devised by Webb & Scholl (1985), Vallenduuk & Moller Pillot (1997) and Proulx *et al.* (2013). These earlier classifications were made for relatively small numbers of species, but with the much larger number of species in the North American fauna they do not cover all the variability seen in these characters and so further modification has been necessary. As well As well Proulx *et al.* (2013) proposed four categories for the PE and a ventromental character is introduced.

<u>The mentum type</u> is defined only by the degree of development of the 4th lateral teeth: Type I - height in same line as the rest of the lateral teeth;

Type II - 4th laterals reduced, height about equal to that of the 5th laterals;

Type III - 4th laterals further reduced, height less than that of the 5th laterals.



From Vallenduuk and Moller Pillot 1997

<u>Central tooth type</u>: The mentum may be further classified by the characters of the central trifid tooth:

Type IA - c2 teeth only partially separate from c1, i.e. as shoulders on c1. (figure a)

Type IB - c2 teeth slightly more separated (figure b)

Type IIA - c1 broad, c2 teeth distinctly separated (figure c).

Type IIB – c1 very broad, c2 less separated (figure d).

Type III - c1 tooth relatively narrow and much higher than the separated c2 teeth (figs e and f).

Type IV - c2 teeth well separated, not much lower than the relatively narrow c1 tooth (figs g

and h)



From Webb and Scholl 1985

Ventromentum



VMR = a/b

Pecten epipharyngis

Proulx *et al.* (2013) recognized 4 types of PE in North American species. These are useful if the teeth are not worn down, as they often are in older larvae.

Type A - fine sharp rather uniform teeth.



Type B - teeth broader but still sharp. Sometimes with one or two fine smaller teeth interspersed.



Type C - rounded and rather uniform, may also have sharp teeth. Worn type B teeth may be mistaken for this type.



Type D - rounded teeth with smaller teeth interspersed (generally found in the subgenera *Lobochironomus* or *Chaetolabis*).



Premandible

Assessment of the premandible can be difficult due to quite excessive wear and distortion in mounting. Where they are in good condition and lying flat, they can be categorised into 5 groups based on the relative widths of the teeth and whether they come to a sharp or a broad point. The teeth are measured as close as possible to their bases. Type A: Both teeth narrow and coming to a fine point.



Type B: inner tooth moderately broad, about 3-5 times wider than, outer tooth. This can be split into 2 subgroups:

B1 – both teeth come to relatively fine points.



B2 - the inner tooth comes to a relatively broad point.



Type C: Both teeth are moderately broad, coming to relatively broad points.



Type D: Inner tooth very broad, outer tooth moderately broad.



Type E: Both teeth very broad and often quite short.



There is also at least one species (*C*. sp. Florida) recorded with a five toothed premandible similar to that of *C*. *javanus*.



Species of Kiefferulus also have a five-toothed premandible, but it is broader.



<u>The mandible type</u> is defined by the degree of darkening and separation of the 3rd inner tooth. Tooth coloration may be more independent of the degree of separation than recognized in Europe or by Proulx *et al.* 2013. As a result, it seems better to consider the two characters separately. Separation: Type I – tooth fused Type II – tooth partially free Type III – tooth completely separated Color: Type A - tooth pale Type B - some degree of pigmentation Type C – as dark as other inner teeth



From Webb and Scholl 1985.

This figure would show IA, IIB, and IIIC respectively

Mandible length and Mdt-Mat

Mandible length is measured from the heel to the tip of the apical tooth.



Hirvenoja and Michailova (1998) illustrated that the distance between the tip of the dorsal tooth and the apical tooth could differ between related species (Mdt-Mat) (blue line in figure below).



However for different sized species it may be preferable to divide this value by the length of the interior teeth (black line) to obtain the MTR, i.e. Mdt-Mat/Len. inner teeth.

Relationship on the FC of the distance between antennal bases and distance between S4 setae This character gives some indication of the shape of the anterior region of the FC: the amount and extent of the narrowing from the anterior end near the antennal bases, and where the S4 setae are in relation to the broadening of the clypeus. Where the anterior narrowing continues almost to the S4 setae the distance between antennal bases will be greater; if the narrowing is only near the antennal bases and the width remains approximately the same until near the S4 setae the distances will be about equal; while if narrowing is quite short and then begins to widen up to the maximum width, the distance between the S4 setae will be greater. Note that this only an approximate measure of these relationships as other features such as the relative width of the FC at its widest point will also affect the relationship.



Frontoclypeus with approximately equal distance between antennal bases and S4 setae Note also the barely visible 'ring organ' of Yamamoto et al. (2015), (more obvious at top) immediately opposite the S5 setae. This is characteristic of the genus *Chironomus*.

Salivary reservoir:

This can sometimes be a useful character for separating species, but can be quite variable, probably due to squashing during slide mounting. Therefore those specimens with the widest opening are the most appropriate to use.

One aim in these studies has been to evaluate as many characters of adults, pupae and larvae as possible in order to evaluate which ones might prove useful for separating the species that can only be recognized at present by the banding patterns of the larval polytene chromosomes.

It should be noted that many of the larval characters referred to in the following descriptions can be quite variable. General size and ventral, lateral and anal tubules can be affected by environmental conditions, as well as by genetic variability. Appearance of mouth parts is also affected by wear, for example a worn type III central trifid tooth can appear to be type II. Genetic variation can also apply to these characters. Consequently, identification may need to be based on agreement of the majority of characters, particularly those that are least variable. This is why identification of larvae on the basis of morphological characters is so difficult.

Keys: Adult males – see Townes (1945)

thummi-cytocomplex

C. annularius sensu Strenzke, 1959 (Species 3d)

Species 9 – Martin (1979) and Wülker, Devai & Devai (1989).

In BOLD Bin: BOLD:ABA9112

Some specimens in this bin are called *C. cingulatus*, but there is no record of that species in North America.

Currently European specimens called *C. annularius* are listed in 3 other BOLD bins: <u>BOLD:AAU4046</u>; <u>BOLD:AAU4047</u> and <u>BOLD:AAW3973</u>

I am grateful to Prof. P. Michailova for pointing out that this North American species is identical to material of *C. annularius* from Russia.

Adult:

Male (Based on description of Strenzke, 1959): AR 4.0 (abt 3.72-4.28) Wing length 3.9 (abt 3.6-4.2) mm. Thorax greyish yellow, bands black or red-brown with more or less darker flecking. Setae – acrostichal abt 18-27; dorsocentral abt. 27-36; prealar about5-8; supra alar abt1-2; scutellar abt 33-48. Legs with tarsi darkened. LR1 abt 1.35-1.45; F1/Ti1 abt 1.04-1.08.

Abdomen with most of seg. I yellow brown, segs II-V darker, II-IV with anal border extended medially.

Hypopygium with abt 4 setae in patch in center of TIX; SVo of E-type (variable); IVo markedly narrowed at the tip, GS narrowing gently over posterior half.



From BOLD database: specimen A08z+1337961340

Female (from specimens in BOLD):

Wing length abt 4.5 (3.5-5.0) mm, VR 1.04

General coloring dark brown; palps brownish black; thorax yellow with bands brown, postnotum dark; abdomen dark brown, with lighter posterior margin on segments 2-4; Legs yellowish with darkening at knees and tarsal segments darkening to black from Ta1.

Antennae brown, darkening towards the tip; AR about 0.36-0.44; A5/A1 about 1.13-1.17; approximate segment lengths (micron) 210 : 140 : 130 : 140 : 242. Approx. lengths of palpal segments 2-5 (micron) 50 : 225 : 275 : 420.

Legs: Approximate lengths and proportions (micron):

	Fe	Ti	Ta1	Ta2	Ta3	Ta4	Ta5	LR	F/T	Ta4/Ti
PI	1525	2925	2275	1080	710	565	225	1.53	0.93-1.15	0.38
PII	1590	1275	1000	500	280	250	195	0.90	1.40	
PIII	1765	1760	1065	690	485	300	205	0.52-0.69	0.92-0.94	

Pupa: some pupal characters known from a late prepupa.

Caudolateral spur on segment VIII (below) with about 3–5 spines only near the tip. Anal fringe with about 94 taeniae in multiple rows.



Fourth instar larva a medium length plumous-melanotus-type (15.3-17.8 mm), very similar to that of Sp. 3b but gular region darker (posterior 2/3 dark to very dark, extending beyond width of mentum and generally higher at edges), FC normally pale, but occasionally very slightly darkened or with a streak along the middle and on edges. Lateral tubules shorter (about 160-240 µm). VT relatively long, about equal or anterior pair slightly longer (ant. 0.92–1.80, 1.42 mm; 0.92–1.80, 1.34 mm). AT relatively short (420-640 µm) with no constriction, about 2 to 3.2 times longer than wide, ventral pair usually shorter.



Anal tubule of *C*. annularius.

Mentum (Figs. e & f, below) with 4th laterals only slightly reduced (type I-ii), central trifid tooth sometimes type IB and others type III, with c2 teeth separated to about 1/3 depth of c1 tooth, which can also look like type IIA if worn.

Ventromental plates (Fig. g, below) about 237-270 μ m long, 3.5-4 times wider than deep, 0.99-1.07 times the mentum width; IPD about 40% of width of mentum, with about 42–43 striae; VMR 0.27-0.30.

Premandible (Fig. b, below) closest to type D with relatively broad inner tooth, about 3.5-4 times wider than the outer; teeth relatively short, only about twice as long as the greatest width. PE (Fig. a, below) with about 16-17 pointed teeth.

Antenna (Fig. c, below) with basal segment about 0.35-0.4 of VHL, about 3.5-3.6 times longer than wide, RO just under half way up the segment; AR about 2.2–2.4, relative length of segments (μ m) 152 : 35 : 9 : 15 : 8.

Distance between the antennal bases less than that between the S4 setae, which are separated by about 78-81% of the FC width.

Mandible (Fig. d, below) with 3rd inner tooth relatively well separated, but still relatively pale (type II-IIIB), 16–19 furrows on outer surface at base; 11-16 taeniae in PecM; MTR 0.21-0.34.



Mouth parts: a. Pecten epipharyngis; b. Premandible; c. Antenna; d. Mandible; e. Mentum - Crooked Lake, Sask.; f. Mentum - East Horsehead Lake, Wisc.; g. Ventromental plate.

Cytology: 4 polytene chromosomes with the thummi arm combination AB, CD, EF, G. Arm G with a median and terminal BR, with a nucleolus and heterochromatin at the other end, separated from the BRs by a distinct constriction. Nucleoli developed subterminal in E (may be a double structure), proximally to the olive in arm A and also proximal in C. Polymorphism in arms A?, C, E and G.

h'annA2: 1a - 2c, 10 - 12a, 13ba, 4a-c, 2g-d, 9 - 4d, 2h - 3, 12cb, 13c -19 i.e. as h'antA1, h'pluA2.

h'annB2: Puff, with distal dark bands (gps 8-7), just distal to typical bands 24-27 as h'annB2.

h'annC2: 1a - 6b, 11c - 8a, 6c - 7d, 16 - 17a, 6hg, 11d - 15, 17b - 22 as p'annC2 n'annC4?: Simple inversion of about 1/3 of arm near the distal end. but could be p'annC1? n'annD3: 1 - 3g. 11 - 13a, 10a, 7a - 4, 10e-b 13b - 16, 8b - 9, 7b-g, 18a-d, 8a, 17a-f, 18e - 24h'annE1: 1-3e, 5a-e, 4hg, 10b-6, 4f-3f, 10c-13 i.e. probably derived from h'annE2 h'annE2: 1 - 3e, 5a - 10b, 4h - 3f, 10c - 13n'annF3: 1 - 4b, 8c - 4c, 17 - 13, 10a-d, 18c -a, 11 - 12, 9f - 8c, 18de, 19 - 23

n'annG3: subterminal nucleolus and median and terminal BR this could be a subterminal inversion of p'annG1 (not p'annG2)



Found: Alberta - Amisk Lake; Astotin Lake, Elk Island N.P. (53.685°N, 112.86°W); Two Hills (53.7104°N, 111.744°W) (BOLD) British Columbia - New Afton Mine, Kamloops (50.663°N, 120.504°W) (BOLD) Manitoba - Riding Mountain N.P. (50.676°N, 99.898°W) (BOLD) New Brunswick - Rothesay Park(45.338°N, 65.996°W) (BOLD). Ontario - Haggersville (42.957°N, 80.051°W); Prairie Smoke, Carden Alvar (44.645°N, 79.095°W; Williamstown (45.146°N, 74.573°W) (all BOLD) Prince Edward Island - Miscouche (46.432°N, 63.864°W)(BOLD). Saskatchewan - Crooked Lake (50.60°N, 102.75°W), Pasqua Lake (50.78°N, 104.00°W) & Round Lake (50.53°N, 102.37°W), Qu'Appelle River; Mission Lake, 2 Km w. Lebret (50.75°N, 103.70°W); Big Quill Lake abt 1 ml s. Dafoe (51.55°N, 104.72°W). Yukon Territory - Lake Laberge (60.958oN, 135.184oW) (BOLD). Indiana - Crooked Lake, Angola. Minnesota - Lake Christina (46.08°N, 95.75°W), Douglas Co. North Dakota - Larimore Dam, Grand Forks Co. (47.90°N, 97.67°W); Warsing Dam, Eddy Co.; and Brewer, Williams, Blacktail, Clearwater Lakes; McVille Dam (Kiknadze et al. 2016). Wisconsin - Booth Lake (Hilsenhoff & Narf 1968), East Horsehead Lake, Oneida Co.; Grand Portage Lake, Iron Co. (Hilsenhoff & Narf 1968); Kegonsa Lake, Dane Co. (Hilsenhoff & Narf 1968); Pine Lake, Pleasant Lake, Walworth Co.

Lakes and dams.

Fourth instar larva very similar to that of sp. 3b but gular region darker, frontoclypeus pale, and lateral projections shorter. Some information on arm F published by Martin (1979), Wülker, Devai & Devai (1989), under the name Species 9. Some larval characters given in

Hilsenhoff & Narf (1968) as *Chironomus* species D. Karyotype described by Kiknadze *et al.* (2012) and (2016)

It is possible the specimen sequenced in Guryev *et al.* (2001) may have been a misidentified *C. muratensis* as it is closest to that species in GenBank accessions.

C. muratensis (Species 3t)

Misidentification in Dinsmore & Prepas 1997, p. 2171. No evidence that this species occurs in North America. See *C. annularius* (species 3d)

Chironomus anthracinus-group

C. anthracinus Zetterstedt 1860 (Species q)

In BOLD Bin: BOLD:ACB1083

The nearest neighbor to this species in the BOLD database is species 5TE from Norway

Adult:

Male (based on Townes (1945) supplemented from Shobanov (1996):

Wing length 5.6 mm, AR 6.0, anterior LR 1.15, body stout.

Blackish with brown body hairs; legs blackish brown with long sparse tarsal beard.

Frontal tubercles of medium size, clypeus very large.

Mesoscutum with a weak median tubercle.

Leg lengths (micron) and proportions (Shobanov 1996):

	Fe	Ti	Ta1	Ta2	Ta3	Ta4	Ta5	LR	F/T	BR
PI	1465	1500	1880	1085	710	600	305	1.00-1.27	0.95-1.00	6.4-8.1
PII	1600	1525	900	575	400	280	220	0.59	1.04-1.05	
PIII	1840	1880	1295	805	550	365	244	0.68	0.98	



Male hypopygium of *Chironomus anthracinus* (from Townes 1945)(left) and of a European specimen (Shobanov 1996)(right)

From the illustrations it appears there are 3-6 setae in about 3 compact pale areas on tergite IX. The anal point and appendages unusually short and broad. SVo as illustrated by Townes (1945) does not exactly fit any of Strenzke's types but is essentially an E-type. It differs from that of Palearctic specimens as described by Shobanov (1996) (above). IVo longer than anal point and to about midpoint of GS, which is quite swollen and narrows sharply over distal 1/3.

Female:

Similar to the male except for the usual sexual differences. Some details of the female were described (in Russian) for European specimens by Rodova (1978). Length 6-6.5 mm., wing 5.5 mm.

Thoracic setae: acrostichal 18-33; dorsocentral 50-65; prealar 8-12; supraalar 1-2; scutellar about 55 in 3 irregular rows.

The ventral terminalia are illustrated (below):



Ventral view of the terminalia of C. anthracinus (Rodova 1978)

Pupa: Based on Langton & Visser (2003): Includes both males and females, but males expected to be smaller. Length 10.3-13.7 mm.; wing 2.27-2.66 mm. Cephalothorax dark brown, abdomen colorless with restricted brownish or golden markings. Cephalic tubercles conical 125x100 (female?)-200x157 (male?) μ m; no secondary cephalic tubercle or frontal warts. Basal ring 155-157 x 55-70 μ m; HR abt 2.24-2.72. Thoracic granulation strong and extensive over anterior half. Hook row of segment II entire, occupying 0.54 of segment width and with 87 (male?)-131(female?) hooks. Shagreen an undivided, moderately extensive patch of strong points, but more or less reduced on seg.VI. Spur of segment VIII elongate with about 3-6 narrow spines. Anal fringe of 119(male?)-159(female?) taeniae.

Fourth instar larva a medium to large thummi-type larva (12.4-20.2 mm) with anterior pair of VT normally longer (Ant. 1.0-1.9 mm; Post. 0.73-2.44 mm); one larva had a small TLt about 20 μ m long. Salivary reservoir about 81-91 x 25 μ m, 3.2-3.6 times longer than wide. Anal tubules mostly 340-520, but up to 1240 μ m long, usually simple but the longest one with a median constriction, 2.9-4.3 times longer than wide.

Ventral head length about 410 μ m (female), 385 μ m (male). Gular region dark over at least the posterior two thirds and commonly right up to the base of the mentum, higher at outer edges and narrowing anteriorly; FC pale but with slightly dark lines along the edges.



From Proulx *et al.* (2013)

Mentum (c, below) width about 250 micron (female), 235 micron (male), about 0.6 of VHL; with a broad c1 tooth, c2 teeth sometimes clearly on shoulders of c1, but in other specimens may be almost distinct teeth (type IB- IIA). Fourth lateral teeth reduced to height of 5th laterals (type II-III).

VM (d, below) about 246-250 μ m wide and 3.5 times wider than deep, slightly wider than the mentum (1.01-1.03), with about 38-42 striae reaching 2/3 of way to anterior margin and reaching closer towards the lateral edge; IPD about 40% of mentum width, with about 37-47 striae; VMR 0.25-0.32. PE (c, below) with about 12-17 teeth of type B, but a few may be reduced.

Premandible with inner tooth broad, about 3.5-3.8 times wider than the moderately broad outer tooth.

Antenna (b, below) with relatively long basal segment, about 0.35 of VHL and 2.7-3.9 times as long as broad; RO about one third to a half up from the base; AR about 1.8-2.1; antennal segments about 150 : 39 : 11 : 16 : 8 micron.

ASA greater than distance between the S4 setae, which are separated by about 0.85-0.87 of the frontoclypeus width. S5 setae about level with the nearby RO.

Mandible (e, below) about 340 µm long, of type II-IIIB; about 16-20 furrows on outer surface near base, 14-15 taeniae in PecM; Mdt-Mat 30-33 µm, MTR 0.29-0.34.



Two specimens from Friebauer Lake, Wisconsin differ in three respects - they possess TLt about 360 μ m in length, the posterior VT are longer, and the FC is darkened. The significance of these differences is uncertain.

Cytology: 4 polytene relatively short chromosomes with the thummi arm combination AB, CD, EF, G.

Arm G unpaired and cloudlike with only one or two clear bands and a nucleolus. Arm F with a median nucleolus in group 9. Polymorphism in arms A and D. See also *C. rempelii* (sp. 2m), which may be a synonym. Some larvae have arm F with one or two

heterochromatic knobs (F1k and F1kk), but whether such knobs are sex linked as in *C. rempelii* has not been determined, and hence there is uncertainty as to the synonymy.

h'ant A1: 1-2c, 10-12a, 13ba, 4a-c, 2g-d, 9-4d, 2h-3, 12cb, 13c-19 i.e. as *plumosus* A2

h'ant A2: 1-2c, 10-12a, 13ba, <u>3f-2h, 4d-9e, 2d-g, 4c-a</u>, 3g-i, 12cb, 13c-19

- n'ant A3: 1 2c, <u>9a-e, 2d-g, 4c-a, 13ab, 12a 10</u>, 8a 4d, 2h 3i, 13c 19 (heterozygote only)
- h'ant B1: not mapped
- h'ant C1: 1 6b, 11c 8, 15 11d, 6gh, 17a 16, 7d 6c, 17b 22
- h'ant D1: 1-3g, 14g-16, 8c-7g, 5d-7f, 18d-17, 8d-10a, 13a-11, 14f-13b, 10b-e, 4-5c, 18e-24
- h'ant D3: 1-3g, 14g-16, 8c-7g, <u>18a-d, 7f-5d</u>, 17f-a, 8d-10a, 13a-11, 14f-13b, 10b-e, 4-5c, 18e-24

- h'ant E1: 1 3e, 5a 10b, 4 3f, 10c 13
- h'ant F1: 1 8e, 9c 23
- n'ant F3: 1 8e, 9c-e, 14h 10, 15 23
- h'antG1 Virtually terminal nucleolus



Found: Alberta - ¿Lake Amisk (Kiknadze *et al.* (2005). Manitoba - ¿Baptist Lake (Kiknadze *et al.* (2005). ¿Lake Winnipeg (Sæther 2012). Ontario - Bat Lake (45.577°N, 78.523°W), Algonquin Provincial Park; Raft Lake (46.42°N, 80.95°W), Hannah Lake (46.45°N, 81.05°W), Pine Lake (46.38°N, 81.03°W), Ramsey Lake (46.47°N, 81.05°W), Silver Lake (46.47°N, 80.95°W), all Sudbury.
Quebec - Osisko Lake (48.25°N,79.00°W), Lake Arnoux (48.15°N, 79.33°W), both Rouyn-Noranda, Quebec City. California - Riverside, Riverside Co. Indiana - Crooked Lake.
New Hampshire - Mirror Lake, Grafton Co. Wisconsin - Pine Lake, Oneida Co.

Found in lakes.

Karyotype described by Kiknadze, Wuelker, Istomina and Andreeva (2005), but some rare sequences from Canada may relate only to *C. rempelii*.

Molecular data suggests that two species are included amongst North American material, one of which corresponds to the Palearctic species (Proulx *et al.* 2013) The status of the second type (Sp. NAI) is currently unclear. It may correspond to *C. rempelii*, but unfortunately no material from larvae with the likely *C. rempelii* sequences have been available for molecular analysis.

i.e. as *cingulatus*

There are differences in the description of the larval type in the literature: Shobanov (1966) illustrates the Palearctic larva as a bathophilus-type, while the Nearctic specimens are a thummi-type larva, and there are reports of a *C. anthracinus* adult emerging from thummi-type larva in England. This may reflect a polymorphism for lateral tubule presence or it may be that some specimens, lacking cytological or barcode confirmation, are another member of the *C. anthracinus*-group. Larger specimens, with posterior VT longer, may be *C.* nr. *anthracinus* (Species 3c).

C. nr. *anthracinus* (Species 3c)

Adult and Pupa: not known.

Fourth instar larva a medium to large (fem. 12.4-19.8 mm; male 17.4 mm) plumosus-type; Head capsule width less than 0.82mm. VT relatively long, posterior pair longer (ant 1.68-1.81, post 1.91-2.44 mm), TLt about 320-360 μ m. AT long, ventral pair with a median constriction, dorsal pair about 500-800 μ m, ventral pair 760-785 μ m, respectively 2.5-4.5 and 2.7-3.1 times longer than wide.

Gular region dark over $\frac{3}{4}$ or more, extending beyond the width of the mentum and lowering towards the edges, FC darkened. Salivary reservoir 68-76 μ m long and about 2-2.5 times longer than wide.

Mentum (c, below) about 0.65 of VHL, with pointed teeth; c1 tooth broad with short parallel sides, c2 teeth relatively well separated (type IIA) about 0.26 of MW; 4th laterals reduced about halfway to height of 5th laterals (type I-II).

VM (d, below) with inner margin downturned, IPD 0.28-0.33; about 3.4 times wider than deep and 1.07-1.27 times the MW; with 45-59 striae; VMR 0.27-0.29. PE (a, below) with about 15-16 somewhat variable teeth, but essentially ty.B.

Premandible of type C, with broad inner tooth about 4-5 times the width of the relatively narrow outer tooth.

Antenna (b, below) with relatively long basal segment about 3.4-4.4 times longer than wide, RO about 40% up from base, AR about 2.04-2.23; segment proportion about 144 : 35 : 11 : 14.5 : 7.5.

Distance between the antennal bases greater than that between the S5 setae, which occupy 0.73-0.75 of the frontoclypeus width. S5 setae at least slightly posterior to the nearby RO. Mandible (e, below) with third inner tooth slightly reduced and moderately colored (type IIB); 15-16 furrows on outer surface at base; 15 taeniae in PecM; Mdt-Mat 43, MTR about 0.45 when not worn.



Cytology: 4 short polytene chromosomes with the thummi arm combination AB, CD, EF, G. Arm G cloudlike with an almost terminal nucleolus and only four or five obvious bands; paired only at the nucleolus. No nucleoli in other chromosomes.

1 - 2c, 10 - 12, 3 - 2d, 9 - 4, 13 - 19

1 - 3e, 5 - 10b, 4 - 3f, 10 - 13

1 - 10, 17 - 11, 18 - 23

Arm A1: Arm E1: Arm F1: i.e. as *holomelas, tardus,* etc. i.e. as *cingulatus, tardus* and sp. 3b. i.e. as in *cucini, tardus* and *tenuistylus*.



Found: Wisconsin - Friebauer Lake, Bayfield Co.

Lakes

Some larval characters given in a brief key of Hilsenhoff & Narf (1968) as *Chironomus* n. sp. C.

C. nr. *anthracinus* (*C.*nr. *aberratus* according to Wülker) (Species 3b) Also called *C.* 'Apple Valley' (Wülker 1980)

Adult and Pupa: not known.

Fourth instar larva a plumosus type larva, although VT length and shape could not be determined. Posterior of gular region and the FC darkened. C1 tooth of mentum relatively wide with well separated c2 teeth; 4th laterals not visibly reduced. 4th tooth of mandible clearly demarcated.

Cytology: 4 relatively short polytene chromosomes with the thummi arm combination AB, CD, EF, G. Arm G partly unpaired with a subterminal nucleolus; only about a dozen visible bands. Nucleolus in arm B and possibly a smaller one in arm F. Polymorphic in arm B.

Arm A1: 1-2c, 10-12, 3-2d, 9-4, 13-19 i.e. as in *holomelas, cucini, tardus, magnus,* etc.
Arm B1: differs from *longistylus* by a distal inversion.

- Arm C1: 1-6b, 15c-e, 8-11c, 15b-11d, 6gh, 17a-16, 7d a, 6f c, 17b-22 as *cucini*, *islandicus*
- Arm D1: proximally similar to *longistylus*
- Arm E1: 1-3e, 5-10b, 4-3f, 10-13

Arm F1: 1 - 23

i.e. as in *cingulatus* and *tardus*. i.e. Standard as in *piger*.



Found: California - Spring Lake, nr. Hesperia, Apple Valley, San Bernadino Co.

High altitude lake.

Cytology described as 'C. species Apple Valley' by Wülker (1980) some information on Arm F given in Fig. 3 of Martin (1979) and a photo of arm C with an incorrect sequence given in Devai *et al.* (1989).

The larva shows similarity to that of *C*. nr. *annularius* (Sp. 3d) but the gular region is not as dark, the FC is darker and the lateral projections longer (Wülker, pers. comm.) From the ecological information for the locality given by Egan and Ferrington (2015), it is likely that this is their "*Chironomus aberratus*".

C. rempelii Thienemann, 1941 (Species 2m)

C. hyperboreus - Rempel, 1936, misdetermination of *C. hyperboreus* Staeger, 1845 Syn. *Tendipes anthracinus* - Townes, 1945

Possibly a synonym of C. anthracinus Zett., but may be a sibling species.

Adult:

Male: Length about 8 mm. Dark species, thorax black, scutal stripes barely visible, abdominal segments black with grey apical margins; legs black, anterior tarsi with beard.

Wing length about 5.93 mm, width 1.33-1.40 mm; 4 SCf, about 44.7 (43-47) setae on squamal fringe.

Head: AR 5.08-6.16, Frontal tubercles about 46-51 x18 μ m and 2,6-2.9 times longer than wide. Relative proportions of palp segments (micron) 90 : 81 : 312 : 320 :; P5/P3 about 345; P5/P4 about 1.07-1.08.

Thoracic setae: perhaps 24 acrostichal; 46-57 dorsocentrals; 10-13 prealars; 2 supraalars; 64-66 scutellars with about 25-28 in posterior row and 38-39 in scattered rows anteriorly.

Leg lengths (micron) and proportions:

	Fe	Ti	Ta1	Ta2	Ta3	Ta4	Ta5	LR	F/T	BR
PI	1880	2030	2395	1420	860	680	350	1.17-1.18	0.92	3.04-3.1; 4.4-6.2
PII	2090	2065	1220	795	540	355	260	0.59	1-1.02	
PIII	2520	2635	1755	1130	775	460	290	0.66-0.67	0.93-1.02	

SensChaet of MidTa1 – abt. 38-43; HindTa1- abt 21-23

Beard appears to be comprised of both a shorter and a longer set of setae.



Male terminalia of *C. rempelii* (From Rempel 1936) Ventral view (left), dorsal view (right)



Male hypopygium (left) and superior volsella (right) of *Chironomus rempelii* from Lake Waskesui, Saskatchewan.

Male genitalia figured by Rempel (1936) and photographed (above); about 6-8 setae in 3-6 clear areas comprising a triangular area (see right figure above) on tergite IX; SVo convex, closest to the E(i)-type of Strenzke (1959); IVo extending beyond the end of the anal point to about 1/3 of the GS length, GS relatively short, narrowing over the posterior quarter.

Female: Generally resembles male, but legs are paler - dark brown, with proximal half of anterior femur somewhat yellowish. Female genitalia figured by Rempel (see figure below). GPVIII crescent shaped; cercus shown with a rounded margin and a slight bulge at anterior of ventral margin.



Female adult of *C. rempelii* (From Rempel 1936) Ventral view (upper left), tergite IX (upper right), lateral view (below)

Pupa: Mean length about 12 mm. Late pupa almost black. Cephalic tubercles with a short seta. Lateral setae on Segments 2 to 4 have 3, 4 and 4 short lateral hairs respectively, while segments V to VII have 4 lateral setae and segment 8 has 5. Shagreen pattern shown in figure below. Posterolateral spurs on segment VIII with about 8 rather long moderately appressed spines.



Egg mass and pupa of *C. rempelii* (From Rempel 1936)

Fourth instar larva a large bathophilus type (see below), length 10.5-22.0 mm.; VT about equal length and longer than posterior prolegs.

Mentum width 220 μ m, with broad c1 tooth, c2 teeth relatively well separated (type I-II); 4th laterals reduced at least to level of the 5th laterals (type II), which in Rempel's material project beyond the level of 3rd to 6th laterals. PE with 16-18 blunt teeth.

Rempel's figure of the premandible suggests the teeth are about equal length and inner tooth is slightly wider (type B1?). Mandible with 3rd inner tooth pale.

Antennal segments in ratio 40 : 10 : 3 : 5 : 1 ; RO towards middle of the basal segment.



Larval parts of C. rempelii from Rempel (1936)

Egg mass: Rempel (1936) figures the egg mass (see above) as globular with two transparent threads traversing it. Can be 650 eggs in the mass.

Cytology: 4 polytene chromosomes with the thummi arm combination AB, CD, EF, G. Arm G generally unpaired, sometimes cloudlike, sometimes with clear bands and heterochromatin cap in the area of the nucleolus. Nucleolus in arm F, which may be heterozygous in males for one (in region 3-4, F1k) or two (other in region 1, F1kk) heterochromatic bands. This polymorphism may be seen in all males (Waskesiu) or only in a small number (Lake Amisk and Baptist Lake). Most common sequence in each arm as in *C. anthracinus*. Polymorphism in arms A, B, C, D and F. Sequences are given the prefix 'ant' to relate them to the sequences as identified by Kiknadze

et al. C. rempeli



antA1: 1-2c, 10-12a, 13ba, 4a-c, 2g-d, 9-4d, 2h-3, 12cb, 13c-19 i.e. as *plumosus* A2 antA2: 1-2c, 10-12a, 13ba, 3f-2h, 4d-9e, 2d-g, 4c-a, 3g-i, 12cb, 13c-19 rare antA3: 1-2c, <u>9a-e</u>, <u>2d-g</u>, <u>4c-a</u>, <u>13ab</u>, <u>12a-10</u>, 8a-4d, 2h-3, 12cb, 13c-19 rare antB1: not mapped. antB2: Simple inversion near distal end. rare antC1: 1-6b, 11c-8, 15-11d, 6gh, 17a-16, 7d-a, 6f-c, 17b-22 antC3: small inversion of region about 17a-6c rare antD1: 1-3g, 14g-16, 8c-7g, 5d-7f, 18d-17, 8d-10a, 13a-11, 14f-13b, 10b-e, 4-5c, 18e-24 antD3: 1-3g, 14g-16, 8c-7g, 18a-d, 7f-5d, 17f-a, 8d-10a, 13a-11, 14f-13b, 10b-e, 4-5c, 18e-24 rare antE1: 1-3e, 5-10b, 4-3f, 10-13 i.e. as *cingulatus*, *tardus* and sp. 3b. antF1: 1 - 8e, 9c - 23 (with variants F1k and F1kk in males only) antF3: 1 - 8e, 9c-e, <u>14 - 10</u>, 15 - 23 rare Found: Alberta - ¿Lake Amisk (Kiknadze et al. (2005). Manitoba - ¿Baptist Lake (Kiknadze et al. (2005) Saskatchewan - Lake Waskesiu, Prince Albert National Park (Type locality)

(53.92°N, 106.08°W). Wisconsin - ¿Pleasant Lake, Walworth Co. (W. Hilsenhoff)

Occurs at depth of 10 m or greater. Life cycle in Lake Waskesiu is two years.



Lake Waskesiu, Prince Alfred National Park, Saskatchewan

Morphology of larva, pupa and adult above are based on the descriptions of Rempel (1936) (as *C. hyperboreus*). Townes 1945 considered the adult was *C. anthracinus* Zett., but Thienemann (1954) still regarded it as a separate species. One difference is that the SVo of *C. rempelii* is a normal convex type, rather than the concave SVo of *C. anthracinus*. The karyotype shows relationship to that of *C. anthracinus* such that Shobanov *et al.* (1996) and Kiknadze *et al.* (2005) have also considered it to be a synonym of *C. anthracinus*. This may well be correct, but there are some aspects that still suggest that it may be a separate subspecies or sibling species: The heterochromatin on arm F and the sequences A3, C3 and F3 have so far only been found in certain Canadian samples, and the location of the MD has

not been determined in any typical *C. anthracinus* populations. The larva described by Rempel (see above) is shown as a bathophilus-type larvae, whereas the true *C. anthracinus* has a thummi-type larva.

Karyotype pictured by Rempel *et al.* (1962) and in more detail by Kiknadze *et al.* (2005). Recent molecular results of Proulx *et al.* (2013) on Canadian lakes indicate that there is a close relative of *C. anthracinus*, which may be *C. rempelii*, although this cannot be proven in the absence of material from the type locality. This material also has a thummi-type larva (see sp. 4x *C.* sp. NAI), so could also be a further member of this anthracinus-group of species.

Chironomus sp. NAI of Proulx et al. (2013) (Species 4x)

also *Chironomus* nr. *anthracinus Chironomus rempelii* – Hamilton (1965) Species 18 of Wülker as *C*. cf. *aberratus*.

In BOLD Bin: <u>BOLD:AAG5476</u>

Some as *Chironomus anthracinus*-group.

The nearest neighbor to this species in the BOLD database is species 5TE from Norway.

Adult: Described as C. rempelii by Hamilton (1965):



Male: AR: 5.23–5.58 (5.37,4) Wing length 4.2–4.9 mm, VR about 1 or slightly higher. LR 1.23–1.44 (3); fore tarsus with a long sparse beard. Black or blackish with grey bands along the caudal margins of each abdominal tergite. Head: frontal tubercles long, about 3x longer than broad., temporal setae in double irregular rows which extend medially to points midway between vertex angles of eyes and mid line of head. Palpal proportions (segs 2-5) 8 : 28 : 26 : 34. Clypeus large with 49-62 setae.

Thoracic setae: dorsocentrals in a double irregular row; prealar – 5-9; scutellum with two irregular rows and some random setae near center line between these two rows. About 10 setae, apparently in a single pale spot on tergite IX. Anal point very broad, SVo curved, E-type of Strenzke (1959) – Hamilton's illustration shows the distal end turned down, and different from the illustration of Townes (1945) for *C. anthracinus*. IVo short, barely longer than the anal point and to about the proximal third of the gonostyle, which narrows relatively gently over the distal third.

Pupa: Described as C. rempelii by Hamilton (1965).

Length 11.1–12.0 mm (7). Cephalic tubercle acute with a short bristle near the apex. Abdomen: Tergite I not shagreened, tergites II-VI with shagreen on central and caudal part of disc (fig. c above), tergite VII with a fine patch of shagreen near each antero-lateral angle, and tergite VIII with two patches of shagreen on the central part of disc; lateral filaments on segments V-VIII usually 4-4-4-5; caudolateral spur of segment VIII with 3–8 fine, appressed spines; anal lobes each with 120–170+ filaments.

Fourth instar larva: A medium sized thummi-type larva, length about 14.8–22 mm (fem. 16.3-18.8; male 14.8-20.5). Gula dark to very dark over at least the posterior 2/3 and covering most of the width of the ventral head, slightly lower at outer edges; FC pale or very slightly darkened. Salivary reservoir 76-98.5 μ m long and 3.6-3.9 times longer than deep. Anterior VT generally longer than posterior (Ant. 0.84-1.82 mm; Post. 0.8-1.7 mm). AT quite long (dor. 340-740 μ m, vent. 340-880 μ m); dorsal pair about 2.9-4.0, and ventral pair about 3.3-4.9 times longer than wide, as illustrated by Hamilton (1965).

Mentum (Fig, d, below) with c2 teeth slightly to moderately separated (type IIB-III), 4th laterals reduced to about the level of the 5th laterals (type II), 6th laterals lower.

VM (Fig, e, below) about 240-273 μ m wide, with smooth outer edge, 3.24-3.6 times wider than deep and 0.95-0.98 of the mentum width, with about 40-49 striae; VMR 0.27-0.32. PE (Fig, a, below) with 10-18 sharp, sometimes irregular teeth.

Premandible (Fig, b, below) of type B1 with moderately broad inner teeth, inner teeth broad at base but narrowing markedly, inner about 3-4.5 times the width of the outer.

Antenna (Fig, c, below) with A1 0.37-0.4 of VHL and about 3.4-3.6 times longer than wide, RO near middle of segment; AR about 1.87-2.10; proportions (μ m): 162 : 46 : 13 : 15 : 8.5. Distance between antennal bases (200-225 μ m) greater than distance between S4 setae (205 μ m), which are separated by about 0.8 of the FC width; S5 setae level or posterior to nearby RO.

Mandible (Fig, f, below) about 323-343 μ m long, with third inner tooth only slightly separated and pale or slightly darkened (1I- IIIB), about 18-23 furrows on outer surface near base; 12-16 taeniae in PecM; Mdt-Mat 35, MTR 0.33-0.34.



Photos from I. Proulx.

Cytology: 4 polytene chromosomes with the thummi arm combination AB, CD, EF, G. Chromosomes quite short. Appearance and sequences essentially as in *C. anthracinus*: Arm G generally paired with a nucleolus about 1/3 from the end. Arm F probably with a nucleolus near the characteristic bands. Nucleoli in arm G, and also in arm F. No polymorphism identified.



This species is very similar to *C. anthracinus*. The population at Marion Lake was originally identified as *C. rempelii*, but Hamilton later provided larvae from the Lake as *C. anthracinus*.

Molecular: This species is largely identified by the mt*COI* DNA sequences, which differ from those of *C. anthracinus* by 22 fixed differences (Table S4 of Proulx *et al.* 2013). GenBank accession numbers: KF278219-20, KF278344 There is no difference in the $gb2\beta$ sequences between this species and *C. anthracinus*. GenBank accession numbers: KF278390-92

Oligotrophic, near-neutral lakes at depth of 4 to 7.5 m. A single generation per year.

Found: British Columbia - Marion Lake, Squamish (49.55°N; -123.18°W). Ontario - Kasten Lake (46.37°N; 80.97°W), Sudbury area (Proulx *et al.* 2013)

C. nr. anthracinus sensu Butler et al. 1995 (see C. atrella).

(End anthracinus group)

C. athalassicus Cannings, 1975 (Species 2v)

This species is in BOLD Bin: <u>BOLD:AAW3972</u> The nearest neighbor to this species in the BOLD data base is *C. atrella* in BOLD:AAG5507

Adult:

Male: Coloration variable from pale to dark.

AR 3.92 - 4.43 (4.14); wing length 4.3-4.7 mm (4.5 mm); VR 1.04-1.09 (1.05); LR 1.07. Frontal tubercles about 40 micron long. Clypeus broad with 25-52 (42) setae. Palp segments 2-5 (micron) 84 : 300 : 265 : 320).

Thorax with median tubercle as a low bump. Setae: Acrostichals 0-5; Dorsocentrals multiserial 30-50 (39); Prealars 15-19 (16); Scutellars 42-70 (53).

Anterior tarsi without beard, but occasional long setae up to 6 times the tarsal diameter. Leg segments (microns):

	Fe	Ti	Ta1	Ta2	Ta3	Ta4	Ta5	LR	F/T	BR
PI	2180	1990	2130	1190	880	690	350	1.07	1.10	6.0 (rare)
PII	2190	2120	1110	740	570	420	270	0.52	1.03	
PIII	2480	2510	1550	980	740	470	290	0.61	0.98	



Hypogygium (left) and superior volsella (right) of a paratype male of C. athalassicus.

Abdominal terga reddish brown with a thin dark basal band. The apical one third to one half of segments 7 and 8 pale. In higher salinities the colors become paler.

Anal point broad, inner edge of SVo only slightly curved and of D(f)-type. IVo relatively long and bending outwards at the end, reaching past the end of the anal point and to about the midpoint of the GS which reduces relatively sharply over posterior quarter.

Female: Color as male, but generally paler.

Wing length 5.0-5.2 mm; VR 1.13 (1.10-1.14) or 0.97 (0.88-0.91); ant. LR 1.14 (1.1-1.15), mid LR 0.52 (0.50-0.54), hind LR 0.63 (0.60-0.68).

Antennal segments (micron) 200 : 150 ; 160 ; 150 ; 260 , AR 0.36-0.43 (0.39); A5/A1 1.2-1.45 (1.3). Frontal tubercles about 25 micron long. Clypeus broad, about 1.7x the antennal pedicel. Palp segments 2-5 (micron) 80 : 225 ; 225 ; 345. Thorax with median tubercle as a bump. Setae: Acrostichals and postalars absent;

Dorsocentrals multiserial 46-74 (56); Prealars 12-16; Scutellars multiserial 62 (47-97). Genital lamina quadrate.

Pupa: (Sexual differences not considered). Total length 11.7mm (10.0-13.8). Cephalic tubercles 240 μ m long, conical and about twice as long as wide at base, subterminal seta about one third of length of tubercle at about 75 μ m long. Respiratory base 110x215 (HR 1.95). Posterior row of curved hooks on segment II comprising 75-90 hooks over 0.7 of the segment width. Caudolateral spur of segment VIII with 6-9 closely applied spines; anal lobes with 115-125 irregularly biserial lateral setae.



Caudolateral spur of segment VIII from a paratype of *C. athalassicus*.

Fourth instar larva a medium to large (fem. 13.5-20.8; male 17.0 mm) melanotus or semireductus-type, possibly depending upon salinity of habitat. TLt relatively short (80-240 μ m); VT short to moderate length, but apparently longer in lower salinity, where the posterior pair of tubules may have a slight coil at the end (saline - about 0.68-0.72 mm; fresh water about 1.12-1.56 mm, anterior generally longer). AT about 2-3 (2.82) times longer than wide (len. 354-633 μ m, width 127-228 μ m) larger in saline conditions and ventral pair generally larger.

Gular region generally darkened in posterior 1/3 to 2/3, extending beyond the mentum width and edges about half the height, FC pale, but darker around antennae and mouthparts. Salivary reservoir 90.9 (71-98.5) µm long and 2.8-4.9 times longer than wide.



Photo courtesy of M.G. Butler
Mentum (c, below) with sharp teeth when not worn; c2 tooth relatively well separated (type III); 4th laterals reduced to about level of 5th laterals (type II).

VM (Fig. d) about 3.1-3.7 time longer than deep and 1.05-1.11 times the mentum width; with about 49.1 (45-54) striae, VMR about 0.32-0.41. PE (a, below) with 10-17 broad sharp teeth, essentially Ty.B.

Premandible with relatively broad inner tooth, about 3-5 times the width of the outer tooth which is narrow along all of its length.

Antenna (b, below) with A1 about 1/3 of VHL, 3.35-3.53 times longer than wide, with RO just below the middle of segment; AR about 2.35 (2.24-2.45); antennal segment proportions (μ m) 156 : 33 : 10 : 16 : 7 (Cannings gives segment 5 as same length as segment 3). Distance between antennal bases sometimes larger than that between the S4 setae, which occupy 75-85% of the frontoclypeal width.

Mandible (e, below) with relatively pale fourth tooth partially separated (type IIB), about 19.6 (17-21) furrows on outer surface near base (arrowed in figure), 15 (13-18) taeniae in PMa; Mdt-Mat 25-45.5 μ m, MTR 0.25-0.42.



Mouth parts of *C. athalassicus*: a. Mentum; b. Antenna; c. Pecten epipharyngis; d. Ventromentum; e. Mandible (striae arrowed).

Cytology: 4 polytene relatively short chromosomes with the thummi arm combination AB, CD, EF, G. Centromeres heterochromatic, forming a chromocenter in BC specimens from saline habitats. Polymorphism in arms B (only in males?), D and F 1.

Arm G relatively short and closely paired, with a nucleolus about in the center. Nucleoli also near the center of arms B and D. Inversions in arms B (only in males?), D and F.

- athA1: 1 2c, 10 12, 3 2d, 9 4, 13 19 i.e. as *holomelas*
- athB1: Nucleolus one third from centromere, puff (group 7) distal as in *atrella*.
- athB2: inversion of about one third of arm near distal end.
- athB3: small inv. just distal of nucleolus.
- athC1: 1 2g, 6b 2h, 11c 8a, 15 11d, 6g-h, 17a 16, 7d-a, 6f-c, 17b 22
- athD1: 1 3, 11 18d, 7 4, 10 8, 18e 24 i.e. as in *longistylus, atrella*
- athE1: 1a 3e, 5a 10b, 4 3f, 10c 13
- athF1: 1 23

- i.e. as *cucini*, *plumosus*, etc.
 - i.e. Standard
- athF2: 1a-d, <u>9 8d, 12 10, 2 3d, 1i-e, 3e 4b, 13 14c, 8c 4c</u>, 14d 23



Polytene chromosomes of *C. athalassicus* N - Nucleolus; BR - Balbiani Ring.

Found: Type locality British Columbia - Springhouse, Alberta - Banff National Park; Elk Island National Park; Jasper National Park (all BOLD)
British Columbia - Barnes Lake; Boitano Creek, and L. Boitano (51.95°S, -122.13°W); Jackson Lake; Sorenson Lake (all Cannings 1975); 10 Km W Kamloops (BOLD).
Yukon Territory - Ivvavik National Park (BOLD)
North Dakota - Clearwater Lake (48.50°S, -120.33°W), Mountrail, Co.; Lake Isabel (46.819°S, -99.750°W), Kidder Co.

Originally described from saline lakes, but the North Dakota localities are freshwater.

Fourth instar larva, pupa and adults described by Cannings (1975), much of whose information is used above. He states that *C. athalassicus* is closest to *C. atritibia*. This is '*C*.

sp. Is Andreeva' of Kiknadze et al. (2004, 2010), the latter reference with a labeled karyotype.

Chironomus atrella-group

C. atrella (Townes, 1945) (Species i)

Tendipes (Tendipes) atrella – Townes,

1945

C. nr. *anthracinus* – Butler *et al.* (1995)

This species is in BOLD Bin: BOLD:AAG5507 Nearest neighbor Bin : ECORG3674-22 C. atrella is very closely related to C. degelenus Seisebaev, Bakhtin & Siirin, 2001 (Karmokov Mukhamed, pers. comm.) There are small number of specimens in BOLD Bin: BOLD:AAI4298 which are

identified as C. atrella. This appears to be a boreal species.

Adult:

Associated adults and pupae are in the collection of J. E. Sublette, now in the museum of the University of Minnesota, St. Paul, MN.

Male (based on Townes (1945):

Wing length 4.1 mm, AR 3.9, anterior LR 1.35, body slender.

Blackish brown; wing vein r-m slightly darker, legs brown, foretarsis with long sparse beard.

Frontal tubercles small, clypeus of medium size.



Male terminalia of C. atrella

About 5 setae in separate pale patches on IXth tergite. Genitalia similar to that of C. decorus but anal point narrower and SVo shorter and stouter (closest to D(e) of

Strenzke 1959); IVo longer than anal point and reaching to about middle of GS which narrows relatively gently over distal third.

Female: not described, but some characters from a photograph in BOLD database: Antennae, thorax and postnotum blackish, scutellum yellowish, abdominal segments dark brown with a light posterior band which becomes larger from segment III; legs yellowish with darkened knees, and slight darkening at distal ends of the segments; wings pale, with slightly darkened crossvein.



Pupa: Caudolateral spur of segment VIII with about one or two major spines but may be two more smaller lateral spines.



Pupal spur of C. atrella

Fourth instar larva a small to medium plumosus type (len. female abt 9.4-16.5 mm; male 10.4-11.5 mm), VT moderately long with posterior pair generally longer (ant. 0.8-2.9 mm; post. 0.90-3.45 mm). Posterolateral tubules well developed, 200-400 μ m. Anal tubules relatively long, often partially bilobed, dorsal and ventral pair approximately the same size, length about 633-660 μ m, and 3.7 to 4.2 times longer than wide. Gular region slightly darkened on posterior third or occasionally half, FC pale.

Mentum (Fig. c, below) with rounded teeth, c1 tooth rather narrow, c2 teeth moderately separated (type IB or III, but often worn); 4th laterals reduced part way or completely to level of 5th laterals (type I-II).

Ventromental plates (Fig. d, below) separated by about 29-33% of mentum width, with about 39-51 striae; VMR about 0.33-0.35. PE (Fig. a, below) with about 11-14 teeth (type B). Premandible with inner tooth 3-4 times wider than outer tooth.

Antenna (Fig. b, below) with basal segment about 3.5-4.7 times longer than wide, RO about 2/5-1/2 up from the base; AR about 2.1-3.2; segment lengths (micron): 139: 33.5 : 10: 12.5 : 7. The relative lengths of the segments varies considerably in larvae from different areas, a New Mexico larva having a much longer antenna, while a Californian rearing had a shorter antenna, the differences not being obviously related to overall larval size.

Distance between the antennal bases generally less than that between the S4 setae, which are separated by 80-90% of the width of the frontoclypeus.

Mandible (Fig. e, below) with 3rd inner tooth only partly separated and colored (type IIB) and about 14-19 furrows on outer surface at base; 10-13 taeniae in PMa; MTR about 0.34.



Cytology: 4 polytene relatively short chromosomes with thummi arm combination, AB, CD, EF, G. Arm G very short and paired only at the terminal nucleolus. Large nucleolus in arm B and a smaller one in arm F at about group 11. Arm C unpaired in some specimens. Polymorphism in arms A, B, C, D, and F.

i.e. as in *aprilinus*



- atrA1: 1a-e, 2d 3i, 12c 10a, 2c 1f, 9e 4a, 13a 19
- atrA2: 1a-e, <u>8a 9e, 1f 2c, 10a 12c, 3i 2d</u>, 7d 4a, 13a 19
- atrB1: Puff (group 7) generally not developed, but is near distal end of arm, nucleolus about middle of arm.
- atrC1: 1 3, 8 11c, 4a-i, 6b 5, 15 11d, 6gh, 17a 16, 7d-a, 6f-c, 17b 22
- atrC2: 1 3, 8 9d, <u>4c-a, 11c 9e</u>, 4d-i, 6b 5, 15 11d, 6gh, 17a 16, 7d-a, 6f-c, 17b 22

atrC3: 1 - 3, 8 - 11c, 4a-i, 6b - 5, <u>12 - 15e</u>, 11h-d, 6gh, 17a - 16, 7d-a, 6f-c, 17b - 22

- atrD1: 1 3, 11 18d, 7 4, 10 8, 18e 24 i.e. as in *longistylus*
- atrD2: 1 3 11 <u>16c 12</u> 16d 18d 7d 4 10 7e 18e 24
- atrD3: 1 3e, <u>5d 4</u>, <u>10</u>, <u>7d 5e</u>, <u>11 15d</u>, <u>18d 15e</u>, <u>3fg</u>, 9 7e, <u>18e 24</u>
- atrE1: 1a 3e, 10b 3f, 10c 13
- atrE2: 1 3e, 10b 9, <u>7 8</u>, 6 3f, 10c 13
- atrF1: 1a-f, 9 3c, 14 10, 1g 3b, 15 23 i.e. inv 3g-14 from 'blaylocki' & sp. b.
- atrF2 1a-f, 9 3c, 14h-a, 16 15, 3b 1g, 10 13, 17 23
- atrF3 1a-f 9 5d 13 14 3c 5c 12 10 1g 3b 15 23
- atrF4 approx: 1a-f 14h-a <u>3c 9 16 15</u> 3b 1g 10 13 17 23 i.e. from F2

Molecular: There is a large amount of MtCOI barcode sequence in the BOLD database

Found: Type locality - Nevada – Reno (Townes 1945) Alberta - Nordegg (Paratype - Townes 1945) British Columbia - Sorenson Lake (abt 51.97°N, -122.52°W), Springhouse area (Cannings and Scudder 1978). Manitoba - Aweme, Caroll (Paratypes - Townes 1945); 6.7 ml s. Erickson (50.48°N, -99.90°W); Lake Winnipeg (Sæther 2012) Ontario - 'Copanspin Farm' Dunrobin (45.42°N, -75.87°W); 0.5 ml e. Dunrobin (45.25°N, -75.86°W); South March nr Mud Lake (44.88°N, -78.27°W); Hogs Back (45.37°N, -75.70°W), Ottawa (all Carleton, Co.). Prince Edward Island - Brackley Beach (abt 46.24°N, -63.13°W), Canadian National Park (Paratype - Townes 1945) Saskatchewan - Floral (Driver 1971)
California - Tahoe City (Paratype - Townes 1945) Lake Davis, Plumas Co.; Spring Valley, San Diego Co.
Colorado - Denver; Fort Collins (Paratypes - Townes 1945); Franklin Creek
Watershed (38.93°N, -104.89°W).(Herrmann et al. 2016).
Indiana - Crooked Lake, Noble Co.
Massachusetts - Oak Bluffs (Paratype - Townes 1945).
Minnesota - Luverne; Nine Mile Creek, Anoka Co. (Paratypes - Townes 1945); Lake Christina (46.08°N, -95.75°W), Douglas Co.; Anderson II WPA wetland, Audubon (46.86°N, -95.98°W), Becker Co.
New Mexico - Eagle Nest Lake (36.55°N, -105.25°W), Colfax Co.
North Dakota - Fuller Lake; Larimore Dam, Grand Forks Co.; McVille Dam, Nelson Co.; Warsing Dam, Eddy Co.
South Dakota - Brookings, Erwin (Paratypes - Townes 1945).
Wisconsin - Reader Farm (45.07°N, -89.42°W), Madison, Dane Co.

Prairie sloughs, pools, and lakes.

It is possible that Townes' type series of *C. atrella* was a mixture of two species: one with a southern distribution e.g. the type from Reno, Nevada; and the other a more Boreal species, e.g. the paratypes from Alberta and Manitoba. This second species is not conspecific with *C.* ?nr. *atrella* (below).

Previously Species Ea of Butler, and then *C*. nr. *anthracinus* of Butler *et al.* (1995). The cytology was described in detail by Martin *et al.* (2006). It seems likely that *C. atrella* is the sister species of *C. degelenus* which is known from Khazakhstan.

C. ?nr. atrella Given manuscript name C. grodhausi by Sublette. (Species 2r)

Adult: Adults may be in the collection of J. E. Sublette, now in the museum of the University of Minnesota, St. Paul, MN.

Male:



TIX with about 7 setae apparently in single spots.

Anal point narrower at the base. SVo closest to D (f) of (Strenzke 1959) and possibly darkened. IVo not reaching the end of the anal point but to about the midpoint of the moderately broad gonostylus which narrows markedly over the distal third to quarter; about 4+2 setae at tip.

Pupa: Only the caudolateral spur on segment VIII has been recorded, with from 1-3 spines at the end of the relatively broad base.



Fourth instar larva a medium sized plumosus-type (no length measurements available). Lateral tubules about 240 μ m long; dorsal anal tubules shorter and thinner than the ventral pair (dorsal 270.5-426 μ m long, 115-200 μ m wide; ventral 270-506 μ m long and 170-180 μ m wide). Salivary reservoir 71-88 μ m wide and 3.5-4.4 times wider than deep.

Gular region darkened over posterior half, wider than the mentum and widest at the posterior margin. FC probably darkened.

Mentum (Fig. c, below) with pointed teeth; c1 tooth relatively broad with diverging sides, c2 teeth, although well separated, virtually continue line of center tooth (type 1B). Fourth laterals only slightly reduced (type I-ii).

Ventromental plates (Fig. d, below) about 212-233 μ m wide and 3.22-4.6 times wider than deep, 1.04-1.16 times wider than the mentum; separated by about 0.37 (0.35-0.39) of mentum width, with about 40.2 (37-45) striae, VMR about 0.22-0.4. PE (Fig. a, below) with about 12.6 (10-16) sharp but relatively broad teeth (type B).

Premandible with outer tooth longer and coming to a fine point, inner tooth broad, about 4.1(3-6) times broader than outer.

Width between antennal bases about equal to, or slightly less than that between the S4 setae, which are separated by 83-90 % of the FC width; S5 setae about level with the nearby RO. Antenna (Fig. b, below) with basal segment about a third of the VHL and 2.8-3.9 times longer than wide, RO from a third to nearly halfway up from base of segment; AR abt 2.0-2.33; relative lengths of segments (micron) 126 : 32 : 8.2 : 12.5 : 7.7.

Mandible (Fig. e, below) with third inner tooth well developed but only partly darkened (Type IIIB), about 18.4 (14-22) furrows on outer surface near the base; 12-13 taeniae in PMa; Mdt-Mat 28.3 (20-35) µm; MTR 0.40-0.42. Possibly only two spines on the inner margin.



Cytology: 4 polytene chromosomes with the thummi arm combination AB, CD, EF, G. Centromeres heterochromatic, showing tendency to stick together to form a centromere. Large BR near center of closely paired arm G, with constriction between BR and centromere; another BR near distal end of the chromosome. Nucleolus in arm B, just distal to the 4 characteristic bands; 1 or 2 nucleoli near the center of arm D. No polymorphism in the small sample examined.



1a-e, 2d-3, 12-10, 14 – 13, 4 - 9, 1f-	2c, 15-19
Nucleolus about 1/3 from centromer	e
possibly 1 – 10, 15 – 11, 16 – 22	i.e. as sp. Apple Valley (sp. 3b)
1 or 2 nucleoli near middle of arm	
1-3e, 5-10b, 4-3f, 10-13	i.e. as <i>cingulatus, tardus</i> and sp. 3b.
1 - 23	i.e. Standard, as <i>piger</i>
	1a-e, 2d-3, 12-10, $14 - 13$, $4 - 9$, 1f- Nucleolus about 1/3 from centromer possibly $1 - 10$, $15 - 11$, $16 - 22$ 1 or 2 nucleoli near middle of arm 1-3e, 5-10b, 4-3f, 10-13 1 - 23

Found: California - Lake Davis, Plumas Co (abt 39.70°N, 120.50°W).

Some information on arm F given in Fig. 3 of Martin (1979). The cytology suggests a possible relationship to species Apple Valley (species 3b).

Chironomus species 3g.

Adult: Based on a reared male: The specimen is in the Sublette collection, now in the museum of the University of Minnesota, St. Paul, MN. Only the hypopygium of this male is available for study:

Tergite IX with 13 setae in individual pale spots within a triangular area. Anal point short and broad, SVo dark and closest to E-type(g) of Strenzke (1959) but the tip more curved and more robust. IVo, with simple setae, reaching well past end of anal point and to about the

midpoint of the gonostylus, which is moderately swollen and narrows genly over the posterior half.



Male terminalia of C. sp. 3g

Pupa: Besides the pupal exuvia of the reared male. there is also a possible pupa from Yankton, South Dakota by P.L.Hudson. Of these exuviae, only photographs of the cephalic tubercles and a pupal spur are available.

The cephalic tubercles have a length/width of about 1-1.6; there are no frontal warts. The spur of segment VIII has two separated spines.



Pupal cephalic tubules (above) and pupal spur of C. sp. 3g

Fourth instar larva (based on one female larva): a small (abt 11.4 mm) plumosus-type; posterior pair VT slightly longer (ant. 1.01 mm; post 1.13 mm). Lateral projections about 280 μ m. AT about 405 μ m long (dorsal) and 380 μ m long and about 2.3-2.5 times longer than wide. Salivary reservoir about 81 x 25 μ m (3.2 times wider than deep).

Gular very slightly darkened on posterior 1/3, and FC pale.

Mentum (Fig. d, below) of type I-ii; with somewhat rounded teeth; c1 tooth relatively narrow with very short diverging sides, then rounded; c2 teeth moderately separated (type IIA-III). Ventromental plates (Fig. c, below) about 211 μ m long and 3.7 times longer than deep; 1.13 times wider than mentum; separated by about 0.22 of mentum width with about 39-40 striae, VMR 0.32-0.35.

PE (Fig. a, below) with 12 short, thick teeth (type B).

Antenna (Fig. b, below) with A1 3.7 times longer than wide, and 0.4 of VHL; RO about 0.40-0.42 up from base; AR 1.73; A2/A1 about 0.22. Segment lengths (μ m): 121 : 36 : 11 : 15 : 9. Distance between S4 setae (154 μ m) just greater than distance between antennal bases (150 μ m), and about 0.87 of FC width at that point.

Mandible (Fig. e, below) with short thickened heel, length about 230 μ m, 16 furrows on outer surface near the base, 3rd inner tooth separated and partly colored (type II-IIIB); PMa with 13-14 taeniae; 3 spines on margin; Mdt-Mat 28; MTR 0.35.



Cytology: 4 relatively short polytene chromosomes with the thummi arm combination AB, CD, EF, G. Arm G short and usually unpaired with a subterminal nucleolus and an obvious median BR. There may be another BR associated with the nucleolus. No nucleolus in longer chromosomes. Arm E often unpaired. Bulb with distal dark bands just near the 4 characteristic bands of arm B.



Found: Minnesota - Eagle Creek, Eagle Bend, Todd Co. (46.13°N, -95.05°W). Possibly also: South Dakota – Yankton, Yankton Co. (42.56°N, -97.20°W)

The cytology suggests that this species may be related to *C. atrella* (species i), however there are morphological differences: pale gula, anterior VT longer than posterior pair, AR apparently lower; and most notably in the cytology is the absence of a nucleolus in arm B.

C. nr. *atrella* of Anderson and Hitchcock (1968) - see *C. quinnitukqut* under *C. decorus*group (below)

(End atrella group)

Chironomus cucini-group

C. cucini Webb, 1969 (Species o)

In BOLD Bin: BOLD: ACQ0844

Adult: Based on Webb 1969.

Webb notes that the adult is closest to C. staegeri

Male:

Color mostly brown, thoracic vittae and postnotum blackish brown, abdomen dark brown with apical quarter of segments I-VIII pale yellow.

Head: AR 3.7; frontal tubercles long (60 μ m); relative lengths palpal segments 2-4: 6 : 20 : 19 : 21. Clypeus 1.3 times antennal pedicel diameter.

Thoracic setae – Acrostichals absent; dorsocentrals extending the length of the vittae; 7-9 prealar; scutellar with numerous setae arranged in 5 transverse rows.

Wing length 4.1-5.2 mm; squama fringed.

Legs dark, tarsal beard very short; proportions:

	Fe	Ti	Tal	Ta2	Ta3	Ta4	Ta5	LR	F/T	Ta5/Ti
PI	32	31	42	24	17	13	6	1.35	1.03	0.19
PII	36	33	23	14	10	7	5	0.89	1.09	
PIII	42	42	31	19	14	9	5	0.73	1.00	

Setae of tergite IX not specified; anal point broad with rounded end; SVo as *C. staegeri* – i.e. closest to E(i) type of Strenzke (1959); IVo broad, extending beyond anal point; GS narrowing over distal third.

Female:

Coloration largely as male, but with just the posterior margin of each tergite yellow. Head: Head, mouthparts and palps fuscous brown. Frontal tubercles large (60 μ m); clypeal width twice the antennal pedicel diameter; palp proportions (segs. 2-5): 6 : 21 :23 : 33.

Setae as in male.

Wing length 5.4-6.3 mm.

Legs dark brown. Leg proportions:

	Fe	Ti	Ta1	Ta2	Ta3	Ta4	Ta5	LR	F/T	Ta4/Ti
PI	34	32	42	22	16	11	6	1.31	1.06	0.34
PII	38	34	22	13	9	6	4	0.64	1.12	
PIII	44	43	31	19	13	8	5	0.72	1.02	

Pupa: (Based on Webb 1969) Length 10.2-11.8 mm. Cephalic tubercles slightly longer than wide, len. about 50 μ m, apical setae as long as tubercle. Tergite I lacking spines or spinules. Shagreen pattern as below. Posterolateral spur of segment VIII with about 4 (2-7) spines. Anal fin with over 50 taeniae.



53. Shagreen pattern of *C. cucini* pupa (Webb 1969)

Fourth instar larva a large (length 12.1-21.3 mm) salinarius or halophilus-type, i.e. posterior VT sometimes present (len. abt 35 mm). AT about 440 μ m long and just over twice as long as wide, dorsal and ventral pair about the same size.



(Picture courtesy of M.G. Butler) In this larva there is some development of the posterior VT.

Gula darkened on posterior half to two thirds, wider than the mentum but somewhat variable: anterior margin vague and may be approximately liner or may be higher at edges; normally wider at base but sometimes a little higher; FC pale or slightly darkened with small dark stripe in posterior part and a lobed dark spot in anterior part.



(Photos courtesy of I. Proulx)

Salivary reservoir about 61-83 µm long and 2.75-4.1 times wider than deep.

Mentum (Fig. c, below) with relatively shallow curve, teeth pointed; center tooth moderately broad, c2 teeth moderately separated (type IIA-III); first laterals slope away from center; fourth laterals definitely reduced (type II-iii).

VM (Fig. d, below) 251.5 (243-259) μ m wide and about 3.2-3.3 times wider than deep; 1.08 (0.92-1.23) times the mentum width; IPD about 0.37-0.45 of the mentum width; 33-43 striae; VMR 0.25-0.30.

PE with 16.2 (11-21) pointed irregular teeth (Fig. a, below) (pointed type D). Premandible with broad inner tooth about 4.5-5 times the width of the outer tooth which narrows markedly along its length.

Antennal segment 1 relatively long (Fig. b, below); 2.94 (2.6-3.2) times longer than wide and markedly wider at base, RO about 0.45 (0.35-0.58) up from base; AR about 2.28 (2.16-2.37); A2/A1 about 0.23-0.25; ratio of segments (μ m) 153 : 37.5 : 8 : 15 : 7 : i.e. A5 about 0.88 of A3.

Distance between antennal bases greater than that between the S4 setae, which are separated by about 0.79 of the FC width at that point. S5 setae mostly anterior to the nearby RO. Mandible (Fig. e, below) with third inner tooth pale, but separated to varying degrees (type II-IIIA-B); 16-21 furrows on outer surface at the base; 12-15 taeniae in PecM.



Larval characters can be quite variable between locations and even in individual larvae – notably the placement of the RO in A1, which may be 0.40 in one antenna and 0.58 in the other.

Cytology: 4 polytene chromosomes with the thummi arm combination AB, CD, EF, G. Centromeres very heterochromatic, forming a chromocenter with all chromosomes attached. Arm G with nucleolus, bounded by dark bands, near centromere, then a constriction followed by two BRs which may be more obvious in Californian populations. No nucleolus in long chromosomes, but a BR may be developed distal to the middle of arm B, particularly in some Californian specimens. No inversion polymorphism has been observed.

- cuc A1: 1 2c, 10 12, 3 2d, 9 4, 13 19 i.e. as holomelas, tardus, etc.
- cuc B1: Commonly a BR towards distal part of arm. Differs from majB1 by a long central inversion.
- cuc C1: 1 6b, 15c-e, 8 11c, 15b 11d, 6gh, 17a 16, 7d-a, 6f-c, 17b 22 i.e. as *islandicus*, sp. 3b.
- cuc D1: 1 3, 11 18d, 7 4, 10 8, 18e 24 i.
 - i.e. as *longistylus, tardus, pilicornis,* etc.
- cuc E1: 1 3e, 5 10b, 4 3f, 10 13
- i.e. as *cingulatus, tardus* and sp. 3b. i.e. as *tenuistylus, magnus & tardus.*
- cuc F1: 1 10, 17 11, 18 23
- cuc G1 Virtually terminal nucleolus and two central BRs.



Note the well-developed chromocenter

Found: British Columbia - Osoyoos (Townes 1945, as *C. atritibia*) Ontario - Clearwater Lake (Proulx *et al.* 2013): Clarke Lake, Kearney Lake, Costello Lake (45.56°N, 78,32°W), (Type locality) all Algonquin Provincial Park ; Lake Nipissing(?)
Quebec - Lake Bousquet, Lake Opasatica, Lake St. Joseph, Lake Vaudray (Proulx *et al.* 2013). California - Lake Davis, Plumas Co.; Castle Lake, Siskayou Co. Indiana - Crooked Lake, Minnesota - Long Lake New York - Kanisko Reservoir, Westchester County (Townes 1945, as *C. atritibia*)

Thick mud in depth 5 m or more in lakes.

Morphology described by Webb (1969), who notes that the adult is most closely related to *C. staegeri*, but also needs to be compared with *C. atritibia*. Can be differentiated from *C. staegeri* most easily by the chromosome number (4 polytenes cf. three) and also in the larvae by the larval type (salinarius or halophilus c.f. plumosus-type in *C. staegeri*; in the adult by the larger size (wing length 4.1-5.2 mm cf. 5-6.3 mm); and the smaller pale apical band of the abdominal segments; in the pupa by the shagreen patterns and the generally smaller number of spines on the spur.

Karyotype figured by Martin (1979) and described by Wülker & Butler (1983). This may be *Chironomus* species B of Hilsenhoff and Narf (1968).

Molecular data:

Sequence of the Barcode region of Mt*COI* was included in Proulx *et al.* (2013) and has GenBank accession numbers KF278284-290 and the *gb2B* sequence has KF278417-420.

C. atritibia Malloch (Species 4c)

Adult: From Townes (1945)

A large dark species, legs brown, no beard on fore tarsi.

Male: Wing length about 5.2 mm, anterior wing veins brown, crossvein somewhat darker; LR about 1.28, AR about 4. Frontal tubercle large and clypeus very large and somewhat elongate, scutum without a median tubercle.



Hypopygium drawn from type by Townes (1945).

From figure of hypopygium the following features can be noted: About 6 setae near center 9th tergite IX. Anal point differs from *C. cucini* in being long and narrow. SVo closest to Strenzke's E(i) type. IVo about as long as the anal point and to middle of GS, which

Female: not known.

Pupa: Not known.

Fourth instar larva has not been seen. Wuelker and Butler (1983) state this species has a salinarius-type larva, quoting a Ph.D. thesis by M.G. Johnson (1969).

is long and relatively narrow, narrowing on posterior quarter.

Cytology: Chromosomes are not known.

Found: Manitoba - Lake Winnipeg (Sæther 2012). Nunavut - (formerly Northwest Territories - Southampton Island, Keewatin (Type locality).

Adult described by Malloch (1934). Note that most specimens listed by Townes (1945), other than the type, are probably *C. cucini*. Townes (1945) notes that the adult should also be compared with *C. biseta* and *C. hyperboreus*.

(End cucini group)

Chironomus decorus-group

C. decorus Johannsen 1905

C. decorus was originally described by Johannsen in 1905, with later additions (e.g. Johannsen, 1937) varying, possibly due to inclusion of other members of the species-group. Sublette examined the holotype specimen in the Johannsen collection at Cornell University (Sublette *et al.* 1998) but did not publish any additional description. However he concluded that this species best fitted Johannsen's material (Wülker *et al.* 2009). This is the species studied by Rothfels and Fairlie (1957).

Johannsen 1905

60. Chironomus decorus n. sp. (P1.23, figs. 7 to 13; pl.29, fig.12)

Larva. The larvae were found everywhere in the ponds and ditches around Ithaca N. Y. They are blood red, and about 12 mm. long. The head is dark blackish brown; the antennae are short, normal. The dorsal sclerite is narrow ovate, posterior end pointed, truncate anteriorly, with three setae along each lateral margin, the first at the extreme anterior end, the last one ball way between the anterior and the posterior end, the second midway between these. Articulated to the cephalic margin, and overhanging the month opening is the labrum. There are two pairs of prominent setae upon its dorsal surface; numerous papillae, two or which are quite promiment on the anterior margin and upon the anterior ventral surface. The arrangement of the setae and the armature of the epipharynx shown in fig.10. The epipharyngeal comb (c) has relatively long and uniform teeth; the lateral arms are dark brown in color. Each eye consists of two distinctly separated pigment spots. The mandibles (fig.7) are black-tipped, with a fringe of apical sche, a prominent lateral spine, and a group of mesad projecting brauched setae; the hypopharynx has its usual papillae upon the fore margin; the maxillae are prominent, each with two lateral setae, the palpus is short and thick. The labium has a black margin with an outline as shown in fig.S. In many specimens the teeth appear to be slightly longer in proportion than shown in this figure. The anterior prolegs have very numerous curved setae. The body is nearly devoid of even minute setae. The anal prolegs are normal, claws dark, bilobed. Anal setae as usual. The eleventh body segment has four long white blood gills on the ventral surface, and candad of the dorsal setae of the twelfth segment are four short ones.

The larva (1) mentioned by Garman (1888) is probably this species.

Pupa. Dusky greenish brown, the colors of the image showing through the integument. Length 7 to 8 mm. Tracheal filaments prominent, white and much branched. Thoras with a few scattered setae. Segments of the abdomen with a seta-pattern as shown in fig. 11 and 12; the pattern more indistinct on the last two segments. On each lateral margin of the fifth to the eighth segment there is a brown longitudinal dash, most conspicuous and half the length of the segment on the fifth. The black chitinized lateral spur of the eighth segment is prominent and without teeth (fig.12). The anal appendage has the usual fringe of matted bairs.

Imago, mals. Length 6 to 7 mm. Head yellow, autennae and proboscis more or less brownish, large basal joint of the antennac and the palpi reddish brown, the latter sometimes fuscous. Thorax greenish yellow with a whitish sheen, the pleura and the scattellum the same color; the three thoracic stripes, some pleural spots, the metathorax and the pectus dull testaceous or reddish, sometimes even brownish; the middle dorsal line divided by a line line. Abdomen hairy, pale yellow or greenish yellow, in life more distinetly green, infuscated toward the tip; each segment with a brownish transverse fuscia slightly in front of the middle. These fascine are widest on the dorsal line, and are obsolete on the last' few segments. Genitatia brownish yellow, hairy, moderately clongated (pl.32, fig.13). Legs including the coxae pale greenish yellow, short haired; tarsi, particularly towards the tip, infus-cated; tips of tibize and of all tarsat joints fuscous, lifth joint wholly fuscous. Tarsal claws simple, pulvilli small, empodium stout, curved, blunt, and pectinate on the convex side. The fore metalarsus about 0.6 longer than its libia. Wings hyuline, cross-vein conspicuously clouded with dark brown, anterior veins yellow, posterior ones hyaline, the two branches of the cubitus and the anal vein accompanied by a faint brown streak. Venation as shown on pl.29, fig.12.

Female. Differs from the male as follows: Slightly shorter, antennne yellow, last joint fuscous; thorax more greenish than yellow, abdomen greenish with dark bands as in the male, but the bands are always wider and usually cover the whole surface of the segment excepting the apical third or fourth. In other respects like the male. This species seems to be very common in many parts of the country. New York, Ohio, Illinois, Iowa, Kansus, Washington State, and Nebraska.



Johannsen's original description (1905)

Townes (1945) listed seven synonyms for this species, however some have subsequently been removed from synonymy and others classed as nomina dubia. *Chironomus anonymus* Dyer 1902 – subsequently recognized as a distinct species (see species 20).

Chironomus attenuatus Walker 1848 – on basis of an adult female. Townes noted it as a member of the *C. decorus* group, but in 1959 revised it to a valid name. It became a senior synonym of *C. decorus* until Sublette & Sublette (1974) classed it as a nomen dubium on the basis that the original description and the fact it was a female, meant it could not be accurately identified.

Chironomus cayugae Tilbury 1913.

Chironomus cristatus Malloch 1915 and Kieffer 1917 – misdeterminations of *C. cristatus* Fabricius

Chironomus distinguendus Kieffer 1917

Chironomus maturus Johannsen 1908 – subsequently recognized as a distinct species (see species d)

Chironomus similis Johnannsen 1905 - on basis of female.

In BOLD Bin: BOLD:AAB7030.

Adult:

Male:

Length 6–7 mm. Wing length about 3.4-4.0 mm; width about 0.91-0.96 mm; VR about 0.91-1.00. LR about 1.39-1.6 (Johannsen, gives 1.6).

Head: Yellow, antennae and proboscis more or less brownish. AR about 3.01-3.43; frontal tubercles about 53-58 μ m long, and 1.8-2.6 times longer than wide, clypeus about 0.52-0.70 of diameter of antennal pedicel with 28-44 setae; palpal proportions (micron) 77 : 66 : 218 : 260 : 352 (P5/P4 1.3-1.4).

Thorax greenish yellow, stripes and postnotum testaceous or reddish.

Setae: Acrostichal – at least 10-29; Dorsocentral – 21-33; Prealar - 6-7; Supra-alar – 1-2; Scutellar – about 24-41 (13-18 in posterior row and 11-23 in about two to three anterior rows).

Wing: Scf on brachiolum 3-4. Setae on squamal fringe 33-36. Distinct brown cloud over r-m crossvein and paler spot over FCu.

Legs greenish, with tips of tibiae and all tarsi brownish, completely so on 5th tarsus. Foretarsus without a beard.

Leg lengths (micron) and proportions:

	Fe	Ti	Ta1	Ta2	Ta3	Ta4	Ta5	LR	F/T	BR
PI	1433	1355	1963	1022	790	710	313	1.39-1.60	1.03-1.07	1.9-3.0
PII	1535	1402	878	500	363	245	172	0.62-0.64	1.09-1.10	
PIII	1725	1700	1292	750	540	337	198	0.77-0.80	1.01-1.03	

Note in PI, Ta4 is about 90% the length of Ta3. Abt 15 SensCh on MTa1; abt 27 on HTa1

The male has saddle-shaped darker markings about a third from base of each abdominal segment, heaviest on TII-IV, becoming more extensive although paler on segments TVI-VIII, occasionally evanescent on TV – these bands are narrower than those of *C. bifurcatus* or *C. blaylocki*.

About 4-10 setae in 1-5 pale spots in center of tergite IX.



Hypopygia of C. decorus - two variants of hypopygium; note also the long superior volsella.

Typical *C. decorus* hypopygium; anal point relatively narrow, essentially parallel sided and coming to a point. SVo (between D and E-type of Strenzke 1959), while

darkened, is paler and longer than that of *C. bifurcatus*. IVo reaching about to the end of the anal point, with setae not forked. Johannsen illustrates the GS as not markedly swollen in the middle and narrowing gently over the distal third. Townes illustrates a similar form but also shows a form with a markedly swollen GS which narrows sharply at the distal third, similar to *C. bifurcatus*. Both types are found from cytologically confirmed material (i.e. reared from egg masses).

Female:

Johannsen notes that the thorax is more greenish and the abdomen greenish with wide dark bands covering most of the segment.

Additional data are available from females reared from egg masses from Wisconsin: Wing length about 3.36 (3.24-3.48) mm; width about 0.89 (0.80-1.02) mm, VR about 0.95-1.0 (or 1.00-1.05); 20.6 (18-23) setae in the squamal fringe and 3-4 SCf on brachiolum.

Head with Frontal tubercles about 45 (28-71) μ m long and 1.5-3.0 times longer than wide. Antennal segments A2-A3 about equal length but with increasing neck length; proportions (micron) and fraction of neck in brackets: 197 (0.31) : 121 (0.34) : 120 (0.38) : 126 (0.43) : 205; AR 0.36 (0.34-0.38); A5/A1 1.04 (0.96-1.11).

Palpal proportions (micron) 57 : 51 : 175 : 228 : 360, A5/A4 1.6. Clypeus about 1.5-2.3x the width of the antennal pedicel; about 47.5 (42-55) clypeal setae.

Thoracic setae: acrostichal - 18-21; humeral 3-5 linear; dorsocentral - 33-41 (incl. humerals - 35-44); prealar - 7; supra-alar - 1-2; scutellar - 23-36 (in about 3 rows, 15-19 in the posterior row and 8-17 in the 2 anterior rows).

Legs as in male. Fore Ta4 slightly longer than Ta3. BR about 1.3-2.1. Lengths (micron) and proportions:

	Fe	Ti	Ta1	Ta2	Ta3	Ta4	Ta5	LR	F/T	Ta4/Ti
PI	1374	1175	1863	953	773	783	333	1.56-1.68	1.16-1.35	0.66-0.69
PII	1428	1341	809	426	320	218	168	0.58-0.62	0.99-1.14	-
PIII	1551	1580	1147	660	507	303	195	0.69-0.76	0.96-0.98	

Abdomen greenish with a dark band 2/3 to 3/4 of segment. About 4-5 setae on GcIX and 7-10 on seg X which is crescent shaped and about 4 times longer than the greatest width.

Pupa: Length of exuviae about 7.4-10.2 mm (fem. 8.17, 7.40-9.30; male. 9.36, 8.13-10.20) mm; inner margin of wing case about 1.57-2.10 mm long, larger in females. Exuvia pale (Johannsen 1905), fine shagreen over most of the abdominal tergites, stronger at distal ends, but only a posterior patch on segment VI.

The pupa has small frontal warts anterior to the CT, normally base wider than height (35.3, 23-68 μ m high and 51.6, 30-775 μ m wide at base) but Wülker et al. (2009) illustrate a female with an unusually high pair (Fig. b, below). The cephalic tubercles differ between males and females. Those of males are generally larger (about 150-196 μ m long and about 1.4-2.4x longer than wide in males; about 65-185 μ m long and 1.0-1.4 times longer than wide in females); a seta up to 105 μ m long arises just before the tip. In males only there is a secondary tubercle, with a small seta, attached at the side (see figure below); secondary tubercle about 81.7 (48-106) μ m high and 62 (30-83) μ m wide at base.



Pupa of *C. decorus*: cephalic tubercles of male above, and female (from Wülker et al. 2009) below. Note the 2ndary tubercles (arrowed), arising at side of the primary tubercles in male and the (unusually) large frontal warts in the female

The respiratory base is slightly kidney shaped, with the basal trunk either kidney shaped or partially constricted in the middle. Length about 148-165 μ m and HR 2.4 (2.2-3.0). There is a rough patch just anterior to the respiratory base about 37-90 x 24-80 μ m which either includes 2 small setae or they are nearby on the lateral side.

About 81 (71-100) recurved hooks at posterior margin of abdominal segment II, central hooks with 2 small knobs on the top and not as downturned as those at either end; hook row continuous, about 57-84% of width of segment (higher values probably due to folding of the abdominal segments of the exuviae).

Pedes spurii B well developed on segment II, usually not seen on segment III; and a large pedes spurii A on segment IV, 191.5 (162-220) μ m long; 106.5 (94-119) μ m wide; about 0.21-0.26 of segment length); other pedes spurii A smaller (about 95-150 μ m) on seg. V; 63-106 μ m on seg VI, with patches of spines. L-setae present on the posterior margin of the preceding segment of the intersegments III/IV and IV/V, may be 70-90 μ m long. Usually 4.6 (range 1-6) closely appressed spines only at the tip of the caudolateral spur of segment VIII (below). Fringe of anal lobe with about 105 (80-138) taeniae, in a double row posteriorly but may become triple at end, on each side.



Fourth instar larva a small to medium sized (fem. 10.7-13.8.; male. 9.4-13.1 mm) (12 mm according to Johannsen, 1905) bathophilus- or melanotus-type (see below); anterior pair of VT slightly longer (ant. 1.30 (0.90-1.72); post. 1.21 (0.80-1.57) mm. In his original description of the larva, Johannsen (1905) did not mention TLt, it was only in his 1937 revision that he mentions them (Johannsen 1937). However this is a polymorphic character, apparently present in more northern populations, but lacking in the more southern populations of Mississippi and New Mexico. AT 2-3 times longer than wide, variable in length - shortest in Mississippi, and longest in Ontario and Wisconsin, often with a constriction in the middle.

Gular region very dark over posterior 1/2 to 2/3, extending beyond the edges of the mentum, higher at lateral margin and wider about a third from posterior margin; FC pale.



Gula region of C. decorus.

Mentum with relatively sharp teeth; c1 tooth relatively broad with parallel sides, c2 teeth relatively well, but not completely, separated (type IIA-III); 4th laterals reduced, often to about height of 5th laterals (type (i)-II).

Salivary reservoir about 70.2 (56-115) μ m wide and 3.5 (2.5-4.6) times wider than deep (if the opening is narrower than 20 μ m it has probably been flattened during mounting). Ventromental plates about 195-263 μ m wide and 3.3-3.8 times wider than deep, and 1.04-

1.25 times wider than the mentum width; IPD 0.28-0.45 of the width of the mentum, with about 39.8 (32-56) striae. PE with about 14.1 (10-16) teeth of type A or sometimes type B. Premandible with the broad inner tooth about 3-5 times wider than the outer tooth which narrows markedly along its length, probably about equal length when not worn.

Antenna relatively short, about 33-41% of VHL; AR about 2.35 (2.0-3.2); basal segment about 3.1 (2.6-3.7) times longer than wide, RO about a third to a half way up the segment from the base; segment lengths (micron) 118 : 27 : 7 : 11 : 6; A5/ A3 is 0.92 (0.82-1.2) i.e. occasionally slightly longer than A3.

Distance between antennal bases 155.2 (132-178 μ m) generally greater than that between the S4 setae 152.0 (129-182 μ m), which are separated by about 0.7-0.9 of the width of the FC at that point. S5 setae about level or slightly posterior to the nearby RO.

Mandible about 259.6 (23-308) μ m long, with the third inner tooth slightly darkened and sometimes slightly separated (type IA-IIB), about 17.8 (12-20) furrows on outer surface near base; 10.4 (8-14) taeniae in PMa; Mdt-Mat 30.6 (20-38), MTR 0.37 (0.25-0.48).

Larvae from southern regions are bathophilus-type, while those from Wisconsin and Ontario seem to be semireductus- or even plumosus-type.



Cytology: 4 polytene chomosomes with the thummi arm combination AB, CD, EF, G. Very polymorphic with inversions in all arms.

Arm G with a nucleolus near one end - Rothfels and Fairlie (1957) indicate it is in a region about 10 bands from the end. However the whole region at the end may appear heterochromatic (see figure below) with no nucleolus obvious. There are three BRs whose relative position depends on the inversion sequence present. Arm G is often only paired at end away from nucleolus. No nucleolus in the long chromosomes.

Chromosome AB is somewhat difficult to recognize from the Keyl pattern, since the "olive" in arm A is not obvious and the 4 characteristic bands of arm B (groups 22–24) are not near the centromere but in variable positions due to inversion polymorphism. A large puff in group 7 is sometimes developed about a third from the end of arm B. Polymorphism occurs for all arms. Rothfels and Fairlie, in their study of 354 individuals, recorded about 30 inversions, including most of those in our smaller studies, with the exception that they found only one sequence for arm F in their Ontario populations, while at least 6 inversions were present in our New Mexico samples. The inversions from our samples are listed below, with comparison to the R & F sequences where possible.

decA1: Pattern difficult to identify as the bands of the typical "olive" are dispersed. decA2: Large medial inversion.

decA3: A distal inversion of A2. May correspond to inversion A of R & F.

decA4: A small medial inversion.

- decB1: Characteristic bands 20-23 not near centromere but near the middle of the arm, puff
- (gp. 7) towards distal end of arm.
- decB2: Inversion of about half of the arm, with proximal breakpoint only 10-12 bands from the centromere.

decB3: Small inversion just proximal to the puff.

- decB4: Small inversion adjacent to the centromere.
- decB5: Small inversion of the more distal region of B2 and apparently sharing the distal breakpoint.
- decC1: Typical groups 3-4 towards the distal end.
- decC2: A very large inversion of about 60% of the arm, with one break distal of the typical bands; as inversion B of R & F.
- decD1: Band groups 16-18 probably towards the distal end of the arm.
- decD2: Large inversion of about 2/3 of arm, proximal breakpoint probably in group 23.
- decD3: Large inversion derived from decD2.



Polytene chromosome arms A-D of Chironomus decorus (sp. 3a)

decE1: 1 - 3e, 8 - 5, 9 - 10b, 4 - 3f, 10c - 13i.e. as maturus, stigmaterus and Inv A of R & F.decE2: 1 - 3e, 8i-e, 3f - 4, 10b - 9, 5 - 8d, 10c - 13i.e. as Inv. S of R & F.decE3: appox. 1a-d, 4 - 3f, 8e-i, 3e-a, 1i-e, 10b - 9, 5 - 8d, 10c - 13i.e. inv of E2decF1: 1a-g, 9 - 2, 10 - 23i.e. as blaylocki, utahensis, etc.decF2: 1a-g, 3d - 9, 3c - 2, 10 - 23from F2decF3: 1a-d, 9 - 3d, 1g-e, 3c - 2, 10 - 23 (?)from F2decF4: 1a-g, 3d - 9, 3c - 2d, 18 - 10, 2a-c, 19 - 23from F2

decF5: 1a-g, 3d - 9, <u>17 - 10, 2 - 3c</u>, 18 - 23

decF6: 1a-d, 9 - 3d, 1g-e, 3c - 2, 10 - 18, <u>23 - 19</u>

dec F3+4: 1a-d, 9 - 3d, 1i-e, 3c - 2d, 18 - 10, 2a-c, 19 - 23

decG1: Subterminal nucleolus and 3 BRs, usually only paired at distal end.

decG2: A small inversion of mid region to just distal of middle BR.

decG3: A larger complex inversion of almost half the arm, towards the distal end, illustrated in Fig. 1(11) of Rothfels & Fairlie (1957)



Polytene chromosome arms E-G of *Chironomus decorus* (sp. 3a)

- Type locality: Johannsen (1905) does not give a type locality but, while noting it is widespread, initially states that it is common in ponds and ditches and, later, in the vicinity of sewage contaminated streams, around Ithaca, New York (1905,1937). Townes (1945) lists all localities in the Johannsen collection as type localities (noted there as "Johannsen1905" although this is not to imply that they are all this species). Other localities from Townes (1945) are not listed as it is not certain what species that actually are.
- Found: Ontario Copanspin Farm, Dunrobin(45.75°N, -75.87°W), and ¿South March nr. Mud Lake (44.88°N, -78.27°W), both Carleton Co.; Don River, Toronto (Rothfels & Fairlie 1957).

from F2 from F3? Arkansas – ¿White River National Wildlife Refuge (Chordas et al. 2004). Colorado - Franklin Creek Watershed (38.93°N, -104.89°W) (Herrmann et al. 2016). Illinois - (Johannsen1905). Iowa - (Johannsen 1905). Kansas - (Johannsen1905). Michigan - Menominee River, Stephenson (45.42°N, -87.61°W), Menominee Co. Mississippi - Belzoni (33.17°N, -90.67°W), Humphreys Co. Nebraska - (Johannsen1905). New Mexico - Eagle Nest Lake (36.55°N, -105.25°W), Canadian River (abt 36.63°N, -104.55°W), Taylor Springs, both Colfax Co.; Rio Grande River (31.75°N, -106,48°W), Dona Ana Co.; Hill Tank (abt. 32.62°N, -104.32°W), Eddy Co.; Pecos River, at Puerto de Luna, Guardalupe Co., also in San Miguel Co.; Gila River, nr. Virden, Hidalgo Co.; Rio Hondo, 2 ml e. Hondo (33.38°N, -105.26°W), Lincoln Co. New York - Ithaca, (Johannsen 1905, 1937), also Cornell University, Ithaca (42.45°N, -78.47°W), Tompkins Co. Ohio - (Johannsen 1905). South Dakota - L. Poinsett, 12 ml n. Arlington; James River, Yankton (42.93oN, -97.33°W), Yankton Co. Washington - (Johannsen 1905). Wisconsin - Murphys Creek; and Arboretum (43.03°N, -89.42°W), and University Houses (43.07°N, -89.42°W), Madison, Dane Co.

Windmill tanks, ponds, ditches, pools in rivers, shallows of lakes. In some localities it occurs along with *C. bifurcatus*. At least where this occurs, the larvae of the two species can be separated by the greater region of gula darkening and the longer AT of *C. decorus* (3a).

Note that most of the data for adult females and the pupae comes from reared egg masses, but there is considerable variation in some characters – mostly these characters are related to overall size of the individual.

Molecular data: (Mt*COI*) Barcode sequence shows that this species is very close to *C*. 'butleri' (sp. 2g), as both are placed in BOLD Bin AAB7030, as also is *C. quinnitukqut*. The sequence for a confirmed *C. decorus* (sp. 3a) is available and indicates that the sequences differ by only two adjacent bases. On the other hand there are about 13 base differences from *C. quinnitukqut*.

The only certain way to separate them is from the polytene chromosome sequences or from the male pupal cephalic tubercles (only species known to have a secondary tubercle), and possibly in the larva by the greater extent of darkening of the gular region.

Some information on arm F is given in Fig. 3 of Martin (1979) and Wülker, Devai & Devai (1989). The karyotype was described in some detail in a Report to New Mexico Energy Institute by Martin, Sublette and Sublette (1979). Also the chromosomes and chromosome polymorphism were described by Rothfels and Fairlie (1957)(below).

C. decorus of Rothfels and Fairlie (1957) (Species 3f)

see C. decorus Johannsen (species 3a)



Only the polytene chromosomes were described

Polytene chromosomes of the C. decorus material of Rothfels and Fairlie

The banding patterns and many of the described inversion polymorphisms correspond to those found in *C. decorus* (sp. 3a).

C. "butleri" (Species 2g)

This species is named for Malcolm G. Butler who published a karyotype photograph.

C. decorus group species 20f Butler et al. 1995

In BOLD Bin: <u>BOLD:AAB7030</u> *C. decorus* and *C. quinnitukqut* are also in this Bin.

Adult: Most adults of this species are in the collection of J. E. Sublette now in Museum of the University of Minnesota, St. Paul, MN.

These specimens are unavailable to me, so I propose as holotype male a reared specimen from Mississippi for which there is pupal data and also Barcode *COI* sequence.

Similarly, the proposed Allotype female is also a reared specimen from Mississippi for which there is pupal data and Barcode *COI* sequence.

Proposed Male holotype: (reared Mississippi specimen UMS.2.1 reared Male DNA1).

BR

3.0

_

1.10

1.01

Wing length 4.0 mm; width: 0.96 mm. VR 1.0. Scf on brachiolum 3-4; 33 &36 setae on squamal fringe. Cross vein and posterior fork slightly darkened.

Head: Antennal ratio: 3.01. Frontal tubercles developed, about 56-58 μ m and 2.6 times longer than width at base. Clypeus about 0.7 of diameter of the antennal pedicel, with about 29 setae.

Thorax brownish yellow with brown vittae; Setae: Acrostichal - 29; Dorsocentral - 33; Prealar - 7; Scutellar: anterior – 23 in 3 rows; posterior - 18 (total 41).

Legs with darkening of the joints of the anterior tarsus and tibia.

Jeg	g lengths (in microns) and proportions as below.									
		Fe	Ti	Ta1	Ta2	Ta3	Ta4	Ta5	LR	F/T
	PI	1490	1450	2050	1065	800	680	325	1.39	1.03

510

653

Leg lengths (in microns) and proportions as below.

920

1190

1440

1694

PII

PIII

1585

1661

Note that for PI, Ta4 is shorter than Ta3 (about 85%).

270

300

190

188

0.64

0.77

360

510

Abdomen: with saddle spots on TII-VI, then whole segment darkened, about 10 setae in a single pale spot on tergite IX. The terminalia are essentially a typical *C. decorus*-type hypopygium: SVo essentially of a D or E type of Strenzke (1959), IVo sometimes turning slightly out at the end and with simple setae; reaching past the end of the anal point to almost 2/3 along the gonostylus which is only moderately swollen, but narrowing significantly over posterior half to third.

There is a small amount of information from a specimen from Lake Waskesui, Prince Albert National Park, Saskatchewan, in the Sublette collection. Essentially only photographs of the terminalia are available (below). This has 11 setae in a single pale area on TIX, SVo variable (from photographs from 3 males, below). Anal point narrowing slightly to distal end. IVo slightly turned out at the end and reaching almost to the end of the anal point and 1/3 of the gonostylus which is only moderately swollen, and narrowing significantly over posterior half to third.



Male hypopygium (left) and SVo showing variation (right) of *C*. 'butleri' from Lake Waskesui, Prince Albert Natl. Park, Saskatchewan. A typical *C. decorus*-type hypopygium

Allotype female (Jackson, Mississippi, specimen UMS.1.1 F DNA1)

Wing: length abt 4.1 mm., width 1.3 mm., VR 0.99; abt 53 setae in squamal fringe, 3 & 4 SCf on brachiolum.

Head: FT abt 46x23 μ m (2.0 times longer than wide). Clypeus abt. 2.1 times wider than the diameter of the antennal pedicel, with about 51 setae. Antenna missing save for A1 which is 225 μ m long. Palp proportions (micron): 73; 67: 213, 271: shriveled. Thorax: Setae – Acrostichal 17+; Humerals 5-9 in group +2-3 linear; Dorsocentrals 43 (dorsocentrals plus humerals 51-54); Prealar 6,7; Supra-alar 1, Scutellar 39 (21 in anterior row, 18 in posterior row).

0											
	Fe	Ti	Ta1	Ta2	Ta3	Ta4	Ta5	LR	F/T	BR	
PI	1390	1290	-	-	-	-	365	-	1.08	1.5	
PII	1620	1490	860	430	340	235	180	0.58	1.09		
PIII	1730	1750	1280	740	580	320	210	0.73	0.99		
			. 1		01	1 . 1 .	T 1				

Legs with darkening of the joints of the anterior tarsus and tibia. Leg lengths (micron) and proportions:

at least 72 SCh on hind Ta1

Most of tergites II-VIII darkened. Abt 10 setae on Seg. X, which has a central oval area and extended lateral extensions, (l/w 4.7); 3 setae on GcIX. Cercus almost square but with a bulge on the ventral posterior corner.



Cercus and segment X of proposed allotype female

Pupa (incl. exuviae of proposed holotype and allotype): Brown cephalothorax and generally pale (yellowish) abdomen. Shagreen over the posterior 3/4-4/5 of TII-V, split into two halves at midline in seg. V, reduced to a small posterior group on TVI.

Female abt. 9.59 mm (2), male about 9.67-9.75 mm (2), in length. Antennal sheath of female about 885 μ m long. Inner margin of wing case 1.64-1.90 mm. Cephalic tubercles large, 114-177 μ m long and 1.2x (fem) or 1.65-1.75 (male, Fig. below) longer than wide; with seta at least 48-51 μ m long. Obvious frontal warts, larger in female (85 x 134 μ m. height 0.63 times the basal width) than in the male (about 32.5x 88 μ m, height only 0.37 times the width at base). Female antennal sheath abt. 890 μ m long. Respiratory scar about 127-170 μ m long and HR about 1.7-2.0; respiratory fibers very narrowed at midpoint; a patch with about 3 small vacuoles just anterior.

Hook row of seg. II with about 110 (94-125) hooks, and occupies abt 0.58-0.84 of segment width. PspB present on lateral margin of segment 2, not seen on segment 3. PspA of segment IV abt 119-245 μ m long and 58-119 μ m wide, occupying 0.13-0.27 of segment length; that of segV of spines about 89-127 μ m long and 42 μ m wide; seg.VI with small patches of spines. L-seta at junction of segments III/IV at least 76 μ m long, that of IV/V not seen.

Caudolateral spur of segment VIII with about 6.8 (4-11) closely applied spines, some arising lower than the head of the spur (see figure below). About 105 (98-113 taeniae on margin of swim fin, in 2-3 rows.



Pupal cephalic tubercles (above) and spur (below) of C. 'butleri'

Fourth instar larva a medium sized <u>bathophilus-type</u>, but sometimes a <u>melanotus-type</u> with TLt about 60-280 μ m long. Length abt. 13.05 (9.3-20) mm, female; 13.12 (9.4-17.4) mm, male; VT may be of fluviatilis-type, with anterior pair usually slightly longer (ant. 1.27 (0.8-1.76 mm; post. 1.22 (0.84-1.96 mm). AT about 300–560 μ m long and about 2-4 times longer than wide, with the dorsal pair sometimes slightly longer than the ventral pair. As well, the longer AT are found in the more northern populations (e.g. Saskatchewan and Kansas) with shorter ones in Mississippi.

Over 2/3 of gular region dark, extending higher at the edges than in the midline and widest anterior to the posterior margin, and FC pale. Salivary reservoir (below) long, about 92 (63-127 μ m), and narrow, about 4.1 (3.4-4.5) times longer than wide.,



Frontoclypeus and gular region of C. 'butleri'. Photos courtesy of I. Proulx

Mentum (a, below) with somewhat rounded teeth; c1 tooth tall and relatively broad, c2 teeth moderately well developed (type IB or III) ; 4th lateral reduced to about level of 5th lateral (type II), although Hilsenhoff & Narf (1968) express that the 5th laterals are raised; 6th laterals generally arising below the level of other teeth.

Ventromental plates (c, below), 3.1-4.0 times wider than deep; 1.08 (0.8-1.15) times the mentum width with about 36.7 (32-42) striae; IPD about a third to a half of the mentum width; VMR 0.29 (0.24-0.33). PE (d, below) with about 14.7 (12-18) relatively blunt teeth (type B), lateral teeth smaller and narrower. Premandible (below)with inner tooth relatively broad, about 3.5 to 7 times wider than the outer tooth which tapers to a relatively fine tip (type B1).



Salivary reservoir (at left), Pecten epipharyngis and Premandible. Photo courtesy Isabelle Proulx.

Basal segment of antenna (b, below) relatively short – only 0.34-0.43 of the VHL, about 3.2 (2.3-3.7) times as long as wide, RO a third to a half up from base of segment; AR 2.34 (2.0-2.6); relative length of segments (micron): 145 : 29 : 8 : 12 : 6.

Distance between antennal bases (143-213 μ m) greater than distance between S4 setae (132-200 μ m), which are separated by 75-88% of the FC width at that point. S5 setae varying from slightly posterior to slightly anterior of the nearby RO.

Mandible (e, below) of type IA to IIB and with about 16.2 (13-22) furrows on the outer surface near the base; about 12.3 (10-14) taeniae in PecM; Mdt-Mat 31.8 (20-43) and MTR 0.38 (0.25-0.46).



Cytology: 4 polytene chromosomes with the thummi arm combination AB, CD, EF, G; centromeres not heterochromatic.

Arm G usually paired only at the end away from the virtually terminal nucleolus; 2 BRs developed-one near the middle, the other near the other end of the arm. No nucleoli in the long chromosomes. Polymorphism in arms A, B, C, F and G - polymorphism may be higher in populations in the northeast.

butA1:	1a-e, 7c - 4c, 3i-f, 17a-h, 12 - 10, 2c - 1f, 15a - 16d, 3e-a, 14	- 13, 4ab, 2k-d, 9 - 7d,
	18 - 19	(Kiknadze)
butA2:	Simple inversion.	
butB1:	Puff near distal end of arm, with dark bands proximal (g	roups 5 and 4).
butB2:	Complex inversion Dark bands distal to puff (groups 4	and 5).
butC1:	1a-e, 3c-2f, 4a-6b, 12b-14c, 12a-11d, 6gh, 17a-16, 7d-a, 6f-c	, 17b-18, 15-14d, 1f-
	2e, 8-11c, 19-22	(Kiknadze)
butC2:	Inversion of about half the arm towards distal end.	
butD1:	1-3e, 17-18d, 14f-10d, 14g-16, 21-18e, 10c-a, 7d-3f, 9-7e, 22	2-24
		(Kiknadze)
butE1:	1 - 3e, 10g-c, 3f - 4, 10b - 5a, 11 - 13	inv from bifE1
butF1:	1a-i, 9 - 2, 13c - 17, 10 - 11d, 13b - 11e, 18 -23	inv from bifF1
butF2:	Inversion of about $1/3$ of arm distal to center.	
butG1:	Virtually terminal N, central and distal BR	
butG2:	Small inversion distal of the distal BR	



DNA analysis:

mt*COI*: Barcode sequence shows that this species is very close to *C. decorus* (sp. 3a), as both are placed in BOLD Bin AAB7030. Comparison of the sequence to that of *C. decorus* indicated only a single base pair difference in the *COI* sequences, while other differences exist as polymorphisms in this species.

This is unexpected in view of the differences in morphology (pupal cephalic tubercles, and possibly by the greater extent of darkening of the larval gular region) and ecology between the species. *C. quinnituqut* is also in the same BOLD Bin, but here there are 13 consistent base differences between the species (with 3 of these including a low level of polymorphism for the base in the other species).

base

				(C. quir	nnituk	qut					
G	А	Т	Т	A/T	G	С	Т	A	A	Т	Т	С
A	G	С	С	A	С. 'ł Т	outleri T	C	G	G	C/T	С	A/T

Found: Ontario - Cactus Field, Point Pelee Natl. Pk. (41.939°N, 82.516°W) (from GenBank) Quebec - St. Charles River (46.82°N, 71.22°W), Quebec City; Lake Adéline (48.20°N, 79.17°W), Lake Dufault (48.28°N, -9.00°W), Lake Duprat (48.33°N, 79.12°W), Lake Fortune (48.18°N, 79. 32°W), Lake Opasatica (48.17°N, 79.33°W), all Rowyn-Noranda.
Saskatchewan - Lake Waskesiu (53.92°N, 106.08°W), Prince Alfred National Park. California - ¿Lake Davis (abt 39.70°N, 120.50°W).
Indiana - ¿Crooked Lake, Angola; Lake Oneotta, Marinette(?); Shafer Lake.
Kansas - Lone Star Lake (38.83°N, 95.38°W); Natural History Reserve, University of Kansas (39.00°N, 95.42°W), both Douglas County.
Massachusetts - Connecticut R., Northfield (42.69°N, 72.452°W), Franklin Co. and Northampton (42.322°N, 72.638°W), Hampshire Co.
Michigan - Saginaw Bay (43.75°N, 83.67°W), Lake Michigan.
Minnesota - Lake Christina (45.08°N, 95.75°W), Douglas Co.
Mississippi - Belzoni (33.17°N, 90.67°W), Humphreys Co.; Jackson (32.50°N, 90.33°W), Hinds Co.
New Mexico - Springer Lake, Colfax Co.; Frio Draw and Weber City cut-off, Curry Co.; Canadian River and Ute Lake, Quay Co.
North Dakota - Kota Ray Dam, Williams Co.; Lake Williams, Kidder Co.
Texas - Quaker Street Plaza, Lubbock (33.64°N, 101.96°W), Lubbock Co..
Vermont - White River (43.80°N, 72.45°W), nr. Sharon, Windsor Co.
Wisconsin - Booth Lake; Friebauer Lake, Bayfield Co.; Lake Oneonta, Marinette Co.; Lake Pepin, Pepin Co.; Pine Lake, Oneida Co.; Booth Lake, Walworth Co.

Found mostly in depths to over 10m in lakes.

This is the *C. attenuatus* of Hilsenhoff and Narf (1968) and *C. decorus*-group species 2 of Butler *et al.* (1995) who provide a karyotype photograph. Wuelker *et al.* (2009) give some cytological comparisons to *C. bifurcatus* and *C. blaylocki*.

The species is closely related to *C. decorus*, as indicated by the Barcode sequence, but shows differences in the chromosome banding patterns and a number of morphological characters, e.g. the frontal warts of the pupa are smaller and lack a seta, while there are usually more spines on the spur (4-11 c.f. 1-6).

C. quinnitukqut Martin, Sublette & Caldwell, 2010 (Species 2n)

This species is in BOLD Bin: <u>BOLD:AAB7030</u> *C*. 'butleri' and *C. quinnitukqut* are also in this Bin.

Adult male identified by Townes as C. atrella, to which the species will key in his 1945 key.

Male:

Wing length 3.70 (2.96-4.31) mm; anterior veins slightly darker; 36 (21-43) setae on squamal fringe; VR about 1. LR 1.16 (1.14-1.22); BR 6.33 (5.71-7.16)

Head, thorax, including most of antepronotum and scutellum paler yellowish; thoracic vittae, postnotum, legs and abdomen largely blackish.

Head – AR 3.57 (3.41-4.70); frontal tubercles 30 (25-42) μ m about 1.7 times longer than wide; clypeal width about the same as width of antennal pedicel; clypeal setae 38 (24-56); palpal proportions (μ m) 75 ; 70 : 203 : 189 : 224.

Thoracic setae – Acrostichals present but not counted; Dorsocentral 29 (25-42) in a single to mostly triple row; prealar 7 (6-7); scutellar 26 (22-42) mostly in a single sl. staggered row laterally becoming double at medial apex.



6 (2-7) setae in individual pale spots on tergite IX; anal point narrows gently to distal end; SVo of the E(i)-type of Strenzke (1959); IVo narrowing towards the tip, longer than anal point, reaching to about the midpoint of the GS, which is only moderately broad and narrowing gently over posterior third.

Female:

Wing length 3.61 mm; 38 setae on squamal fringe; VR 1.12. LR 1.24. Color similar to male but paler abdominal incisures slightly more pronounced. Antennal proportions: 150 : 85 : 85 : 105 : 270; AR 0.64. A5/A1 1.8. Palpal proportions (segs. 2-5)(μ m) 75 : 210 : 190 : 240; about 38 clypeal setae. Thoracic setae: Acrostichal not counted; Dorsocentrals (including humerals) 41 extending anteriorly to near the antepronotum; Prealar 7; Scutellar 38.

Pupa: No pupae were present in our samples, but some pupal characters were visible from a late prepupa. The spur has about three spines, progressively along the outer edge, in the available specimen. About 115 taeniae, multilayered, on each side of anal fringe.



Fourth instar larva a small to medium sized bathophilus-type, length about 12.1 (11.2-13.2) mm; Anterior VT, 0.61 (0.38-1.04) mm., longer than the posterior pair, 0.49 (0.28-0.96) mm. AT relatively short but varying between localities, longer at Truro; ventral pair usually

slightly longer (mean 364.3 μ m c.f. 325.7 μ m) but about the same width; however they can vary from about half as long as wide up to twice as long as wide.

Gular region slightly dark to dark: where dark usually covering most of the gula and widest at posterior edge; FC pale but sometimes with slightly dark lines alongside it or around the antennal base. Salivary reservoir about 68.7 (63-76) μ m wide and 3.03 (2.17-4.15) times longer than deep.

Mentum (d, below) with somewhat rounded teeth; c1 tooth broad and relatively tall, c2 teeth may be little more than notches (type IB) or may be better developed (IIA – fig. d, below); fourth laterals sometimes only slightly reduced but more usually reduced to just above the fifth laterals (type i-II); sixth laterals often arising lower than other laterals.

Ventromental plates (e, below) with a smooth anterior margin and about 208.1 (197-219) μ m wide and 3.2-3.8 times wider than deep; 1.02-1.2 times wider than the mentum; with 41.7 (38-46) striae; VMR about 0.26 (0.22-0.30). Premandible (a, below) with broad inner tooth at least 3–5.5 times wider than the sharp rapidly narrowing outer tooth. PE (c, below) with about 15.4 (11-18) sometimes irregular teeth but basically type B.

Antenna (b, below) with basal segment moderately long (39-44% of the VHL); about 2.6 (2.0–3.2) times longer than wide; RO abt 1/3 to 1/2 way up from base of segment; AR 2.14 (1.85-2.60); antennal segments (μ m): 109 : 24 : 7 : 13 : 7 : i.e. A5 close to, or as long as, A3 (A5/A3 = 0.95 (0.82-1.20).

Distance between antennal bases on average (160.7 μ m) larger than that between the S4 setae (158.9 μ m), but reversed in about 40% of individuals; S4 setae separated by 0.79-0.87 of the FC width at that point; S5 setae usually level with the nearby RO, but may occasionally be slightly anterior to it.

Mandible (f, below) about 253.2 (247-264) μ m long, with third inner tooth relatively well developed and almost completely separated, but not as darkly colored as the other teeth (type II-IIIB), with about 14.5 (12-18) furrows on the outer surface at the base; 12.8 (10-15) taeniae in PecM; Mdt-Mat 26.4 (20-28) μ m; MTR about 0.31 (0.29-0.35).



Cytology: 4 polytene chromosomes with the thummi arm combination AB, CD, EF, G. Keyl pattern difficult to recognize particularly for chromosome AB, where the characteristic bands (groups 24 to 27) of arm B are away from the centromere and the "olive" (groups 6 and 7) of arm A is not obvious. In the CD chromosome, arm D is relatively shorter than arm C, as the result of a fixed asymmetrical pericentric inversion which moves band groups D24 to 21 into arm C, while only group C22 is added to arm D. Arm G may be fully paired, but is usually unpaired towards the sub-terminal nucleolus (see fig. below); a central BR and one near the other end. A nucleolus is sometimes developed about 1/3 from distal end of arm B. Polymorphic in arms A, C, D, E, F and G: polymorphism in C is small and terminal; apparently 3 sequences in arm D, one of which appears to be complex. qutA1:

- qutA2: Some distal homology to A1 of *C. decorus* (sp. 3a)
- qutB1: Characteristic bands towards the middle of the arm; nucleolus, when present, at distal end of these bands.
- qutC1: 1 2e, 12b, 6b 2f, 12c, ?, ?, 21, (D)21 24
- qutC2: 1 2e,
- qutD1:
- qutD2: differs by a simple inversion of distal half of the arm.
- qutE1: possibly 1 3e, 10b 5, 11b 10c, 3f-4, 11c 13 i.e. inv. 4-11b from uliginosus, etc.
- qutE2: possibly 1 2c, <u>5 8, 3e 2d</u>, 11b 10c, 3f-4, 11c 13
- qutF1:
- qutF2:
- qutG1: subterminal nucleolus and posterior BR
- qutG2: small simple inversion of the region around the distal BR



Found: Connecticut - South Cove, Old Saybrook, Middlesex Co. Middlesex Co.

Massachusetts - East Harbor (Truro), Cape Cod National Seashore, Barnstable Co.

Tidal mud flats. However a specimen with an identical COI sequence has been found at Meadow Lake, Flushing Meadow, Indiana (BOLD). Length of VT and AT appears variable from sample to sample or locality to locality, perhaps dependent upon the salinity or other environmental factors at the time. At Truro it was found with an apparently related species C. sp. Cape Cod (sp. 4k).

DNA sequence: Sequence for the mt*COI* gene has been obtained from these samples and also from GenBank (access.nos. HQ979113, HQ979133). These indicate that this species is closely related to *C*. 'butleri' and *C. decorus*, with all three species in the same BOLD Bin. Comparison of the sequences indicates *C. quinnitukqut* differs at 13 bases (with 3 of these including a low level of polymorphism for the base in the other species).

base

				(C. qui	nnituk	qut					
G	А	Т	Т	A/T	G	С	T	А	А	Т	Т	С
					<i>C</i> . 'ł	outleri	1					
А	G	С	С	A	Т	Т	С	G	G	C/T	С	A/T

Perhaps at least partially isolated as a result of adaptation to estuarine waters.

This species was identified as *C. atrella* by Townes and Roback (Hitchcock & Anderson, 1968), but is cytologically quite distinct from other *C. atrella* populations. In general the cytology suggests it could be a member of the *C. decorus*-group, with similarities to *C. decorus* (Sp. 3a) and *C.* sp. Cape Cod. It differs from *C. decorus* by the presence of a fore tarsal beard, but the only information for sp. Cape Cod is that it may be smaller than that species.

The biology was described by Anderson & Hitchcock (1968). Described as *C. quinnitukqut* by Martin *et al.* (2010), including description of the polytene chromosomes.

C. species 'Cape Cod' (Species 4k)

Probably a member of the C. decorus-group and closely related to C. quinnitukqut.

This species is in BOLD Bins: <u>BOLD:AAV7387</u> and <u>BOLD:AAV7388</u> (due to error in 5' region of one sequence)

Adult

No adult is definitely associated, but slightly larger males than those of *C. quinnitukqut*, reared from fluviatilis-larvae which occur at the same location, may belong to this species. Some information is provided:

Male:

Wing: Length 3.18 mm. Anterior veins darkened. Scf with abt 21 setae, VR about 1.



Frontal tubercles about 38 μ m and about twice as long as wide. Palpal proportions (units): 9 : 10: 34 : 31 : 39. P5/P4 1.26; P5/P3 1.15.

Legs dark, and darker tibia, tarsi almost black.

Proportions of anterior leg (units)

	Fe	Ti	Ta1	Ta2	Ta3	Ta4	Ta5	LR	F/T	Ta5/Ti
PI	72	75	90	48	37	30	15	1.20-1.21	0.96	0.2
]	PII Scł	1 - 15;	PIII So	ch – 14		



Photo by J. E. Sublette

Abdomen with dark bands, with darker center-line but pale distal margin. TIX with about 6-11 setae in individual pale areas within larger triangle.



Male hypopygium (left) and SVo (right). Photo by J. E. Sublette

SVo closest to E(i) type of Strenzke (1959). IVo longer than anal point, which is narrower at the base, and extending to about a third or mid-point of the gonostylus, which is only moderately expanded and narrows gently over posterior half.

Female:

The only information available for a possible adult female is a photograph of the female terminalia (below).



Female terminalia of C. sp. Cape Cod. Photo from J.E.Sublette.

Cercus relatively square, but posterior margin shorter, no obvious bulge. About 14 setae on seg. X, which is about twice as long as its greatest width. Pupa: not known.

Fourth instar larva: A small to medium sized (fem. abt. 11.7-13.2 mm) bathophilus-type. VT short, abt equal length (0.52-0.86 mm) or posterior shorter (0.52-0.96 mm). AT about 320 μ m long and twice as long as wide (dorsal) and 340 μ m long and 2.4 times longer than wide (ventral). Salivary reservoir about 61 μ m wide and 3.5 times wider than deep.

Mouthparts mostly quite worn (see figure below). Gula very dark on post 2/3 or almost to the base of the mentum, widest just before posterior margin; FC pale, but some darkening towards rear of head and darker at base of antenna.

Mentum with 4th lateral often only slightly reduced (type I) but may be greater possibly due to wear, 6th lateral at least slightly lower than 5th lateral; c2 teeth usually little more than notches on side of c1 tooth (type IB) but may be well separated (type IIA).

Ventromental plates (d, below) about 211 μ m wide and about 3.6-3.8 times wider than deep, about 1.03 times the mentum width; IPD about 27-30% of the width of the mentum, with about 41 (38-44) striae; VMR about 0.27 (0.25-0.30). PE (a, below) of about 20 (16-24) usually worn type B teeth, sometimes with multiple small teeth in center.

Premandible usually with inner tooth longer (due to wear), and at least 3-4.3 times wider than the outer tooth, which narrows only gently along its length, both coming to relatively broad points (Type D).

Antenna (b, below) with relatively squat basal segment, about 2.8-3.2 times as long as wide, but 35-41% of the VHL; RO about a third to almost halfway up from the base; AR about 2.45 (2.21-2.74); A2/A1 about 0.20 (0.17-0.22); A5/A3 about 0.95 (0.83-1.0); proportions of the antennal segments (micron) 116.5 : 23 : 6 : 11.5 : 6 ; A5 is normally slightly shorter than A3, although of about equal mean value - A5/A3 about 0.95 (0.83-1.00).

Distance between antennal bases (174 μ m) greater than that between S4 setae (155 μ m), which are separated by about 84% of the FC width at that point; S5 setae about level or just slightly anterior to the nearby RO.

Mandible (e, below) about 257 (244-276) μ m long, with fourth inner tooth only partially developed and pale (type I-IIA) although this is often unclear as the 4th inner tooth is often broken off; with about 12.2 (10-16) furrows on the outer surface near the base; about 11.8 (10-14) taeniae in the PecM; Mdt-Mat about 30 μ m, MTR about 0.36.



Cytology: Four polytene chromosomes with the thummi-arm combination AB, CD, EF, G. Arm G with a sub-terminal nucleolus, next to a heterochromatic cap; BR immediately distal of nucleolus; homologs either unpaired or paired at the heterochromatic cap. Second nucleolus about 2/3 along arm D. No polymorphism in the specimens examined. Arm A1:

Arm B1: No obvious large puff. Bands 24-28 removed from usual position close to centromere, as in B1 of *C. quinnitukqut*.

Arm C1:

- Arm D1: Nucleolus distal of middle of arm
- Arm E1:
- Arm F1: May differ by only a small median inversion from F1 of *C. quinnitukqut*.



Found: Massachusetts - East Harbor (Truro), Cape Cod National Seashore, Barnstable Co.

Marine, organic sediments.

DNA sequence: Sequence for the mtCOI gene has been obtained.

This species has been found in association with *C. quinnituqut*, for which the larva is very similar. Two characters that seem to help distinguish between them is that sp. 'Cape Cod' has a higher number of teeth in the PE (more than 16), the outer tooth of the premandible only narrows gently towards the distal end, and the center teeth of the mentum are usually type IIA, while in *C. quinnitukqut* the PE has less than 16 teeth, the outer tooth of the premandible narrows sharply, and the center teeth of the mentum are usually type IB.

C. species 3j. (C. decorus-group sp.)

Adult: Not associated.

Pupa: Not known.

Fourth instar larva a small to medium <u>bathophilus-type</u> (about 11.7-14.1 mm: fem. 14.1 mm.; male 12.0 mm); VT relatively long, anterior pair longer (ant 1.44-1.56 mm; post. 0.96-1.40 mm). Sometimes a <u>melanotus-type</u> larva with TLt slightly developed (up to 160 μ m). AT size variable – dorsal 100-380 μ m long (l/w 1.0-1.02), ventral 140-420 μ m long (l/w 1.75-2.6).

Gula dark over more than the posterior half, FC pale.

Salivary reservoir about 70 µm wide and 3.86 times wider than deep.

Mentum (Fig. c, below) with somewhat rounded teeth; c1 tooth relatively broad with c2 teeth closely applied, not well separated (type IB-IIA); 4th laterals slightly reduced (type I-II). PE (Fig. a, below) with about 11 relatively broad, but irregular teeth.

Ventromental plates (Fig. d, below) about 3.4 times wider than deep; 1.04 times the mentum width, and separated by about 43% of mentum width; about 33-36 striae; VMR 0.22-0.25. Premandible (note can be seen across the central tooth of mentum in Fig. c, below) with outer tooth shorter (due to wear?) and inner tooth about four times the width of the outer tooth. Antenna (Fig. b, below) A1 about 1/3 the VHL and 2.93 times longer than wide, with RO from 1/3 to just below middle of A1, AR about 1.85; relative length of segments (micron): 111 : 30 : 9 : 13 : 8, i.e. A5 only a little shorter than A3.

Distance between the antennal bases greater than that between the S4 setae. Mandible (Fig. e, below) about 250 μ m long, with third inner tooth only slightly separated and darkened (type IA), about 16-17 furrows on outer surface near the base and 10-11 taeniae in PecM; Mdt-Mat 33 μ m, MTR about 0.41.



Cytology: 4 polytene chromosomes with the thummi arm combination AB,CD, EF, G. Arm G at least partly paired, with a subterminal nucleolus and a BR about $1/_3$ along the chromosome. No nucleolus in the long chromosomes. Olive in arm A not obvious, puff not usually developed in arm B.

ArmA1: could be as decA1

ArmB1: typical bands (groups) at least 15 bands away from centromere. Could be close to decB2+3.

ArmC1:

ArmD1:

ArmE1: 1-3e, 8-5, 9-10b, 4-3f, 10c-13 i.e. as *decorus, maturus, stigmaterus*, etc.

ArmF1:

ArmG1: Nucleolus further from end than in *C. decorus*.



Found: Saskatchewan - North Saskatchewan River (53.25°N, 105.08°W) (P.G.Mason) (CS-03-1).

Shows relationship to C. decorus (sp. 3a).

C. bifurcatus Wülker et al. 2009 (Species a)

A member of the C. decorus-group

Also C. species B1 = C. decorus-gr sp.1 of Butler *et al.* 1995.

In BOLD Bin: BOLD:AAG5453 (Gp. 1)

Adult:

The adults and other life stages were described by Wülker *et al.* (2009). However, these descriptions are probably a mixture of specimens of *C. bifurcatus* and C. 'proulxi', so need to be revised.

Male:

The adult male is a typical member of the *C. decorus*-group. Rather similar to those of *C. maturus* Johannsen in coloration and in the darkened SVo of the male genitalia but the SVo is shorter and darker than that of *C. decorus*.

Coloration: Head and thorax light to dark brown, postnotum blackish, scutellum paler, median and lateral vittae weakly separated by lighter color; abdomen strongly vittate, with the basal dark bands occupying half or more of each tergum, except on tergum

VI where the dark marking is more saddle-shaped, as *C. decorus*; legs pale yellowish brown.



Abdomen of C. bifurcatus (Wülker et al. 2009)

Head. AR 4.14 (3.33–4.21); temporal setae 32 (29–32); clypeus about 0.84 width of antennal pedicel; clypeal setae 32 (27–40); FT length 42–86 μ m; Palp segments II-V (μ m): 62: 226: 273: 383; P5/P4 1.40, P5/P3 1.69.

Thoracic setae – Acrostichals abt. 16; dorsocentral 27, mostly in 2 rows; prealar 6; supraalar 1; scutellar 30, with 15 in posterior row, rest scattered anteriorly. Wing length 2.87-3.95 mm; width 0.92-1.00 mm; VR 0.99 (0.98-1.02). Legs: LRI 1.58; LRII 0.62; LRIII 0.77; BR 3.0.

Leg proportions (micron)

	Fe	Ti	Ta1	Ta2	Ta3	Ta4	Ta5	LR	F/T	BR
PI	1190	1190	-	-	-	-	-	1.51-1.68	1.0	2.31-3.57
PII	1385	1150	730	385	230	190	150	0.61-0.66	1.18-1.20	
PIII	1385	1190	1150	615	405	365	-	0.71-0.80	1.0-1.16	

Some measurements of a confirmed specimen can be provided from the photograph of a male in the BOLD database; and for the hypopygium, from an adult from the same egg mass as the Holotype:



Wing length 3.8 for certain–4.1 mm, width 0.9-1.0 mm. Basal dark band at anterior of abdominal tergites, broader at mid-line and becoming more extensive until segs. VI-IX are almost completely dark. Tergite IX with 12 (8-16) setae in separate pale patches.



Male terminalia of C. bifurcatus

A typical *C. decorus*-type hypopygium, with SVo similar to that of *C. maturus*. Anal point relatively broad, SVo dark in type egg mass but paler in some other specimens, of D-type of Strenzke (1959); IVo reaching just beyond the end of the anal point and to about the midpoint of the GS, which is moderately swollen and narrows sharply over posterior third.

Female: - most information from some data extracted from photographs on BOLD



BIOUG03114-A06+1373481716

Coloration basically similar to male; abdomen with dark band on basal half of segment. Legs pale with some darkening at knees and on apical part of tibia, particularly on fore legs.

Wing length about 3.48-4.1 mm, width abt 1.02 mm; VR 0.92-0.95; abt 21 setae on squamal fringe; 3-4 SCf on brachiolum.

Antennal proportions (micron) 190 : 120 : 125 : 115 : 185; AR - 0.34; A5/A1 - 0.97. Frontal tubules about 28-32 μ m long and about 1.4-1.6 times longer than wide. Clypeus about twice the width of the antennal pedicel, about 39 clypeal setae. Palpal proportions (micron) 65 : 50 : 190 : 230 : 310; A5/A4 about 1.35; A5/A3 1.63. Thoracic setae: Acrostichal 17-18; Humeral 7-8 in patches + abt 4 linear; Dorsocentral abt 23-24 if H setae are recognized, otherwise abt 30-35 DL+H; Prealar 6-7; Supraalar 1: Scutellar 15 scattered anteriorly, 14 in posterior row. Leg proportions (approx.)(micron):

	Fe	Ti	Ta1	Ta2	Ta3	Ta4	Ta5	LR	F/T	Ta4/Ti
PI	1455	1310	-	-	-	-	-	-	1.10	-
PII	1500	1630	-	-	-	-	-	-	0.92	-
PIII	1800	1850	1600	800	-	-	-	0.73-0.86	0.93-0.97	-

Pupa is typical of most of the species in the *C. decorus*-group, in lacking the secondary cephalic tubercle on the frontal apotome found in *C. decorus* itself. It also lacks frontal warts. Length 7.56 mm (6.45–8.08) (11). Cephalothorax mostly dark; cephalic tubercles black, remainder of frontal apotome pale; abdomen pale with faint lateral vertical stripes on either of TI; TII–TIV with heavy lateral dark, vertical stripes; TVI, TVII with much weaker lateral stripe, reaching only about halfway to the posterior margin of each tergum; lateral margin of V–VIII darker, heaviest on TVIII, which grades into the dark posterolateral spur; lateral margins of anal lobe darkened.

Cephalothoracic tubercles weak; prealar tubercle elongate, not strongly produced. Abdominal shagreen moderately developed occupying slightly more than the apical half of TII; TIII–TVI with a quadrate shagreen patch which is slightly expanded laterally on TIII–TV and less so on TVI; TVII with a weak patch on each side near the base; TVIII with heavier shagreen than VII.

TII with a continuous row of 78–118 posterior hooks. Caudolateral spur normally with about 2-3 apical spines but varying from 1 to 6, essentially appressed. It differs from *C. maturus* in the usually higher number of spines.

Fringe of anal lobe with 71–93 taeniform setae.



a, c-e - Pupal characters of *C. bifurcatus*. b. Frontal apotome of *C. decorus* (sp. 3a) showing the frontal warts not found in many other species of the *C. decorus*-group. d. Spur from pupa from same egg mass as the Holotype

The pupa is very similar to that of its sister-species *C*. 'proulxi', although overall apparently slightly larger. Unfortunately the available data took no note of the sex of the exuviae, but the largest specimens are larger than the largest females of *C*. 'prouxli', the number of recurved hooks on segment II is higher (78-118 c.f. about 62-84) and there may be more spines on the spur (up to 6 c.f. an occasional 3 in *C*. 'prouxli').

Fourth instar larva small to moderate size, length 12.3 (10–15) (45); (fem. 10.2-13.8, 16); (male 11.4-13.4, 4) mm; essentially of the bathophilus-type but sometimes a melanotus-type with some development of TLt (0-160 μ m) which may be variable within larvae from the same egg mass (see below re. Type egg mass), suggesting it is genetic in origin. VT essentially the same length although the anterior pair have a slightly higher mean length (ant. 1.41 (0.87–2.28), post. 1.35 (0.87–2.04) mm); those of mid-west larvae are of fluviatilis-type (this may be a characteristic of larvae from deeper waters). AT with a medial constriction, dorsal and ventral pair essentially the same size (354-620 μ m long, and 2.5-6.2 times longer than wide).

Gula (below) dark on posterior half to 2/3 extending beyond the width of the mentum, usually higher at the outer edges and widest about the midpoint, FC pale or only slightly

darkened (in Michigan larvae). Salivary reservoir about 50.5-73 µm wide, and 2-4.5 times longer than wide (higher values apparently due to flattening in preparation).



Gula region of *C. bifurcatus* – from a larva from the type egg mass (The anterior border of the darkening probably faded in clearing)

Mentum (Fig. d, below) with pointed teeth, c2 teeth sometimes only partly separated from the c1 tooth (type IB-III), 4th laterals reduced at least halfway to the level of 5th laterals (type I-II).

VM (Fig. e, below) about 185-220 μ m wide and 3.30-3.69 times wider than deep; 0.97-1.19 times the width of the mentum; with about 35.24 (30-49) striae, IPD a third or more (0.28-0.45) of the mentum width, VMR about 0.22-0.35. PE (Fig. a, below) with about 12.3 (11-15) generally sharp, even teeth (type B). Premandible (Fig. b, below) with moderately broad inner tooth about 3-5.5 times the width of the outer tooth, which narrows markedly to a sharp point.

Antenna (Fig. c, below) relatively short, about 30-40% of VHL; basal segment about 2.6–4.1 times as long as wide; RO a third to almost half way up (0.31-0.47) segment 1; segment proportions (microns) 112 : 29 : 7 : 12 : 6.5; AR 2.05 (1.68–2.76); A3 shorter than A4 (A4/A3 abt. 1.4-2.2), and sub-equal or slightly longer than A5 - 1.06 (0.82-1.37).

Distance between the antennal bases is equally greater or smaller than the distance between the S4 setae, which are separated by about 80-90% of the FC width at that point. S5 setae usually about level with or occasionally slightly anterior to the nearby RO.

Mandible (Fig. f, below) with the third inner tooth partially separated and pale or slightly darkened (type IA-IIB), about 19.1 (18–22) furrows on outer surface near base; PMa with 11.7 (10-13) taeniae; Mdt-Mat 26.7 (20-33), MTR 0.33 (0.27-0.39).



Mouthparts of C. bifurcatus larvae from the type egg mass.

Cytology: 4 polytene chromosomes with thummi arm combination AB, CD, EF, G. Arm G usually at least partly unpaired, with a terminal nucleolus and BR. The nucleolar end is always unpaired, giving the forked appearance referred to in the name. The whole arm may be unpaired, which may be due to the presence of inversion polymorphism. No other nucleoli. Puff with distal dark bands usually developed near center of arm B. Polymorphic in A, B, C, D, E, F and G (at least band polymorphism, but also an inversion). At least three new heterozygous inversions have been identified since the published map of Wülker *et al.* (2009).



- bif A1: 1a-e, 14-15, 7b-4c, 13a-f. 8d-9, 2d-3, 17h-a, 3f-i, 12-10, 2c-1f, 8c-7c, 4ab, 16a-d, 18-19
- bif A2: 1a-e, 14-15, 7b-4c, 13a-f, 8d-9, 2d-3, <u>16d-a, 4ba, 7c-8c, 1f-2c, 10-12, 3i-f, 17</u>-19
- bif A3: 1a-e, 14a-g, <u>3-2d, 9-8d, 13f-a, 4c-7b, 15d-a, 14ih</u>,17h-a 3f-i, 12-10, 2c-1f, 8c-7c, 4ab, 16a-d, 18-19
- bif A4: 1a-e, 14a-g, 3-2d, 9-8d, 13f-a, 4c-7b, 15d-a, 14ih, <u>4ba, 7c-8c, 1f-2c, 10-12, 3i-f</u>, <u>17a-h</u>, 16a-d, 18-19
- bif B1: Large puff (group 7) near the middle of the arm.
- bif B2: Puff in more distal position
- bif C1: 1 6b, 12b 15, 8 11c, 12a 11d, 6gh, 17a 16, 7d-a, 6f-c, 17b 22 as C1 of *blaylocki*
- bif C2: 1 4h, <u>15 12c, 6b-4i</u>, 8 11c, 12a 11d, 6gh, 17a 16, 7d-a, 6f-c, 17b 22
- bif D1: 1 3e, 18d 13d, 7e 10, 4a-c, 13c 11, 3gf 18e-g, 7d 5, 19 24
- bif D2: 1 3e, <u>10 7e, 13d 18d</u>, 4a-c, 13c 11, 3gf 18e-g, 7d 5, 19 24
- bifD3: approx. 1 3e, 10 9, <u>16 13d, 7e 8</u>, 17 18d, 4a-c, 13c 11, 3gf 18e-g, 7d 5, 19 24

i.e. as *aberratus*, sp. 2b

- bif E1: 1 3e, 5 10b, 4 3f, 10c 13
- bif E2: 1 3a, <u>10e-c</u>, <u>3f 4</u>, <u>10b 5</u>, <u>3e-b</u>, 11 13
- bif F1: 1, 9 7, 14-17, 11e 13, 11d 10, 2 6, 18 23 (WW)
- bif F2: approx. 1, 9 7, <u>19 18, 6 2, 10 11d, 13 11e, 17 14</u>, 20 23
- bifF3: approx. 1, 9 7, 14 17, 11e, <u>5 2, 10 11d, 13 12</u>, 6, 18 23

- bifG1: terminal nucleolus and usually a large median BR (in type 2) and a smaller almost terminal one.
- bifG2: a small inversion around the median BR.
- Found: Alberta Astotin Lake, Elk Island Natl. Pk.(53.685°N, 112.89°W) (BOLD database) Nova Scotia - Point Pleasant Park, Halifax (44.623°N, 63.569°W) (from GenBank). Ontario - Lac Deschêne (45.37°N, 75.85°W) (Type locality), Arboretum, (45.40°N, 75.87°W), both Ottawa; Copanspin Farm, Dunrobin (45.75°N, 75.87°W); 0.5 ml Dunrobin; South March (45.40°N, 75.87°W), all Carleton Co.; Costello Creek, Algonquin Provincial Park, Nipissing Co. (abt. 45.58°N, 78.70°W); Georgian Bay Islands N.P. (44.7418°N, 79.8501°N)(BOLD); Catholic Central H.S. (42.9869°N, 81.239°W) and Oakridge Secondary School (42.978°N, 81.312°W), both London (BOLD).
 Quebec - L. Adéline (48.20°N, 79.17°W); L. D'Allembert (48.38°N, 79.02°W), L.

Arnoux (48.25°N, 79.33°W), L. St. Joseph (2007) (46.8°N, 71.63°W), L. Duprat (48.33°N, 79.12°W) and L. Opasateca (48.17°N, 79.33°W) (Proulx *et al.*). Massachusetts - Lake Pleasant (43.56°N, 72.51°W), nr. Montague, Franklin Co. Minnesota - Turtle Lake, Becker Co. (M.G. Butler).

Localities not clearly identified as C. bifurcatus or C. 'proulxi':'

Manitoba - Lake Winnipeg (Sæther 2012)

New Brunswick - Kouchibouguac Natl. Pk. (48.858°N, -64.975°W). Ontario - Bear Creek (45.37°N, -75.47°W), Carlsbad Springs, Carleton Co.; Algonquin Provincial Park, Nipissing Co. (abt. 45.58°N, -78.70°W). Quebec - Brewery Creek, Hull (45.43°N, -75.73°W), Gatineau. Kansas - Lone Star Lake (38.83°N, -95.38°W), Douglas Co. Michigan - Lake Michigan, Epoufette (46.05°N, -85.17°W). Minnesota - Anderson Lake, Clearwater Co.; Bad Axe Lake, Hubbard Co. (M.G. Butler); Eagle Creek, Eagle Bend, Todd Co. (46.13°N, -95.05°W).

Creeks, pools, the shallows to the profundal of lakes. In some locations it co-occurs with *C. decorus* (species 3a). At least where this occurs, the larvae of the two species can be separated by the greater region of gula darkening and the longer AT of *C. decorus* (3a).

In common with many widely distributed species, many characters are variable in different geographic areas but, as noted above, the egg mass data indicates that there are genetic differences even within localities.

All stages (except female) and the salivary gland chromosomes described by Wuelker *et al.* (2009) (but see note above). Some information on arm F given in Fig. 3 of Martin (1979) and a photograph of the chromosomes in Butler *et al.* (1995) as *C. decorus*-gr. species 1.

DNA analysis: Sequence for the mitochondrial *CO1* and the nuclear $gb2\beta$ genes are available. The data of Proulx *et al.* (2013) suggested that the species may comprise two closely related components. Additional data, mostly from GenBank, confirms there are distinct base differences between the groups at 10 bases (see Table below).

Locality	# specimens		base no. 1 2 3 3 3 4 4 5 5 6 9 7 3 4 8 7 9 1 6 2								
		1	2	3	3	3	4	4	5	5	6
		9	7	3	4	8	7	9	1	6	2

		7	1	6	2	1	7	8	6	4	1
Group 1											
Halifax NS	1	С	С	т	т	A	т	С	С	A	?
L. D'Allembert QC	1	С	С	т	т	А	т	С	С	А	А
L. Adéline QC	2	С	С	т	т	А	т	С	С	А	А
L. Arnoux OC	1	С	С	т	т	А	т	С	С	А	
L. Duprat QC	4	С	С	т	т	А	т	С	С	А	А
L. Opasatica QC	3	С	С	т	т	А	т	С	С	А	А
L. St Joseph QC(07)	1	С	С	т	т	А	т	Α	С	Α	А
GeoBayIsNP ON	1	С	С	т	т	А	т	Α	С	Α	А
Algonquin Pk ON	1	С	С	т	т	А	т	С	С	Α	А
Ottawa ON	1	С	С	т	т	А	т	С	С	Α	А
London CCHS ON	1	С	С	т	т	А	т	Α	С	Α	?
London OSS ON	1	С	С	т	т	А	?	?	?	?	?
Sudbury ON	2	С	С	т	т	А	т	С	С	А	А
ElkIsNP AL	1	С	С	т	т	А	т	С	С	А	А
Pleasant L. MA	1	С	С	т	т	А	т	С	С	А	А
Kaibab Nat.For. AZ	1	т	т	А	т	А	т	А	С	А	С
Group 2											
Pr.Ed.Is NP. PEI	1	т	А	Α	С	т	Α	т	т	А	?
L.St Joseph QC(06)	3	т	G	Α	С	т	Α	т	т	т	С
L.Kinojévis, QC	1	т	G	Α	С	т	Α	т	т	т	С
McFarlane L. ON	3	т	G	Α	С	т	Α	т	т	т	т
Tilton L. ON	1	т	G	А	С	т	А	т	т	т	т
Kawatha ON	1	т	G	А	С	т	А	т	т	т	С
Kawatha ON	2	т	G	т	С	т	А	т	т	т	т
Brockville ON	1	т	G	А	С	т	А	т	т	т	?
Kitchener ON	1	т	А	А	С	т	А	т	т	А	?
London BSPS ON	1	т	т	А	С	т	А	т	Т	А	?
London ABLSS ON	1	т	А	А	С	т	А	т	т	?	?
Meaford CS ON	1	т	А	А	С	т	А	т	т	А	?
Peterborough ON	1	т	А	А	С	т	А	т	т	А	С
Puslinch Twn ON	1	т	А	G	С	т	?	?	?	?	?
Rouge NUP ON	1	т	А	А	С	т	А	т	т	?	?
Whitby ON	1	т	А	А	С	т	А	т	т	?	?
Madison WI	2	т	А	А	С	т	А	т	т	А	С
Pr.Alf. NP SK	1	т	А	А	С	т	А	т	Т	А	С
Grasslands NP SK	1	т	А	А	С	т	А	т	Т	А	?
Gulf Is NP BC	2	т	С	А	С	Т	Т	Т	Т	А	?
Kamloops NAM BC	2	т	G	А	С	Т	А	Т	Т	Т	?
Kelowna CS BC	1	т	G	А	С	Т	А	Т	Т	Т	?
Riffle Ck OR	1	Т	G	Α	С	Т	A	Т	Т	т	С

Bases that differ between the two groups of C. bifurcatus in the COI tree

? - base unknown in short sequences from BOLD database

DeSalle *et al.* (2005) suggested that for closely related species, such specific base differences are a better indicator of specific status than distance measures. Martin (2011) noted some

examples amongst the Australian species and the distinction between *C. frommeri* (sp. 2d) and the other members of the *C. staegeri*-group is a further example.

Initially the split of *COI* sequence of specimens identified as *C. bifurcatus* was recognized in the BOLD database by placing them in separate bins, although BOLD has subsequently amalgamated the two Bins, probably as a result of confusion from shorter Barcodes accepted in the database (a large percentage of the sequences in this BOLD bin are less than 600 bp). As seen in the Table above (where sequences shorter than 500bp were not included), some of these sequences can be missing up to four of the critical bases.

There is no indication of the two types in the gb2ß sequences as there is a very low level of polymorphism overall.

As well there is some suggestion of alternative banding sequences on some chromosome arms (particularly arms B and F) of those relatively few specimens for which both cytology and DNA sequence is available. Those of group 1 correspond to those of the type egg mass (see Table below).

\ Arm	Α	В	С	D	Е	F	G(med BR)
Specimen \							
Group 1			some C2				
Pleasant L. MA 21	A2	B1	C1	D2	E1	F1	abs
Algon Pk ON 21f	A3	B1.2	C1	D1	E1	F1	pres.
L.Dupret QC 10	A1	B1		D1	E1	F1	abs
L.Arnoux QC 11	A1.2	B1	C1	D1.2	E1	F1	abs
L.D'AlembertQC 12	A1	B1	C1	D1	E1	F1	abs
Group 2							
Madison WI em#8	A3	B2	C1	D2.3	E2	F2	pres.
Madison WI em#7	A3	B2	C1	D2	E2	F2	pres.
McFarlane L.ON 7	A1	B2	C1	-	E1*	F2	pres.
Tilton L.ON 8	-	B2	-	-	E1?	F2	pres.
Type material							
Type egg mass	A1&3	B1	C1	D1	E1	F1	abs.

* it is just possible that this is E1.2

Sequences of Chironomus bifurcatus specimens used in COI analysis

It is possible that where the two types occur together there is some level of interbreeding. For example a specimen from Copanspin Farm, Dunrobin, Ontario had the karyotype A2.3, B1.2, C1.2, D2.2, E1.1, F1.2, G1.1.

In case this is supported, the following information is given based on material from the same egg mass as the Holotype:

Adult: No additional data other than the hypopygium shown above.

Pupa: Caudolateral spur of known pupa has only 3 spines, as shown below.



Fourth instar larva: Length 10.2–13.8 mm, TLt sometimes absent, but up to 186 μ m in length in others. Posterior ventral tubules longer in half the available larvae, but equal or anterior tubules longer in others. AT up to 3 times (2.5-2.8) longer than wide.

Gula darkened on posterior half to 2/3 (as in figure above), frontoclypeus pale.

Mentum of type II (4th laterals reduced almost to level of 5th laterals), central tooth of variable type (IA,B, IIA or III), with c2 teeth relatively separated.

About 30-43 striae on ventromental plates, which are separated by a third or more (0.32-0.43) of the mentum width and 3.38-3.69 times wider than deep.

Antenna relatively short; AR about 2.09 (1.7–2.8); A1 about 2.6–4.1 times longer than wide; RO almost half way up segment 1 (0.32-0.45); segment proportions (microns) 111 : 25 : 7 : 12 : 6.

11-15 sharp teeth (type B) in PET; premandible with inner tooth about 3-4 times wider than the outer tooth.

Third inner tooth of mandible hardly separated and with little color (type IA), about 18-22 furrows and 10-13 taeniae in the PMa; MTR 0.27-0.39.

This egg mass has the sequences bifA1 and A2, bifB1, bifC1, bifD2, bifE1, bifF1, bifG1 with no median BR.

C. 'proulxi' (Species 2b)

C. bifurcatus Type 2 (Proulx et al. 2013).

Was in BOLD Bin: BOLD: AAW4003

but BOLD operators have amalgamated it with the Bin containing *C. bifurcatus*, <u>BOLD:AAG5453</u>, probably because of the confusion caused by short sequences, although the distance graph shows two peaks.

Adult:

Male

Description of a potential type male (UWI.5.2 em#8, reared male 3)

Coloration not clear as specimen had been in ethanol for 40 years. Abdomen appears to have a brown band over the anterior 2/3 of segments II-IV, heaviest in the midline, then most of segments V-VIII.

Wing length 3.36 mm, width 0.81 mm, VR 0.98; 4 SCf on brachiolum, about 34 setae on squamal fringe.

Head: AR 3.14; frontal tubercles well developed, but appearance variable: 35-45 µm

long and about 2-3.5 times longer than wide; seta not visible. Palp proportions (micron) 95 : 50 : 190 : 230 : abt 300; P5/P4 1.3, P5/P3 1.58. Clypeus about 0.7 of the width of the antennal pedicel, with 32 setae.

Thoracic setae: Acrostichal 16; dorsocentral 21-25 beginning at anterior end of lateral vittae; prealar 6,7; Supra-alar 1,1; Scutellar 11 in 2 approximate anterior rows, 14, mostly larger, in posterior row (total 25).

	Fe	Ti	Ta1	Ta2	Ta3	Ta4	Ta5	LR	F/T	Ta5/Ti
PI	1245	1200	1785	945	710	635	300	1.48	1.04	0.25
PII	1320	1265	735	420	310	210	150	0.58	1.05	0.12
PIII	1520	1550	1165	670	495	285	190	0.75	0.98	0.12

Leg lengths and proportions (micron):



7 setae probably in a single patch in a pale oval on tergite IX. SVo closest to E(g) of Strenzke (1959); IVo with simple setae, reaching just beyond end of anal point and to about the middle of the gonostylus which tapers sharply over posterior 2/3.

Some additional information can be provided from other males from two egg masses, and from a male (see below) from a photograph in the BOLD database:



C. ' proulxi ' male from Arizona

Coloration much as C. bifurcatus, but knees and anterior tarsi darkened. Basal dark band at anterior of abdominal tergites, broader at mid-line and becoming more extensive until segs. VI-IX are almost completely dark.

Wing length 3.34 (2.78-3.8) mm, width 0.80 (0.68-0.9) mm, VR 0.98-1.03; 3.6 (3-4) SCf on brachiolum, about 28.25 (19-34) setae on squamal fringe.

Head: AR 3.23 (3.04-3.50); frontal tubercles 38.3 (35-45) µm long and about 2.5 (1.8-3.5) times longer than wide. Palp proportions (micron) 68 : 50 : 173 : 211 : abt 263: P5/P4 abt 1.25; P5/P3 1.51. Clypeus about 0.7 (0.5-0.75) of the width of the antennal pedicel, with 31 (28-33) setae.

Thoracic setae: Acrostichal at least 13.8 (9-16); dorsocentral 24.2 (21-28) beginning at anterior end of lateral vittae; prealar 6.9 (6-8); Supra-alar 1; Scutellar 11 (11-12) in 2 approximate anterior rows; 13.3 (12-14), mostly larger, in posterior row (total 24.7 (24-25)).

	Fe	Ti	Ta1	Ta2	Ta3	Ta4	Ta5	LR	F/T	Ta5/Ti
PI	1115	1120	1700	880	710	625	290	1.48-1.56	0.85-1.09	0.25-0.29
PII	1265	1175	720	405	280	190	145	0.58-0.66	1.03-1.18	
PIII	1405	1445	1110	650	460	280	180	0.75-0.80	0.93-1.0	
BR 1.4-2.0										

Approximate leg lengths (micron) and proportions:

About 7-8 probably in a single patches in a pale oval on TIX. SVo closest to E(g) of Strenzke (1959); anal point (below) broad, narrowing to distal end and with rounded end; IVo with simple setae, reaching to about the middle of the gonostylus which tapers noticeably over posterior third.



Anal point (note taper and rounded end) and IVo

Female

Coloration essentially as in the male.

Wing length about 3.40 (3.31-3.48) mm, width abt 0.95 (0.91-1.02) mm; VR 1.05 (1.02.-1.08); abt 19.3 (15-25) setae on squamal fringe; 3.2 (3-4) SCf on brachiolum. Antennal proportions (micron) and proportion of neck in brackets) 165 (0.24) : 106 (0.45) : 112 (0.55) : 112 (0.58) : 184; AR - 0.4 (0.34-0.46); A5/A1 – 0.40 (0.97-1.40). Frontal tubules about 34.5 (28-400 μ m long and about 1.4-2.3 times longer than wide. Clypeus about twice the width of the antennal pedicel, about 35.7 (33-39) clypeal setae.

Palpal proportions (micron) 59 : 51 : 193 : 230 : 300; P5/P4 about 1.35; P5/P3 about 1.49-1.63).

Thoracic setae: Acrostichal 15-18; Humeral 10-16, usually 7-10 in cluster + abt 4-6 linear; Dorsocentral abt 29.8 (28-35) if H setae are recognized, otherwise abt 34.5 (33-37 DL+H; Prealar 6-7; Supraalar 1: Scutellar 16 (15-17) scattered anteriorly, 13 (9-16) in posterior row - total 29.3 (26-33).

	Fe	Ti	Ta1	Ta2	Ta3	Ta4	Ta5	LR	F/T	Ta4/Ti
PI	1320	1095	1865	975	750	730	330	1.68-1.76	1.19-1.23	0.64-0.69
PII	1340	1275	760	415	305	200	155	0.58-0.60	1.02-1.07	
PIII	1445	1555	1135	645	500	275	180	0.72-0.74	0.91-0.94	

Approximate leg proportions (micron):

BR about 1.82

9.35 (6-15) setae on seg. X which is crescent shaped, about 3-3.8 times longer than the greatest width, and 3-4 setae on GcIX. Cercus 'helmet' shaped; margin curved with a basal bulge on ventral margin (although not seen in one specimen).

Pupa: Length of exuvia about 5.53-7.80 mm, females larger than males: female 7.54 (7.25-7.80); male 6.88 (5.53-7.48). Inner margin of wing case 1.38-1.64 mm.: female 1.61 (1.57-1.64); male 1.51 (1.38-1.59). CT simple, those of male (105-137 μ m and 1.3-2.1x longer than width at base) (see below) much larger than those of female (50-94 μ m and 0.7-1.5x longer than wide); seta at least 50-90 μ m long. No frontal warts or secondary CT. Respiratory scar 124-152 x 48-68 μ m, slightly larger in females, and narrowed in middle to kidney-shape, HR 2.3 (1.3-3.3); a patch of ovals and setae just anterior and lateral to scar. Shagreen of sharp points, strongest on posterior 2/3, but at posterior only of segment VI and not obvious on segment VII. About 73 (45-84) recurved hooks, higher in females (79-84) than in males (64-79), in continuous row at rear of abdominal segment II, occupying about 80-90% of the width of the segment.

Clear PsB on segment II, and PsA on segment IV larger in females (170-185 μ m) than males (95-172 μ m); (i.e. about 0.20-0.26 of the segment length) and about 58-81 μ m wide; that on segment V is mainly spines, perhaps 0.85 the size of that on segment IV; on segment VI only spines and about 0.38 of the size of that on segment V. L-setae present on anterior margin of intersegments III/IV and IV/V, most coiled or broken, but one on IV/V about 106 μ m long. Caudolateral spur (below) about 170 μ m long with 1-3 spines, and sometimes with an additional smaller spine. Fringe of anal lobe with about 79 (66-89) flattened taeniae (males 66-79; females 82-90) on each side, initially in single row but increasing to 2 or 3 rows posteriorly.



Cephalic tubules of male (above), and spur with single spine (below)

The pupa is very similar to that of its sister-species *C. bifurcatus* although overall apparently slightly smaller, with lower maximum numbers of recurved hooks and spines on the spur (see note in *C. bifurcatus*, above).

Fourth instar larva a small to medium (about 11.5 (10.0-15.6 mm; sexed females: 10-13.3 mm) melanotus-type (lateral projections only slightly developed, 0-160 μ m); VT essentially straight, 0.68-1.92 mm long, with posterior pair usually longer (means: ant. 1.37; post 1.44 mm.). Anal tubules about 360-580 μ m (dorsal), 300-540 μ m (ventral), 2.4-4.7 times longer than wide (longer in Wisconsin than in Canada). A single parasitized male was measured at 13.6 mm long, ant.VT 1.55 mm. post. VT 1.81 mm, lateral tubule 160 μ m, suggesting it approaches the females in overall size.

Dark posterior half to two thirds of gular region, extending wider than the mentum width, higher at the edges and widest part way up (see below), FC usually pale, but may have very slight darkening. Salivary reservoir 61.5 (55.5-76) μ m long and 3.2 (2.9-3.8) times longer than wide (values above this are apparently due to flattening during preparation).



Gula region of C. 'proulxi' - courtesy of I. Proulx

Mentum (Fig. b, below) with generally rounded teeth; c1 tooth fairly broad, c2 teeth only moderately developed, generally type IIA, occasionally type III, but with wear may appear as type IB; fourth laterals usually reduced almost to level of 5th laterals (type II).

VM (fig. c, below) about 180-221 μ m wide and 2.95-3.72 times wider than deep; 1.06-1.19 times the width of the mentum; with about 45 (41-52) striae, IPD about a third or more (0.27-0.40) of the mentum width, VMR about 0.31 (0.25-0.45).

PE (Fig. a, below) of type B with about 13 (10-14) teeth. Premandible with teeth about equal or outer shorter (wear?), inner tooth about 3-5 times wider than inner tooth, which narrows markedly along its length.

Antenna (Fig. e, below) relatively short, about a third to a half VHL; basal segment about 2.7–3.8 times as long as wide; RO a third to half way up (0.29-0.50) segment 1; segment proportions (microns) 116 : 29 : 9 : 11.5 : 6; AR 2.06 (1.74–2.42); A3 shorter than A4 (A4/A3 abt. 1.1-1.8), but slightly longer than A5 (A5/A3 abt. 0.63-0.86).

Distance between antennal bases variable with respect to that between the S4 setae – in Canada it tends to be greater, but in Wisconsin it is less; S4 setae separated by 0.78-0.88 of the FC width. S5 setae generally slightly anterior to the nearby RO, occasionally level. Mandible (Fig. d, below)with 3^{rd} inner tooth only partially separated and pale (IA), but occasionally may be more separated or slightly darker (i.e. IB or IIA); about 19.7 (15-25) furrows on outer surface at the base; about 11.5 (10-13) taeniae in PMa; MTR about 0.35 (0.26-0.45).



Cytology: 4 polytene chromosomes with the thummi arm combination AB, CD, EF, G. Arm G partly paired and possibly submetacentric or with a block of heterochromatin about one third along the arm; nucleolus almost terminal on short arm, BR about one third from end of long arm and possibly another close to the nucleolus. No nucleolus on any other arm. Polymorphism in arms A, B, and F, and perhaps also G.



prxA1 1a-e, 14a-g, 3-2d, 9-8d, 13f-a, 4c-7b, 15d-a, 14ih,17h-a 3f-i, 12-10, 2c-1f, 8c-7c, 4ab, 16a-d, 18-19 i.e. as bifA3 prxA2: probably as bifA1 prxB1: Puff (gp. 7) with proximal dark bands, about 1/3 from distal end. i.e. as bifB2 prxB2: probably as bifB1 prxC1: 1 - 6b, 12b - 15, 8 - 11c, 12a - 11d, 6gh, 17a - 16, 7d-a, 6f-c, 17b - 22 i.e. as bifC1 prxD1: 1 - 3e, 10 - 7e, 13d - 18d, 4a-c, 13c - 11, 3gf 18e-g, 7d - 5, 19 - 24 i.e. as bifD2 prxE1: 1 - 3e, 5 - 10b, 4 - 3f, 10c - 13 i.e. as *aberratus*, bifE1. prxF1: probably 1, 9 - 7, 14 - 17, 11e - 13, 11d - 10, 2 - 6, 18 - 23 i.e. as bifF1 prxF2: small inversion a few bands distal of the characteristic bands.

prxG1: heterochromatin block and/or a BR, about one third from end with nucleolus, and a BR about one third from other end.

Found: British Columbia - North Pender Island, Gulf Islands N.P. (48.781°N, -123.301°W)(BOLD); Kelowna C.S., Kelowna (49.863°N, -119.454°W)(BOLD). New Brunswick - Kouchebouguac National Park. Ontario - Bear Creek, 0.5 ml s.e. Carlsbad Springs (45.37oN, -75.47oW); abt 5 km NE Bobcayeon, Kawartha (44.57°N, 78.5°W) (BOLD); Brockville (44.589°N, -75.689°W)(BOLD); A.B.Lucas SS (43.033°N, 81.248°W) London (BOLD); 4 ml e. Sudbury (46.52°N, -80.90°W); Sudbury area (Proulx et al.); McFarlane L. (46.42°N, -80.95°W) and Tilton L. (46.35°N, -81.07°W), Peterborough (44.271°N, -78.394°W)(BOLD). Prince Edward Island - Bubbling Springs Trail PEI Nat. Pk. (46.411°N, -63.093°W)(BOLD). Quebec - L. St. Joseph (2006) (46.88°N, -71.63°W); L.Kinojévis (48.13°N, -78.90°W) (Proulx et al.). Saskatchewan - Grasslands Nat. Pk. (49.001°N, 106.557°W); Prince Albert Nat. Pk., (53.85°N, -106.078°W)(both BOLD) Arizona - at 2033 meters Kaibab National Forest (35.939°N, -112.121°W) (BOLD) Oregon - Rolling Riffle Creek, e. Dexter, Lane Co. (43.90°N, -122.8°W). Tennessee – 10-Mile Creek, nr. Knoxville, Knox Co. (35.90°N, -84.07°W). Wisconsin - Greene Prairie, Arboretum (43.03°N, -89.42°W) and University Houses (43.07°N, -89.42°W), Madison, Dane Co.

Possibly also:

Ontario – Bear Creek, nr. Carlsbad Springs (45.37°N, -75.47°W).

Molecular sequences:

Mt*COI*: This species can be separated from the closely related *C. bifircatus* at the molecular level on the basis of consistent differences at 10 base pairs:

	base no.													
1	2	3	3	3	4	4	5	5	6					
9	7	3	4	8	7	9	1	6	2					
7	1	6	2	1	7	8	6	4	1					
т	A/G	A	С	Т	A	Т	Т	A/T	C/T					

Note that 6 of these 10 sites are in the 3' part of the Barcode sequence and so may not be included in the short sequences in the BOLD database.

Lakes, pools and creeks.

The potential type locality for this species is Madison, WI (UWI.5.2 and UWI.6.5) where reared egg mass specimens are available.

C. blaylocki Wülker et al., 2009 (Species l)

A member of the *C. decorus*-group, described as *C. tentans* by Blaylock, Auerbach and Nelson (1964).

Adult: Presumptive male described by Wülker et al. 2009:

Male: Head, thorax and abdomen with extensive dark markings, legs infuscate. Wing length about 2.87 mm, VR 0.98, 22 setae in squamal fringe; AR 3.86; LR 1.73. Head with frontal tubercles about 32 μ m long, 28 clypeal setae, palp segments (2-5)(micron): 47 : 203 : 265 : 351.

Thoracic setae: Acrostichal abt 16; dorsocentral 27; prealar 6; supraalar 1, scutellar 15 strewn on anterior face, 15 in posterior row.



Male terminalia of presumptive C. blaylocki (left); superior volsella (right)

10-11 setae in individual pale patches on TIX; SVo of D-type of Strenzke (1959); IVo reaching beyond the end of the relatively broad anal point and to about the middle of the GS, which narrows relatively gently over posterior third.

Female and pupa: not described.

Fourth instar larva a medium sized plumosus-type (length about 12.2–13.9 mm female, 11.9 mm male). VT long, posterior pair usually longer (3.0-3.76 mm, cf. 2.44-2.80 mm). TLt well developed, about 300-420 micron. AT with a medial constriction. Gular region dark over posterior third and a pale or slightly darkened FC.



Mentum with rounded teeth, 4th laterals not reduced (type I); c1 tooth moderately broad with well-developed c2 teeth (type III).

VM about 4 times wider than deep with about 36-40 striae, about same width as the mentum, VMR about 2.76; MTR about 0.33. PE with about 13 to 16 teeth (type B), the most lateral ones being somewhat narrower.

Premandible with outer tooth shorter (may be due to wear), inner tooth about 2.5 times wider. Antenna with relatively long basal segment, about 3.7 times longer than wide; RO about 1/3 to 1/2 way up from base of segment; AR about 2.36; segment proportions (microns) 150 : 38 : 10 : 13 : 8 ; A1/VHL about 0.45; A2/A1 about 0.25.

Distance between the antennal bases greater than that between the S4 setae.

Mandible with 3 spines on the inner margin; about 15-16 furrows on outer surface near the base; 3rd inner tooth clearly separated but only moderately darkened (type II-IIIB); PMa with about 13 taeniae.

Cytology: 4 polytene chromosomes with thummi arm combination AB, CD, EF, G. Arm G generally paired with a subterminal nucleolus and nearby BR. No nucleoli in the long chromosomes. Bulb about middle of arm B with distal dark bands. Polymorphic in all arms in areas subject to radioactive effluent (Blaylock *et al.* 1964), but no endemic inversions recorded for arms A, E and G.

- blaA1: 1a-e, 8 9, 2d 3e, 15 14, 2c 1f, 16a-d, 7 4, 13a-f, 10 12, 3i-f, 17 19
- blaB1: Puff (group 7) with distal dark bands about the middle of the arm. Possibly as B2 of Sp. b
- blaB2: Formed by overlapping inversions from B1, such that the puff is near the distal end of the arm, with the dark bands proximal to it.

- blaC1: 1 6b, 12b 15, 8 11c, 12a 11d, 6gh, 17a 16, 7d-a, 6f-c, 17b 22 as *C*. *bifurcatus*.
- blaC2: 1 2e, <u>12b, 6b 2f</u>, 12c 15, 8 11c, 12a 11d, 6gh, 17a 16, 7d-a, 6f-c, 17b 22
- bla D1: 1 3, 11 12, 18 19, 13 17, 4 7, 10 8, 20 24
- bla D2: 1 3, <u>19g 18, 12 11</u>, 19h, 13 17, 4 7, 10 8, 20 24
- bla E1: 1 3e, 8 5, 9 10b, 4 3f, 10c 13 i.e. as maturus & stigmaterus.
- bla F1: 1, 9 2, 10 23
- bla F2: 1, 9 5d, <u>16 10, 2 5c</u>, 17 23
- Found: Tennessee White Oak Creek (Type locality) (36.03°N, 84.27°W), and McCoy Branch, Clinch River, nr. Oak Ridge, Knox Co; Ten Mile Creek (35.90°N, 84.07°W), Knox Co.

Pools in creeks.

Fourth instar larva, presumptive adult and the salivary gland chromosomes described by Wuelker *et al.* (2009). Chromosomes mapped, as *C. tentans*, by Blaylock *et al.* (1964). The identity of this species as a member of the *C. decorus* group was noted by Wülker *et al.* (1968). The sequences of arms E and F were given in Wülker *et al.* (1989) and of arms C1 and D1 in Kiknadze *et al.* (2004). This species is very close to *C.* sp. b, as they share many chromosome banding sequences.

C. decorus group (Species b) "Chironomus parablaylocki"

This species is cytologically closely related to *C. blaylocki*, with which it shares banding sequences. It is suggested that this species be named *Chironomus parablaylocki*. The suggested holotype would be the slide of larval body and polytene chromosomes from Copanspin Farm, Dunrobin, Ontario, Canada CO.23.15 81M. A photograph of the polytene chromosome complement of this larva was published in Wuelker *et al.* (2009). The male below would be a paratype male.

Adult:

A reared adult male (Ontario CO.23.11 reared male) is in the Canadian National Insect Collection

Male: The adult male is a typical member of the *C. decorus*-group. At least 6 setae, probably in individual pale spots, on TIX. The GS is relatively short and stout compared to those of *C. bifurcatus* or *C. blaylocki*, narrowing sharply over distal quarter. The SVo shows similarities to that of C. *blaylocki* i.e. S-type, closest to Strenzke's figure c. IVo with simple setae, reaching to about middle of the gonostylus.



Male terminalia of *C*. species b Note relatively short GS and the SVo similar to that of *C*. *blaylocki*.

Female: No information available.

Pupa: (Male) Caudolateral spurs (below) with about 5 spines along the outer edge.



Fourth instar larva a moderate sized (female 15.9 (14.4-17.2) mm; male 13.9 (12.0-15.3) mm.) plumosus-type with long VT, posterior pair normally longer (anterior 1.97 (1.6-2.3) mm; posterior 2.29 (1.9-2.8) mm. TLt about 256.9 (170-360) μ m. AT relatively long, about 4.4 times longer than wide, with a median constriction; length about 599 (557-680) μ m, ventral pair may be narrower.



Gular region darkened on posterior half, FC normally darkened, occasionally pale.



Salivary reservoir about 67 (55.5-86) μ m long and 1.2-4.3 time longer than deep (lower values possibly due to flattening during slide mounting).

Mentum (see c, below) width about half the ventral head length; with somewhat rounded teeth, c2 teeth of center trifid tooth well separated (type III or IB if worn); fourth laterals not reduced (type I).

Ventromental plates (see d, below) about 221 (210-233) μ m wide and 3.73 (3.54-4.09) μ m wider than deep; about 1.22 (1.17-1.29) times the mentum width; separated by about one third of the mentum width, with about 45 (44-47) striae; VMR about 3.2, increasing towards midline.

PE (see a, below) with about 14 (12-16) relatively broad teeth (type B). Premandible (see b, below) with broad teeth, often broken or worn, inner tooth about 4.3 (3-6) times the width of the outer which comes to a fine point (Type B2).

Antenna (see f, below) with basal segment 0.36-0.41 of VHL and about 3.6 times as long as wide; RO just less than halfway up A1; A3 just shorter than A4, and just longer than A5; proportions (μ m): 128 : 33.5 : 10 : 11.5 : 8. AR about 2.03 (1.87-2.12).

Distance between the antennal bases $(154.2 (134-170) \mu m)$ slightly less than the width between S4 setae $(160.3 (135-182) \mu m)$ which is 0.8-0.85 of the FC width at that point; S5 setae about level with nearby RO (see above).

Mandible (see e, below) about 230-250 µm long, with 3rd inner tooth partly darkened, but not well separated (type IIA-B), with about 15.3 (14-20) furrows on the outer surface at the

base; about 13 (11-14) taeniae in the PecM; Mdt-Mat about 21(20-22.5) μm.; MTR about 0.3 (0.25-0.36).



Cytology: 4 polytene chromosomes with thummi arm combination AB, CD, EF, G. Arm G normally paired with subterminal nucleolus and BR about one third from other end. No other nucleoli. Bulb with proximal dark bands near end of arm B. Polymorphic in A, B, D, E & F.

- pbl A1: 1a-e, 4d-a, 13-15, 3e-2d, 9-8, 5-7, 16d-a 1F-2c, 10-12, 3i-f, 17-19 (Wülker)
- pbl A2: 1a-e, 4d, <u>10a-e</u>, <u>2c-1f</u>, <u>16d-a</u>, <u>7-5</u>, <u>8-9</u>, <u>2d-3a</u>, <u>15-13</u>, <u>4a-c</u>. 11-12, <u>3i-f</u>, 17-19
- pbl B1: Puff with proximal dark bands (groups 7 and 8) near distal end of arm.
- pbl B2: Puff with <u>distal</u> dark bands (groups 8 and 7) about 1/3 from end of arm, as B of *blaylocki*
- pbl C1: possibly as C2 of *blaylocki*
- pbl D1:

pbl F2:

- pbl D2: Simple inversion of proximal half of arm.
- pbl E1: 1 3e, 8 5, 9 10b, 4 3f, 10c 13 i.e. as maturus, blaylocki & stigmaterus.
- pbl E2: 1 3e, <u>6 8</u>, 5, 9 10b, 4 3f, 10c 13

1, 9 - 2, 10, 17 - 11, 18 - 23

pbl F1: 1a-f, 9 - 2, 10 - 23

- i.e. as *blaylocki*
- (Wülker)
- pblG1: Nucleolus subterminal as in *C. blaylocki*, but with the largest BR much further away and closer to a smaller distal BR.


Found: New Brunswick – Kouchibouguac River, Kouchibouguac National Park (abt 46.50°N, 65.33°W)
 Ontario – Copanspin Farm, Dunrobin (45.42°N, 75.87°W), Carleton Co.; Bear Creek, Carlsbad Springs (45.37°N, 75.47°W), Carleton Co.
 Wisconsin - Arboretum, Madison, Dane Co. (43.03°N, 89.42°W).

Shallow pools, including in creeks.

This species is very close to *C. blaylocki*. Wolfgang Wülker suggested it was a subspecies, but the larval antennal proportions at least are different (largely a longer A1 in that species). A brief description of the larva and a photograph of the polytene chromosomes are given in Wülker *et al.* (2009).

C. harpi Sublette, 1991 (Species 2z)

This species is in BOLD Bin: BOLD:AAJ4275

The nearest neighbor in BOLD is <u>BOLD:AAG5472</u> an unidentified species mainly from central and western North America.

Adult: Described by J.E. Sublette in Wülker, Sublette & Martin (1991).

Holotype male: Ground color of head and thorax yellowish, antennal pedicels, thoracic vittae, postnotum, sternoplueron, postnotum and abdomen blackish brown. AR 4.32, frontal tubercles 15 μ m; palpal proportions (segs 2-5) 55 : 195 : 234 : 234.

Clypeus with 36 setae. 29 temporal setae.

Wing length 3.20 mm.; VR 0.99; r-m crossvein darkened; 29 setae in squamal fringe in partial double row.

Thorax with small but distinct mesonotal tubercle. Setae: Acrostichal - 17; Dorsocentral - 28; Prealar - 5; Supraalar - 1; and Scutellar - 20.

Leg ratios: PI 1.40; PII 0.56; PIII 0.72; BR 3.26. Sensilla chaetica PII – 9; PIII – 7. Setae on TIX - 5 in single patch; SVo long, narrow and blunt tipped with a small recurved hook, closest to E(g)-type of Strenzke (1959); IVo reaching to about end of anal point and about 2/3 along the GS, which is quite swollen and narrows sharply about posterior third.

Other males: as Holotype but abdominal color seasonally variable with late spring specimens with only a basal dark band and distal yellowish band increasing from 1/3 on TII to 2/3 on TV. Legs also seasonally variable from all dark to F, Ti and Ta1 pale on all legs.

Wing length 3.20 (2.63-3.67) mm.; VR 0.99; r-m crossvein darkened; 26 (23-41) setae in squamal fringe.

Head: AR 3.88 (3.69-4.32); frontal tubercle 15-31 μ m; clypeus 0.75 width of ant. pedicel, with 32 (28-40) setae.

Setae: Acrostichal - 19 (16-20); Dorsocentral - 25 (21-30); Prealar - 5 (4-6); Supraalar - 1; and Scutellar - 29 (23-39).

Leg ratios: PI 1.39 (1.33-1.43); PII 0.59 (0.57-0.62); PIII 0.72 (0.71-0.73); BR 3.36 (2.2-4.67).



From Wülker et al. 1991

Setae on TIX 4 (2-9) in single patch; about 7+1 setae at tip of gonostyle.

Female:

Original description from Allotype female, expanded by additional specimens from Illinois.

Coloration as in males but darker, legs blackish.

Wings: length 3.17-4.17; width 1.02 mm, VR 0.94-0.95; anterior wing veins darkened. 3 Scf on brachiolum, 28-43 setae in squamal fringe.

Head: Frontal tubercles conical, 23-31 μm long. Antennal proportions (micron) (fraction of neck in brackets) 193 (0.29) : 125 (0.31) : 115 (0.33) : 110 (0.28) : 252; AR 0.46; A5/A1 1.36. Palps (micron) 65 : 70 : 211 : 257 : 351; P5/P4 - 1.37; P5/P3 - 1.66.

Clypeus width abt 1.5-1.7 times the diameter of the antennal pedicel with about 21-39 setae.

Thorax with small but distinct mesonotal tubercle; Setae: Acrostichal about 21; humeral 11; dorsocentral 25-27 (dorsocentral plus humeral 37-48); prealar 5-10; supraalar 1-2; scutellar about 16 large in posterior row and 18 smaller in 2 anterior rows (total 24-34).

<u> </u>	<u> </u>	<u> </u>	/	-						
	Fe	Ti	Ta1	Ta2	Ta3	Ta4	Ta5	LR	F/T	Ta4/Ti
PI	1365	1290	1975	935	720	720	345	1.45-1.53	1.04-1.07	0.56
PII	1445	1350	785	430	310	205	175	0.54-0.55	1.04-1.10	
PIII	1525	1635	1125	665	515	300	195	0.68-0.70	0.89-0.98	

Legs lengths (micron) and proportions:

BR 1.79; Sensilla chaetica on Ta1: PII 67; PIII 68.

Genitalia of the allotype female, illustrated in Wülker *et al.* (1991), shows only the labium and ventrolateral lobe.

From other females, segment X is wider than usual for a greater part of the length (see figure below), dorsal end hard to see, about 4.75 times longer than its greatest width with 11-18 setae; the cercus has the dorsal margin slightly shorter than the ventral margin which has a basal bulge, so that the dorsal margin is longer than the somewhat rounded ventral margin.



Cercus (right) and segment X (left) of C. harpi

Pupa: Described by J.E. Sublette in Wülker, Sublette & Martin (1991), but with additional information:

Coloration: Exuvial cephalothorax blackish-brown to yellowish-brown; muscle scars dark yellow-brown; abdominal terga I-V with dark shagreen over each tergum; VI with basal transverse band and 2 apical patches of dark; VII-VIII largely pale, fins dark, with a longitudinal dark stripe that becomes progressively broader on VIII.

Total length abt 7.90 (7.84-7.96) mm in males, and 8.86 (7.82-9.51) mm in females; inner margin of wing case abt 1.77 mm; antennal sheath of females abt 56 μ m. Cephalic tubercles wider than long (63-66 x 94-96) μ m from 1 female; with a seta about 56 mm long), no secondary tubercle or frontal wart. Respiratory base abt 152 x 71 (HR 2.14) with the basal trunk slightly contracted; a patch of about 2-5 lacunae with 2 setae is just anterior to the base and a large rugose patch about 250 μ m long more ventral on each side.

About 64-101 recurved hooks on TII, occupying about 70% of segment width. Pedes spurii B on segment II present, that on III not seen; large pedes spurii A on segment IV, about 253 x 121 μ m and about 0.28 of the segment length; that of segment V about 164 μ m long and of

segment VI about 100 μ m long; L-seta at junction of segments III/IV at least 50 μ m, and that of segments IV/V at least 110 μ m long.

Posterolateral spur of segment VIII with 4 (1-8; 14) spines; swim fin with 82 (62-103; 8) taeniae initially in a single row, then becoming double and triple over posterior quarter of length.

Fourth instar larva a small to medium (male 11.2-12.5 mm; female 12.5-16 mm) plumosusor thummi-type (may be pH dependent?), where present the lateral tubules range from 150-280 μ m (mean 175.6 μ m), with posterior VT usually slightly longer (ant 1.34 (0.90-2.56) mm; post 1.46 (0.90-2.40) mm). Anal tubules relatively long, ventral pair longer (dors. 480, vent. 540 μ m), and 4.8 cf. 5.4 times longer than wide. Gular region slightly dark-dark over posterior third, sloping down so wider than mentum at base; FC pale with occasional slight darkening just outside the edge. Salivary reservoir about 80 (66-96) μ m wide and 2.9-5.1 times wider than deep.

Mentum (Fig. c, below) with relatively pointed teeth; c1 tooth relatively broad with short parallel sides, c2 teeth moderately well separated (type IB-III); 4th lateral reduced part way to level of 5th lateral (type i-II).

Ventromentum (Fig. d, below) about 185-201 μ m wide and about 3.6-3.8 times wider than deep and 1.06-1.11 times wider than the mentum; with about 45-51 striae; VMR about 0.24-0.32; IPD about 27-38% of the mentum width.

PE (Fig, a, below) with about 14 (13-17) teeth of type B, although sometimes somewhat irregular in length. Premandible (Fig. f, below) with inner tooth relatively broad, about 3.7-5.1 times wider, both coming to relatively fine points (Type B1).

Distance between the antennal bases (137 μm) less than that between the S4 setae (147 μm), which are separated by about 83-88% of the FC width; S5 setae slightly anterior to the nearby RO.

Antenna (Fig. b, below) with basal segment just over a third of the VHL, about 2.5-3.2 times longer than wide; RO variable around a third up from base of the segment, AR about 1.58-2.0; segment lengths (micron) 108 : 38 : 6 : 14 : 8.5, i.e. A5 longer than A3.

Mandible (Fig. e, below) length about 218-258 μ m; of type I-IIB, with about 17.3 (15-18) furrows on the outer surface near the base, 14.3 (13-16) taeniae in the PecM; MTR about 0.32 (0.27-0.34).



Cytology: 4 polytene chromosomes with thummi arm combination AB, CD, EF, G.

Arm G usually closely paired with a BR about 1/3 from one end, although specimens from

South Dakota had a nucleolus immediately distal to the BR. Nucleolus is near the centromere in arm D. Polymorphism in arm G near distal end, which is homozygous in South Dakota specimens and instead may be heterozygous for an inversion of about 2/3 of arm G. Polymorphism for arm F also occurs in South Dakota.

- harA1: 1a-e, 8-9, 2d-3, 15-13, 4-7, 3f-i, 12c-10, 2c-1f, 16-19 from *utahensis* by In7-8
- harB1: Puff, with distal dark bands (groups 7 8), at distal end of arm, but lacks proximal BR found in *utahensis*.
- harC1: rearranged c.f. C. utahensis
- harD1
- harE1: 1-3e, 5-7c, 10c-12, 3f-4, 10b-7d, 13 from utahensis by In12-10c
- harF1: 1, 9-4c, 14-13c, 2a-4b, 15-17, 10, 13b-11, 18-23
- harF2: approx. 1, 9-4c, 14-13c, <u>11-13b, 10, 17-15, 4b-2a</u>, 18-23 (South Dakota)
- harG1: BR about 1/3 from one end
- harG2: inversion near distal end
- harG3: inversion of 2/3 arms from harG2



Found: New Brunswick – N. Brunswick Mines and Health Steel Mines (paratypes) (Wülker et al. 1991)

Ontario – Point Pelee National Park (41.959°N, 82.518°W) (NCBI) Quebec – Lake Arnoux (pH 2.7-3.8), Rouynn-Noranda, Quebec City (48.25°W, 79.33°W) (Proulx *et al.* 2013) Arkansas - 40 km sw. Little Rock, Saline Pa. (Type locality) (Bauxite open pit lakes) (paratypes) (plumosus-type) Illinois - Bradley's Acid Pit (37.90°N, 89.53°W), Jackson Co. (paratypes) (thummitype) New York - 2-2.5 km e. Middleport (43.22°N, 78.47°W), Orleans Co. (paratypes) (plumosus-type) South Dakota - 3.5 km w., 5 km s. Lake Andes, Charles Mix Co.

Pools with low pH.

Morphology of all life stages, and cytology described by Wülker, Sublette & Martin (1991). Some ecological data given by Harp and Campbell (1973) as *C. plumosus*; Harp and Hubbard (1972) as *C.* n.sp., and Bates and Stahl (1985) as *C.* nr. *maturus*. The South Dakota population may represent a distinct species, however more samples are required to clarify this. In the meantime it is assumed that this is just geographic differentiation.

Molecular Data:

The Barcode sequence of the mitochondrial COI gene, for 2 larvae (including one collected along with the paratypes from Bradley's Acid Pit), are available on the BOLD database (CotW024-08 and 067-20). Further sequences from Pt. Pelee National Park, Ont., Canada are available in NCBI (incl. KJ165251).

C. mozleyi Wülker 2007 (Species 3u)

Originally C. species Michigan A - Wülker, Devai and Devai (1989).

Adult

The adults of this species are not known for sure, but adults reared from fluviatilis-larvae in the area are similar to those of *C. decorus*-group species (see 'fluviatilis-type male' below).

Pupa - not known.

Fourth instar larva of fluviatilis-type, i.e. ventral tubules scimitar shaped with relatively pointed end. .Length 5.4-15.9 mm (presumably the very small specimens are third or early fourth instar); no TLt; VT longer than width of abdominal segments, posterior pair longer. AT well developed, sometimes with a median constriction. Head capsule of normal width, with gula sometimes slightly darkened at posterior, frontoclypeus pale.

Mentum (below) with central trifid tooth tall, c1 and c2 teeth moderately separated (type III), with the fourth laterals about as high as the fifth laterals (type II); relative height of sixth lateral unclear from figure.

About 30 (29-31) striae on each ventromental plate. Antenna (below) with basal segment 120 (115-127) μ m about 2.5-2.9 times longer than wide (41-47 μ m), RO about a third of the way up from base of the segment; AR 2.1-2.3; A2 length 24 (27-32) μ m., 0.25 of A1 length. Mandible about 300 (285-315) μ m long, with all inner teeth darkened (Type IIIB).



Antenna and mentum of C. mozleyi from Wülker (2007)

Cytology: 4 polytene chromosomes with the thummi arm combination: AB, CD, EF, G. Centromeres not heterochromatic. Arm G with a nucleolus at one end and a constriction near

the other. Distal of the constriction, and also near the middle of the arm are two BRs. No nucleolus in the long chromosomes. Sometimes a puff is developed in arm B. Polymorphic for arms A, B, C, D, E and F.

mozA1:	1a-e, 10-12,	, 3if-, 15a-e,	3e-2d, 9e-a,	7-4, 8g-a,	14-13, 2c-1f, 1	6-19
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- mozA2: 1a-e, 8a-g, 4-7, 9a-e, 2d-3e, 15e-a, 3f-i, 12-10, 14-13, 2c-1f, 16-19
- mozB1: Obvious dark bands at distal end.
- mozB2+3: Differs from B1 by an included inversion, such that dark bands are now proximal, but median section of arm is in same order as in B1
- mozC1: approx. 1-2c, 17a-16, 6b-5c, 8-11c, 15-13, 5b-2d, 7d-6c, 17b-22
- mozC2: approx. 1-2c, 17a-16, <u>abt. 14-15, 11c-8, 5c-6b</u>, abt. 13f-a, 5b-2d, 7d-6c, 17b-22
- mozD1: 1-3, 11-12, 15-13d, 4(?)-8,13c-a(?), 16(?), 9-10, 17-24
- mozD2: 1-3. 11-12, <u>23a-17, 10-9, 16(?), 13a-c(?), 8-4(?), 13d-15</u>, 23b-24
- mozE1: 1-3e, 5-10b, 4-3f, 10c-13 i.e. as *aberratus*, bifE1, etc.
- mozE2: 1-3e, 5a-6, <u>12c-10c</u>, <u>3f-4</u>, <u>10b-7</u>, 12d-13
- mozF1: 1a-i, 9-5d, 16-17, 10a-d, 13b-11, 13cd, 18-19b, 2-5c, 15-14, 19c-23
- mozF2: 1a-i, 9-5d, 16-17, 10a-d, 13b-12, <u>19dc, 14-15, 5c-2, 19b-18, 13dc, 11a-i</u>, 20-23



Slightly modified from Wülker (2007)

Found: Michigan - Lake Michigan (Type locality).

From depths of 12-15 m in fine sand sediments

Larva is most easily distinguished from the co-habiting *C. winnelli* by the wider head capsule, the mentum type, and the higher number of VM striae. This species is a member of the *C. decorus*-group. Sequences for arms A, E and F given in Wülker, Devai and Devai (1989), as *C.* species Michigan A.

Description given by Wülker (2007). Holotype in Zoologische Staats Sammlung, Munich.

Adult males reared from fluviatilis-type larvae from same locality as *C. mozleyi* and *C. winnelli.* (from Wülker, 2007).

These adults were reared from unsorted larvae so it is uncertain whether they were *C*. *winnelli* or *C*. *mozleyi*.

Color faded in Euparal specimens, but TII-IV with strong longitudinal bands and weaker transverse bands, similar to other broader decorus-group species. (note Wülker refers in the paper to *C. utahensis*, but this is a member of the cytologically defined decorus-group, not the morphologically defined group); foreleg without a beard.

AR 2.95 (2.73-3.33)

Wing length 2.92 (2.80-3.10) mm.

Thoracic setae: acrostichals 91-92; dorsocentrals 28; prealars 6; scutellar 25.

Legs: Fore leg – LR 1.52 (1.43-1.60); BR 1.81 (1.45-2.20).

8 (4-14) setae in a single patch on TIX. SVo closest to E(i) type of Strenzke (1959); IVo, apparently with simple setae, probably longer than the broad anal point and about 1/3 of the length of the GS which is moderately swollen and narrows relatively gently over distal third.



Male hypopygium, from Wülker (2007)

C. winnelli Wülker, 2007 (Species 3v)

Originally C. species Michigan B - Wülker, Devai and Devai (1989)

Adult

The adults of this species are not known for sure, but adults reared from fluviatilis-larvae in the area are similar to those of *C. decorus*-group species (see 'fluviatilis-type male' above). Since the larvae of this species were more frequent in the samples, it is possible that this adult is that of *C. winnelli*.

Pupa - not known.

Fourth instar larva of fluviatilis-type, i.e. ventral tubules scimitar shaped with relatively pointed ends (posterior pair longer), with no TLt.



Posterior end of holotype larva of *C. winnelli*, from Wülker (2007)

Length 5.2–12.5 mm (presumably the very small specimens are third or early fourth instar). VT (above) longer than width of abdomen, posterior pair shown as longer. AT well developed, figured (above) as at least 4 times longer than wide, sometimes constricted at midpoint, ventral pair longer. Head capsule unusually narrow (width 0.39-0.49 mm), gula sometimes darkened distally, or may be pale; FC pale.

From published figure (below), fourth laterals of mentum appear to be only slightly reduced (type I-ii), and center trifid tooth type IIA; it appears the 6th laterals arise at the same level as other laterals.

About 24 (23–25) striae on each ventromental plate.

Antenna (below) with basal antennal segment 101 (94 -121) μ m; about 2.7-3.5 times longer than wide (30–39 μ m), RO just over a quarter way up from base of the segment; AR 2.1-2.3; A2 length 21–30 μ m, about 0.31 of A1.

Mandible with all inner teeth darkened (type IIIB).



Antenna and mentum of C. winnelli, from Wülker 2007.

Cytology: 4 polytene chromosomes with the thummi arm combination: AB, CD, EF, G. Centromeres not heterochromatic. Arm G with terminal nucleolus and BR near middle of the arm. No nucleoli in the other chromosomes. Sometimes a puff developed in arm B and C. No polymorphism recorded.

win A1: 1a-e, 9a-e, 2d-3e, 17-16, 1f-2c, 13-14, 15e-a, 3f-i, 12-10, 4-7, 8g-a, 17e-19

- win B1: Puff with dark bands (groups 7-8) near middle of the arm, with typical groups 24-28 just proximal to them.
- win C1: typical group 2d-5b near middle of the arm, a puff developed near the centromere
- win D1: 1-3, 11-12, 15-13d, 4(?)-8,13c-a(?), 16(?), 9-10, 17-24 i.e. as mozleyi D1
- win E1: 1-2b, 11b-10c, 3f-4, 10b-5, 3e-2c, 11c-13
- win F1: 1a-1, 9-5d, 14-17, 10a-d, 13b-11, 13cd, 18-19b, 2-5c, 19c-23
- winG1: terminal nucleolus, median BR



Slightly modified from Wülker (2007)

Found: Michigan - Lake Michigan (Type locality).

From depths of 6-12 m in fine sand sediments

Larva is most easily distinguished from the co-habiting *C. mozleyi* by the narrower head capsule, the mentum type, and the lower number of VM striae.

This species is a member of the *C. decorus*-group. Sequences for arms A, E and F given in Wülker, Devai and Devai (1989), as *C.* species Michigan B. Description given by Wülker (2007). Holotype in Zoologische Staats Sammlung, Munich.

C. decorus group species c. (Species c)

Adult:

There should be a reared adult male in the Canadian National Insect Collection (Carlingwood, Carleton Co., Ontario, CO.22.2, egg mass #1, reared male 1).

Male: The adult male is a typical member of the *C. decorus*-group.



Male terminalia of *C*. species c. A typical *C*. *decorus*-type hypopygium

About 13 setae in multiple associated pale patches on TIX, SVo closest to E(g)-type of Strenzke (1959) i.e. longer than most decorus-group species. Anal point relatively broad with approximately parallel sides. IVo slightly longer than the anal point and to about the mid-point of the gonostylus which narrows markedly over the distal third.

Pupa: The pupa (a male exuvia and the spur from a prepupa)) has small frontal warts (see figure below) on the frontal apotome but smaller than those found in *C. decorus* itself; cephalic tubercles about 1.9 times longer than width at base. Caudolateral spur on segment VIII has about 4-5 closely appressed spines on a long base. About 65 taeniae on anal fringe.



Fourth instar larva of the semireductus-type with moderately long tubules (abt 1.2 times segment width), on average of equal length (anterior 0.99 (0.71-2.28) mm; posterior 0.94 (0.62-1.84 mm). Larval length, females 12.59 (9.0-14.1); males 11.9 (9.5-12.3) mm. TLt relatively short, 154.8 (31-250) micron. AT about 3-6.6 times longer than wide, dorsal pair usually slightly longer (mean 448.5 cf. 397.8) and with a constriction about a third from the base. Salivary reservoir (background of Fig. a, below) about 68.3 (61-76) µm wide and 4.3

times longer than wide. ASA (155 (129-175) μ m) generally slightly less than S4A (157.3 (129-177) μ m). Gular region darkened over most of central region, wider than the mentum and widest about a third up from Po, FC pale.



Mentum (Fig. c) with pointed teeth, c2 teeth only partly separated from the square sided c1 tooth (type IB or sometimes approaching type III); 4th laterals reduced part way or about to level of 5th laterals (type I-II); width about 0.55-0.69 of VHL.

VM (Fig. d) about 209.2 (197-212.5) μ m wide and 3.26-3.82 times wider than deep and 1.06-1.08 times the mentum width; with about 36.2 (30-43) striae; IPD abt. 58-69.5 μ m (0.29-0.38 of mentum width); VMR about 0.23-0.29. PE (Fig. a) with 13.7 (11-16) sharp pointed teeth (type A).

Premandible with teeth about equally long, coming to narrow points, outer tooth about 2.5-4.2 times wider than inner tooth.

Distance between the antennal bases on average (155 (129-175) μ m), about the same as that between the S4 setae (157.3(129-177) μ m).

Antenna (b) with basal segment 0.36-0.39 of VHL; AR abt. 2.14 (1.92-2.39); basal segment about 2.9-3.5 times longer than wide; A4/A3 about 1.85 (1.5-2.5), i.e. A3 relatively short (see fig. b); relative lengths antennal segments (micron) 117 : 27 : 8 : 12 : 7.

Mandible (e, below) with the third inner tooth slightly colored and not separated (type IA or B) and about 16.6 (14-20) furrows on outer surface near base; PMa with about 11.7 (11-13) taeniae; Mdt-Mat about 35.7 (30-39) μ m, MTR 0.45 (0.43-0.47).

Cytology: 4 polytene chromosomes with thummi arm combination AB, CD, EF, G, these arm combinations difficult to identify.

Arm G normally paired with the nucleolus clearly subterminal, BR present but often difficult to see. No other nucleoli? Band groups 22–24 of arm B in normal position close to the centromere. Quite polymorphic, including in arms A, D and F, and sometimes with complex inversions.



Arm E1: 1 - 3a, 5 - 10b, 11b - 10c, 3f - 4, 11c - 13The inv4-11b in arm E suggests a connection to *C. quinnitukqut*.

Farm pools and pools in rivers.

Found: Ontario - Dunrobin (45.45°N, 76.00°W), Carlingwood, Ottawa (45.37°N, 76.00°W); Rideau River, and Black Rapids (45.32°N, 75.82°W), all Carleton Co.; Vittoria, Norfolk Co.
Florida – Lake Ruby, Winter Haven.
Vermont - White River, Sharon (43.80°N, 72.45°W), Windsor Co.

C. decorus group species j (Species j)

Reared specimens are in the J. E. Sublette collection in the Museum of the University of Minnesota, St. Paul, MN. Specimens labelled Q.26.1 reared males 1 and 2 should be in the Canadian National Insect Collection.

Adult male:

Only a photograph of the terminalia is available.



Male terminalia of C. sp. j.

About 9 setae in a central pale patch on TIX, probably in separate patches. A typical *C. decorus*-type hypopygium: anal point approximately parallel sided, SVo similar to that of *C. bifurcatus* and *C. maturus* (D-type of Strenzke, 1959). IVo appears slightly curved, extending beyond the end of the anal point to about midpoint of GS which narrows sharply from half to two thirds along its length.

Female: No information available.

Pupa: postero-lateral spur on segment VIII with about 4 spines.



Fourth instar larva a medium size (fem. 12.9-14.1; male 12.1-13.6) thummi-type, with anterior VT longer (ant. 1.68 (1.30-1.99); post. 1.56 (0.93-1.91)). Lateral tubules absent. AT about 455-530 μ m long and 3-3.5 times longer than wide, with a slight constriction in the middle, ventral pair thinner than the dorsal pair (hence with higher length to width ratio). Dark posterior half to 2/3 of gular and extending virtually whole lateral width, with a slightly

convex anterior margin and usually widest anterior to the posterior margin of the head capsule; FC pale or with darkened edges.

Salivary aperture about 74.4 (63-84) µm long and 3.3-4.7 times longer than wide. Mentum (Fig. d, below) with rounded teeth, probably due to wear; 4th laterals reduced almost to height of 5th laterals (type II); c1 tooth high and fairly narrow, c2 teeth moderately separated (type III, but may appear to be type IB (as Fig. d, below) or IIA due to wear).



Ventromental plates (Fig. b, above) 210.4 (195-229) μ m wide and 3.2-3.6 times wider than deep, 1.09-1.18 times the mentum width; with about 45.5 (39-48) striae; IPD about 0.26-0.4 of mentum width; VMR 0.22-0.36. Pecten epipharyngis with about 9-13 relatively broad teeth.

Premandible (Fig. c, above) with inner tooth about 3.3-4.8 times the width of the outer tooth, which narrows markedly along its length. PE (Fig. a, above) with 9.6 (8-16) teeth, of type B when not worn.

Antenna (Fig. b, above) with A1 relatively short, only about 0.3-0.4 of the VHL, about 2.7-3.5 times longer than wide, RO about 0.3-0.4 up from base; AR about 1.89 (1.63-2.24); A4 about 20-50% longer than A3, which is usually longer than A5; proportions (μ m) 112.5 : 30 : 9 : 12 : 7.

Distance between the antennal bases (mean 158.8 μ m) about the same as that between the S4 setae (156.9), i.e. both are equally likely to have a higher value. S4 setae separated by 83.3 (76-88)% of the FC width. S5 setae usually about level with the nearby RO, and equally likely to be anterior as to be posterior to it.

Mandible (Fig. e, above) with 3rd inner tooth slightly colored and partly separated (type IIB); about 18.0 (14-22) furrows on the outer surface at the base, 12.1 (10-13) taeniae in PecM, Mdt-Mat 27.4 (20-33), MTR 0.33 (0.24-0.39).

Cytology: 4 polytene chromosomes with thummi arm combination AB, CD, EF, G. Arm G largely unpaired, although may be paired in the center half. Nucleolus subterminal to an apparently heterochromatic centromere, BR subterminal at the other end. No nucleolus on other arms but large distal bulb (gp. 7) in arm B. Polymorphism in arms A, B and D.

Arm A1:

- Arm A2: Simple inversion near the centromere.
- Arm B1: Group 7 distal.
- Arm B2: Simple inversion of about 1/3 of arm just distal to middle of arm.
- Arm C1:
- Arm D1:
- Arm D2: Could be a complex inversion.
- Arm E1: possibly 1 3e, 9 10b, 4 3f, 10c 11a, 5 8, 11b 13
- Arm F1:
- Arm G1: Subterminal nucleolus.



Arm G and part of heterozygous arm A of C. sp. j

Found: New Brunswick - Kouchibouguac National Park (46.858°N, 64.975°W)
 Ontario - Algonquin Provincial Park (45.58°N, 75.70°N), Nipissing Co.; Bear Creek, Carlsbad Springs (45.37°N, 75.47°W), Carleton Co.
 Quebec - Notch Road, Gatineaus (45.92°N, 75.60°W)

Slow moving creeks and pools.

<u>C. species 2a</u>. Possibly C. decorus group (Species 2a)

Only two larval specimens of this species are known for certain, and only one was available for detailed morphological examination, and a further specimen represented by the polytene chromosome squash.

Adult and Pupa not known.

Fourth instar larva a small to medium (abt. 11.5 mm) plumosus-type, VT of moderate length. AT relatively long, ventral pair longer (ant. 506; post. 582 μ m) 5 times (ant) and 5.1 (post) times longer than wide.

Gular region dark over posterior half, slightly wider than the mentum, and a slightly darkened FC. $76 \,\mu\text{m}$ long and 3.75 times longer than deep.

Mentum (Fig. c, below): width about 0.58 of VHL; c1 tooth fairly narrow with short almost parallel sides, c2 teeth fairly well separated (Type III); 4th laterals reduced about to level of 5th laterals (type II) and 6th lateral arising lower than the other lateral teeth.

Ventromental plates (Fig. d, below) about 210 μ m wide and 3.46 times wider than deep, 1.17 times the mentum width; IPD about 0.28 of the width of the mentum; with about 47 striae; VMR about 0.33. PE (Fig. a, below) with about 11-12 relatively broad teeth (type B). Premandible teeth broken, but inner tooth about 3.3 times the width of the outer tooth.

Antenna (Fig. b, below) with basal segment only about 35% of the VHL and about 3.1 times longer than wide, RO about 0.4 up from base of A1; AR abt 1.52; antennal proportions (μ m) 109 : 39 : 12 : 12.5 : 8; i.e. A4 barely longer than A3.

Distance between the antennal bases about the same as the distance between the S4 setae, which are separated by about 88% of the width of the frontoclypeus; S5 setae about level with the nearby RO.

Mandible (Fig. f, below) about 235 μ m long, with 3rd inner tooth not completely separated and slightly darkened (type IIB); 14-16 furrows on outer surface at the base; about 12-13 taeniae in the PMa; Mdt-Mat 28 μ m, MTR 0.34.



Cytology: 4 polytene chromosomes with the thummi arm combination AB, CD, EF, G. Arm G usually paired, nucleolus subterminal with a BR nearby and a further BR about one third from other end. Small nucleolus (probably usual bulb or BR of group 7) about center of arm B. Centromeres apparent but not heterochromatic. Polymorphic in A.

- Arm A: Inversion covers over half the arm to within about 10 bands of the distal end.
- Arm B: Bulb with proximal dark bands (groups 7 & 8) near middle of the arm.
 - Characteristic bands (21-28) near centromere.
- Arm C: Characteristic constriction about a third from the centromere.
- Arm D:
- Arm E: ???, 4 3f, 10c 13
- Arm F: 1a-i, ???, 17 23.
- Arm G: Subterminal nucleolus with nearby large BR, a smaller BR followed by a constriction about a third from the other end.



Found: Ontario - Bear Creek, Carlsbad Springs, Carleton Co. (45.37°N, 75.47°W).

Pools in creek.

This species is recognized on the basis of the polytene chromosomes, with a relatively short, closely paired arm G which has a subterminal nucleolus closely followed by a large BR, then a second large BR followed by a constriction.

C. decorus-group species 3h (Species 3h)

Adult:

Male: from a specimen in the Canadian National Insect Collection Wing length abt. 3.3 mm. Fore LR about 1.50. Frontal tubercles large. Abdominal coloration as in *C. maturus*, but paler. Superior volsella very slightly darker than the rest of the terminalia. Anal point broad.

Pupa: Not known.

Fourth instar larva a medium sized 15.08 (13.7-16.3) mm plumosus-type; TLt about 240-360 μ m. VT moderately long and about equal length (ant. 2.02 (1.34-2.56) mm; post. 2.02 (1.98-2.44) mm). AT about the same size, 2-2.5 times longer than wide (len. 329-481 μ m), without a constriction. Gular region dark on posterior half, wider than the mentum, often with a

convex anterior margin and widest point anterior to the posterior margin; FC usually pale, but may be slightly darkened at posterior end.

Salivary reservoir 66-98.5 μ m wide and 3.1-3.7 times wider than deep.



Mentum (Fig. c, above) with somewhat rounded teeth; c1 tooth relatively narrow, top sometimes rounded; c2 teeth slightly separated (probably type IB if not worn, some may be type III); 4th laterals generally only slightly reduced (type I, or occasionally approaching type II).

Ventromental plates (Fig. d & c, above) about 230 (215-242) µm wide and 3.5-3.8 times wider than deep, 1.06-1.15 times wider than the mentum; IPD about 0.31-0.34; about 44.6 (42-46) striae reaching about half way to anterior margin; VMR about 0.21-0.38.

PE (Fig. a, above) with about 14.3 (12–18) relatively broad (type B) teeth. Premandible with inner tooth slightly longer and about 3.7-4.8 times the width of the outer tooth, which narrows markedly to the tip.

Antenna (Fig. b, above) with A1 about a third to 2/5th of the VHL; RO between a third and half way up A1 which is 2.95-4.3 times longer than wide; AR about 2.05 (1.90-2.31); relative length of segments (μ m) 129 : 32 : 9 : 14 : 7.

Distance between the antennal bases usually greater than the distance between the S4 setae, although the means are quite similar (173.6 c.f. 171.38), setae separated by about 81-85% of the FC width; S5 setae slightly posterior to, or level, with the nearby RO.

Mandible (Fig. e, above) with third inner tooth usually moderately separated but pale (type IIB), but degree of separation variable; PMa with about 12.6 (11-14) taeniae; about 16.0 (14-17) furrows on outer surface at the base; Mdt-Mat about 31.6 (30-35) μ m; MTR about 0.35 (0.32-0.41).

Cytology: 4 polytene chromosomes with the thummi arm combination AB, CD, EF, G. Arm G relatively short and thick with a sub-terminal nucleolus, probably in terminal heterochromatic cap, and a BR near the center, followed by a definite constriction and dark bands; commonly only paired at the nucleolus. Arm B may have a nucleolus just near the center of the arm, also with a bulb (group 7?) near the end of the arm with some dark bands proximal. No polymorphism in available specimens.

A1: possibly 1 - 2c, 10 - 12, 2d - 3, 9 - 4. 13 - 19

B1: ??, 7-8, ???, 22-27.

C1: Constriction near distal end of arm.

D1:

E1: probably 1 - 3e, 8 - 5, 9 - 10b, 4 - 3f, 10c - 13 i.e. as *maturus, blaylocki*, etc.

F1: 1 - 6, ?? , 11 - 23



Polytene chromosome complement of C. sp. 3h. One homolog of arm G is broken.

Found: New Brunswick - Kouchibouguac National Park (48.8583°N; 64.9750°W). Ontario - ¿South March nr. Mud Lake (44.88°N, 78.27°W), Carleton Co.

Pools and rivers.

Another species recognized on the basis of the polytene chromosomes, where arm G is short with a virtually terminal nucleolus followed by a constriction following which it is likely to be unpaired. Appears to show relationship to C. sp. u.

C. decorus-group species 3i (Species 3i) Only 3 larvae are known for certain.

Adult:

Male

This specimen not necessarily associated as other species were also present.



Male terminalia of C. ?sp. 3i (left) and superior appendage (right)

About 6 setae on TIX, possibly in separate pale areas. Anal point relatively broad. SVo of E-type (g) of Strenzke (1959) - i.e. as *C. cingulatus*; IVo about as long as the anal point and to about 1/3 of the gonostylus, which narrows relatively sharply over distal third.

Female: Not known.

Pupa: Not known

Fourth instar larva a medium sized plumosus-type (about 10.1-15.3 mm); the larva from Sudbury was the smallest, but it was also parasitized; VT relatively long, posterior pair longer (ant. 1.20-2.64, post. 1.30-2.86 mm). Gular region slightly dark to dark on posterior1/3-1/2, slightly wider than the mentum and widest at the posterior margin; FC pale. Salivary reservoir 63-83 µm wide and 3.3-4.2 times wider than deep.

Mentum (Fig. c, below) with somewhat rounded teeth, probably due to wear, c2 teeth of center trifid tooth not well separated (type IA-IIB, and III at Sudbury); 4th laterals reduced (type II-III).

Ventromentum (Fig. d, below) about 171-187 μ m wide, 3-3.4 times wider than deep, and 1.02-1.04 times the width of the mentum; with 36-43 striae; VMR 0.29-0.32. PE (Fig. a, below) with about 11-12 sharp but broad teeth (type B).

Premandible with inner tooth relatively narrow, 3.4-4.5 times wider than the outer tooth, both teeth narrowing to a point.

Antenna (Fig. b, below) with A1 relatively short, only about 0.33-0.35 of VHL; about 2.92 (2.71-3.19) times longer than wide, RO about a quarter to half way up from base; AR about

1.9 (1.75-2.12); antennal proportions (micron) about 103 : 28 : 7 : 11.5 : 7 ; i.e. A5 is only slightly shorter or longer than segment 3.

Distance between the antennal bases (144 μ m) on average the same as that between the S4 setae (144 μ m), which occupy about 81-91% of FC width, but may be greater or smaller. S5 setae either level with or slightly posterior to nearby RO.

Mandible (Fig. e, below) 207-243 μ m long, with third inner tooth at most partly separated and partly colored (type I-IIB); 16.5 (15-18) furrows on the outer surface at the base; 13.2 (10-16) taeniae in PecM; Mdt-Mat about 20-30 μ m when not badly worn; MTR 0.28-0.43.



Cytology: 4 polytene chromosomes with thummi arm combination AB, CD, EF, G. Centromeres obvious but not markedly heterochromatic.

Arm A with groups 4-6 approximately as in pigA1 (could be groups 1-6 at that end); arm B with bulb (gp 7?) in proximal third, but apparently separated from the dark bands of group 8. Distinguishing bands of arm C about the middle of the arm. Arm G closely paired with 2 BRs near middle of the arm. Nucleolus in arm D near centromere.

Arm E: 1 - 3e, ?? 7 - 6, 9 - 8, 12 - 13 Arm F: ?? 13 - 11, 16 - 23



Found: Ontario - 4 m E Sudbury (46.52°N, 80.90°W). Pennsylvania - vicinity Pittsburgh (40.43°N, 79.97°W), Allegheny Co.

Pools or small streams.

This species was not found in the Proulx *et al.* (2013) samples from the Sudbury area, possibly because these were samples from lakes.

Shows some similarities to *C. stigmaterus*, which might indicate that the male (above) belongs to that species (although the species has not been recorded from Canada). The larva can be distinguished by the normally darkened FC; longer A1 (3-3.94 longer than wide) and the more fully developed 3rd inner tooth of the mandible of *C. stigmaterus*. It also shares the only nucleolus being near the centromere of arm D, but other sequences, particularly arm A are different. The only other known Nearctic species with a nucleolus in arm D is *C. utahensis*, which is considered a member of the cytological *C. decorus* group, but not morphologically similar (Wülker, Sublette & Martin, 1991).

C. decorus-group (?) species 4l (Species 4l).

Known from three larval specimens with polytene chromosomes.

Adult and Pupa not known.

Fourth instar larva: A medium sized melanotus-type larva (length (2) 12.7-13.5 mm), VT well developed and about equal in length (ant. 1.24-1.56; post 1.32-1.52.); TLt about 140-160 μ m. Anal tubules 2-4 times as long as wide probably because the dorsal pair are longer (abt. 470 μ m) while the ventral pair are shorter (abt. 320 μ m). Gular region dark over posterior

1/2 to 2/3, wider than the mentum and with the widest point anterior to the posterior margin of the head capsule; FC pale. Salivary reservoir about $68-81\mu$ m wide and 3.9-4 times wider than deep.

Mentum (Fig. a, below) with 4th laterals reduced to about the level of 5th lateral (type II); center trifid tooth either type IB or III.

VM (Fig. b, below) about 202-210 μ m wide, 3.15-3.61 times wider than deep; 1.07-1.23 times the mentum width; with about 31-34 striae; VMR abt. 0.25 (0.24-0.26). PE (Fig. c, below) with about 14-16 mostly even sized, sharp teeth, but distal teeth smaller.

Premandible with inner tooth slightly longer and about 3.3-4 times the width of the outer tooth, both teeth narrowing markedly to a sharp tip.

Antenna with basal segment relatively long and narrow, 0.4 of VHL; about 3.3-4.3 times as long as wide; RO about 30-40% up from base; AR about 2.24-2.53; A2/A1 about 0.19-0.22; A4/A3 about 1.5-2.4; segment lengths (micron) about 105 : 22.5 : 8 : 10 : 6.

Distance between the antennal bases probably about the same as that between the S4 setae, which are separated by about 77-86% of the frontoclypeus width.

Mandible (Fig. d, below) about 240-250 μ m long, with 3rd inner tooth pale and almost completely fused (type IA); 18-19 furrows on the outer surface near the base; 10-11 taeniae in the PecM; Mdt-Mat about 30 μ m and MTR 0.33-0.36.



Cytology: 4 polytene chromosomes with thummi-cytocomplex arm combination, AB, CD, EF, G.

Arm G often largely unpaired and with a virtually terminal nucleolus and a BR separated by a further 7-10 bands. Polymorphism at least in arm B near the centromere and arm E. Bulb (seg7) subterminal in arm B with some dark bands immediately proximal.



Found: Kansas - Mill Creek (38.95°N, 94.80°W), nr. Craig, Johnson Co.. Louisiana - Bar ditch, Many, Sabine Parish.

C. decorus-group (?) species 5u (Species 5u).

An adult and pupal exuvia reared from a larva should be in the Canadian National Insect Collection. It shows many features common to species of the *C. decorus* group but differs in some characters.

Adult: This adult male was probably measured by J. E. Sublette and the details may be in his laboratory books of *Chironomus* species.

Male: Only photographs of the male hypopygium are available. These do not show the setae of TIX. The most obvious difference from other known species of the decorusgroup is the gonostylus which is moderately swollen but narrows only a little over the posterior third, while other species narrow quite markedly. The SVo is moderately darkened and of D-type of Strenzke (1959), similar to that of *C. bifurcatus*. The anal point is quite broad and blunt-ended, much longer than the IVo which barely reach the mid-point of the gonostylus.



Female: Not known.

Pupa: The cephalic tubercles are short and broad, barely longer than the width at the base, like those of *C. decorus* (see figure of that species, above), but frontal warts are absent. The spur of segment VIII has about 3 closely appressed spines.



Pupa of species 5u: Cephalic tubercles (above), note absence of frontal warts; Spur of segment VIII (below)

Larva: not known but may be those of Species z, which were collected at the same place at the same time (see Part 2).

Found: Ontario - Bear Creek (45.37°N, -75.47°W), Carlsbad Springs, Carleton Co.

The male hypopygium is closest to those of males raised from Lake Michigan and assumed to be associated with *C. mozeleyi* or *C. winnelli*. However the larvae of this species are from amongst weeds at the side of a shallow creek.

C. utahensis Malloch, 1915 (Species 2p)

Tendipes (Tendipes) utahensis, Townes 1945: 127.

Adult redescribed by Townes (1945) and by Sublette in Wülker, Sublette and Martin (1991). Male: Wing length 3.77 (3.10-4.53), LR 1.18 (1.07-1.27), fore tarsi with a long dense beard. AR 4.26 (3.71-4.70).

Palpal proportions (micron): 55-70 : 62-70 : 211-265 : 195-245 : 179-350; P5/P4 1.10; P5/P3 1.02.

Frontal tubercles 40-50 μ m long, up to twice as long as wide, although they may be only 12.5 μ m long and only 0.8 as long as wide. Clypeus 0.87 (0.68-1.00) of width of antennal pedicel, but the larger values may be due to squashing in slide preparation, about 43 setae.

Thorax yellowish-brown to dark brown, with vittae, postnotum blackish brown. Thorax: mesonotal tubercle distinct; Setae -15 (12-20) acrostichals; 36.5 (26-46) dorsocentrals in two to partially 3 rows; 7-13 prealars; 1-2 supra-alars; 40 (32-77) scutellars, with 2 rows heavy setae posteriorly and several strewn rows anteriorly. Wing width 1.01 mm; with 3 Scf on brachiolum; 24 (20-41) setae in squamal fringe, anterior veins darkened; VR 0.99.

Legs with femora and tibiae yellowish brown; tarsi largely blackish; basal third of TA1 on PII and PIII, slightly paler; fore tarsus heavily bearded.

	Fe	Ti	Ta1	Ta2	Ta3	Ta4	Ta5	LR	F/T	BR
PI	1430	1480	1670	1035	635	480	270	1.07-1.27	0.97	5.2-7.75
PII	1555	1600	860	580	405	265	190	0.55-0.62	1.01	
PIII	1845	2000	1365	885	580	345	205	0.68-0.72	0.92	
PIII	1845	2000	1365	885	580	345	205	0.68-0.72	0.92	

Leg lengths (micron) and proportions:

Sensilla chaetica: PII 9 (6-10); PIII 11 (6-13).

Abdomen largely blackish-brown, terga II-VIII with a slightly paler brown, narrow apical fascia; genitalia dark.



Male hypopygium from Wülker et al. (1991)



Variation of SVo between California (left) and New Mexico (right)

Tergite IX with 7 (0-19) setae, usually in one paler patch, occasionally in two smaller patches, or lacking. Anal point short and moderately broad at the base, not strongly downcurved. There appear to be some differences in other structures between populations, particularly the SVo. The SVo is usually slightly curved (D(e) type of Strenzke 1969) (as left, above), while that of males from Upper Abbott Lake, New Mexico (right, above) is much straighter and turned slightly out at the end (closest to D(f) type of Strenzke (1969)(possibly as the illustration in Townes (1945), although that figure is not particularly clear) reaching only to end of anal point and base of the gonostylus. IVo quite broad, moderately capitate in side view, with simple setae and reaching to 1/3 to 1/2 way along the GS, which is abruptly tapered over distal third.

Female: Color similar to male, but thorax more yellowish, with vittae more distinct. Wing length 3.89-4.53 mm; squama with 33-41 marginal setae.; LR 1.07-1.21. Head – antennal proportions (μ m): 179 : 133 : 133 : 125 : 211; AR = 0.37; A5/A1 about 1.18.

Palpal proportions (Segs 2-5)(in μ m): 62 : 195 : 195 : 250; P5/P4 & P5/P3 1.28. Clypeal base 1.73 times the width of antennal pedicel, with 74-88 setae. Thorax with distinct mesonotal tubercle. Setae – Acrostichal abt 17-18; dorsocentral 45-48 in two to three rows at widest (number of Humeral unknown); prealar 17-18; scutellar 75-77.

Femora of all legs brownish, only apices blackish; anterior tibia and tarsi black; Mid and hind tibiae brownish, with only a narrow apical and basal darkening. Mid LR 0.51-0.54; Hind LR 0.66-0.67. 111-123 sensilla chaetica on mid leg, 114-120 on hind leg.

Pupa: Length 8.22-9.67 mm. Cephalothorax brownish-black, papillose; abdomen pale, very weakly dark longitudinal stripes laterally; posterolateral spur and margin of anal lobe dark. Cephalic tubercles relatively long and curved. Hooks of tergum II pale, mean 90 (82-91); postero-lateral spur with 6 (4-8) appressed spines; fringe of anal lobe with 94 (88-100) flattened taeniae.



Fourth instar larva a medium sized bathophilus- or melanotus-type (i.e. some larvae have TLt up to 120 μ m long), length 13-15.8 mm (fem); 10.6-13.7 (male); VT about equal length, although anterior generally slightly longer (ant. 1.40-2.68; post. 1.20-2.64). TLt and VT lengths vary between populations: e.g. both are relatively longer at Klamath Falls than in California, and in California only 3 of 9 larvae had TLt up to 40 μ m, while all larvae from Klamath Falls possessed TLt, varying from 40-200 (ave. 95) μ m. AT a simple lobe, with some variability in length between populations; dorsal pair generally longer and varying from about 2-3.5 times longer than wide in California and South Dakota to 4-4.2 times longer than wide in Oregon.

Dark to very dark posterior 2/3 of gular region with V-shaped anterior margin, wider than the mentum width with the widest point anterior to the posterior margin of the head; pale FC or slightly darkened (in California); and antennal pedicels characteristically darkened. Wülker et al. (1991) give the ventral head length as 186-198 μ m but this appears incorrect as in other measured larvae it is over 325-368 μ m. Salivary reservoir relatively small, about 58-71 μ m wide and 3.3-3.5 times wider than deep.

Mentum with somewhat rounded teeth; c1 tooth relatively narrow with short parallel sides, c2 teeth well separated (type III but can appear as IB if worn); 4th laterals reduced at least to the level of the 5th laterals (type II-III).

Ventromental plates about 220-228 μ m wide 3.3-4.1 times wider than deep; with about 38 (34-44) striae reaching about 2/3 to the anterior margin; VMR about 0.32 (0.27-0.38). PE with 11-15 relatively uniform broader teeth (type B). IPD about 0.38-0.45 of mentum width. Premandible with inner tooth relatively broad, about 3.2 (2.5-3.8) times wider than the slightly longer outer tooth, which narrows sharply along its length to a point (type C).

Basal segment of antenna about 30-40% of VHL and 2.8-3.7 times longer than wide, RO between 1/3 and $\frac{1}{2}$ way up from base of segment; AR about 2.3 (1.8-2.6); A2/A1 about 0.21-0.28; segment lengths (micron) 137.5 : 32 : 7.5 : 12.5 : 7; i.e. A3 only slightly longer than A5.

Distance between the antennal bases (170 (149-192) μ m) and that between S4 setae (172 (154-187) μ m) generally similar, but can vary in either direction; S4 setae separated by about 81-86% of FC width.

Mandible with 3rd inner tooth generally partly separated, but sometimes well separated (as fig. f below); and relatively pale (type II-IIIB); about 16 (11-20) furrows on outer surface at the base; PMa with 12.5 (11-14) taeniae; Mdt-Mat 28-33, MTR about 0.28-0.43.



Cytology: 4 polytene chromosomes with the thummi arm combination AB, CD, EF, G. Centromeres partly heterochromatic, particularly for CD chromosome. Arm G closely paired, without a nucleolus, but with a BR towards one end, the position varying due to an inversion. The end near the BR is almost square due to a constriction, while the other end is fanned. Nucleolus in arm D. Polymorphic in arms A, D, E, F and G. The basic sequences (A1-G1) appear common to all populations, but the type and frequency of polymorphisms is variable and appears limited to particular areas.

$u_{111111111111111111111111111111111111$

- utaA2: approximately 1a-e, 7 4, 13 15, 3e-b, <u>9a-g, 2d 3a</u>, 8?-a, 3f-i, 12 10, 2c 1f, 16 19. (Oreg)
- utaB1: Puff with distal dark bands (groups 8-7) at distal end of arm, smaller BR in proximal third of arm.
- utaC1: 1-6b, 12b-15, 18d-17b, 6c-f, 7a-d, 16-17a, 6hg, 11d-12a, 11c-8, 18e-22
- utaC2: approximately 1, <u>14-12b, 6b-2</u>, 15a-e, 18d-17b, 6c-f, 7a-d, 16-17a, 6hg, 11d-12a, 11c-8, 18e-22 (Calif & Oreg)

(Silver Lake, Calif)

- utaC3: approximately 1-2e, <u>3-2f</u>, 4-6b, 12b-15, 18d-17b, 6c-f, 7a-d, 16-17a, 6hg, 11d-12a, 11c-8, 18e-22 (Calif)
- 1-3e, 17b-13d, 12-13c, 4c-a, 10-9, 17c-19b, 11a-c, 3gf, 8-5, 19c-24 utaD1:
- utaD2: 1-3e, 17b-13d, <u>19b-17c, 9-10, 4a-c, 13c-12</u>, 11a-c, 3gf, 8-5, 19c-24 (Calif) (Oreg)
- large inversion, limits not defined utaD3:
- utaD4: 1, 15-17b, 3e-2, 14-13d, 12-13c, 4c-a, 10-9, 17c-19b, 11a-c, 3gf, 8-5, 19c-24 (New Mexico)
- 1-3e, 5-7c, 12-10c, 3f-4, 10b-7d, 13a-g i.e. differs from *aberratus* by Inv 12-7d utaE1:
- utaE2 Inversion of middle part of the arm
- utaF1: 1a-i, 9-2, 17-13c, 11-13b, 10d-a, 18-23
- utaF2: approximately 1a-i, <u>14-13c</u>, <u>2-9</u>, <u>15-17</u>, 11-13b, 10d-a, 18-23. (Calif. & Oreg) i.e. differs from F1 by two overlapping inversions 14-13c and 9-17.
- utaG1: Obvious BR about one third from centromere.
- Inversion of about two thirds of the arm, taking the obvious BR to near the distal utaG2: end. (Calif & Oreg)



Modified from Wülker et al. (1991)

Shallows of lakes or other pools, particularly where much algal growth, in western and central North America.

Found: Alberta - Elk Island & Lesser Slave Lake (Townes 1945)

Arizona - Shultz Pass Tank and Lower Lake Mary, near Flagstaff, Conico Co. (Shaller & English 1976); Williams (Townes 1945).

California – Antioch, & West Pitsburg, Contra Costa Co.: L. Crowley Bishop. Inyo Co.; w. Litchfield, Lassen Co.; Lancaster, Los Angeles Co.; Stranglehold, Modoc Co.; Convict Creek, & 1.7 ml Benton Hot Springs (37.48°N, 118.30°W), Mono Co; nr Spring Valley Lake, Apple Valley, San Bernadino Co.; Alkali Lake in Antelope Valley, Kern Co. (Townes 1945); Lake Davis, Silver Lake & near Storrie, Plumas Co.; Sheepy Creek, Siskiyou Co. (Wülker *et al.* 1991).

Colorado - Fort Collins (Townes 1945); Franklin Creek Watershed (38.93°N, 104.89°W) (Herrmann et al. 2016).

Minnesota - Sand Lake (Townes 1945)

Montana - 2 ml s. Ronan, Lake Co. (Wülker et al. 1991).

Nevada - Reno & Wells (Townes 1945)

New Mexico - Mineral Springs, Taylor Springs; Eagle Nest Lake (36.55°N, 105.25°W), Miami Lake, & Stubblefield Lake, all Colfax Co.; Upper Abbott Lake 36.15°N, 104.20°W), Harding Co.; Charette Lake, Mora Co.; Morphy Lake, S. Mora Co.; 10 m. e. Dora, & 10 mi. e. Dora, Roosevelt Co.; Morphey Lake, Sapallo River & Storrie Lake, San Miguel Co.; La Plata River, & Morgan Lake, s. of Fruitville, San Juan Co. (Wülker et al. 1991).

North Dakota - Dead Colt Creek Dam; Crystal Springs, Kidder Co. (Wülker et al. 1991).

Oregon – Eagle Ridge Park; Klamath River Hwy 97; Fremont Bridge; Upper Klamath Lake (42.47°N, 121.95°W), 1 ml Williamson River, all Klamath Co. South Dakota –L. Andes; Wagner; Lake Francis Case, Platte Lake; Picktown: all Charles Mix Co.; Campbell, Campbell Co.; 4 ml. n. Yankton, Yankton Co. (Wülker et al. 1991).

Utah - Kaysville (Type), Bear River Bay, Great Salt Lake, Honeyville, Magna & Plain City (Townes 1945); Goshen & Utah Lake, Goshen, Utah Co.; Sevier Bridge Res. 10 m. n. Gunnison; 6 m. w. Smithfield, both Sanpete Co. (Wülker et al. 1991). Wisconsin - Cyn Lake, n. Stratford, Marathon Co. (Wülker et al. 1991).

Morphology and cytology described by Wülker, Sublette & Martin (1991), which corrects a minor error in the arm E sequence in Martin, Sublette, Sublette (1979). A photograph of the karyotype was also published in Schaller & English (1976) and Kiknadze *et al.* (2010). The cytology places *C. utahensis* as a member of the karyosystematically defined (but not the morphologically defined) 'decorus-group'.

The adult male is recognized by the dark color, heavy fore-tarsal beard, broad anal point and the almost capitate IVo. Smaller specimens can be similar to larger specimens of *C. atrella* but are darker and have a heavier beard (Townes 1945).

Known as the "Klamath midge" because of the large numbers causing nuisance in the vicinity of the Klamath lakes.

The larvae are quite variable between localities and, taken along with the apparent clines of inversion polymorphism (Wülker *et al.* 1991), and possible differences in the SVO, raises the question as to whether there may be incipient species involved.

Chironomus alchichica Acosta & Prat 2017 (Species 5w)

BARCODE data shows it is a member of the *C. decorus*-gp probably close to *C.* 'proulxi' and *C.* 'butleri'.

In BOLD Bin: BOLD:AAB7030

Adult

Male: Total length 4.34 (3.95-5.07) mm; Wing length 3.53 (2.99-4.25) mm. Coloration: Head yellowish brown, thorax dark brown. Abdomen pale brown with dorsal dark markings on TI-VII, wider on TVIII. Legs uniformly pale yellow. Head: AR 3.79 (3.75-3.83); clypeus with 15-20 setae; 30-40 temporal setae. Palps (segs 2-5) 47 : 171 : 189 ; 245; P5/P4 1.30; P5/P3 1.43.

Wings transparent without setae, anterior veins pale brown, r-m crossvein and C, M, R_1 , R_{4+5} dark brown. Squama with 20-26 setae, 2 SCf on brachiolum. VR 0.94 (0.91-0.95).

Leg lengths (meron) and proportions.										
	Fe	Ti	Ta1	Ta2	Ta3	Ta4	Ta5	LR	F/T	BR
PI	1088	1081	1579	860	580	470	220	1.03-1.27	0.97-1.01	1.9
PII	1224	1223	715	440	321	203	148	0.56-0.60	1.01-1.08	
PIII	1446	1519	995	609	440	263	164	0.66-0.71	0.93-0.96	

Leg lengths (micron) and proportions:

Setae on TIX not clear; anal point narrowing to end; SVo of type E of Strenzke 1959); IVo about to end of anal point, apparently with simple setae. Gonostylus moderately swollen and narrowing significantly over distal third, 6-7 setae at distal end.



Male hypopygium of C. alchichica from Acosta et al. 2017

Female: Not described.

Pupa: Length of abdomen 5.4 (4.9-6.2) mm. Cephalic tubercles (males?) conical 145.2-209.7 μ m; no mention of frontal warts. Basal ring of thoracic horn 122.4 (107.4-137.6) μ m. Thorax scarcely granular. Abdomen without dorsal shagreen patches evident. Hooks at posterior of

segment II continuous but not counted. Pedes spurii B and A well developed on segments II and IV respectively.



Pupal spur of *C. alchichica* from Acosta *et al.* 2017

Spur of segment VIII with 5-6 closely applied teeth; more than 115 taeniae in anal fringe, apparently in multiple rows.

Fourth instar larva a plumosus-type about 9-10 mm in length, VT appear relatively short. Ventral head length 0.28 (0.26-0.31) μ m. Gula slightly darkened, slightly wider than the mentum and widest part way up from posterior margin.



Larval mouthparts of C. alchichica from Acosta et al. 2017

Mentum of type II and median tooth of type III; ventromentum with a smooth outer margin, VMR perhaps about 0.17, striae not counted.

Premandible with outer tooth very narrow and coming to a sharp point, inner tooth about 6 times wider but still coming to a relatively sharp point (type B2). PE with 13-14 teeth (type A).

Antenna with basal segment about 3 times longer than wide, RO about 1/3 up from base (0.23, 0.18-0.29). Segment proportions 118 : 30 : 8.5 : 16 : 8, AR 1.9 (1.69-2.39). Mandible of type IIB, number of taeniae in PecM not given, nor the presence or number of furrows at the base, MTR about 0.43.

Cytology: Four polytene chromosomes with the thummi-cytogroup combination, AB, CD, EF, G. Centromeres clearly visible and heterochromatic. Arm G with 2 BRs and an NOR, partially unpaired; Bulb of arm B near the telomere.

NORs also on arm C (in some cells) and arm D, both near the centromere.

- alchA1: 1, 13 16, 2c, 10 12, 3 2d, 9 4, 17 19.
- alchB1: Puff with proximal dark bands)(7-8) near telomere.
- alchC1: Constriction (2-5b) near distal end of arm.
- alchD1: NOR near centromere.
- alchE1: 1 3e, 10c 3f, 4 10b, 5, 11 13 Inv5-10c from *maturus* 'proulxi', etc.
- alchF1: 1 8c, 12 17, 10- 8d, 11, 18 23.
- alchG1: terminal NOR, nearby small BR and a large subterminal BR near the other end.



Polytene chromosomes of C. alchichica from Acosta et al. 2017

Molecular:

mt*COI*: Barcode sequence of seven specimens are in GenBank: MG603057-63. These sequences indicate a relationship to species of the *C. decorus*-gp, particularly, *C.* 'proulxi' and *C.* 'butleri'.

Found: (Type locality) Lake Alchichica (19.412°N, -97.400°W), Puebla, Mexico.

Other *C. decorus* relatives - (see also *pseudothummi*-cytocomplex: sp. z, sp. 2t & sp. 3r; and *maturus*-cytocomplex: *C. maturus* & *C. whitseli*)
End decorus-group

Chironomus decumbens-like

C. decumbens(?) (Species 2x)

Could be *C. decumbens* Malloch.

Adults of this species are in the collection of J.E. Sublette, at the University of Minnesota, St. Paul, MN. He noted that the adults show some relationship to *C. atritibia* and *C. pilicornis*.



Hypopygium, anal point and superior volsella of C. spec. 2x.

Description of *Chironomus decumbens* from information in Townes (1945) (known only from type specimen)

Male: Wing length 4.7 mm; fore LR 1.27; BR about 4.0; antennal ratio 4.5. Body of medium stoutness.
Frontal tubercles of moderate size, clypeus rather small.
Middle portion of pronotum hardly widened; mesoscutum without a tubercle.
Fore tarsus with a rather short depressed beard, arising at about 40° to 45°.
Blackish brown, legs slightly paler.
Hypopygium with very narrow anal point reaching about to midpoint of GS, with IVo about the same length; superior volsella of Strenzke's E-type.
Townes figure appears to show about 17 setae on tergite IX, Sublette's adult has only about 10 in a number of pale spots.



Female: Unknown.

Pupa: Caudolateral spur of segment VIII with one large and perhaps two smaller spines.



Fourth instar larva a medium sized (abt. 13.2 mm) salinarius type. Gular region sl. dark – dark, at least on posterior third. AT moderately long, dorsal shorter ($400 \times 300 \mu$ m), ventral relatively longer ($600 \times 120 \mu$ m), about 2.7 times longer than wide

Mentum (Fig. b, below) relatively flat, c1 tooth relatively broad with parallel sides, c2 teeth quite well separated (Type IIA); 4th laterals slightly reduced (essentially Type I). Ventromentum (Fig. c, below) with about 45 striae; VMR about 0.28. PE (Fig. a, below)

with about 13 teeth, an occasional one reduced in size.

Premandible with outer tooth shorter than the inner tooth, which is about 2.7 times wider. Antenna (Fig. d, below) with basal segment about 3.6 times as long as broad; AR about 2.6; A1/A2 about 4.3, A4/A3 about 1.14; antennal proportions μ m) 152 : 35 : 9 : 10 : 5. Development of 3rd inner tooth of mandible not clear.



Cytology: 3 polytene chromosomes with a modified thummi arm combination AB, CD, GEF.

Arm G region unpaired with nucleolus near end of arm, although it could also be at the end of the other arm. Possibly a nucleolus also in the CD chromosome, just proximal to the characteristic constriction of groups 3-4.



Found: Alaska - Harding Lake.

The type locality of *C. decumbens* Malloch is Nunavut (formerly North West Territories) - Southampton Island, Keewantin.

The cytology of this species suggests close relationship to *C. islandicus* and the species noted as *C.* nr. *decumbens* (species h) in that it has a small unpaired arm G and multiple nuclei in the long chromosomes.

Chironomus sp. NAIII of Proulx et al. (2013) (Species 4u)

In BOLD Bin: BOLD:AAV3582

Adult specimens in same Bin labelled *C*. c.f. venustus This presumably refers to *C. venustus* sensu Pinder 1978, not sensu Staeger 1839 which has an S-type SVo. The Pinder species probably requires a new name, pending further investigation (Spies & Sæther 2004).

Adult:

based on photos associated with the BOLD Bin. A dark species, with lighter patches on sides of thorax. Male:



SVo closest to E(g) of Strenzke (1959), but not tapering towards the end; IVo extending to about the end of the anal point. GP relatively long and narrow, tapering gradually over posterior third.

Female:



Wing length about 5.25 mm. Rear F/T about 1. Appears haltere may be white.

Pupa: Not known.

Fourth instar larva: A medium sized salinarius-type larvae; length about 10-18 mm. The frontoclypeus is pale and the gular region (below) is strongly darkened on posterior half. Salivary reservoir (f, below) with relatively long margins, and opening about 2.9 times longer than wide and support structure with shoulders.



The c2 teeth of the mentum (Fig. b, below) trifid tooth are partially separated from the c1 tooth (type IB). The mentum 4th lateral teeth are reduced (type II).

The anterior margin of the ventromental plate is smooth (fig. e, below), with about 36-42 striae reaching to about center of the plate and then with fewer striae reaching about 2/3 of way to anterior margin.

PE with 9-15 pointed and rather uniform teeth. Premandible (Fig. a, below) with broad inner tooth (type 2B1), inner tooth about 2.5-3.2 times wider than the outer tooth.

Antenna (Fig. c, below) with ratio of A1/A2 from 3.1-3.8; which is slightly lower than that of the very similar *C. cucini* (3.6-4.3); AR about 1.77 (1.58-2.03); A1 about 2.95 (2.02-3.38) times longer than wide; ratio of antennal segments (μ m) 126 : 36 : 10 :15 : 8. The 3rd inner tooth of the mandible (Fig. d, below) pale and fused to the lower margin (type 1A), at least 13-21 furrows on the outer surface at the base.



(Photos courtesy Isabelle Proulx)

Cytology: 3 polytene chromosomes with the modified thummi-complex combination AB, CD, GEF.

Centromeres are heterochromatic. A nucleolus is located near the junction of the arm G with arm E and a BR is located towards the distal end of arm G. Arm G closely paired.



This species was found in oligotrophic to mesotrophic lakes at depths varying from 5-12 m and at pHs varying from 7.1-7.9.

Found: Ontario - Lake McFarlane (46.42°N, 80.95°W), Hannah Lake (46.45°N, 81.03°W), Ramsey Lake (46.47°N, 80.95°W) - all nr. Sudbury (Proulx *et al.* 2013); St. Charles College, Sudbury (46.516°N, 80.926°W (BOLD). Quebec - Lake D'Alembert (48.38°N, 79.02°W), nr. Rouyn-Noranda. Norway – Finnmark (70.1545°N, 23.7212°W)(BOLD)

Cytology of this species suggests that this species might be related to *C. decumbens* The *C.* sp. NAIII larval morphological description is comparable to that of *C. ?decumbens* (sp. 2x), but the chromosomes of the only known larva of that species do not have heterochromatic centromeres.

End decumbens-group

Chironomus hyperboreus/aberratus-group

C. hyperboreus Staeger, 1845 (Species x)

Possible synonym – C. polaris Kirby 1821 (but more likely a syn. of C. pilicornis)

Molecular sequence from Canada is under the name *C*. species TE13.

In BOLD Bin: BOLD:AAC0596

Adult

Male (from Townes, 1945 and photo in BOLD):



C. hyperboreus male from BOLD (left) and hypopygium from Townes 1945 (right) Wing length 4.2-5.2 mm; fore LR 1.25-1.27(1.27 in co-type); antennal ratio 5.0. Body moderately stout.

Blackish brown, legs brown (blackish in type description), darker towards the apices. Frontal tubercles rather small, clypeus small.

Middle portion of pronotum slightly broadened; mesoscutum without a tubercle.

Wings with anterior wing veins brown, crossvein somewhat darker, posterior veins not pigmented.

Fore tarsus with a long dense beard, BR about 4.

Superior volsella rather unusual, not easily fitted into the Strenzke groups. Townes notes that the specimen described has paler legs than the types.

Leg lengths (micron) and proportions:

	Fe	Ti	Ta1	Ta2	Ta3	Ta4	Ta5	LR	F/T	BR
PI	1200	1440	1720	710	550	475	400	1.20-1.34	0.83	abt 4
PII	1000	1640	880	720	360	280	200	0.54-0.62	0.63	
PIII	2000	2200	-	-	-	-	-	0.69-0.72	0.91	

Abdominal segments blackish at base, with a brownish band across the distal ¹/₄ to 1/3. Townes shows about 11 setae in a number of pale patches on TIX. Anal point narrow, extending beyond midpoint of gonostyle; SVo rather unusual, not easily fitted into the Strenzke groups, closest to E(i) but more curved and often not widened towards its distal end. The Townes illustration (above) is more robust than the variants illustrated by Pedersen (1978) from Staeger's specimens. IVo not reaching to end of anal point, but to about midpoint of GS, which is moderately swollen and narrows gently over posterior third, shown 4+1 setae at tip.

Female:



From a photograph in the BOLD database (above), the following characters can be adduced:

Wing length about 3.1 mm.

Thorax, scutellum, etc., blackish, abdominal segments blackish at base, with a brownish band across the distal $\frac{1}{4}$ to $\frac{1}{3}$; bases of anterior and mid legs brownish, rest of legs blackish.

Leg lengths (micron) and proportions:

	Fe	Ti	Ta1	Ta2	Ta3	Ta4	Ta5	LR	F/T
PI	885	914	-	-	-	-	-	-	0.97
PII	940	-	-	-	-	-	-	-	-
PIII	1157	1343	1050	514	385	214	157	0.78	0.86

Pupa: no information available, although described by Andersen (1937).

Fourth instar larva a medium sized (male 13.3 (12.2–14.5) mm) salinarius-type. AT relatively long (dorsal 375x140, ventral 401x185 μ m), dorsal about 3 times longer than wide, the ventral pair about 2.2 times longer. Head capsule brown rather than yellowish. Gular region, FC and sometimes other parts of the head, dark brown; gula darkening over posterior half, wider than the mentum, anterior margin rounded, and more extensive at lateral edges. Salivary reservoir about 61-79 x 18-25 μ m (l/w 3.1-3.5).



Mentum of all specimens badly worn, width about 202-218 μ m; center teeth are well separated (type IIA) and fourth laterals reduced (type II).

Ventromentum abt 194-205 μ m wide and abt 3.15 (3.1-3.3) times wider than deep; slightly narrower or the same width as the mentum; abt 42-44 striae; VMR about 0.24-0.28.

PE with 8-14 teeth, sometimes irregular and broad (see Fig. a, below) an abnormality also seen in *C*. species v, which sometimes occurs in the same pools..

Premandible with broad teeth about equal in length, inner tooth about 3-4 times wider than outer tooth, coming to relatively broad point (Ty. B2).

Antenna with basal segment about 3.15 (3.1-3.3) times longer than wide, RO about 0.35-0.40 up from base; AR about 1.80 (1.66-1.94); A2 about 0.29-0.32 of A1; segment proportions (micron) 126: 37: 10.5: 14: 7.5.

Distance between antennal bases about the same as the distance between the S4 setae, which are separated by about 0.78 of the FC width at that point.

Mandible abt 245-266 μ m long, with 3rd inner tooth usually well developed (type IIIA-B), but may be broken, and with about 14.8 (14–16) furrows on the outer surface near the base; PMa abt 13 (12-14); Mdt-Mat abt 19.3 (15-28) μ m, MTR 0.23 (0.19-0.28).



Mouthparts of larvae of *C. hyperboreus*. The worn nature is common to all available larvae. a and b show alternative forms of the PE teeth.

Cytology: 4 short polytene chromosomes with the thummi arm combination AB, CD, EF, G, but Keyl pattern hard to recognize. Centromeres heavily heterochromatic, often forming a chromocenter.

Arm G short, often unpaired, with a nucleolus near the centromere and possibly a BR near the other end. Polymorphic in arm A. Polymorphism also occurs in arm F, but only F2 is present at Ellesmere.

Preliminary sequences according to Wülker:

- hyp A1: 1-2c, 10-12, 3-2d, 9-4, 13-19 i.e. as *holomelas* I
 - A2: 1-2c 10-12 7-9 2d-3 6-4 13-19 (Greenland)
 - A3: 1-2c 3-2d 9-7 12-10 6-4 13-19 (Ellesmere)
- hyp B: as *riihimakiensis*.
- hyp C1: 1-2c, 3-2d, 9-7, 12-10, 6-4, 13-19 (Wülker) or
- 1-6b, 11c-8, 15-11d, 6gh, 17a, 16h-a, 7d-a, 6f-c, 17b-22 (Kiknadze)
- hyp D1: 1-3, 11-18d, 7-4, 10-8, 18e-24
- hyp E1: 1 3e, 5 10b, 4 3f, 10c 13
- -24 i.e. as *longistylus, tenuistylus,* etc. 13 i.e. as *aberratus,* bifE1, etc.
- hyp F1: 1 6, 12 7, 13 23 (Greenland)
- hyp F2: 1 2, 14 13, 7 12, 6 3, 15 23



Polytene chromosome complement from Hazen Camp Pond, Ellesmere Island, N.W.T.

Found: Alberta - Corral Creek old road Banff National Park (51.407°N, 116.154°W), NE Jasper Lake, Jasper National Park (both as sp. TE13) (BOLD)
Manitoba - Launch Rd Bluffs, Churchill (58.765°N. 94.013°W) (as sp. TE13) (BOLD)
Nunavut (was Northwest Territories) - Hazen Camp Pond, Skeleton Lake and its inlet marsh, Hazen, Ellesmere Island (Pond 33 & Tarn 34 in Oliver & Corbet, 1966). Quebec - Quarry Island, Mingan Archipelago National Park Reserve (50.2135°N, 63.7979°W) (BOLD)
Value Territories Whiteheres (C0.50540N 124.0520W) (as an anticipation of the second se

Yukon Territory - Wolf Creek, Whitehorse (60.5954°N, 134.953°W) (as sp. TE13)(BOLD)

Greenland - Lake 517, Stoe Kvaneso, West Greenland (Type locality); Egedesminde (Townes 1945); Lake 547, Kurnefield; Zackenberg Research Station, Northeast Greenland (GenBank & BOLD).

Arctic lakes and pools.

Chromosomes pictured and briefly described by Wülker & Butler (1983), redescribed by Wülker & Martin (2000).

Molecular sequences:

mt*COI*: A number of sequences are available in GenBank or BOLD, under the names *C*. *hyperboreus* or *C*. sp. TE13 (from Arctic Canada).

Chironomus species h. Similar to *C. decumbens* but may be new species (Species h)

In Bold Bin: <u>BOLD:AAP3010</u> (Mined from GenBank KF278349 – see below) The nearest neighbor Bin in the BOLD database is <u>BOLD:AAI4308</u> Identified as *Chironomus islandicus* Adult: There are at least 4 adult males in the collection of J. E. Sublette, in the Museum of the University of Minnesota, St. Paul, MN, or in the collection of the Canadian National Insect Collection in Ottawa.



Male: Only a photograph of the male hypopygium is available.

Male hypopygium of adult male (left) and spur of pupal segment VIII (right)

Anal point slim. A small number of setae in pale circles on tergite IX. SVo of E-type (j) of Strenzke (1959), IVo to about the tip of the narrow anal point, GS moderately swollen, narrowing relatively sharply over posterior third.

Female – not known.

Pupa: Caudolateral spur of segment VIII with about 4 closely spaced spines, but with other fine spines on base to the spine, particularly on the inner side (see figure above).

Fourth instar larva of the plumosus-type, medium to large size (fem. 14.2–17.5 mm; male 13.2-16.7 mm), lateral projections from 130–280 μ m. Anterior pair of VT usually longer (ant. 0.72-1.60 mm; post. 0.72-1.30). AT long (1.06-1.52 mm), about 3.8–6.4 times longer than wide, ventral possibly longer; apparently longer in deeper water. Gular region usually darkened on posterior third to half with slight wing-like extensions but may be pale; and FC pale or occasionally slightly darkened.

Mentum (Fig. b, below) with pointed teeth; c1 tooth broad, possibly with a flanged edge, c2 teeth moderately separated but tend to continue edge line of c1 tooth (type IB); 1st laterals sloping outwards, 4th laterals reduced almost to level of 5th laterals (type II).

VM about 3.5 times longer than deep, with about 44-49 striae reaching almost to anterior margin; VMR 0.25. PE with about 13-14 sharp, broad teeth (type B).

PreM (Fig. b, below) with teeth about equal in length, inner tooth about 2.5-3 times wider than outer tooth.

Distance between antennal bases about the same as that between the S4 setae, which are separated by about 77-81% of FC width at that point.

Antenna long 3.5-4 times longer than wide, RO about 2/5 to 1/2 up from base of A1; AR about 2.04-2.09; A2 about 0.25 the length of A1; segment lengths (μ m) 145 : 37 : 10 : 15 : 8. Mandible with 3rd inner tooth distinct but only partially colored (type II –IIIA/B); about 19-21 furrows on outer surface near the base; 12-14 taeniae in PMa.



Cytology: 4 relatively short polytene chromosomes with markedly heterochromatic centromeres; thummi arm combination AB, CD, EF, G.

Arm G very short, bands indistinct and normally only paired at the almost terminal nucleolus. Nucleoli also in arms B, C, D (2 or multiple nucleoli) and F. No polymorphism in known specimens.



Arm A1: 1 - 2c, 10 - 11? 9, 2d - 3, 12, 8 - 4, 13 - 19 (Wülker)

- Arm B1: 1-?, 20 - 18, ?, 9(?) - 13, ?- 8b, 17 - 14, 23 - 28 i.e. derived from *heteropilicornis*
- Arm C1: Nucleolus about one third from centromere.
- Arm D1[.] Nucleoli near middle of the arm
- Arm E1: 1 - 3e, 5 - 10b, 4 - 3f, 10c - 13 i.e. as *aberratus*
- 1a-f, 9 1g, 11 23? Arm F1: nucleolus about group 15.
- Virtually terminal nucleolus. Arm G1:
- Found: North West Territories Cache Lake, Tuktut Nogait National Park (from BOLD) Ontario - Bat Lake (45.577°N, 78.523°W), Algonquin Provincial Park.



Bat Lake, Algonquin Provincial Park.

Amongst deep organic litter in a highly eutrophic lake.

This species shows clear relationship to C. islandicus: they have a similar SVo (although that of *C. islandicus* is longer and IVo is shorter; pupal spur has fewer spines); arm G is small and lacking a nucleolus, with multiple nucleoli on the long chromosomes some of which appear to correspond. It also shows relationship to C. ?decumbens, which also has a small arm G and multiple nucleoli.

DNA sequence:

mtCOI- in BOLD, also GenBank, accession no. KF278349 (see Proulx et al. 2013)

Chironomus islandicus (Kieffer 1913) (Species 5s)

In BOLD Bin: BOLD: AAI4308

Some specimens identified as C. islandicus from Iceland and also from North America. This is the first indication that this species may occur in the Nearctic, but unfortunately no morphological data are given. The nearest neighbor is C. sp. h in BOLD Bin: BOLD:AAP3010

Adult: The species was redescribed by Pedersen (1978) along with the cytology. Wülker and Butler (1983) also described the polytene chromosome sequences and some larval characters and Stur and Ekrem (2020) gave further information on the adults and photographs of some larval characters.

Male:

Wing length 4.9 (3.7-5.5) mm. AR 5.0 (4.5-5.5); VR about 1; LR1 1.12 (1.06-1.17); LR2 0.58 (0.53-0.60); LR3 0.67 (0.62-0.72). BR 5.7 (5.1-6.3).

Head black. Thorax with black ground color, with pruinose areas on antero-lateral and posterior-median parts of scutum and some pruinescence on the pleural sclerites. Scutellum and postnotum black, halteres dirty white.

Thoracic setae: acrostichal 21 (13-34), dorsocentrals 58 (34-84), prelars 12 (8-16). Legs black-brown with tarsal segments a little paler.



Male hypopygium of *C. islandicus* (BOLD GL25+1315334716)

Abdomen black, posterior margins of segments paler. TIX with about 9 setae in individual pale spots.

Anal point relatively broad; Svo long E(i) type of Strenzke (1950); Ivo long, beyond the end of the anal point and about to midpoint of GS, which is relatively narrow and narrowing markedly over terminal quarter to fifth.

Female: not described.

Pupa: (From Langton & Visser 2003): Apparently includes both males and females. Hence smaller values are likely males and larger values likely females (except for cephalic tubercles). Exuvial length 9.0-12.0 mm.

Exuviae golden brown to dark brown, without marked contrast in color between thorax and abdomen; outer margin of the anal lobes colored except at base.

Cephalic tubercles conical, $80x105-160x120 \mu m$; frontal setae 45-72 μm long. Basal ring of thoracic horn 145x80-175x90 μm , HR 1.81-1.94. Thorax less distinctly granulate, anterior granules small and distinct, medially most are somewhat flattened, producing an irregular reticulate pattern, or evanescent.

Hook row of segment II entire, 0.47-0.57 of the tergite width, with 97 (male?)-137 (female?) hooks.

Armament of tergites II-VI not so strongly waisted and with the posterior transverse band not successively increasing in extent to tergite VI; usually reduced on VI. Shagreen of sternite II continuous from postero-lateral corner to seta V5, the region between containing points of similar size to those on each side. Both sternites III and IV with lateral longitudinal bands of shagreen. Sternite IV postero-laterally bare, without a patch of enlarged points. Pleura of

segment IV spinulate. Caudolateral spur of segment VIII long with 2-7 stout teeth. Fringe of anal lobe with 102 (male?)-137(female?) taeniae.

Fourth instar larva: (From Wülker & Butler 1983) A salinarius-type larva, although small humps may be present at rear of 11th segment. Posterior of gula darkened, a small dark stripe at rear of frontoclypeus and lobed dark spot near front, but high individual variability. Antenna – A1 201; A2 30; A1 about 4.2 times longer than wide.

In Svalbard specimens the proportions are about 78 : 27 : 7.5 : 14 : 6; AR abt 1.6. RO about a third up from base of segment; A1 about 3 times longer than wide.



BOLDBJ149-556

Mentum width about 140 μ m, with central tooth type IIA; 4th lateral not reduced (type I). PE with about 8-9 teeth; mandible with third inner tooth separated and partly colored (Ty IIIB); MTR about 0.27.

Cytology: Four polytene chromosomes with the thummi arm combination AB, CD, EF, G. Centromeres relatively heterochromatic. Arm G small, with a virtually terminal nucleolus but it is not the major nucleolus and is not always developed; with at least three other BRs – the major one proximal in arm B and two other minor ones in arm E, at least one of which may not always be developed. Arm G is usually unpaired and sometimes attached to the centromere of chromosome CD.

- isl A1: 1a 2c, 10a 12c, 3i 2d, 9e 4a, 13a 19f i.e. as holomelas, cucini, magnus, etc.
- isl B1: Nucleolus proximal in arm B, banding unclear.
- isl C1: 1 6b, 15c-e, 8 11c, 15b 11d, 6gh, 17a 16, 7d-a, 6f-c, 17b 22 i.e. as *cucini*, sp. 3b.
- isl D1: distal bands identical to those of *tardus, cucini, cucini, mangnus,* but unspecified changes nearer the centromere.
- isl E1: possibly 1 3e, 5 10b, 4 3f, 10c 13 but difficult to be certain due to the 2 nucleoli i.e. possibly as *aberratus, tardus, cucini, hyperboreus,* sp. h, etc.
- isl F1: possibly 1-10, 17-11, 18-23 i.e. as *cucini, tenuistylus, tardus,* etc.

isl G1: May have nucleolus developed at centromere and a heterochromatic band near middle of the arm.



Arm G, with nucleolus undeveloped, attached to CD centromere

Found: Nunavut – Kitikmeot (69.2187°N, -104.926°W) (BOLD)
Iceland (Type locality); Stoedurvatned, Kvisker (68.9838°N, -16.4371°W) (BOLD); Thingvallavatn; Myvaten (Wülker & Butler 1983).
Norway – Svalbard, Bear Island (74.4814°N, 19.0048°E); Jan Mayen Island (abt. 72°N; -7.5°W) (Stur & Ekrem 2020); Lebesby, Finnmark (70.441°N, 26.805°E); Melhus, Sor-Trondelag (63.2171°N, 10.3076°E), (all BOLD)

The cytology indicates that this species is clearly closely related to a group of northern species, such as *C. hyperboreus, C. cucini, C. magnus* (Wülker & Butler 1983) and *C.* species h, as all have heterochromatic centromeres and a small arm G and most with multiple nucleoli in the long chromosomes, some of which appear to correspond. As well, the males of both species have a similar SVo. The adult is very similar in coloration to *C. hyperboreus*, but can be differentiated by the lower LR.

Langton & Visser (2003) distinguish the pupa by the lack of setae on postero-lateral of sternite IV (the couplet seems to imply that this means there is no pedes spurii A).

C. jonmartini Lindeberg & Wiederholm 1979 (Species 4e)

As new name for *C. neglectus* Lindeberg, 1960 (junior synonym) A member of the *Chironomus aberratus* species group.

There are two specimens, from Iceland, listed in the BOLD database but they lack DNA sequence and so are not allocated to a BOLD Bin.

Adult: (from Lindeberg's (1960) description of his *C. neglectus* from Finland and the Lindeberg & Wiederholm (1979) redescription)

Male: Wing length 3.0-5.0 mm (smaller specimens in late summer). AR 4.0-4.4 Entirely black, sometimes with legs and scutellum paler, posterior margins of tergites often greyish, haltere knob dirty white.

Frontal tubercles well developed (greater than 30 µm long).

Fore leg proportions: 35 : 34 : 43 : 22 : 15 : 12 : 6, LR 1.18-1.38; F/T 1.03: BR 4.5-7.

Illustration shows 4 setae in a single spot on tergite IX. Anal point slender and parallel sided or tapered. SVo an E-type, closest to 'g' of Strenzke (1959), but ending bluntly. IVo reaching to end of anal point (abt 1/3 of GS length) with recurved setae only on apical half; GS only moderately swollen and narrowing relatively gently over distal third.



Larva and male hypopygium of C. jonmartini from Lindeberg 1960

Female: No available information.

Pupa (from Langton & Visser 2003): Probably incudes both males and females. Length 7.6-9.4 mm (9.0-11.0 mm in Finland). Contrast in color between thorax and abdomen not so marked; outer margin of anal lobes colored except at base.

Cephalic tubercles 70x44 (female?)-160x130 (male?); frontal setae 34-72 μ m long. Basal ring of thoracic horn 125x50-160x60 μ m, i.e. HR 2.5-2.7. Thorax extensively granulate. Hook row of segment II with 70 hooks, extending 0.46 of width of segment (n=1). Armament of tergites II-VI not so strongly waisted and with posterior transverse band not successively increasing in extent to tergite VI; usually reduced on VI. Spur of segment VII with 1-5 (3-7 in Finland) stout teeth which may be long. Anal lobe with 75-130 taeniae.

Fourth instar larva of melanotus-type (Lindeberg calls larva a plumosus-type, although his illustration (above) shows VT do not show the 'plumosus-type' bending and coiling), with dorsal AT much smaller than the ventral one. Head with dark gular region and dark head stripe on the FC. Mentum with 4th laterals reduced almost to level of 5th laterals (type II), C1 tooth of mentum moderately wide with c2 teeth moderately separated (type IB). Premandible with teeth about equal in length, inner tooth about twice as wide as the outer tooth.

Mandible possibly type IIB or C, about 17 furrows on outer surface near base.

Cytology: 4 polytene chromosomes with the thummi-cytocomplex arm combination (AB, CD, EF, G). Centromeres heterochromatic.

Arm G often unpaired, with a subterminal nucleolus after a heterochromatic terminal band, and an apparently heterochromatic interstitial band. At least one BR, but not clear enough to be sure if others are developed. Polymorphism in arms A, D and F in the Palearctic, but only arm F known to be polymorphic in North American populations. However, arm A seems to be the sequence A3, which is less common in the Palearctic.





- h'jon A3: 1 2c, 10 12, 4 5, 3 2d, 9 6, 13 19
- h'jon B1: 1 7, 8b 15, 22 28 according to Hirvenoja & Michailova (1997), but this leaves many bands unaccounted for.
- h'jon C1: 1 6b, 11 8, 15 11d, 6gh, 17a 16, 7d-a, 6f-c, 17b 22 as Kiknadze *et al.* 2004, 2016
- h'jon D1: 1-3, 11-18d, 7 4, 10 8, 18e 24
- h'jon E1: 1 3e, 5 10b, 4 3f, 10c 13
- h'jon F1: 1 10, 17 11, 18 23
- h'jon F3: 1 2, 5 3, 6 10, 17 11, 18 23
- as Kiknadze *et al.* 2004, 2016 i.e. as *aberratus*, pluE1, etc. i.e. as *cucini*, *tenuistylus*, *tardus*, etc. from Kiknadze *et al.* 1996, 2016

as Wülker 1991b

Found: Northwest Territories - tundra pond FBV, Horton River area (M.G.Butler) Also: Finland (type locality); Russia – Altai, Yakutia; Sweden; Iceland.

In Europe in more or less saline rock pools.

C. jonmartini was proposed by Lindeberg & Wiederholm as a new name for *C. neglectus* Lindeberg. However it is not certain that the present material is identical to that of Lindeberg & Wiederholm, which was partly cytologically described by Wülker (1973) as arm G may differ. However, studies from other parts of the Palearctic indicate that the larval morphology and chromosomal banding patterns are variable (Kiknadze *et al.* 1996, Rakisheva *et al.* 2001). The North American material seems to be identical to that described by Kiknadze *et al.* (1996) and most of the larval description above is based on their description.

Chironomus species 2u (Species 2u)

Near C. hyperboreus, C. aberratus or C. sororius and C. sp. u.

This species is in BOLD Bin: <u>BOLD:AAI4297</u> The nearest neighbor Bin in BOLD is BOLD:ACN3382 which is an unidentified species

Adult:

Adults and rearings of this species are in the Sublette Collection, now in the Museum of the University of Minnesota, St Paul, MN.

Male: Some information can be noted from photographs in the BOLD database:



Wing length about 4.2-4.8 mm., VR about 0.86-0.97; anterior wing veins and crossvein slightly darkened. AR probably quite high (perhaps 5?) Thorax dark brown, vittae, scutellum, legs, etc., blackish with postnotum pale. Abdomen dark brown, narrow pale band distal on segments VI and VII, sometimes very narrow on preceding segments.

Approximate leg proportions (micron):

	Fe	Ti	Ta1	Ta2	Ta3	Ta4	Ta5	LR	F/T	BR
PI	1615	1530	-	-	-	-	-	-	1.05	-
PII	1580	1500	1000	630	475	315	210	0.63	1.03-1.05	
PIII	1775	2000	1260	940	645	410	290	0.78	0.89	



Apparently a small number of setae (perhaps 6 in individual pale areas) on TIX. Anal point stout, narrowing slightly to distal end which is rounded (above, right); SVo rather stout and blunt, most similar to D(d)-type of Strenzke 1959; Setae of IVo simple, shorter than anal point and to about midpoint of the gonostyle, which narrows relatively gently over posterior third.

Female:

Wing length about 5 mm Generally dark color; vittae, scutellum and postnotum and sternoplueron blackish. Abdominal sternites brown with yellowish posterior band. Photos suggest femurs may be a lighter brown.

Pupa: Cephalic tubercles about 1.3 times longer than wide. Caudolateral spur of segment VIII with about 3(1-4) closely applied spines. Fringe of anal lobe with about 62 taeniae in a double row on each side.



Fourth instar larva a medium size plumosus-type 13.7 (11.5-16.5) (female 12.7-16.5 mm); TLt about 305 (200-440) μ m; anterior VT 2.0 (1.10-2.48) mm, posterior 2.15 (1.30-2.56) mm, posterior pair generally longer but some variability in Saskatchewan larvae. AT about 442 (405-500) μ m (dors), 434 (359-600) μ m (vent) and 2-3 times longer than wide, dorsal pair relatively longer (2.3-3.1 times) than the ventral pair (1.9-2.6 times).

Gular region (below) generally dark on posterior third to half and higher at outer edges, widest up from posterior margin, and in rare cases reduced to a 'V'; FC usually slightly dark to dark, often as a thick stripe. Ventral Head Length 356 (295.5-396) μ m with overlap between sexes.



Salivary reservoir 79.5 (63-91) μ m in length and 3.66 (2.27-4.5) times longer than wide. Mentum (Fig. c, below) abt 0.53-0.63 of VHL with relatively rounded teeth; central trifid tooth usually type IB but may also be IIA; 1st laterals sloping outwards; 4th laterals reduced (type I-II), sometimes only slightly in Calgary specimens, but generally almost to level of 5th laterals in Saskatchewan.

Ventromental plates (Fig. d, below) 229.4 (210-245) μ m wide and 3.63 (3.26-3.91) times wider than deep, 1.13 (1.06-1.26) times wider than mentum; separated by about 0.3 to 0.4 of the width of the mentum, with 45.5 (37-52) striae; VMR about 0.35 (0.29-0.43).

PE (Fig. a, below) with about 12.3 (9-14) relatively broad teeth (type B).

Premandible (Fig. a - blue rectangle, below) with inner tooth 3.1 (2-4.5) times wider than the outer tooth, both coming to relatively sharp points, perhaps type B2.

Antenna (Fig. b, below) with basal segment only 0.35-0.45 of VHL; abt 3.5 (2.9-4.0) times longer than wide, RO a third to half way up from base of segment; AR 2.0 (1.72-2.63); antennal proportions 139: 36: 11: 13: 7.

Distance between the antennal bases, 155.3 (142-187) μ m, generally less than that between the S4 setae, 172 (163-197), but occasionally greater. S4 setae separated by 0.87 (0.80-0.94) of FC width at that point. S5 setae may be slightly anterior, level or sl. posterior to nearby RO.

Mandible (Fig. e, below) generally with 3rd inner tooth partly to well developed and at least partly darkened (type IIB-IIIB/C), about 19.2 (14–24) furrows on outer surface near base, about 13.1 (10-16) taeniae in PMa; Mdt-Mat 25-33 μ m, MTR 0.28-0.42.

i.e. as *aberratus*, *cucini*, etc.



Cytology: 4 polytene chromosomes with the thummi arm combination AB, CD, EF, G. Centromeres moderately heterochromatic.

Arm G mostly unpaired, with a virtually terminal nucleolus and a BR (possibly 2 close BRs) near the other end. No nucleoli in the long chromosomes. Arm B with a BR near the 4 characteristic bands. A small distal inversion in arm D heterozygous in one specimen.

- Arm A1: perhaps as *piger* i.e. 1 19?
- Arm B1: Puff (group 7) with distal dark bands near distal end of arm. Possibly by simple inversion from B2 of *C. atrella*; close to *aberratus*.
- Arm C1: Groups 3-4 near middle of arm, possible inversion 3–6.
- Arm D1: Groups 15 16 near middle of arm. Probably as *aberratus*, etc.
- Arm D2: Small inversion in distal third.
- Arm E1: 1 3e, 5 10b, 4 3f, 10c 13
- Arm F1: 1, 5 2, 6 10, 17 11, 18 23. i.e. inv2-5 from *aberratus*, *sororius*, etc.
- Arm G1: Partly unpaired, virtually terminal nucleolus, large BR near other end.



Found: Alberta - Edmonton (53.508°N, -113.486°W); Wood Bison Trail, Elk Island Natl. Park (53.567°N, -112.851°W); Ecole Agnes Davidson ES, Lethbridge (49.672°N, -112.813°W); New Horizons School, Ardrossan (53.558°N, -113.259°W); Spruce View School, Innifail (52.028°N, -113.948°W); nr Jasper Lake, Jasper Natl. Park 53.193°N, -117.954°W) (all BOLD); Huntington Hills, Calgary (51.08°N, -114.08°W).

British Columbia - New Afton Mine, 10 km W Kamloops 50.643°N, -120.517°W) (BOLD).

Manitoba - Churchill (58.771°N, 93.851°W); Grasslands Natl. Park (both BOLD); abt 8 Km nw Theodore (51.75°N, 102.95°W).

Nunavut - Water Lake, Kitikmeot (69.132°N, -105.063°W) (BOLD) Ontario - 4 ml e. Sudbury (44.88°N, 78.27°W).

Saskatchewan - Frenchman River, Grasslands Natl. Park (49.149°N, -107.53°W); Lucky Lake School (50.98°N, -107.135°W); St. Peters School, Unity (52.444°N, -109.153°W); M.C. Knoll Elementary School, Yorkton (51.217°N, -102.433°W); Vanscoy School (52.003°N, -106.977°W); Whitewood School (50.328°N, -102.258°W);Yellow Grass School (49.671°N, -103.864°W); Wapusk Natl. Park (both BOLD); 5 ml. w. Theodore (51.75°N, 102.95°W).

Yukon Territory - Auriol Trail, Kluane Natl. Park (60.714°N, -137.432°W); Lake Laberge (60.958°N, -135.184°W); Nilsson-Lammers Research Forest, n. Whitehorse (60.841°N, -135.208°W) (all BOLD)

A related species occurs at South Dakota - 3 ml. w Yankton, Yankton Co. (abt 42.88°N, - 97.40°W) (see below)

Prairie pool, about 60cm deep, with dark mud and much organic matter and slimy green covering.

Molecular sequences:

mt*COI*: Sequence is available from the known localities, other than the South Dakota variant. There are numerous specimens in the BOLD database.

Although the adult is similar to *C. hyperboreus* amongst North American species, the larvae and cytology are different and the cytology and mt*CO1* sequence suggest a closer relationship to Palearctic species such as *C. aberratus* Keyl 1961 and *C. sororius* Wülker 1973.

Species nr. Sp. 2u.

Known from a single larva collected by Patrick. L. Hudson (it is possible that there is another specimen in the collection of J.E. Sublette, in the Museum of the University of Minnesota).

Fourth instar larva: Larval type not known, as only the head capsule was mounted on the slide. Ventral head length 316 μ m; Mentum (Fig. d, below) of type I (4th laterals only slightly reduced), central tooth not fitting any of usual types because c2 teeth slope towards the c1 tooth perhaps closest to IV(fig. h).

Ventromental plates (Fig. e, below) separated by about 49% of mentum width, about 3.3 times wider than deep and abt 1.3 times the mentum width, with 43-45 striae; VMR perhaps 1.9.

PE (Fig. a, below) with 14 teeth, very worn, possibly type B.

Premandible (Fig. b, below) broader than usual even if relatively worn, with inner tooth about 3 times wider, between types D and E.

Antenna (Fig. c, below) with segment A1 about 2.9 times longer than wide, RO about halfway up from base; AR 2.40; antennal proportions (micron) 125 : 23 : 8 : 11 : 7. Distance between the antennal bases less than that between the S4 setae.

Mandible (Fig. f, below) with third inner tooth separated and darkened (type IIIC); 12 furrows on outer surface near base, 9-10 taeniae in PMa; MTR about 0.33



Cytology: Four relatively short polytene chromosomes with the thummi arm combination AB, CD, EF, G. Centromeres moderately heterochromatic.



i.e. as *aberratus*, *cucini*, etc.

- Arm A1: 1-2c, 10-12, 3, 8-9, 2k-d, 4-6c, 7-6d, 13-19
- Arm B1: Puff (group 7) not developed. Distal end (about bands 1-9) may be as in *piger*.
- Arm C1: Groups 3-4 about middle of arm (may be groups 1-9, from telomere). Possibly as *aberratus*.
- Arm D1: Groups 15 16 about 1/3 from centromere. Perhaps 16-24 to centromere, but possible small inversion at distal end.
- Arm E1: 1 3e, 5 10b, 4 3f, 10c 13
- Arm F1: 1, 5 2, 6 10, 17 11, 18 23. i.e. inv2-5 from *aberratus*, *sororius*, etc.
- Arm G1: Paired at the terminal nucleolus, followed by a constriction and with a large BR relatively close.

Found: South Dakota - 3 ml. w Yankton, Yankton Co. (abt 42.88°N, -97.40°W)

C. nr. *tuxis* (Species u)

For *C. tuxis* Curran, see species 40 (below).

This species is closely related to C. species 2u.

There is no BARCODE sequence matching that of this species currently in the BOLD database.

Adult:

The adults and pupal exuviae of this species are in the Sublette Collection, now in the museum at the University of Minnesota, St. Paul, MN. Only the male terminalia can be given here.

Male:



Male hypopygium (left) and SVo (right) of C. sp. u

About 5 setae within a patch on TIX. SVo closest to E(g)-type of Strenzke (1959); IVo extending almost to end of anal point and 1/3 to 1/2 way along GS which narrows gently over posterior half. Anal point appears narrowed at base.

Pupa: Cephalic tubercles of only specimen relatively small (female?), postero-lateral spur with 1-2 long closely applied spines and possibly a shorter one arising lower down the spur.



Fourth instar larva a medium sized plumosus-type: length 10.5-16.5 mm (single male 10.5 mm; female 14.4 (12.2-16.5) mm). Specimens from Manitoba smaller than those from Alberta and Saskatchewan. TLt about 236 (200-290) μ m; anterior VT 1.64 (1.0-2.38) mm, shorter than the posterior pair 1.75 (1.18-2.12) mm. Anal tubules a single lobe, ventral pair 427 (280-600) μ m, usually longer than dorsal pair 379 (240-455) μ m, and 2-3.5 times longer than wide.

Salivary reservoir 81.7 (73-94) μ m wide and 20.3 (18-23) μ m deep, i.e. 4.09 (3.2-5.3) times wider than deep.

Gula (below) broadly darkened but less in mid line (below) (as in *C. staegeri*) and widest about a third up from posterior margin; FC with a thick dark stripe (also in *C.* species 2u). VHL about 369.5 (368-371) μ m (female) and 325.5 (318-333) μ m (male).



Mentum (c, below) with 4th laterals reduced almost to level of 5th laterals (type I-II) median tooth with c2 teeth quite well separated (type IB in Alberta but maybe type III in Manitoba – differential wear?). Mentum width about 0.58 (0.54-0.62) of VHL.

Salivary reservoir 81.7 (73-94) µm long and 3.7-5.3 times longer than wide.

Ventromental plates (d, below) about 220.8 (205-232) μ m wide, 3.65 (3.3-4.0) times wider than deep and 1.09 (1.02-1.12) times the mentum width; with about 45.9 (41-52) striae; VMR about 0.37 (0.32-0.40); separated by about 0.28-0.4 of mentum width. PE (a, below) with about 12.8 (11-16) relatively broad teeth (i.e. type B).

Premandible with inner tooth relatively broad, 3.5-5 times wider than the outer tooth which narrows to a sharp point, while inner tooth narrows to a broad point (Type C).

Antenna (b, below) with basal segment about 0.33-0.40 of VHL and 2.9 (2.3-3.4) times longer than wide; AR about 1.87 (1.62-2.14); RO from one third to almost a half up from base of segment; antennal segments (micron): 127 : 36.5 : 11 : 13 : 9. Segment A1 sometimes quite wide, but this may be due to being squashed onto slide during mounting. Distance between antennal bases 149.5 (106-170) µm less than that between S4 setae 170.5 (142-192) µm; distance between S4 setae about 87.5 (81-92)% of FC width. S5 setae level or slightly posterior to the nearby RO.

Mandible (e, below) with 3rd inner tooth moderately colored and mostly partially separated (type i-IIB); about 21.1 (18-24) furrows on the outer surface near the base; and 12 (11-14) taeniae in PecM; Mdt-Mat 30.7 (28-35) μ m, MTR 0.35 (0.29-0.44).



Mouth parts of the larva of *Chironomus* nr. *tuxis* a. Pecten epipharyngis; b. Antenna; c. Mentum (showing reduction of 4th laterals); d. Ventromentum; e. Mandible.

Cytology: 4 relatively short polytene chromosomes with the thummi arm combination AB, CD, EF, G. Arm G short and generally paired only at the virtually terminal nucleolus; BR about 5 bands from the other end. No nucleoli in the longer chromosomes. Puff in arm B (groups 7 & 8) near end of arm, with distal dark bands. No inversion polymorphism in the available specimens. The chromosome preparations are not clear because they are quite contracted, but it is possible that many sequences are the same as those of *C*. species 2u, while arm G is much shorter.



Chromosome complement of *C*. nr. *tuxis*. N - Nucleolus; BR - Balbiani Ring.

- Arm A; Possibly as C. sp. 2u.
- Arm B: Likely an inversion of the distal part of the arm.
- Arm C: Possibly as *C*. sp. 2u.
- Arm D: Possibly as *C*. sp. 2u.
- Arm E: Possibly 1 3e, 10b 3f, 10c 13
- Arm F: Possibly as *C*. sp. 2u.
- Arm G does not always show this end to end pairing at the nucleolus

Found in a large pool with mud bottom at depth of about 60 cm.

Found: Alberta - Huntington Hills, Calgary (51.08°N, 114.08°W). Manitoba - 6.7 ml s. Erickson (50.48°N, 99.90°W).

Molecular data:

Mt*COI*: Sequence has been obtained from only 1 larva from Manitoba but, as noted above, there is no matching sequence in BOLD or GenBank.

Chironomus tuxis Curran, 1930 (Species 40)

Adult



Male hypopygium of *Chironomus tuxis* Drawn from type by Townes, 1945 (left) and Sublette 1966 (right)

Male (from Townes 1945; Sublette 1966, Sæther 2012): Wing length 3.4 mm (type 3.46 mm, VR 1.02; fore LR 1.63 (type 1.67); antennal ratio 3.22-3.8 (type 3.92). Body moderately slender.

Frontal tubercles small, clypeus small.

Middle portion of pronotum hardly at all broadened.

Fore tarsus without a beard.

Blackish brown and pruinose, legs brown towards their apices, pale towards the base. 6–8 sensilla chaetica on mTa1, 7 on hTa1.

Acrostichal setae in single staggered row; dorsocentrals in double staggered rows; 5 prealars; 1 supra-alar; scutellars with 14-16 setae strewn in anterior row, 12 erect in posterior row (total 26-28).

	Fe	Ti	Ta1	Ta2	Ta3	Ta4	Ta5	LR	F/T	BR
PI	780	700	1170	580	440	380	160	1.63-167	1.11	absent
PII	780	780	460	260	200	120	80	0.59	1.00	
PIII	900	920	720	410	300	180	90	0.78	0.98	

Leg proportions:

Abdominal segments without the yellowish apical segmental bands seen in *C. riparius*. TIX figured with about 10 setae in a single pale area.

Genitalia similar to those of the *C. decorus*-group, but D(e)-type SVo shorter and stouter. Anal point approximately parallel sided with slightly rounded distal end. IVo reaching almost to end of anal point and midpoint of the GS which reduces relatively gently over the distal third. Setae of IVo shown as simple.

Pupa and Fourth instar larva: Not known

Cytology; Not known

Found: Manitoba - (Oliver *et al.* 1990); Lake Winnipeg (Sæther 2012) Ontario - (Oliver *et al.* 1990) Florida - (Caldwell *et al.* 1997) Georgia - (Caldwell *et al.* 1997) Maine - (Oliver *et al.* 1990) Michigan - (Oliver *et al.* 1990) Minnesota - (Oliver *et al.* 1990) New Jersey - Lakehurst; Riverton (Townes 1945) New York - Tuxedo (Type locality); Hudson; Moorestown; Prattsville; Westchester Co. (all Townes 1945)

Found in lakes (Hudson et al. 1990)

Adult redescribed by Townes (1945), Sæther (2012), and from type by Sublette (1966).

C. hyperboreus sensu Rempel (see C. rempelii, above)

End hyperboreus/aberratus-group

C. longistylus Goetghebuer 1921 (Species 3n)

In BOLD Bin: <u>BOLD:AAI4305</u> Nearest neighbor BIN: <u>BOLD:AAJ3011</u> an unnamed species (Species 5T) from Canada and Greenland.

Adult:

(based on European specimens in Wülker 1991a)

(NB: Langton & Visser note that there are differences between the male (sensu Wülker) and those shown on Goeghebuer's original figures)

Male:

Black, with a dark SVo. Anal point may be less slender than that of *C. tenuistylus*. Wing length 4.22 (4.15-4.30) mm.

AR 3.8-4.5; anterior LR 1.33-1.53; BR 2.41-3.1

Frontal tubercle 30-40 µm in length.

Setae: acrostichal 19-25; dorsocentral 33-44; prealar 7-14; scutellar34-53.

5-14 setae on TIX, possibly in individual pale areas.

The SVo of this form is shorter and more club-shaped than the longer narrower type figured by Goetghebuer (e.g. Fig. 144C of Pinder 1978, below); dark and closest to E(g) type of Strenzke (1959); IVo reaching almost to end of the parallel-sided anal point, or about halfway along the gonostyle, which is moderately swollen and narrows relatively gently over posterior third. Anal point essentially parallel-sided.



Female:

Wülker (1991) Fig. 9 shows the cercus to have a relatively pointed posterior end, with a significant ventral bulge.



Pupa: (based on Palearctic specimens from Langton & Visser, 2003)(probably includes both sexes):

Exuvial length 9.7 mm (7.2(male?)-11.8(female?)) mm. Color golden brown to dark brown, difference in color between thorax and abdomen not marked, outer margin of anal lobes colored except at bases.

Cephalic tubercles 108x110 (female?) – 200x185(male?) µm; frontal setae 30-82 µm long. basal ring of thoracic horn 176×77 (120x60-200x80) µm; HR 2.28 (2.13-2.50). Hook row of segment II entire, occupying 0.47-0.53 of the segment width, with about 68 (male?)-99 (female?) hooks. Armament of tergites not extensive, that of tergites II-VI in form of an undivided, usually extensive patch of strong points, the points in each segment increasing in size posteriorly, the point patches increasing in size from II-V; points of VIII mainly isolated and irregularly arranged. Seta D4 of TVI closer to midline than seta D3. Lateral spur of segment VIII elongate with 1-4 stout apiculate teeth. Anal lobes weakly rounded with 110 (66 (male?)-140 (female?)) fringe taeniae, and 1(2) dorsal taenia.

Fourth instar larva of the plumosus-type but smaller than *C. tenuistylus*. Gular region darkened only at posterior, FC pale, ring organ about 0.3 of the distance from the base of antennal segment 1. The Yamal specimens have a mentum of type II and central tooth of type IIA. The mandible appears to be II-IIIB, with heavy basal flanges.

Cytology: 4 polytene chromosomes with the thummi arm combination AB, CD, EF, G. Centromeres heterochromatic.

Arm G generally unpaired with a nucleolus near the heterochromatic centromere, the other end forming a fan-like structure terminated by dark bands, 2 BRs near the nucleolus. A nucleolus is also present in arm C. Polymorphism known in arms B (Holarctic), D, F & possibly E (Nearctic).

lonA1:	1 - 2c, 10 - 12, 3 - 2d, 9 - 4, 13 - 19	i.e. as holomelas, tenuistylus, etc.						
lonB1:	1 – 8a, 21 - 16?, 8b – 15, 22 – 25i.e. as <i>staegeri</i> , 'tigris'B1(Kiknadze)							
lonC1:	1-6b, 11c-8, 15-11d, 6gh, 17a-16, 7d-a, 6f <i>tenuistylus</i> , etc.	-c, 17b-22 i.e. as <i>aberratus</i> ,						
lonD1:	1 - 3, 11 - 18d, 7 - 4, 10 - 8, 18e - 24	i.e. as tardus, cucini, pilicornis.						
lonE1:	1 - 3e, 5 - 10b, 4 - 3f, 10c - 13	i.e. as <i>tenuistylus</i> , etc.						
lonF1:	1 - 23	i.e. Standard, as <i>piger</i> , sp. 3b, etc.						
Found:	Newfoundland & Labrador - Torngat Mounta 62.7982W) (BOLD)	ins National Park (58.4505N, -						
	Northwest Territories - Nailoicho, Nahanni National Park (61.606°N, -125.758°W)							
	(BOLD)							

Yukon Territory - Ivvavik National Park (69.162°N, -140.155°W); Kluane National Park (60.748°N, -137.513°W) (both BOLD)

Alaska - No name Lake, Haul Road, South Slope.

Also: Yamal Peninsula, Arctic zone of Russia (? same species as in North America, may be *C. riihimakiensis*).

Morphology and cytology described by Wülker (1991a) but the morphology of North American specimens is not known. There is obviously some confusion as to the true *C. longistylus* of Goetghebuer (1921) as specimens with differing mt*COI* sequences are known, and see comment from Langton and Visser 2003), above re. Wülker's adult description.

Chironomus species 5t.

In Bold Bin: BOLD:AAJ3011

Adult:

Male: (Specimen from Greenland). Wing length about 4.3 mm, anterior veins only slightly darkened.

Thorax largely black but probably a pale haltere. Abdomen dark brown with narrow yellow band at posterior margin of tergites IV-VIII, terminalia dark; legs black or dark brown.

Female: (From photograph in BOLD)

Wing length about 3.7 mm., anterior veins only slightly darkened. Basic color a brownish yellow, with black thoracic vittae, postnotum and mesonotum, scutellum brown; abdomen brown with narrow yellowish band at posterior margin of tergites IV-VIII; legs with femurs yellow brown with dark knees, hind tibia also yellow brown, other segments missing.

Pupa, fourth instar larva and cytology: not known.

Found: Manitoba - 12 Km ESE Churchill (58.7541°N, -93.9974°W) (BOLD) Newfoundland and Labrador – Torngat Mountains National Park (58.45°N, -62.8°W) (BOLD) Also Greenland – Narsarsuaq (61.1511°N, -45.3977°W), Kujalleq (BOLD).

C. magnus White & Ramsey, 2015 (Species 2q)

New Name for C. major Wülker & Butler, 1983 (junior homonym)

Currently no identified material in BOLD database

Adult: Morphologically similar to C. plumosus (Epler 2001).

Male – Wing length greater than 7 mm, slightly longer than *C. plumosus*. BR <6.0. Thorax dark brown; abdominal segments entirely light brown without dark bands or other markings.

Hypopygium illustrated by White & Ramsey (2015). SVo about twice as long as in *C. plumosus*. Gonostyle swollen just beyond half way, then narrowing sharply.

Pupa: Not described.

Fourth instar larva of type series of halophilus type, but most other reports refer to salinarius type. Very large species, length from 30 mm to over 55 mm (Epler 2001). Where VT present the anterior pair are 1.04-1.11mm long, generally shorter than the posterior pair which are 1.09-1.11 mm long. Details of AT are available only for the halophilus-type larvae, where they are about 567-784 μ m long (means: dorsal pair 625, ventral pair 641) and about 2.2-3.1 times longer than wide.



Gula (below) darkened on posterior half to three quarters and extending up to margin on either side of the mentum, FC pale. Salivary aperture (Fig. b, below) about 2.18 times longer than wide.



Mentum (Fig. e, below) with 4th laterals reduced at least to level of 5th laterals (type II); central tooth relatively broad with c2 teeth relatively well separated (type IIA). VM (Fig. f, below) about 3.65-4.0 times longer than deep, but only about 0.90-0.96 of the

mentum width and separated by about 0.42 (0.38-0.49) of mentum width, about 48.5 (41-68) striae, VMR 0.31-0.40.

PE (Fig. a, below) with 12.7 (10-15) broad (type B), often somewhat irregular due to wear, teeth. Premandible (Fig. d, below) with broad teeth, inner tooth about 2.8-5 times the width of the outer tooth, probably about equal in length, but outer badly worn in most of the available material.

Distance between antennal bases greater than that between the S4 setae, which are separated by about 0.75 of mentum width at that point.

Antenna (Fig. c, below): Segment 1 relatively long, 2.36-3.78 times longer than wide, RO about 0.45 (0.38-0.47) up from base of segment; A_2/A_1 about 0.18-0.25; AR about 2.46

(2.22-3.01); ratio of segments (µm) 173 : 38: 9 : 14 : 8.

Mandible (Fig. g, below) with third inner tooth often only partly separated and partially colored but may be completely separated and dark (type IIB-IIIC; about 25 (21-31) furrows on outer surface near the base; 11.6 (10-13) taeniae in the PMa.


Cytology: 3 polytene chromosomes with a modified thummi arm combination AB, CD, GEF. Centromeres heterochromatic and may have a large vacuolated region and may form a chromocenter. No nucleolus in arm G, only nucleolus in arm A. Two BRs in arm G region. Arm B sometimes shows a bulb (group 7) with distal dark bands near distal end.

- mgsA1: 1-2c, 10-12, 3-2d, 9-4, 13-19 i.e. as in *holomelas, cucini* and *tardus*.
- mgsB1: Puff and distal dark bands (groups 7-8) near distal end. Differs from tardusB1 by small inversion just distal of middle of arm.
- mgsC1: Differs from neocoraxC1 by small distal inversion.
- mgsD1: Differs from cucD1 by small distal inversion.
- mgsE1: 13a-10c, 3f-4, 10b-5, 3e-1, 13b-g Long inv. from *cingulatus*
- mgsF1: 1-10, 17-11, 18-23 i.e. as in *cucini, tardus* and *tenuistylus*.
- mgsG1 Attached to distal end of arm E, with 2 BRs in middle of the arm.



Salivary gland chromosomes of *C. magnus* (modified from Wülker and Butler 1983. B - Balbiani rings

Found: Alabama - locality not recorded. Georgia - 5 ml w. Athens (33.97°N, -83.50°W), Clarke Co. (Type locality); Lake Sinclair, nr. Milledgeville, Putnam Co.; Lawrenceville, Gwinnett Co.; Lullwater Lake, nr. Atlanta, DeKalb Co. Kentucky - Kentucky Lake, Livingston Co. (Balco et al. 2004). Ohio - Buckey Lake, Perry Co. (Bolton 2012). Oklahoma - Lake Texoma (abt. 33.80°N, 96.55°W), Buncombe Creek. Cove, Marshall Co. Tennessee - Reelfoot Lake, Lake Co. (Epler 2001)

Lakes and ponds in southern U.S.A.

Described by Wülker and Butler (1983) as *Chironomus major* (although a junior homonym), some information on arm F given in Fig. 3 of Martin (1979).

Chironomus pilicornis-group

C. pilicornis (Fabricius 1787) (Species m)

as Tipula pilicornis

Proposed synonyms by Kiknadze et al. (2016): C. conformis, Malloch (1923)(new name C. sanctipauli Sublette 1966, see species 5g); C. dolens, Walker (1856); C. moerens, Walker (1848); C. niveipennis, Fabricius (1805); C. obscurus, Zettersted (1838); C. polaris, Kirby (1824) (also Townes 1959, but usually considered a nomen nudum); C. ridis Zettersted (1838); C. tristis Weidemann (in Meigen, 1818); C. sp. Ya1, Kiknadze et al. (1996).

While possibly all correct, it is also possible that some of these names are the valid

name for some of the species incorrectly called C. pilicornis - e.g. Sp. 5e (see below).

It is possible that the specimens in BOLD Bin: <u>BOLD:ACX5781</u> belong to this species – although none are from North America.

Adult:

North American adults of C. pilicornis were described by Townes (1945).

Male:

AR 6.5. Frontal tubercles rather small, clypeus large.

Wing length 5.5 mm. "Body stout, legs somewhat inflated". Body coal black, legs pale by comparison.

LR 0.95-1.0. Fore tibia with a long dense beard. Legs brown.

Anal point narrow (unusual among dark species). Gonostyle only narrows, sharply, in the distal 1/5 (as in Townes' figure).

Townes (1945) claimed that *C. pilicornis* was unique in the genus in having an LR of about 1. This has since proven to be incorrect, with at least 6 species having a similar LR (*C. pilicornis, C. heteropilicornis, C. jonmartini, C. sanctipauli, C.* sp.2u, *C.* sp.5e). An unfortunate consequence has been that any adult male from the Nearctic with an LR of about 1 has been called *C. pilicornis*. (see also below)

Since it is uncertain that Townes had the correct species, or even just material of a single species, the description of the cytologically characterized Palearctic material from Wülker (1996) is given as well:

AR 4.67-5.87.

Wing length 3.80-4.45 mm. Body black, including setae of thorax and abdomen; only a rectangular or quadratic field at posterior of abdominal segment VII is light; halteres brownish.

Thoracic setae: acrostichal – 0-12; dorsocentral – 37-64; prealar – 8-24; scutellar - 45-70.

LR 0.97-1.11. Foretarsus with long very dense beard: BR 4.0-8.0.



Fig. 3. C. pilicornis, male adult, hypopygium. a) upper parts, dorsal view, b) lower parts, dorsal view. (Wuelker 1996)

About 0-8 setae in group on tergite IX. SVo curved, reaching to the end of the anal point and about halfway up length of IVo, closest to Strenzke's type E(h). Anal point narrowed at base (shared with *C. heteropilicornis*). However the nature of the gonostyle appears to be more uncertain. Wülker's figures show it narrowing sharply over posterior third (similar to Townes figure).

Female: Not described but inferred to be as Palearctic material described in Russian by Rodova (1974):

Length 8 mm. Wing length 6 mm. Dull black with dark-brown halteres. Frontal tubercles with thick pubescence vary in size. Antennae dark-brown, first segment black; proportions (units) 25 : 15 : 16 : 13 : 31; AR about 0.42, A5/A1 about 1.24. Palps brown, third segment slightly longer than fourth.

Pronotum, mesonotum, scutellum and thoracic vittae black.

Thoracic setae – Acrostichals - nil; Dorsocentrals (incl. Humerals) 60-115; Prealar – 20; Supraalar - 2; Scutellar – approx. 100. About 9 Scf on stem vein.

Legs black brown. LR and other leg proportions (micron) given by Wülker (1996): Leg lengths (micron) and proportions:

	Fe	Ti	Ta1	Ta2	Ta3	Ta4	Ta5	LR	F/T	Ta4/Ti
PI	1432	1506	1630	888	543	420	272	1.06	0.95	0.27
PII	1531	1630	914	543	370	272	247	0.56	0.93	0.17
PIII	1852	2099	1481	790	617	321	247	0.71	0.88	0.15



Characters of female head and abdomen from Rodova (1974)

Abdomen black-brown, with light colored setae. Tergites and sternites light-brown along posterior edge; 8th sternite black, with 2 large lighter colored tubercles (GcIX), unevenly covered with setae. Two spermathecae located at the boundary of segments VII and VIII. Cercus with a highly arched dorsal surface, coming to a short curved posterior margin and with a large ventral bulge. The figure indicates about 10 setae on segment X.

Pupa: At least one adult male was reared from an egg mass from Calgary, Alberta (in Sublette collection) and photographs exist of the cephalic tubercles and the spurs of two pupal exuvia. The cephalic tubercles are about 2.5-3.3 times longer than wide and the spurs have 3-4 closely applied fine sharp spines.

Additional information for a Palearctic specimen from Langton and Visser (2003): Length 9.0 mm. Color golden brown to dark brown; contrast between thorax and abdomen not so strongly marked; outer margin of anal lobes colored except at base. Cephalic tubercles (male below, of Palearctic specimen not stated) 175x85 μ m: frontal seta 35 μ m long. Basal thoracic ring 165x85 μ m (HR 1.95). Anterior thorax hardly granulate; dense small granulation begins just behind level of insertion of the thoracic horn and continues to posterior margin of the wing sheath base, rapidly evanescing from there. Hook row II entire with 77 hooks. Armament of tergites reduced, the points in each segment increasing in size posteriorly. Armament of tergites II-VI not strongly waisted and with posterior transverse band not successively increasing in extent, usually reduced on VI. Armament of tergite VI less extensive posteriorly. Posterior points of TIII & IV much larger than the median points on the same tergite. Points of TVIII arranged mainly in distinct short rows of 2 to 5. Conjunctives IV/V and V/Vi armed with points or spinulate points. Spur of segment VIII with 1-4 closely applied, fine elongate teeth (below). Anal lobes weakly rounded, with one taeniate dorsal seta; anal fringe of 86 taeniae.



Fourth instar larva: a medium sized plumosus-type although none of the available specimens were able to be measured. TLt about 260 μ m, VT quite long, anterior pair longer (2.5 mm. cf. posterior 2.3 mm). AT about 506-555 μ m long and 2.8-2.9 times longer than wide. Dark posterior 1/3-1/2 gula (just under region of mentum) and FC slightly dark-dark. Salivary reservoir about 106 x 30 μ m (3.5 times wider than deep).

Mentum (c, below) with pointed teeth, c1 tooth quite broad and parallel sided, c2 teeth well separated and pointed (usually type III but sometimes closer to IB); 4th laterals reduced nearly to level of 5th laterals (type II).

VM plates (d, below) about 3.6-3.7 (one was 5.5) times longer than deep and separated by about 0.26-0.39 of mentum width; about 52 (37–63) striae which reach about halfway to the anterior margin; VMR about 0.36 (0.32–0.41) but increasing to about 0.4-0.42 towards the medial margin for those with lower values.

PE (a, below) with about 14 (12-18) relatively broad teeth (type B), sometimes with smaller teeth at outer edges. Premandible with inner tooth about 2-4 times the width of the outer tooth, both coming to a fine point (possibly type A).

Antenna (b, below) with A1 about 3.3 (2.87-3.97) times longer than wide, RO about a third to a half up from base of segment; AR about 2.20 (1.97-2.46), ave. length of segments (μ m) 165 : 35 : 12 : 15 : 8.

Distance between antennal bases usually less than that between the S4 setae, but sometimes may be slightly larger; S4 setae separated by about 88% of the frontoclypeal width.

Mandible (e, below) with third inner tooth partly to completely separated, and moderately darkened (type II-IIIB); about 20 (14-24) furrows on outer surface near the base; PMa with about 15.4 (14-18) taeniae; Mdt-Mat 40, MTR 0.48.



Wülker (1996) described the larva of Scandinavian specimens as a plumosus-type, about 15 mm in length. Head with diffusely dark clypeus, hind part of gular with a dark oval spot. Lateral tubuli on abdominal segment VII less than 1/5 of the length of the segment.

Cytology: 4 relatively short polytene chromosomes with distinct heterochromatic centromeres. Arm combination is thummi-cytocomplex, AB, CD, EF, G. Arm G closely paired with a constriction near the heterochromatic end, and a dark group of bands near the middle of the arm. No distinct N in G, but N probably sub-terminal as in Palearctic material, probably 2 BR towards the other end of the chromosome. Nucleoli developed in all three larger chromosomes, in arm B (although may not be obvious), 2 proximal in F, 2 in D and a terminal nucleolus is sometimes present in C (see below). In the Palearctic, polymorphism has been reported in arms A, B, and D as well as B chromosomes in some populations (Kiknadze *et al.* 1996b, 2016). In North America, so far, polymorphism has only been reported in arm D in the region of the nucleoli. Kiknadze *et al.* (2016) give a different interpretation of the arm D sequences, with their D2 being closest to the NA sequence.



pilA1: 1 - 3, 12 - 4, 13 - 19 i.e. as *pseudothummi* 1-2d, 3-2e, 20-18, 21-22, 6-8a, 5-4, 8b-13, 17-14, 23-28 pilB1: 1-6b, 11c-8, 15-11d, 6gh, 17a-16, 7d-a, 6f-c, 17b-22 i.e. as *aberratus, tenuistylus* pilC1: pilD1: 1 - 3, 11 - 18d, 7 - 4, 10 - 8, 18e - 24 i.e. as longistylus, tardus, cucini pilD2 (Pal): 1 - 3, 11 - 16, 9 - 4, 10a-e, 17 - 24 (Kiknadze et al., 2016) 1a - 3e, 10b - 3f, 10c - 13g i.e. as in aprilinus, atrella, athalassicus pilE1: pilF1: could be 1 - 6, 12 - 7, 13 - 23 i.e. as Palearctic pilicornis pilG2 (Nearctic): Inversion of distal half compared to Palearctic pilG1.

Found:

Alberta - Rosebud (51.30°N, -112.95°W); Huntington, Calgary (51.08°N, -114.08°W); ¿Waterton Lakes (adult – Townes 1945). Manitoba - 7 ml S of Erickson (50.48°N, -99.90°W); ¿Southern Indian Lake (57.17°N, -98.50°W) (Rosenberg *et al.* 1984). Northwest Territories - ¿Trough Pond, Horton River area (M.G.Butler) Ontario - 4 ml e Sudbury, Sudbury Co. (46.52°N, -80.90°W). Saskatchewan - ¿Oxbow, Saskatoon (Townes 1945). Alaska - ¿Point Barrow (71.39°N, -156.48°W); ¿St Paul Island, Bering Sea (57.18°N, -170.27°W) (*Chironomus sanctipauli*, see species 5g); (both Townes 1945) South Dakota - ¿Emanuel Cr., 2 ml W of Springfield (Sublette, pers. comm.). (some of these localities may refer to related species) ¿Greenland - Nedre Midsomer Sö. Also found in the Palearctic, Type locality: Kiel, Germany.

Prairie sloughs and pools.

The chromosomes have been described for Palearctic material from Europe by Wülker (1996) and from Siberia by Kiknadze *et al.* (1996) (as *C.* species Ya1) and Kiknadze *et al.* (2002, 2004, 2016). The chromosomes of North American specimens are essentially identical in sequence to Palearctic *C. pilicornis* in 5 of the 7 arms. As noted above, present data suggests there may be several species amongst the material considered to be *C. pilicornis* but for which there is no cytological confirmation.

There may be Barcode sequence from Europe to help confirm identity (the specimens identified as *C. heteropilicornis* from Scandinavia may be misidentified *C. pilicornis* (see below). It is possible that *C. pilicornis* (at least the species recognized cytologically) may not have an Arctic distribution.

There is no evidence that the very similar *C. heteropilicornis* occurs in the Nearctic as there is no Nearctic data in the BOLD database or GenBank that matches the BOLD Bin of cytologically identified *C. heteropilicornis* from South Caucasus (Kamokov 2019).

Molecular data:

Mt*COI*: There is BARCODE sequence in the BOLD database that is attributed to North American *C. pilicornis*, however this is almost certainly a misidentification, as the larva shown is a salinarius-type rather than a plumosus-type. The sequence matches species 5e (see below). There is no available sequence from cytologically identified North American specimens of *C. pilicornis*. However, as noted above, there may be specimens from Scandinavia.

Species 5g. *Chironomus sanctipauli* Sublette 1966 New name for *C. conformis* Malloch 1923

Adult:

Original description:

"ô. Deep back, opaque. Antennal plumes fuscous; thorax with slight indications of 3 longitudinal grayish pruinescent lines; abdomen with faint brownish posterior margins to segments; legs fuscous, tibiae and tarsi yellowish; wings slightly brownish, veins distinct, cross vein darkened; halteres yellowish brown; hairs on body black, on legs brownish.

"Hypopygium similar to that of *decorus* Johannsen, the superior process and apical portion of lateral arm as in Plate XIII, Figure 13. Basal joint of fore tarsi very little longer than fore tibia (82: 78), second joint very much longer than third (52:30); fore tarsi and mid and hind legs with very long and rather dense hairs. Radius slightly arcuate apically, ending as far before apex of wing as does media behind it.

"?. Agrees in color with male. Fore tarsi without long hairs; basal joint about as long as fore tibia.

Length, 8-9 mm."

To this may be added:

Holotype male, number 26470, St. Paul Island, Bering Sea, August 16, 1914, E. A. Preble.

Head and thorax dark brown to blackish-brown, abdomen dark brown; legs paler brown. Back of head whitish pollinose. Postocular bristle very dense, multiserial. Antennal segments not clearly discernible on dry mount. Frontal tubercles minute, 0.041 mm long. Palpi four-segmented but distorted so measurements were not made. Clypeus slightly narrower than antennal pedicel.

About fifteen prealar bristles, posteriorly becoming two rows. Dorsomedial bristles in two rows. Dorsolateral bristles multiserial. Pronotum rather strongly projecting anteriorly. Mesonotum with a slight tubercle.

Leg ratios: foreleg, 1.00; middle leg, 0.58; hind leg, 0.65. Beard eight times as long as tarsal diameter; at almost right angles to leg.

Anterior wing veins brown; r-m darker. Venarum ratio, 1.04. Wing length, 5.33 mm. Halteres pale.

Description of C. sanctipauli, including original description as C. conformis, from Sublette (1966).

From these can be derived:

Male: Wing length 5.33 mm; VR 1.04. Frontal tubercles 41 μ m. Clypeus slightly narrower than diameter of antennal pedicle. Mesonotum with slight tubercle. Acrostichal setae in two rows; dorsomedial multiserial; prealar about 15 Leg ratios: Ant 1.0-1.05; Mid 0.58; Hind 0.65. BR 8.0.

Tergite IX with about 8 setae in a subdivided pale patch.



Hypopygium of holotype male (from Sublette 1966)

Anal point moderately wide and parallel sided, closest to fig. i; IVo just longer than the anal point, to about middle of gonostyle and bending outwards at the end; gonostyle only moderately swollen and narrows noticeably over posterior third.

A possible additional male specimen from Alaska was collected by P.L. Hudson and A. Namayanden. Available information from this specimen is: Color dark brown. Wing length 5.20 mm, width 1.1 mm, >40 setae on squama. AR 3.50. About 8 setae on TIX, in individual pale areas; SVo closest to D(e) type of Strenzke (1959), reaching only about halfway along the length of the IVo which has simple setae, but restricted more to the distal end than shown by Sublette (1966); gonostylus moderately swollen, reducing over posterior third and with 5 setae at the tip. Anal point about the same length as the IVo and could be narrowed at the base.



Hypopygium of possible male (from Hudson & Namayanden)

Female: Length 8-9 mm; LR about 1; no beard.

Pupa, Fourth instar larva and Cytology: Unknown

Found: Alaska - St. Paul Island, Bering Sea (57.18°N, -170.27°W) (Type locality); Margaret Lake, near Beaver Pond, Revillagigedo Island (55.33°N, -131.33°W).

Townes (1945) considered *C. conformis* to be a synonym of *C. pilicornis* but, in renaming it, Sublette noted that it differed in significant ways: the anal point is broader, the SVo more conspicuously hooked, the gonostyles more distinctly tapered apically and the ninth tergite with a conspicuous patch of setae, so considered it a new species closely related to *C. pilicornis*. It would also be closely related to Species 5e, but there is no mention of a pale scutellum.

Species 5e. Chironomus sp. 5e

In BOLD BIN: <u>BOLD:AAG5437</u> (some entries incorrectly under the name *C. pilicornis*)

Adult:

Torbjørn Ekrem and Elisabeth Stur (pers. comm.) state that the adult of this species matches the Wülker (1996) description of *C. pilicornis*. In the case of the female this is not quite true, as *C. pilicornis* is described as having a completely black thorax, while the female below clearly has a yellowish scutellum. The chromosomes are also different from those of *C. pilicornis*. The species is therefore another member of the pilicornis-group for which one of the names listed as synonyms of *C. pilicornis* may be the valid name. From photographs in Bin BOLD:AAG5437

Male LR about 1.0.



OIII BOLD GL

About 13 setae, mostly in separate pale spots, central on tergite IX. SVo long and curved, closest to type E(i), reaching at least halfway up the IVo, which is closest to E(i) type of Strenzke (1959). Gonostyle relatively narrow and narrows sharply in distal quarter.

Female



From BOLD BIOUG16470-A05

Blackish species, pale band at posterior of abdominal segments; halteres and scutellum yellow. Wing length about 5.2 mm, width about 1.4 mm; VR about 1.07; anterior veins

moderately darkened.

Legs yellowish, with darkened knees.

Pupa: Not known.

Fourth instar larva:



From BOLD HLC-30527 (labelled as *C. pilicornis*)

A medium sized (12.3-13.2 mm) salinarius-type larva. Dorsal ATs shorter but wider than the ventral pair (480x300 vs. 610x210 µm) (below).



Head capsule generally brownish, gula slightly dark to dark on posterior third to half, FC slightly dark, also slightly dark outside it.

Mentum (Fig. d, below) with 4th laterals slightly reduced (type I-II), although worn in available specimens, the c1 tooth is high, with relatively well separated c2 teeth (i.e. type IB).

Ventromental plates (Fig. e, below) separated by about 0.4 of the mentum width, about 3.36 times longer than deep and 1.03 times the mentum width, with about 46-56 striae; about 7 notches (or teeth?) at the inner margins, VMR about 0.29-0.36.

PE (Fig. a, below) with about 10-12 relatively broad (but worn) teeth, probably type C. Premandible (Fig. c, below) with relatively broad teeth (type D), about equal in length; inner tooth about 2.5-3 times wider than the outer tooth.

Antenna (Fig. b, below) has A1 about 3.5-4 times longer than wide; RO from 0.15-0.36 up from base; AR about 1.75-2.51; antennal proportions (μ m); 125-147 : 32-40 : 9-11 : 13-15 : 6-10.

Distance between antennal bases sometimes up to 21% greater and sometimes about the same as that between the S4 setae; setae separated by about 0.72 of the FC width.

Mandible (Fig, f, below) with 3rd inner tooth partially or completely separated, but only partly darkened (type II-IIIB); about 12-13 furrows on outer surface near the base and about 12-13 taeniae in the PMa.



Larval characters, particularly of the antenna, are quite variable. While nothing is known of the larval habitats, it is possible that this is due to ecological difference which may influence the length of the life cycle, which is known to extend up to 7 years in some high northern species Butler 1982), i.e. there may be members of different cohorts.

Cytology: 4 polytene chromosomes with the thummi-cytocomplex arm combination AB, CD, EF, G.

Centromeres obvious, but not heavily heterochromatic as in *C. pilicornis*, and at least 3 nucleoli - one near the centromere of arm A, one near middle of arm C, a third subterminal in arm G (probably the major one) and probably an additional one subterminal at the other end of the arm.

Possible polymorphism distal in arm D, which also differs by a medial inversion from that of *C. pilicornis*.



Molecular sequence:

Mt*COI*: The sequence of this species is not found in GenBank, but in the BOLD database it matches the specimens incorrectly identified as '*C. pilicornis*'.

The male hypopygium is quite similar to that of *C. islandicus*, but the chromosomes are relatively different (e.g. larger arm G which is paired, and centromeres less heterochromatic).

 Found: Greenland - Nedre Midsomer Sö, Peary Land, sample GP8 (82.63°N, -32.50°W), Zackenberg (74.4701°N, -20.576°W)(BOLD).
 Nunavut - Devon Is. (HLC-30527); Quttinirpaaq Natl. Pk, Ellesmere Is. (81.402°N, -76.873°W)(BIOUG16470-A05) (both from BOLD)

End *pilicornis*-group

Chironomus plumosus-group

C. balatonicus (Species 3q)

Misidentification in Dinsmore & Prepas 1997, p.2171 See *C. annularius* (species 3d)

C. entis Shobanov, 1989 (Species 30)

In BOLD Bin: (<u>BOLD:ADD4190</u>), now in Bin: <u>BOLD:ADM7020</u> as is *Chironomus plumosus*.

Adult essentially similar to *C. plumosus*. Shobanov shows some differences exist in Palearctic specimens, but these have not been confirmed in the Nearctic. No description of the adults or pupae of the Nearctic specimens appears to have been published, so the data for Palearctic specimens from Shobanov (2005) will be used.

Male:

Body length generally larger than *C. plumosus*, but ranges overlap. AR 5.52 (5.09-6.29).

Clypeal setae 75.9 (61-92). Palp segs (3-5) µm: 339 : 375 : 534; P5/P4 1.42, P5/P3 1.42.

Thoracic setae: Dorsocentral 90.3 (77-115); Scutellar 101.4 (79-130). Selected leg measures (mm) and ratios:

	Fe	Ti	Ta1	LR	F/T
Fore	2.07-2.34	2.30-2.54	2.83-3.12	1.15-1.31	0.90-0.92
Mid	2.41-2.66	2.39-2.68	1.39-1.59	0.58-0.59	0.99-1.01
Hind	2.85-3.32	3.00-3.45	2.05-2.37	0.68-0.69	0.95-0.96

About 9-13 setae on TIX, mostly in individual clear spots; SVo possibly closest to E(g) of Strenzke (1959); IVo relatively long, extending just beyond the end of the anal point and about two thirds of the length of the GS, which narrows relatively sharply over posterior third.



From Shobanov (2005)

Female – no information.

Pupa: Based on Palearctic material from Langton and Visser (2003): Length of exuvia 16.3–18 mm. Color golden to golden brown. Cephalic tubercles 220x220-250x250 μ m; frontal setae 126-140 mm long. Basal ring of respiratory horn 300x160-320x160 μ m, i.e. HR=2. Thoracic granulation strong and extensive.

Hook row entire, occupying 0.52-0.56 of the segment width, with 110-115 hooks. Armament of TII-VI not so strongly waisted and with posterior band not successively increasing in extent to TVI, usually reduced on VI. Median point patches of tergites III-V, lack marmoration.

Spur of segment VIII with 10-16 narrow pointed teeth. Fringe of anal lobe 180->250 taeniae.

Fourth instar larva is relatively variable in different areas, possibly due to different ecological conditions. Large (13.7-28 mm; fem. 21.3-28 mm; male 18.3-25.4 mm), generally semireductus-type, anterior VT straight and taper only at end (Butler, unpubl.) The VT (below) are generally shorter (ant. 0.26-0.80 mm; post. 0.17-0.68 mm) than those of *C. plumosus* with the anterior pair longer. The VT also seem to be quite variable in length between localities, with those from Lake Waskesiu, Saskatchewan much shorter (ant. 0.26 μ m; post. 0.17 μ m) than those of other localities for which measurements are available. TLt relatively short (90-220 μ m).

Gula dark-very dark on basal 2/3 and up to ventromentum, with a rough edge; frontoclypeus pale.

Mentum with 4th laterals hardly reduced (type I) and the central tooth with separated c2 teeth, either type IIA or possibly a worn type III.



Short VT and gula region of larva of *C. entis.* (Picture courtesy M.G. Butler) (Courtesy of I. Proulx)

AT well developed, about (759-911 μ m) and 2.5-2.75 times longer than wide.



Anal tubules of C. entis

Pale FC, dark to very dark gular region. Russian workers report differences in head coloration pattern to those of *C. plumosus*, but these have yet to be confirmed in North American material. Salivary reservoir apparently wider (sometimes arched) than that of *C. plumosus*, about 4 (3.56-5.6) times longer than wide.

Mentum (c, below) of type I tending to type II (i.e. 4th laterals reduced slightly but not down to level of 5th laterals); 1st laterals sloping outwards.

Ventromental plates (d, below) about 3.9-4.7 times wider than deep; wider than mentum width about 4.1-4.7 times longer than deep, separated by about 0.33-0.41 of width of mentum, and about 1.06 (1.02-1.15) times wider than the mentum; anterior edge, particularly near the middle, appears rough due to outer hooks (f, below) projecting past the edge; and with about 85-93 striae (lower than Palearctic specimens where the mean is 103) reaching to the anterior margin; VMR 0.24 (0.20-0.26).

Premandibles (b, below) with relatively narrow teeth coming to a narrow sharp point; inner tooth about 1.5 to 4.5 times as wide, and slightly longer. PE (a, below) with about 14 (10-17), often irregular, teeth.

Distance between antennal bases generally greater (288 μ m)than that between the S4 setae (261 μ m), which are separated by about 0.81 (0.79-0.81) of FC width at that point.

Basal segment of antenna (g, below) about 3.25-3.7 times longer than wide; AR about 2.32-3.1; segment lengths (microns) 210; 40; 12; 16; 9.

Mandible (e, below) with third inner tooth well developed and dark (type IIIC), about 29 (24-35) furrows on outer surface near the base; about 15.2 (13-18) taeniae in PMa; Mdt-Mat 44.5 (30-56).



Mouth parts of C. entis

Cytology: 4 polytene relatively short chromosomes with thummi arm combination AB, CD, EF, G. Very similar to *C. plumosus* (species p)

Arm G more commonly partly paired, with a large virtually terminal nucleolus; BR near middle of arm below the nucleolus. Arm A most commonly with A4 sequence. Polymorphism in arms A, D, E and E

rorymorphish	Γ in arms A, D, L and Γ .
h'entA4:	1-2c, 10-12a, 13ba, 4a-c, 2g-d, 9-4d, 2h-3, 12c-b, 13c-19
n'entA11:	1-2c, 10-12, 14f-13, 3-2h, 4d-9, 2d-g, 4c-a, 14g-19
n'entA12:	1-2b, <u>12a-10, 2c</u> , 12bc, 14f-13, 3-2h, 4d-9, 2d-g, 4c-a, 14g-19
n'entA13:	1-2c, 10-12, 14f-13, 3-2h, 4d-9, 2d-g, 4c-a, 14g-i, <u>17d-15</u> , 17e-19
n'entA14:	1-2c, 10-12, 14f-13, 3-2h, 4d-9, 2d-g, 4c-a, 14g-i, 17-15, 18-19
n'entA15:	1-2c, 10ab, 7d-9, 2d-g, 4c-a, 13ab, 12a, 11-10c, 7c-4d, 2h-3, 12cb, 13c-14, 17
	15, 18-19
h'entB1:	BR near distal end of arm.
n'entC3:	1a, 11h-d, 6gh, 17a, 16h-a, 7d-a, 6f-c, 2c, 5-6b, 11c-8, 15-12, 1b-2b, 4-2d,
	17b-22
h'entD1:	1-2d, 15e-16c, 18cd, 8-10a, 13a-12, 18ba, 7-4, 10e-b, 13b-15d, 2e-3, 11a-c,
	16d-17, 18e-24
n'entD4:	1-2d, 15e-16c, 18cd, 8, <u>19-18e, 17-16d, 11c-a, 3-2e, 15d-13b, 10b-e, 4-7,</u>
	<u>18ab, 12-13a, 10a-9</u> , 20-24
h'entE1:	1-2e, 10g-c, 3f-4, 10b-5, 3e-a, 11-13 i.e. as <i>muratensis</i>
(alt. E1):	1-2b, 11b-10c, 3f-4b, 3b-e, 10b-4c, 3a, 2e-c, 11c-13 (see Kiknadze et al.
	1998c)
n'entE5:	1a-g, <u>10c-11b, 2b-1h</u> , 3f-4, 10b-5, 3e-2c, 11c-13 (from alt E1)

n'entEF(E6+F5): simple pericentric inversion of about Egp6 - Fgp7

h'entF1: 1a-d, 6-1e, 7-10, 17-11, 18-23

n'entF4: 1a-d, 6-1e, <u>19-18</u>, <u>11-17</u>, <u>10-7</u>, 20-23 i.e. as *plumosus* F3

- h'entG1: Terminal nucleolus
- n'entG3 Small distal inversion

Found: British Columbia - Near Opposite Crescent, Bechers Prairie, Cariboo and Chilcotin Parklands (Canning);

Manitoba - Winnipeg (P. Chang); Lake Winnipeg (Sæther 2012).

Ontario - Bay of Quinte, Belleville (O. Johannsen); White Lake, Three Mile Bay (48.70°N, 85.67°W) (D.R.Oliver);

Quebec - Lake D'Alembert (48.38°N, 79.03°W), Lake Dasserat (48.28°N,

79.42°W), Lake Marlon(48.27°N, 79.07°W), Lake Opasatica (48.17°N, 79.33°W) and Lake Pelletier (48.22°N, 79.05°W).

Saskatchewan - Lake Waskesiu (53.92°N, 106.08°W), Prince Albert National Park; Crooked Lake, Pasqua Lake, and Round Lake, Qu'Appelle River (all W. Warwick). Colorado - Vega Reservoir (39.23°N, 107.79°W), Mesa Co. (Kiknadze *et al.* 2000) Indiana - Crooked Lake (41.40°N, 85.02°W), Angola Co.; Manitou Lake (41.03°N, 86.11°W), Fulton Co.

Michigan - Saginaw Bay, Lake Huron (43.45°N, 83.67°W), Bay Co.

Minnesota - Lake Itasca (R. Hellenthal), Clearwater Co.; Lake Christina (46.08°N, 95.75°W), Douglas Co., Turtle Lake (46.79°N, 96.28°W) Kiknadze *et al.* 2000). North Dakota - Blacktail Dam (48.26°N, 103.44°W), Williams Co.; Brewer Lake (47.25°N, 97.77°W); Fuller Slough (47.32°N, 97.77°W), and South Golden Lake (47.31°N, 97.50°W), both Steele Co.; Crooked Lake (47.39°N, 100.54°W), McLean Co.; Dead Colt Creek Dam (46.43°N, 97.68°W), Ransom Co.; McVille Dam (47.77°N, 98.17°W), Nelson Co.; Lake Elsie (46.04°N, 96.53°W), Richland Co.; Lake Isabel (46.49°N, 99.40°W), and Lake Williams (47.06°N, 90.17°W), both Kidder Co.; Lake Metigoshe (48.57°N, 100.22°W), Bottineau Co.; Red Willow Lake (47.88°N, 98.40°W), Griggs Co.; Silver Lake (46.05°N, 97.95°W), Sargent Co.; Warsing Dam (47.83°N, 99.12°W), Eddy Co.; Wilson Dam (47.06°N, 99.40°W), Dickey Co.

Oklahoma - Buncombe Creek, Marshall County.

South Dakota - Lake Alice (44.53°N, 96.38°W), Deuel Co. Wisconsin - East Horsehead Lake (45.42°N, 89.37°W), and Pine Lake (45.49°N, 89.55°W), both Onieda Co.; Grand Portage Lake (46.10°N, 90.80°W), Iron Co.; Green Lake (43.72°N, 89.00°W), and Little Green Lake, (43.44°N, 80.59°W), both Green Lake Co.; Lake Kengonsa (42.977°N, 89.205°W), Dane Co.; Pepin Lake (44.26°N, 92.09°W), Pepin Co.; Pleasant Lake (42.47°N, 88.33°W), Walworth Co.; Winnebago Lake 44.01°N, 88.27°W), Calumet Co. (W.Hilsenhoff); Yellow Lake (45.55°N, 92.24°W), Burnett Co.

Many localities from Kiknadze et al. 2000).

Found in lakes, often with C. plumosus.

Cytology described by Kiknadze *et al.* (2000a and b) and arm A revised by Golygina and Kiknadze (2008); larvae described by Shobanov (1989a and b).

Kiknadze *et al.* (1991) describe the outer hooks on the anterior margin of the VM as being longer and sharper than those of *C. plumosus* in Palearctic populations, but does not seem to apply in North America - besides being very difficult to see. Although the VT are generally shorter than those of *C. plumosus*, and where the two species occurred together at Lake Itasca, MN, the two species could be accurately separated on this character, there is considerable overlap and could only be used if the VT were less than 0.4 mm in length. Shobanov (1989b) notes that the basal antennal segment of *C. entis* (abt 210 μ m) is longer than that of *C. plumosus* (abt 167 μ m). The characters of the Palearctic pupae suggest that most characters of the two species broadly overlap, but there are differences in the median point patches of tergites III-V, which lack marmoration in *C. entis*.

C. entis and *C. plumosus* cannot be separated on the basis of the DNA "barcode" sequence of *COI*, or *CytB* (Guryev *et al.* 2001) but can be separated by the sequence of the globin gene gb2ß (Guryev and Blinov 2002).

C. plumosus (Linn. 1745) (Species p)

A Holarctic species.

Doubtful synonym: *C. vancouveri*Michailova and Fischer 1986a (Butler *et al.* 1999) (but see Species 5m)

It might also be noted that Spies (2011) re-examined the available types and found them to be a species of the former subgenus *Camptochironomus*.

In BOLD Bin: <u>BOLD:ADM7020</u> as is *Chironomus entis*.

Adult essentially similar to *C. entis*. Shobanov claims differences exist in Palearctic specimens, but these have not been confirmed in the Nearctic.

Townes (1945) description probably includes *C. entis*, but is likely correct in gross details, given the two species are so similar. No description of the adults or pupae of the Nearctic

specimens appears to have been published subsequent to the identification of *C. entis*, so the data for Palearctic specimens from Shobanov (2005) will be used.

Male:

Wing length abt 5.9 mm (Townes (1945); Body length generally smaller than *C. entis*, but ranges overlap; AR 5.12 (4.79-5.48).

Clypeal setae 33-49. Palp segs (3-5) µm: 289 : 299 : 426.

Thoracic setae: Dorsocentral 51.2 (41-65); Scutellar 57.9 (47-69).

Selected leg measures (mm) and ratios:

	Fe	Ti	Ta1	LR	F/T	Ta4/Ti
Fore	1.73-2.02	1.83-2.07	2.12-2.61	1.16-1.26	0.91-0.94	0.4-0.46
Mid	1.83-2.29	1.93-2.32	1.10-1.29	0.56	0.95-0.99	
Hind	2.29-2.71	2.34-2.76	1.56-1.85	0.67	0.98	

About 13-14 setae on TIX, mostly in individual clear spots; SVo possibly closest to E(i); IVo only to about the end of the anal point and about half-way along the length of the GS which narrows more gently over posterior third to half. A comparison with Shobanov's figures suggests that the illustration of the hypopygium in Townes (1945) (below) is that of *C. plumosus*.



Female:

Townes (1945) simply states: "Similar to male except for the usual sexual differences" However, Johannsen (1926) in his notes on *C. ferrugeovittatus*, an agreed synonym of C. plumosus, notes that the setae on fore Ta1 are much shorter than the diameter of the segment, i.e. BR less than 1.

Specimens from BOLD have wing length 7.3 mm; LR 1.21-1.25.

Rodova (1978) provided some information (in Russian) for European specimens and illustrated some characters (below):



Some characters of European females (Rodova, 1978)

Pupa: (from Langton & Visser (2003) (Palearctic specimens):

Length 16.5 (14.3-19.4) mm. Exuvia golden to golden brown, rarely brown.

Cephalic tubercles $213x177-275x225 \ \mu\text{m}$; frontal setae $87-110 \ \mu\text{m}$. Thoracic base $250x100 - 370-x155 \ \mu\text{m}$.

Hook row of segment II entire, covering 0.54-0.61 of segment width; 63-113 hooks. Armament of TII-VI not strongly waisted, posterior transverse band not increasing in length to TVI; usually reduced on TVI.

Spur of segment VIII with 12-26 elongate teeth; fringe of anal lobe with 211 (82-285) taeniae.

Fourth instar larva are relatively variable in different areas, possibly due to different ecological conditions. Large (13.7-29.9 mm: fem. 27.2-29.9; male 20.3-25.4) semireductus-to plumosus-type; anterior VT often with a flexure in basal half (Butler, unpubl.), and often longer than posterior pair (ant. 0.4-2.07 mm; post. 0.4-1.29 mm), but in New Mexico specimens the VT are longer and the posterior pair are nearly equal or may be longer. TLt variable, from 90-550 μ m in length. Anal tubules quite long (759-911 μ m) and about 2.2-3.0 times longer than wide (ventral pair often narrower than dorsal pair). Gular region dark to very dark, covering most of the region, but often with a slightly

scalloped anterior margin (up and around the outer edges of the mentum), FC pale.



(Picture courtesy of M.G. Butler)

In this larva the ventral tubules are relatively long, showing the inflection of the anterior pair and tendency for posterior pair to coil.

Mentum with pointed teeth and fourth laterals hardly reduced (Type I); c1 tooth relatively narrow, c2 teeth well separated (Type III, or IIA if worn).

VM with a slightly jagged edge, particularly near the center, due to the presence of protruding outer hooks (Shobanov (1989b) notes that these hooks have the shape of an isosceles triangle), striae reaching to margin, VMR difficult to measure because of the striae in the anterior region, 0.23-0.37; separated by about 1/3 of mentum width. PE with about 12-20 broad, normally sharp, teeth (type B).

Antenna with relatively long narrow basal segment, about 3.7 (3.1-4.2) times as long as wide (lower figures, e.g. 2.62, may be obtained where the mandibles have not been dissected off, probably due to increased squashing of the antennae), RO near middle of the segment (0.32-0.54); AR 2.08-3.47; relative lengths of segments (μ m) 167 : 38 : 11 : 15 : 9, but segment 3 may be only as long as segment 5.

Distance between the antennal bases about the same as that between the S4 setae, but can vary in either direction. S4 setae about 238-278 μ m apart, about 0.73-0.84 of FC width. Mandible with third inner tooth well developed and darkened (type IIIC), with about 28-34 furrows near base and 11-14 taeniae in the PMa.



Cytology: 4 relatively short polytene chromosomes with the thummi arm combination AB, CD, EF, G. Banding pattern often unclear.

Arm G usually unpaired, with a large virtually terminal nucleolus - often with a constriction just before the nucleolus; at least one BR near the other end. No nucleoli in other chromosomes but a BR often developed in arm B, mostly near the 4 characteristic bands near the centromere, but sometimes towards the end of the arm due to polymorphism. Arm A generally with sequence n'pluA9, but often with h'pluA2, possibly latitude or depth dependent. Polymorphism in arms A, B, D, and E including pericentric inversion E4/F4. h'pluA2: 1 - 2c, 10 - 12a, 13ba, 4a-c, 2g-d, 9 - 4d, 2h - 3, 12c-b, 13c - 14f, 15a - 14g, 15b -19 1 - 2a, <u>17 - 13c</u>, <u>12bc</u>, <u>3 - 2h</u>, <u>4d - 9</u>, <u>2d-g</u>, <u>4c-a</u>, <u>13ab</u>, <u>12a - 10</u>, <u>2cb</u>, <u>18 - 19</u> n'pluA9: n'pluA10: 1a-e, 3e-a, 2k-h, 4d-9, 2d-g, 4c-a, 13ab, 12a-10a, 2c-a, 1k-f, 3f-i, 12cb. 13c-14f, 15a-14g, 15b-19 n'pluA11: 1-2a, 17-15b, 14g-15a, 14f-13c, 12bc, 3-2h, 4d-9, 2d-g, 4cb, 19d-18, 2bc, 10-12a, 13ba, 4a, 19ef from n'pluA9 h'pluB1: 1 - 4c, 20c - 23a, 20b - 19, 15 - 17, 6d - 4d, 6e - 8b, 19a - 18, 8c-13, u, 23b - 28 BR proximal, near the 4 characteristic bands; puff in group 7 not developed. approx. 1 - 4c, 20c - 23a, 20ba, 13 - 8c, 18 - 19a, 8b - 6e, 4d - 6, 17 - 15, 19a-i, h'pluB2: 23b - 28 BR distal due to large inversion of B1 (heterozygous only in Nearctic) BR proximal, inversion of region 5-10 (heterozygous only) n'pluB4: n'pluB5: BR about middle of arm due to complex inversion (heterozygous only) h'pluC2: 1-2c, 6c-7, 16-17a, 6hg, 11d-12, 4-6b, 11c-8, 15-13, 3-2d, 17b-22 h'pluD2: 1-3, 10b-e, 4-7, 18a-d, 8-10a, 13a-11, 13b-17, 18e-24 n'pluD6: 1-3, 10b-e, 4-7, 18g-e, 17-13b, 11-13a, 10a-8, 18d-a, 19-24 1-3, 10b-e, 4-7, 15d-13b, 11-13a, 10a-8, 18d-a, 15e-17, 18e-24 n'pluD7: 1-3, 10b-e, 4-7, 18ab, <u>11-13a, 10a-8, 18dc</u>, 13b-17, 18e-24 n'pluD8: n'pluD9: 1-3, 10b-e, 4-7, 18ab, <u>9-8, 18dc, 10a, 13a-11, 13b-17, 18e-24</u> n'pluD10: 1a-d, <u>2f - 1e, 2gh, 3a-g</u>, 10b-e, 4 - 7, <u>18a-d, 8a</u> - 10a, 13a - 11a, 13b - 17, 18e -24 n'pluD11: 1-3, 10b-e, 4ab, <u>18ba, 7-4c, 18cd</u>, 8-10a, 13a-11, 13b-17, 18e - 24 1 -3a, <u>4c - 10b, 3e-b</u>, 4b - 3f, 10c - 13 h'pluE1: 1 - 3e, 10b - 3f, 10c - 13 h'pluE2: n'pluE3: 1 - 3e, 10b - 5c, 4e - 5b, 4d - 3f, 10c - 13n'pluE4: 1 - 3c, 4c - 10b, 3ed, 4b - 3f, 10c - 13 [F20d - 23] (Kiknadze *et al.* 2016) h'pluF1: 1a-d, 6 - 1e, 7 - 10b, 18ed, 17 - 11, 18a-c, 10dc, 19 - 23 n'pluF4: 1a-d, 6 - 1e, 7 - 10b, 18ed ,17 - 11, 18a-c, 10dc, 19a - 20c -/- arm E

h'pluG1: as Palearctic specimens

Numerous lakes in Canada and U.S.A.

Found:

Alberta - Amisk Lake (54.50°N, 112.65°W) (Kiknadze *et al.* 2016) and South Baptiste Lake (54.72°N, 113.57°W) both Athabasca Co.
British Columbia - Near Opposite Crescent, Bechers Prairie, Cariboo and Chilcotin Parklands (about 52.13°N, 122.14°W) (Cannings).
Manitoba - Delta Marsh Beach (50.20°N, 98.20°W), Portage la Prairie (Kiknadze *et al.* 2016); Lake Winnipeg (about 52.10°N, 97.25°W) (from figures of Sæther 2012)

Ontario - Arboretum (45.38°N, 75.70°W), Ottawa; Bay of Quinte, Belleville (44.15°N, 77.25°W) (O. Johannsen); Millhaven Bay (44.20°N, 76.75°W) (P. Rueffel); White Lake, 3 Mile Bay (D.R.Oliver); Kelly Lake (46.45°N, 81.07°W), Sudbury (Proulx *et al.* 2013).

Quebec - Lake Saint Augustin (46.75°N, 71.40°W), Quebec City; Lake D'Alembert (48.38°N, 79.03°W), Lake Duprat (48.33°N, 79.12°W), Lake Fortune (48.18°N, 79.32°W), Lake Kinojévis 48.13°N, 78.90°W), Lake Marlon (48.27°N, 79.07°W), Lake Osisko (48.27°N, 79.00°W), Lake Pelletier (48.22°N, 79.05°W), and Lake Rouyn (48.17°N, 78.95°W), all Rouyn-Noranda (Proulx *et al.* 2013). Saskatchewan - Lake Waskesiu (53.92°N, 106.08°W), Prince Albert National Park.

Alabama - Farm pond, Auburn (32.58°N, 85.48°W), Lee Co.

California - Clear Lake; Lake Merced (37.72°N, 122.495°W), San Francisco. Colorado - Crawford Lake (38.40°N, 107.35°W), Delta Co.; Kettering Reservoir (39.57°N, 105.02°W) (1641m), Broomfield Co. and Littleton, Littleton Co. (39.629°N, 105.01°W)(1631 m), Jefferson Co. (Kiknadze *et al.* 2016); Miramonte Lake (37.58°N, 108,20°W), San Miguel Co.; Vega Reservoir (39.13°N, 107.47°W), Mesa Co.

Georgia - Lagos Pond (33.97°N, 83.33°W), Athens, Clarke Co..

Indiana - Crooked Lake (41.68°N, 85.05°W), Angola, Stueben Co.; Crooked Lake (41.44°N, 85.80°W), and Sylvan Lake (41.50°N, 85.35°W), both Noble Co.; Manitou Lake (41.06°N, 86.19°W), Fulton Co.; Shafer Lake (40.83°N, 86.80°W), White Co. Kentucky- Lake, Campbell Co.

Massachusetts - East Longmeadow Pond (42.07°N, 72.51°W) (Kiknadze *et al.* 2016).

Michigan - Saginaw Bay (43.45°N, 83.67°W), Lake Huron, Bay Co. Minnesota - Anderson Lake (47.29°N, 95.25°W) (Kiknadze *et al.* 2016); Lake Itasca (47.23°N, 95.21°W), Clearwater Co. (R. Hellenthal); Lake Christina (46.08°N, 95.75°W), Douglas Co.; Spearhead Lake (47.37°N, 94.96°W), Hubbard Co. (Kiknadze *et al.* 2016).

New Mexico - Eagle Nest Lake (36.55°N, 105.25°W), Colfax Co.; Upper Abbot Lake (36.25°N, 104.33°W), and Lower Abbot Lake (36.25°N, 104.33°W), both Harding Co.

North Dakota - Blacktail Dam (48.26°N, 103.44°W), Williams Co.; Brewers Lake (47.15°N, 97.46°W), Cass Co; Dead Colt Creek Dam (36.26°N, 97.41°W), and McVille Dam (47.77°N, 98.18°W), both Ransom Co. (Kiknadze *et al.* 2016); Fordville Dam (48.18°N, 97.77°W), and Larimore Dam (47.93°N, 97.60°W), both Grand Forks Co. (Kiknadze *et al.* 2016); Fullers Lake (47.19°N, 97.46°W), and South Golden Lake (47.31°N, 97.50°W), both Steele Co. (Kiknadze *et al.* 2016); Red Willow Lake (47.88°N, 98.40°W), Griggs Co.; Silver Lake (46.02°N; 97.57°W), Sargent Co.; Warsing Dam (47.83°N, 99.12°W), Eddy Co.; White Earth Dam (48.45°N, 102.74°W), Mountrail Co.; Williams Lake (47.06°N, 99,40°W), Kidder Co. and Wilson Dam (47.06°N, 99.40°W), Dickey Co. (Kiknadze *et al.* 2016); Ohio - (Bolton 2012)

Oklahoma - University of Oklahoma Biological Station (33.89°N, 96.83°W), Willis, Marshall Co.

South Dakota - Lake Kampeska (44.93°W, 97.21°W), Codington Co.; Burke Lake (43.11°N, 90.17°W), Gregory Co. (Kiknadze *et al.*).

Wisconsin - Allequaush Lake (46.04°N, 89.62°W), Vilas Co. (Kiknadze *et al.* 2016); East Horsehead Lake (45.70°N, 89.62°W), Onieda Co. (W.L.Hilsenhoff); Grand Portage Lake (46.10°N, 90.80°W), Iron Co. (W.L.Hilsenhoff); Green Lake

(43.72°N, 89.00°W), and Little Green Lake (43.44°N, 80.59°W), both Green Lake Co.; Lake Kengonsa (42.977°N, 89.205°W, Mendota Lake (43.08°N, 89.39°W), Lake Wingra (43.05°N, 89.42°W), and Murphy's Creek (43.05°N, 89.42°W), Madison, all Dane Co.; Lake Koshkonong (42.83°N, 89.00°W), Rock Co., Lake Onalaska (43.87°N, 91.31°W), La Crosse Co. (J. Kawatski); Pepin Lake (44.50°N, 92.30°W), Pepin Co.; Pine Lake (45.68°N, 89.40°W), Oneida Co.; Pleasant Lake (42.79°N, 88.55°W), Walworth Co. (W.L.Hilsenhoff); Yellow Lake (45.55°N, 92.24°W), Burnett Co. (J.E. Sublette). Many localities from Butler *et al.* (1998b).

Lakes up to considerable depths (up to 23 m).

One North American population has been described as *C. vancouveri* by Michailova and Fischer (1986), but most have considered it a synonym of *C. plumosus* (Butler *et al.* 1998b, Kiknadze *et al.* 2016). Other workers (e.g. Hilsenhoff and Narf 1968) have suggested the existence of more than one species on ecological grounds. The broad range of some measurements, along with the tendency for extreme specimens for one measurement to be near one or other extreme for other measurements, but not related to north/south distribution, would also be consistent with presence of two forms.

Cytological studies indicate the presence of *C. entis* (see Species 30), separated in part by differences in polymorphism and location of BRs. The two species are often found in the same lake. The form with 2 generations per year in general has better quality chromosomes. The cytology of North American *C. plumosus* has been described by Butler *et al.* (1999, 2000) and arm A revised by Golygina and Kiknadze (2008).

Kiknadze *et al.* (1991) describe the outer hooks on the anterior margin of the VM as being shorter and blunter than those of *C. entis* in Palearctic populations, but does not seem to apply in North America - besides being very difficult to see. Although the VT are generally longer than those of *C. entis*, and where the two species occurred together at Lake Itasca, MN, they could be accurately separated on this character, there is considerable overlap and it can only be reliably used if over 1 mm in length. Shobanov (1989b) notes that the basal antennal segment of *C. plumosus* (abt 167 μ m) is shorter than that of *C. entis* (abt 210 μ m), and available data suggests this is only partly true in North America- the mean is lower but the ranges overlap. The characters of the Palearctic pupae suggest that most characters of the two species broadly overlap, but there are differences in the median point patches of tergites III-V, which lack marmoration in *C. entis*.

Molecular: *C. plumosus* and *C. entis* cannot be separated on the basis of the DNA "barcode" sequence of *COI*, but can be separated by the sequence of the globin gene gb2ß (Guryev and Blinov 2002).

Chironomus vancouveri Michailova & Fischer 1986a (Species 5m)

While this species is generally considered to be a synonym of *C. plumosus* (e.g. Kiknadze *et al.* 2016), there are a number of features that indicate some degree of separation, such that Oliver *et al.* (1990) recognized it as a separate species. It is known only from one small collection and many comparisons to *C. plumosus* are made to Palearctic material rather than Nearctic specimens, which may obscure the true degree of differentiation from nearby populations.

Adult:

Male: Thorax, abdomen and legs light brown, but brownish middle spots on abdomen.

	Fe	Ti	Ta1	Ta2	Ta3	Ta4	Ta5	LR	F/T	Ta5/Ti
PI	2130	2100	2680	1805	1025	885	435	1.27-1.29	1.01-1.03	0.21
PII	2300	2240	1415	885	625	410	305	0.61-0.65	1.01-1.04	
PIII	2665	2745	1845	1160	850	525	345	0.67	0.96-0.98	
BR 1 44										

Leg measurements (micron) and ratios:

Hypopygium with a rounded pale area with about 18 setae. Anal point narrow, downcurved; SVo closest to D(g) type of Strenzke (1959); GS long, relatively narrow and narrowing over posterior third; Ivo slightly out curved and extending to about the end of the anal point.

Female brown. Gonosternite VIII with darkly colored edges and long setae; Gonopophysis VIII with short setae.

Pupa: Length of exuvia 14-14.5 mm. Cephalic tubercles conical, about 191 (179-203) μ m long and 153 μ m wide at base, ending with short seta about 92.5 μ m in length. Basal ring of respiratory horn kidney shaped with two fused tracheolar branches; length 240-242 μ m; width 110-115 μ m.

Tergite I without shagreen; tergite II with several setae and the hook row with about 80 hooks. Tergites II-IV with shagreen covering the whole of the tergite; tergite VII with fine point patches anteriorly and laterally a long seta on each side; on tergite VIII the shagreen is divided into left and right sections with none in the middle. The postero-lateral spur of segment VIII is brown and strong, with 8-10 teeth. The fringe of the anal lobe comprises about 70-80 taeniae.

Larva: About 23-25 mm. long; VT essentially same length; TLt present. Frontoclypeus and other regions of the dorsal head, pale. Gula darkened virtually to base of mentum and extending beyond the width of the mentum, which is 291-300 μ m wide, longer than the VM where both measures are available; VM is 279.1 (257-324) μ m in length and 0.87 the width of the mentum.

Mentum with c1 and c2 teeth well separated (type IIA); fourth laterals do not appear to be reduced (type I). Salivary reservoir shorter but wider than that of *C. plumosus* – about twice as long as wide in *C. vancouveri* compared to 3.8-5.5 times longer in *C. plumosus*.

Antennal segment I about 3 times longer than wide (2.16-3.53), with RO about the middle of the segment (0.39-0.53 up from base of segment); AR about 2.66 (2.47-2.84); relative length of segments (micron) 187 : 40: 8 : 10: 8.

Ventromentum with about 88 striae; VMR 0.25-0.27. PE with about 13-15 moderately wide teeth (type B).

Distance between the antennal bases slightly greater than that between the S4 setae, which are separated by about 0.62 of the frontoclypeus width at that point.

Premandible possibly with outer tooth slightly longer when not worn; inner tooth almost 3 times wider than the outer.

Mandible with all inner teeth dark (type IIIC); distance between Mdt and Mat about 40 μ m; about 17-18 taeniae in PMa.

Cytology: Four polytene chromosomes with the thummi arm combination AB, CD, EF, G, as *C. plumosus*. Nucleolus essentially terminal in arm G, with a BR near the other end of the arm. Major banding sequences appear the same as those found in Nearctic *C. plumosus*, but the major differences come in incomplete pairing of similar sequences in hybrids with Nearctic *C. plumosus* (Michailova & Fischer 1986a & b) and quite different C-banding patterns: *C. plumosus* has C-banding only at the centromeres while *C. vancouveri* has numerous C-banded sites along the length of the chromosomes (Michailova 1994, 2014), but the centromere bands are thinner.

vanA1: 1-2c, 10-12a, 13ba, 4a-c, 2g-d, 9-4d, 2h-3, 12cb, 13c-14, 15b-19 i.e. as h'pluA2

vanB1: not mapped on the Devai et al. system. i.e. as h'pluB1 vanC1: 1 – 2c, 6c-f, 7a-d, 16 – 17a, 6hg, 11d – 15, 8 – 11c, 6b – 2d, 17b – 22 i.e. as h'pluC2 vanD1: 1-3g, 10b-e, 4-7, 18a-d, 8 - 10a, 13a - 11, 13b - 17, 18e - 24 i.e. as h'pluD2 vanE1: 1 – 3e, 10b – 3f, 10c – 13 i.e. as h'pluE2 vanF1: 1a-d, 6 - 1e, 7 - 10, 18ed, 17 - 11, 18a-c, 10dc, 19 - 23 i.e. as h'pluF1 vanG1: essentially as h'pluG1, central region often unpaired, distal bands often unclear.

Although the sequences in *C. vancouveri* are similar to sequences in Nearctic *C. plumosus*, the latter have high frequencies of n'pluA9, which has not been found in *C. vancouveri*. However, h'pluA2 is common in some Nearctic populations of *C. plumosus*, so it is possible that this is a situation similar to that of the species pair *C. riparius* and *C. piger*, although the appearance of the bands is not as markedly different, being generally thinner bands including the centromere in *C. vancouveri*.

In addition to the cytological studies of the hybrids, Michailova & Fischer (1986b) note that hybrids between *C. vancouveri* and Nearctic *C. plumosus* develop at about 90% of the rate of intra-form crosses. The ability to hybridize does not necessarily indicate that they represent a single species, but simply that *C. vancouveri* has not had any opportunity to mate with Palearctic specimens and so has not developed any isolating mechanisms.

Some differences exist between larval characters of *C. vancouveri* and those of *C. plumosus,* notably in the relative lengths of the antennal segments, those of *C. vancouveri* being more similar to those of *C. entis,* but differing from both in that segments 3 and 5 are about the same length. As well, the tip of the dorsal tooth of the mandible of *C. vancouveri* is further from the tip of the apical tooth (Mdt-Mat 40 μ m) than that of *C. plumosus* (Mdt-Mat 30 μ m).

These data raise questions as to the status of *C. vancouveri*. Kiknadze *et al.* (2016) consider it a highly divergent Nearctic population of *C. plumosus*, but this is on the basis that the Palearctic and Nearctic populations of that species are considered a single species, which is really irrelevant to the status of *C. vancouveri*. The differences in pairing of hybrids and the significantly different C-banding patterns indicate that *C. vancouveri* must have had some degree of isolation, and presumably for a significant period of time, from *C. plumosus*, so that the accumulation of heterochromatic regions could evolve. There are no molecular data for this material.

It is unfortunate that the natural distribution of *C. vancouveri* is not known and that no further specimens have been found near Vancouver, raising the possibility that this sample was the result of a chance migration from a source population of unknown location. The status of Vancouver as an international air- and sea-port offers the opportunity for such migration from numerous regions. The finding of other populations could help confirm the status of this 'species'.

Found: British Columbia - Deer Lake (49.24°N, -122.98°W), Burnaby, Vancouver.

End *plumosus*-group

<u>Chironomus prior-tardus-group</u>

C. prior Butler, 1982 (Species 3z)

All information based on Butler (1982) and Wülker & Butler (1983) Adult

> Male: Dark species, AR 4.89 - 5.48 (mean 5.20); frontal tubercles 20 - 40 μ m. Wing length 4.44 (4.04-4.80) mm. Weak or absent scutal tubercle Abdominal segments dark, but often pale on posterior margins. Fore leg with moderate beard, BR 4 - 7. LR₁ 1.16 (1.10 - 1.22); LR₂ 0.60 (0.58 - 0.63); LR₃ 0.69 (0.65 - 0.71). Sensilla chaetica: Leg₂ 8 - 21; Leg₃ 5 - 15 (lower than *C. tardus*; and when taken in conjunction with the higher LR, is useful for separating males of the two species)



Hypopygium and SVo variation of *C. prior* (from Butler 1982)

Anal point relatively narrow; SVo of the D-type; IVo turns slightly outward over distal third; 2 - 17 setae on segment IX. Female: Thoracic color slightly lighter than that of the males, base color light to medium brown, with scutal stripes, postnotum, etc., dark brown. Abdomen similar color to males.

Wing length 4.48 (3.98-4.91) mm. LR₁ 1.17 (1.10-1.24); LR₂ 0.61 (0.60-0.63); LR₃ 0.73 (0.71-0.76).

Pupa not studied in detail, but noted that the hooks on abdominal segment II are sickle shaped, generally with a smooth outer margin. In this regard they differ from those of *C*. *tardus* which have a relatively straight point at quite a sharp angle, and the convex margin often has several small teeth.

Fourth instar larva a salinarius type. Gular region and FC completely brown, with darkening of the rest of the head capsule except for a pale area just posterior to the eye spots. VHL 309 - 342 (mean 325) μ m.; head width 529 - 618 (mean 569) μ m. Head capsule smaller and darker than that of its sibling *C. tardus*.



Cytology: 4 polytene chromosomes with the thummi arm combination: AB, CD, EF, G. According to Butler (1982) there are differences in arms F and G compared to *C. tardus.* pri A1: 1 - 2c, 10 - 12c, 3 - 2d, 9 - 4, 13 - 19 pri E1: 1 - 3e, 5 - 10b, 4 - 3f, 10c - 13 *i.e.* as *tardus* A1 *i.e.* as *tardus*, *islandicus, cucini*, etc.

priF1:

Found: Alaska - Pond J, Barrow; Loon Pond, Prudhoe Bay.

Tundra ponds.

Description and a brief comment on the karyotype in Butler (1982).

This species is the sister species to C. tardus.

C. tardus Butler 1982 (Species 3s)

Information based on Butler (1982) and Wülker & Butler 1983).

Adult

Male: Dark species, AR 5.39 (5.09-5.78); frontal tubercles 20-40 μ m. Weak or absent scutal tubercle Abdominal segments dark, but often pale on posterior margins. Fore leg with moderate beard, BR 4-7. LR₁ 1.01-1.14; LR₂ 0.56-0.64; LR₃ 0.67-0.73.

Sensilla chaetica: Leg₂ 21-46; Leg₃ 15-34 (higher than *C. prior*; and when taken in conjunction with the lower LR, is useful for separating males of the two species)



Hypopygium and SVo variation of *C. tardus* (from Butler 1982)

Segment TIX with 8-31 setae. Anal point relatively narrow; SVo of the D-type; IVo parallel over entire length (unlike *C. prior* where they turn outward over the distal third) and slightly longer than the anal point and about to the midpoint of the gonostyle, which is relatively narrow and narrows only slightly over the posterior third.

Female: Thoracic color slightly lighter than that of the males, base color light to medium brown, with scutal stripes, postnotum, etc., dark brown. Abdomen similar color to males.

Pupa not studied in detail, but noted that the hooks on abdominal segment II have a relatively straight point at quite a sharp angle, and the convex margin often has several small teeth. In this regard they differ from those of *C. prior* which are sickle shaped, generally with a smooth outer margin.

Fourth instar larva a salinarius type. Very dark gular region, dark FC, but rest of head paler. VHL 351 (320-386) μ m; head width 662 (629 - 717) μ m Head capsule larger and paler than that of *C. prior*.



Cytology: 4 polytene chromosomes with the thummi arm combination: AB, CD, EF, G. Centromeres heterochromatin zed. Nucleolus terminal on arm G and proximal on arm D. No polymorphism known.

- tarA1: 1 2c, 10 12, 3 2d, 9 4, 13 19 i.e. as holomelas, cucini, magnus, etc.
- tarB1: similar to *magnus* except for a small inversion about 1/3 from distal end.
- tarC1: may be similar to *neocorax*; inverted *c.f. islandicus* and *cucini* just distal to middle of arm.
- tarD1: 1 3, 11 18d, 7 4, 10 8, 18e 24 i.e. as in *longistylus, cucini,* etc., other than for the nucleolus.
- tarE1: 1 3e, 5 10b, 4 3f, 10 13 i.e. as in *cucini, magnus, tenuistylus, hyperboreus,* etc.
- tarF1: 1 10, 17 11, 18 23 i.e. as in *cucini, magnus, tenuistylus, hyperboreus,* etc.
- tarG1: terminal nucleolus



Found: Alaska - Pond S, Barrow; Loon Pond, Prudhoe Bay (type locality).

Tundra ponds.

Description of larva in Butler (1982) and cytology given by Wülker & Butler (1983).

This species is the sister species to C. prior.

End prior-tardus -group

Chironomus riparius-group

C. riparius Meigen, 1804 (Species y) Syn: C. thummi thummi Meigen – Credland (1973a) C. serus Malloch, 1915 C. cristatus Branch 1923 C. militaris Johannsen 1937

This species is in BOLD Bin: BOLD:AAA7263

Adult



Male: Wing length about 3.7-4.3 mm., width abt. 1.05 mm. AR 3.2–3.6. LR about 1.6-2.95.

Ground color pale to dark brown, thoracic markings red-brown to blackish brown; legs pale green to light brown towards their bases, tarsal segments darker; abdomen brown to blackish, apical 0.25 of each tergite pruinose and pale. Specimens from colder habitats are darker.

Head: Frontal tubercles small 10-30 x 15 μ m (l/w 1.5-2), clypeus rather small (0.66-0.72 of diameter of antennal pedicel) with 34-39 setae. Palpal proportions (micron): 69 : 69 : 129 : 130 : 191 (P5/P4 – 1.47).

Some thoracic setae: Dorsocentral -27-33; Prealar -6-7; Supraalar -1-2; Scutellar - about 24 setae; 9 in 2 rough anterior rows and 15 larger setae in posterior row. Leg lengths (micron) and proportions (1 specimen):

	Fe	Ti	Ta1	Ta2	Ta3	Ta4	Ta5	LR	F/T	BR
PI	1530	1465	2005	1745	825	760	340	2.95	1.04	2.05-2.95
PII	1595	1595	925	530	385	260	185	0.58	1.0	
PIII	1870	1975	1330	810	610	365	220	0.67	0.95	

M sensCh 13-14; H sensCh 12-14



Terminalia of adult male

About 3–10 setae in individual pale spots (which may be closely packed) on tergite IX. The SVo is Strenzke's S(b) type. IVo long, reaching to about middle of gonostyle, with simple setae. Gonostyle only moderately swollen and narrows gently from about midpoint or more rapidly over posterior 2/3. Anal point narrow at base and widening at distal end.

Female: According to Townes (1945), similar to male except for usual sexual differences. About 13 setae on the crescent shaped segment X, which is about 3.3 times longer than the greatest width. Morisch & Wülker (1987) Fig. 1 shows the cercus to have a relatively pointed posterior end, with a significant ventral bulge.



Pupa: (partly from Palearctic material of Langton & Visser, 2003) Length of exuvia 8.1-10.0 mm long; inner margin of wingcase 2.02-2.05mm. Color brown to dark brown.

Cephalic tubercles conical, narrower in males than in females, $95x85-137x107 \mu m$ (Nearctic sizes about the mean of this range, male $106x67 \mu m$); frontal setae $33-37 \mu m$. Lacking frontal warts.

Basal ring 133-180x63-89 µm (Nearctic specimens near largest measures); HR 1.91-2.33. Hook row entire, length row 0.51-0.65 of segment width, 87 (67-97) hooks.

Armament of tergites II-VI in the form of an undivided patch of strong points, the points in each segment increasing in size posteriorly. The point patches increase in size from TII-V, but on TVI the patch is more or less reduced. Armament of TII-VI not strongly waisted and with posterior transverse patch not successively increasing in extent to TVI, usually reduced on VI.



Pupal cephalic tubules, and caudolateral spur of segment VIII

Spur of segment VIII with 1-5 (mostly 3-4) closely appressed spines. Anal lobes weakly rounded; 130 (97-164) (mean 149 in N.A.) taeniae in up to four rows.

Fourth instar larva a medium sized thummi-type, length 10.5-16.5 mm (female 14.78 (12.6-16.5) mm; male 12.1 (10.5-14.4) mm). Gular region dark, FC and other parts of the head capsule darkened. VT variable in length but anterior pair longer than the posterior pair, anterior about 1.42 (0.46-2.21) μ m; posterior 1.53 (0.92-2.21) μ m. TLt lacking. Mentum (c, below) with pointed teeth and of type I; c1 tooth moderately broad with almost parallel sides, c2 teeth well separated, notches almost vertical (Type III); fourth lateral essentially in line with third and fifth laterals.

VM (d, below) with about 43 striae. PE (a, below) with about 13-14 irregular teeth (essentially Ty. A).

Aperture of the salivary reservoir relatively wide, 63-104 μ m long, 18-28 μ m wide, 3.57-4.0 times longer than wide.

Premandible (a, below) with relatively broad teeth, inner tooth about 3-5 times the width of the outer tooth, coming to a relatively broad point (type B2).

Mandible (e, below) with third inner tooth pale and only partially separated (type IIB).


Cytology: 4 polytene chromosomes with the thummi arm combination AB, CD, EF, G. Centromere regions distinctly heterochromatic.

Arm G normally paired with a subterminal nucleolus and generally with 2 BRs near the center, the second followed by a constriction and a dark band. No nucleoli in long arms. Sequences as in the European synonym *C. thummi*. In European populations the MD is located on arm F in the vicinity of groups 1-2 (Hägele 1985, Kraemer & Schmidt 1993), although it could be closer to the centromere at about F11-15, the site found in some individuals of the Australian *C. oppositus* (Martin 2010).

individuals 0	1 uic / tusu
ripA1:	1 - 19
ripB1:	1 - 28
ripC1:	1 - 22
ripD1:	1 - 24
ripE1:	1 - 13
ripF1:	1 - 23

Found: Manitoba s- Southern Indian Lake (Rosenberg *et al.* 1984); Lake Winnipeg (Sæther 2012).

Ontario – Amherstview (Lake Ontario?) (44.22°N, -78.65°W); Etobicoke Creek nr. Brampton (43.68°N, -79.75°W) (Shrivastava & Loughton 1970); Stratford; Windsor; 2.5 ml. S & W Clarence Creek (45.50°N, -75.22°W), Russell Co. Northwest Territories - Smoking Hills (Jernelov 1981) Kansas - Douglas Co. (Townes 1945); Mill Creek, nr. Craig, Johnson Co. Maryland - Baltimore, Baltimore Co. Missouri - Columbia, Boone Co. New York - Adams Center, Jefferson Co. (Branch 1923); Cornell University, Ithaca, Tomkins Co. South Carolina - Liberty, Pickens Co. South Dakota - 1 ml W, 2 m N Yankton, and 3 ml W Yankton, Yankton Co. Tennessee - Oak Ridge (abt 36.00°N, -84.16°W), Anderson Co. Wisconsin - Badfish Creek, Dane Co.; Trout Lake Limnological Station (46.03°N, -89.67°W), Vilas Co. Wyoming - Hawk Springs, Goshen Co. Greenland - (Lindegaard 2015) (but see species 5c and Sp.51). Also: Many European countries and Iran (Raskt).

Creeks and pools, particularly where polluted, and especially where polluted with milk waste.

Molecular sequences:

MtCOI: There are numerous sequences from 10 countries in the BOLD database.

Adult re-described by Townes (1945). Larvae described by Johannsen (1937) as *C. militaris*, and biology and all life stages (including some photographs) described as *C. cristatus* by Branch (1923).

Cytology given by a number of authors in North America (e.g. Poulson and Metz 1938, Blaylock 1971) and in Europe as *C. thummi* Kieffer (e.g. Keyl and Keyl 1959, Devai *et al.* 1989).

Credland (1973b) established a method for maintaining this species in the laboratory.

C. riparius group (Species f)

In BOLD Bin: BOLD: AAM6280

Adult:

Some data on the adults can be obtained from photographs in the BOLD database.

Male - Antennae are not available, fore tarsi are not available, so AR, LR and BR are not known.



Thorax yellow, with vittae, etc., brown. Wing length about 3.35 mm. Femur and tibia yellowish, but with dark knees. Leg proportions (approximate) (micron):

	Fe	Ti	Ta1	Ta2	Ta3	Ta4	Ta5	LR	F/T	BR
PI	1300	1050	-	-	-	-	-	I	1.24	-
PII	1370	1275	-	-	-	-	-	-	1.07	
PIII	1660	1535	-	-	-	-	-	-	1.0	

Abdominal tergites dark brown, with just a narrow distal stripe on segments III-VII.

Female - Antennae are not available, fore tarsi are not available, so AR and LR are not known. Darker than the male, thorax yellowish brown, vittae dark brown. Wing length about 4.7 mm.

Legs with anterior half yellow, then darkening, and tibiae and tarsi brown. Leg proportions (approximate) (micron)

	Fe	Ti	Ta1	Ta2	Ta3	Ta4	Ta5	LR	F/T	BR
PI	2050	1610	-	-	-	-	-	-	1.28	-
PII	2050	1750	-	-	-	-	-	-	1.17	
PIII	2160	2160	-	-	-	-	-	-	1.0	

Abdomen dark brown.

Pupa: Not known.

Fourth instar larva of the plumosus-type, length about 16 mm; VT quite long. Gula slightly darkened, FC pale or slightly darkened.

Mentum (c, below) with teeth pointed, c1 tooth with square sides, c2 teeth well separated (type IV); 4th laterals slightly reduced (type I-II).

VM (d, below) about 3.6 times longer than deep, with about 39-40 striae, VMR about 0.33-0.41. PE (a, below) with about 14 sharp but irregular teeth (type C variant).

Antenna (b, below) with basal segment about 4 times longer than wide; AR about 2.

Mandible (e, below) with third inner tooth pale but separated (type II-IIIA). AT quite long.



Cytology: 4 polytene chromosomes with thummi arm combination AB, CD, EF, G. Arm G mostly paired, although ends may be unpaired, with a median BR and relatively heterochromatic centromere. Nucleoli in arms A and D. BR in arm E. Polymorphism in arm G.

- Arm A1: nucleolus near bands 4-6 making arm hard to identify.
- Arm B1: Bulb, with proximal dark bands (groups 7 & 8), almost terminal.
- Arm C1: Groups 3-4 proximal of center of the arm.
- Arm D1: Nucleolus median in arm.
- Arm E1: 1 3e, 5 10b, 4 3f, 10c 13 i.e. as *aberratus*, bifE1, etc.
- Arm F1: 1a-d, ? ?, 7 9, 2 1e, 16 17, 11 14?, 6 4? 19?, 23 20
- Arm G1: Approximately median BR
- Arm G2: Inversion of most of the length of the arm (see figure below).





DNA analysis:

Mt*COI* – there are numerous sequences in the BOLD database, which are unidentified but belong in BOLD BIN AAM6280.

Found: Alberta - Jasper National Park (from BOLD) British Columbia - Mount Revelstoke National Park (from BOLD) New Brunswick - Grand Bay-Westerfield (from BOLD); Kouchibouguac Natl. Pk.; Gros Morne National Park (from BOLD). Newfoundland & Labrador - Gros Morne National Park; Terra Nova N.P., Newman Sound (48.543°N, -53.978°W)(from BOLD) Nova Scotia - Cape Breton Highland Natl. Pk. (from BOLD); Kejimkujik Natl. Pk. (from BOLD).
Ontario - Clarence Creek, Russell Co. (45.50°N, -75.22°W), Barron Creek, nr. Squirrel Rapids (45.87°N, -77.90°W), Algonquin Provincial Park.
Prince Edward Island - Prince Edward Island Natl. Pk. (from BOLD)
Quebec - King Mt. (45.43°N, -75.73°W), Gatineaus; La Mauricie National Park; Mingan Archipelago National Park Reserve (from BOLD)
Saskatchewan - Prince Albert National Park.

Snow melt pools and other small pools.

Chironomus sp. Julianehåb. (Species 51)

This species has been considered in Greenland to be *C. riparius* but this is not correct. It is known from a single larva.

There is no equivalent BARCODE sequence in the BOLD Database or in GenBank.

Adult and Pupa: Not known.

Fourth instar larva: A melanotus-type with a very small LP ($60\mu m$). Length abt 11.7 mm, VT about 0.72 mm, equal in length, AT with dorsal pair longer (dorsal 400 μm , 4x longer than wide; ventral 340 μm , 2.8x longer than wide).



Gula and FC pale. Aperture of the Salivary reservoir (Fig. b, below) $81x23\mu m$ (l/w 3.6), shorter and wider than that of *C. plumosus* or *C. entis*.

Mentum (Fig. d, below) width 177 μ m about 0.59 of VHL; central tooth type IIA, 4th laterals reduced almost to level of 5th laterals (ty. II); width c1 23 μ m, between c2 33 μ m, c1+2 53 μ m, between 1st lats. 66 μ m.

VM (Fig. e, below) width 192 μ m, about 3.4x wider than deep; 1.09x MW, and separated by about 0.34 of mentum width; VMR abt 0.33-0.38; about 48 striae.

PreM with inner tooth about 4.7x wider than outer tooth, coming to a broad point (type B2). PE (Fig. a, below) with 16 broad, somewhat irregular teeth (type C).

Distance between S4 setae (116 μ m) less than that between antennal bases (132 μ m)and about 0.66 of FC width at that level. S5 setae slightly posterior of RO.

Antenna (Fig. c, below) with A1 about 3.34x longer than wide, about 0.41 of VHL; RO about 0.38-0.43 up from base; AR about 1.73, A2 about 0.29 length of A1; segment proportions (micron): 123 : 36 : 13 : 13 ; 9.

Mandible (Fig. f, below) with 3rd inner tooth relatively pale and only partly separated (type IIA), about 17 furrows on outer surface near base; about 11 taeniae in PMa; MTR about 0.28



Cytology: Polytene chromosomes of only available specimen relatively poor, but 4 chromosomes probably with the thummi-cytocomplex arm combination, although only arms E, F, and G were identified. Arm G with a terminal nucleolus.

Molecular sequence:

mt*COI*: A partial BARCODE sequence has been obtained. It will be submitted to the BOLD Database when more sequence is available.

Found: Greenland – Julianehåb (now Qaqortoq) (60.72°N, -46.03°W).

(see also *pseudothummi*-cytocomplex: sp. e & sp. 2c)

End riparius -group

Chironomus staegeri-group

C. crassicaudatus Malloch, 1915 (Species 2j)

This species is a member of the staegeri-group erected by Wülker et al. (1971).

In BOLD Bin: BOLD:AAP2996

Original description of Malloch (1915):

Male.—Yellowish green, opaque. Head yellow; scape of antennae and base of flagellum yellowish, the remainder fuscous, plumes pale brown; palpi brownish yellow. Mesonotum with grey or brown vittae, the disc with faint whitish pruinescence; sternopleura reddish except on upper margin; postnotum brown. Abdominal segments each with the basal half blackish brown, the dark color usually extending posteriorly on median line, or with a broad brown band on basal half which does not extend to the extreme base. Legs greenish yellow, knees, and apices of tibiae and of the tarsal joints narrowly brown. Wings as in *stigmaterus* (?).

Frontal tubercles large; palpus as in Figure i, Plate XXXII. Hypopygium as in Figure 13, PlateXXXIII; lateral view as in Figure 13, Plate XXXIV. Surface hairs on fore tarsi and mid and hind legs shorter than in *stigmaterus* (?); basal joint of fore tarsi slightly less than one fourth longer than fore tibiae (98:80). Venation as in *stigmaterus* (?). Length, 8 mm.

Female and early stages unknown.

The male of this species is readily separated from any other species of the plumosus group by the form of the hypopygium.

Adult Adult redescribed by Townes (1945), with some additional data by Sublette and Dendy (1957) and Sublette and Sublette (1971).

Male: Wing length - 4.87 (3.77-6.0) mm; VR - 1.03 (1.00-1.05) or 0.97 (0.95-1.00). AR - 4.52 (4.15-4.87); LR - 1.25 (1.11-1.35).

Large and very stout. Ground color light or pale brown, thoracic markings ochraceous brown.

Head - frontal tubercles moderately large - 57 (35-82) μ m; Clypeus of moderate size, clypeal setae - 60 (44-76)

Thoracic setae - dorsocentral - 47 (29-59); prealar -10 (8–13); scutellar 48 (36-62). Legs - pale brown, apices of tibae and of tarsal segments brown. Fore tarsus with short sparse beard, fore LR - 1.28; mid LR - 0.48; hind LR - 0.54. Leg proportions (units):

	Fe	Ti	Ta1	Ta2	Ta3	Ta4	Ta5	LR	F/T
PI	130+	220	290	130	80	85	40	1.23-1.33	?
PII	240	210	110	60	40	30	25	0.42-0.53	1.00-1.14
PIII	290	300	170	90	80	-	-	0.50-0.57	0.97



Male hypopygium of *C. crassicaudatus* (left) and superior volsella (right)

Abdominal tergites each with a central brown transverse band with indefinite edges leading to the appearance of alternate yellow and brown stripes, VI and following segments mostly brown.

Genitalia large and heavy, with SVo essentially an E-type, closest to "i" of Strenzke (1959), but with a blunter, more rounded end. IVo bending inwards towards each other and extending well up to past the mid-point of the gonostyle, which is broad and narrows evenly from about a third of its length.

Female: Townes notes as "similar to male except for the usual sexual differences."

Pupa: Cephalothorax pale yellowish with brown markings, abdomen pale yellowish with brown markings. Cephalic tubercles acutely tipped, subterminal seta about 70 µm long. Length 11.7-14.9 mm (11 mm from Sublette & Dendy, 1957) (Female: about 12.7-13.1 mm, Male: about 11.7-14.9 mm).

About 92-124 recurved spines in complete row at apex of tergite of segment II; caudolateral spur of segment VIII with 12-18 spines (female), 9-19 spines (male) (14-16 from Sublette & Dendy 1957).

Abdominal tergites covered with a variable amount of fine shagreen, incomplete on segs 1 and 7, almost complete covering tergites 2-6 and 8 (Sublette & Dendy 1957).



Abdomen of pupa of *C. crassicaudatus* from Sublette & Sublette (1971)

About 110 taeniae on each side of swim fin (Sublette & Dendy 1957).

Fourth instar larva a large plumosus-type, some characters variable in different regions. Length 21.4 (17.3-22.8) mm. TLt 370 (300-440) μ m; LP – ant. 2.45 (1.84-3.00) mm; post. 2.06 (1.64-2.48) mm; AT 530 (480-640) μ m. Very dark gular region over posterior 2/3, over most of ventral width and higher at edges; pale FC.

Oesophogeal opening about 71-94 μ m long and 2.8-3.4 times longer than wide. Illustrated in figure 30 of Wülker et al. (1971).

Mentum with pointed teeth, c1 tooth broad, c2 teeth well separated and sharp pointed (type III); fourth laterals slightly reduced (type I-ii).

VM about 238-258 µm wide and 3.62-4.48 times wider than deep, about 1.08-1.09 times the mentum width; with finely crenulated anterior margin, median edges sharply downturned; striae reaching about 2/3 towards anterior margin, then replaced by numerous very fine striae to the margin; VMR 0.19-0.28.

PE with 17 (13-20) irregular, conspicuous large and small teeth (ty. D). Premandible with inner tooth about 3-4.5 times wider than the inner tooth; teeth about equal in length and narrowing markedly along their length.

Antenna with basal segment about 3.2 times as long as wide (3.09-3. 50); RO between one third and halfway up from base of the segment; antennal proportions 141 : 30 : 9 : 13; 6. Mandible 260-330 µm long; polymorphic for third inner tooth, which may be type IA or IIB; about 23-26 furrows on the outer surface at the base, Mdt-Mat 25-28 µm, MTR 0.27-0.31; about 16 taeniae in the Pma.

Cytology: 3 polytene chromosomes with a modified thummi arm combination. AB, CD, GEF. Arm G normally paired with a nucleolus near attachment to arm E, with nearby BR and sometimes an additional puff. Nucleolus in arm B near 4 characteristic bands. Polymorphism in arms A, B, C and D; that in arm A appears closely linked to the MD in a Kansas population.

crcA1:	1 - 2c, 10 - 12, 3 - 2d, 9 - 5e, 17d - 13, 4 - 5d, 17e - 19
crcA2:	1a-e, <u>12 - 10d, 13 - 17d, 5e - 9, 2d - 3, 1f - 2c, 10a-c</u> , 4 - 5d, 17e - 19
crcB1:	Puff with distal dark bands (groups 8-7) about $1/3$ from distal end.
crcB2:	Puff with proximal dark bands (groups 7-8), still about $1/3$ from distal end.
crcC1:	
crcC2:	
crcD1:	
crcD2:	
crcE1:	3e - 1, 3f - 10b, 12e - 10c, 12f - 13
crcF1:	1 - 6a, 6d - 9 6b-c, 17 - 11d, 19 - 18, 10 - 11c, 20 - 23
crcG1:	

Molecular data. There is mtCOI sequence in GenBank (e.g. KR085208), as well as specimens in the BOLD database.

The molecular sequence, along with the modified male hypopygium, indicates that this species is less close to the other three species of the staegeri-group.

Found: Manitoba - Lake Winnipeg (Sæther 2012)

Ontario -- Trenton, Ottawa & Point Pelee (41.959°N, -82.518°W) (Townes 1945) Alabama – Auburn (Sublette & Dendy, 1957) Arkansas - Marianna, Lee Parish; White River National Wildlife Refuge, Arkansas (Chordas et al. 2004). Florida - Cannon Lake, Polk Co., Lake Apopka, Orange Co. (Frouz et al. 2002), Miccosuccee Lake; Little Manatee River; Myakka River S.P.; Kissimmee Prairie Preserve S.P. (all BOLD). Georgia - 5 miles w. Athens, & Lagos Pond, Athens . Clarke Co.; Lullwater Lake, DeKalb Co. Illinois - Mahomet, Champaign Co.; Peoria, Peoria Co. (Type), Homer Park, Champaign Co. (Townes 1945) Indiana - Shafer Lake. Iowa - Davenport & Kossuth Co. (Townes 1945) Kansas - Potters Lake (38.95°N, -95.23°W), Lawrence, Douglas Co.; State Park, Leavenworth, Leavenworth Co. (Townes 1945) Louisiana - Cane River Lake, Natchitoches, and Grand River, Grand Ecore, both Natchitoches Pa. (Sublette & Dendy, 1957) Maryland - Fenwick, Charles Co. (Townes 1945) Michigan - Midland Co. (Townes 1945) Minnesota - Hennepin Co. & Traverse Co. (Townes 1945) Nebraska - no locality (Townes 1945) New Jersey - Wildwood (Townes 1945) Ohio - Maumee Bay, Toledo (GenBank KP954640) Oklahoma - Buncome Creek Bay, Lake Texoma, Marshall Co.; Oklahoma City (Townes 1945) Pennsylvania - Philadelphia (Townes 1945) South Dakota – Yankton; Lake Francis Case; and Gavins Point National Fish Hatchery, Yankton Co. Tennessee - Oak Ridge, Anderson Co.; Reelfoot Lake. Texas - Lake Lomalta and Katherine (Paratypes), Brownsville & Lake Refugio at Twin Mott (Townes 1945) Wisconsin - Lake Wingra (43.05°N, 89.42°W), Dane Co.; East Horsehead Lake, Oneida Co.

Lakes and other lentic habitats, where it can be a pest (Frouz *et al.* 2002). To depth of 5.5 metres (Sublette & Dendy, 1957).

Identification: The larva of this species can be recognized by the combination of characters: large plumosus-type; very dark gula but pale frontoclypeus; greater than 80 striae on ventromentum with a crenellated anterior margin. Possibly it may be differentiated by the fine striae in the anterior 1/3 to the margin from *C. staegeri* or *C. frommeri*.

Although placed in the staegeri-group, the species differs more in both morphology and cytology. This suggests that the fusion leading to the reduced chromosome number occurred much earlier than those leading to *C. staegeri* and *C.* 'tigris'.

This species was placed in the subgenus *Camptochironomus* by Beyer (1941), but was not included in that subgenus by later workers. Morphology and cytology described by Wülker *et al.* (1971). Sublette and Sublette (1971) note that the only species that the adult male could be confused with is *C. tentans*, but this should also include *C. pallidivittatus* and *C. vockerothi*, the latter particularly since Rassmussen (1984) queried whether that species might be a hybrid between *C. tentans* and *C. crassicaudatus*.

The species can be bred in the laboratory (Hein and Schmulbach 1971; Frouz et al. 2002).

C. frommeri Sublette & Sublette 1971 (Species 2d)

Syn. *Chironomus* species 2 - Morrow, Bath, and Anderson 1968 *Chironomus* species 2 - Bath and Anderson 1969 Initially attributed to Atchley and Martin 1971, but that use of the name did not constitute a valid description.

This species is a member of the staegeri group erected by Wülker *et al.* (1971). In BOLD Bin: <u>BOLD:AAP3004</u>

However, this bin number is currently shared with C. staegeri and C. "tigris".

Adult: (Information from Sublette & Sublette 1971)

Male - Wing length 3.69-6.22 (5.41) mm; VR 1.00-1.05; AR 4.66 (4.10-5.30); LR 1.24.

Ground color pale yellowish brown; thoracic markings dark brown, abdominal tergites with a dark central spot, particularly on segments 2 to 5.

Frontal tubercles about 44-82 μ m. Clypeus broad, clypeal setae - 48-76. Palpal proportions (segs 2 to 5) - 5 : 16 : 15 : 25.

Thoracic setae - Acrostichals in a staggered row; 28-76 dorsocentrals; 8-14 prealars; 48-78 scutellars.

Legs pale, some progressive darkening of tarsal segments. Fore tarsi with a sparse beard.

Leg proportions:

	Fe	Ti	Ta1	Ta2	Ta3	Ta4	Ta5	LR	F/T	BR
PI	38	39	50	27	17	15	8	1.16-1.35	0.97	4
PII	42	42	23	14	11	7	5	0.54-0.65	1.00	
PIII	50	51	35	21	15	9	6	0.65-0.79	0.98	

Abdominal tergites with darkening as on thorax, essentially saddle shaped on segments II-III, central in segments IV- V. About 9 setae in a single clear area near middle of tergite IX.



Male abdomen (a) & hypopygium of lectotype (b) of C. frommeri

from Sublette & Sublette (1971)

SVo long, of Strenzke's E-type, closest to his figure i, but tending to a knob at the distal end. IVo long, extending beyond end of anal point to about halfway along the GS, which is moderately swollen and narrows only very gently towards the end.

Female – General coloration as male but with dark markings more extensive. Wing length 6.08 mm; VR 1.05.

Antennal proportions 25 : 17 : 20 : 20 : 35. AR 0.43, A5/A1 1.4.

Length frontal tubercles 75 μ m. Clypeus broad, 2.2 times width of antennal pedicel. Palpal proportions (segs. 2-5): 5 : 16 : 15 : 22.

Thoracic setae: Acrostichal in one staggered row; dorsocentral – 70; prealar – abt 9; supra-alar 2;Scutellar – anteriorly abt. 60 in strewn pattern, posterior row about 40. Leg proportions:

	Fe	Ti	Tal	Ta2	Ta3	Ta4	Ta5	LR	F/T	Ta4/Ti
PI	40	40	49	25	16	14	8	1.22	1.0	0.35
PII	45	45	24	13	9	6	6	0.62	1.0	
PIII	53	55	38	21	16	9	6	0.69	0.96	

Cercus with an essentially curved margin, with a large ventral and a small dorsal bulge - that of the paralectotype female looks a little like a helmet on its side.

Pupa: Length 9.19-14.0 mm (Female: about 12.9-14.0 mm, Male: about 9.2-13.3 mm). Cephalothorax brown, abdomen pale yellowish with brown markings.



Pupal abdomen of *Chironomus frommeri* from Sublette and Sublette (1971)

Frontal tubercles short and conical, subterminal seta about 35 μ m long. About 74-129 recurved spines at apex of tergite of segment II; caudolateral spur of segment VIII with 7-11 spines (female), 6-12 spines (male), closely applied. Fringe of anal lobe with about 130 flattened taeniae.

Fourth instar larva: Larval characters quite variable between localities. A large plumosustype (length: female - about 17.8-22.8 mm; male - about 16.2–20 mm); VHL about 290 micron (330-476). VT long, usually anterior pair longer (Female: Ant. 2.52 mm (2.20-3.00), Post. 2.15 mm (1.68-2.48); Male: Ant. 2.31 mm (1.84-2.64), Post. 1.98 mm (1.64-2,32)). TLt about 300-440 μ m long. AT may be short and relatively cone shaped, or may be considerably larger, ventral pair generally longer (520-708 μ m cf. 480-607 μ m), 1.83 (1.3-2.3) times longer than wide.

Dark to very dark posterior 2/3 gular region, slightly higher at edges, pale FC.



Photo courtesy I. Proulx

Mentum with pointed teeth; c1 tooth with parallel sides, c2 teeth well developed (type IIA); 4th laterals slightly reduced (type I-II).

VM plates about 305 (281-375) μ m wide and 4.3-4.7 times wider than deep, 1.04-1.16 times the width of the mentum, and separated by 0.31-0.37 of mentum width, with finely crenulated anterior margin; about 80 (59–110) striae clearly visible about half way to margin, but actually extending almost to margin.



Salivary aperture and Pecten epipharyngis

PE with about 15 (11-20) broad teeth, larger near the center, diminishing in size laterally (type B-C). Premandible with teeth about equal in length, broad inner tooth about 2.7 (2.1-3.4) times wider than the relatively narrow outer tooth.

Salivary aperture 86-124 μ m in length, quite deep and widest at the middle, l/w 1.79-2.93. Antenna with basal segment 2.7–5.5 times longer than wide. At least in the Klamath Lake, Oregon population this character appears to be dimorphic, with some less than 3 times longer than wide (unless an artefact of slide mounting – heavy pressure to flatten the head capsule -, as values less than 3 were only found in this population), others around 4 times longer than wide (4.0–4.3); AR 2.47 (2.32–2.65); antennal proportions 162 : 36 : 8 : 12: 7; A3 shorter than A4, and usually slightly longer than A5.

Distance between antennal bases greater than the distance between S4 setae, which is 0.82-0.87 of the FC width at that point.

Mandible, with small heel, with third tooth relatively distinct, but usually pale (type IIA-IIIB), about 26 (19–35) furrows on the outer surface at the base; distance Mdt-Mat 23-43 and MTR 0.17 (0.10-0.37).

i.e. as aprilinus,

Identification: The larva of this species can be recognized by the combination of characters: large plumosus-type, dark gula and pale FC, about 60-90 striae on VM, which has a crenulated anterior margin. This does not separate it from *C. crassicaudatus* or *C. staegeri*. In general this is not a problem as *C. frommeri* is normally only found in the western U.S.A. except for a small population in Quebec which was probably transferred by aircraft. It can be differentiated from *C. staegeri* by the more extensive darkening of the gular region.

Cytology: 4 polytene chromosomes with the thummi arm combination AB, CD, EF, G. Arm G closely paired with a virtually terminal nucleolus and a central BR; chromosome narrows from BR to nucleolus. Nucleolus also in arm B, just distal to the 4 characteristic bands. Inversion polymorphism in arms C, D and rarely in F.

- fro A1: 1a-e, 7 5e, 3 2d, 9 8, 13 17d, 1f 2c, 10 12?, 4 5d, 17e 19 multiple inversion steps from homA1
- fro B1: Puff with proximal dark bands (groups 7 and 8) at distal end of arm. Nucleolus about group 21.
- fro C1: not clarified, typical group 3-4 about the middle of the arm.
- fro C2: not clarified
- fro D1: not clarified
- fro D2: not clarified
- fro E1: 1 3a, 10b 3f, 10c 13

etc.

- fro F1: 1 6a, 6d 9, 6b-c, 17 10, 18 23 by transp. 6bc from *obtusidens*
- fro F2: 1 5, <u>17, 6c-b, 9 6d, 6a</u>, 16 10, 18 23
- froG1: virtually terminal nucleolus and median BR



Found: Quebec - near Trois-Rivières.

California - Antioch, Contra Costa Co.: 4 ml e. Olive, Los Angeles Co.; O'Neil Forebay and San Luis Reservoir, Merced Co.; Lake Crowley, Bishop, Mono Co., Greenfield, Monterey Co.; 1 ml s. Napa, Napa Co.; Lake Elsinore, Riverside Co. (type locality); Lake Merced, San Francisco Co.; San Mateo Co.; Lake Dalwigk, Vallejo, Solano Co. Oregon - Upper Klamath Lake, 1 ml n. Williamson River (42.47°N, 121.57°W); Smith Loop Road, 7 ml. s. Corvallis (44.50°N, 123.25°W).

Lakes, oxbows and permanent ponds.

The morphology and cytology have been described by Wülker *et al.* (1971). Sublette & Sublette originally designated the species authors as Atchley and Martin, 1971, but Spies (2000) designated a lectotype and changed the author designation.

Molecular

Although in the same BOLD Bin as C. staegeri and C. 'tigris', C. frommeri differs at 7 bases:

Species	# specimens	base no.
		1 4 5 5 5 6 6 9 5 6 7 8 0 4 7 5 1 3 8 9 5
C. frommeri	2	TGGTTTT
C. staegeri C. 'tigris'	7 4	C A T/G C C C C C A T C C C C

C. staegeri Lundbeck 1898

Described from Greenland, but may not be in the U.S.A.

C. staegeri sensu Townes 1945 (Species s)

Synonym: C. fasciventris Malloch 1915

Note: Lindegaard (2015) notes that the larval type of *C. staegeri* Lundbeck 1898 from Greenland is different from that described here (see below). This raises the likelihood that the current species is not *C. staegeri*, in which case it would become *C. fasciventris* Malloch 1915.

Lundbeck's original description provides little information that would enable identification of his species, other than that it is like *C. hyperboreus* but the beard of the foretarsus is shorter. Townes examined a male and a female of the type series and noted that both were very dark, and the superior volsella of the male was shorter than in North American specimens.

The North American species is a member of the staegeri-group erected by Wülker *et al.* (1971).

In BOLD Bin No. <u>BOLD:AAP3004</u> i.e. the same as *C. frommeri* and *C.* "tigris" Adult redescribed by Townes (1945) and also by Sublette and Sublette (1971), who fortunately based their redescription on the Lectotype and Allolectotype of *C. fasciventris*, providing a better confirmation that this name relates to the present species.



Male: Wing length 5.58 (3.86–6.30) mm, width about 0.55 mm; AR 4.70 (3.64–5.83); LR 1.40 (1.30–1.54). Body moderately slender, fore tarsus with a short beard on its outer side, appressed and all in one plane. Ground color light pruinose brown with greenish tinge, with dark brown markings.

Head: frontal tubercles large 53 (30-75) μ m long, clypeus rather large, 48 (26-74) clypeal setae. Palpal proportions (segs. 2-5) 4 ; 18 : 18 : 14 (*C. fasciventris* lectotype) - the segment 5 measurement appears low, but not noted as shriveled, as in female. Measurement of another adult suggests it should be about 36 μ m, i.e. P5/P4 and P5/P3 both about 2.0.

Thoracic setae: acrostichals - not stated; dorsocentrals 37.9 (31-48), in three rows anteriorly; prealar 9.1 (5-12); scutellar 40.3 (32-56) in a strewn pattern.

Wings: VR 1.01 (0.96–1.05)); about 17 setae on squamal fringe.

Legs pale brown with apices of segments darker brown. BR 3.52 (2.5-4.7), mid LR 0.59 (0.55-0.70), hind LR 0.71 (0.67-0.76). 19 sensilla chaetica on pII and 13 sensilla chaetica on pIII

Abdominal tergites I-VI with a submedian transverse dark band occupying 0.3 to 0.7 of the segment, tergite VII and anal segment completely dark. About 9-11 setae in broken patches (1 or 2 setae per patch) in center of tergite IX.



Male hypopygium of the lectotype of *C. fasciventris* Malloch From Sublette & Sublette (1971)

Hypopygium: There is some difference between the figures of the hypopygium in Townes (1945) and Sublette & Sublette (1971), notably in the length and thickness of the anal point which may be attributed to mounting differences. SVo long and curved, ending bluntly, closest to E(i)-type of Strenzke (1959); IVo reaching to about midpoint of the gonostyle which is moderately swollen and narrows gently over the posterior half.

Female (measurements from lecto-allotype of *C. fasciventris*): Coloration generally as in male, but with posterior pollenose bands on abdominal segments; genital lamellae reddish brown, rather small.

Wing length 4. 88 mm. Antennal proportions 22:15:15:30. AR – 0.45; A5/A1 – 1.36. Frontal tubercles 82 μ m. Palpal proportions (segs. 2-5) 7:26:34:30 (last somewhat shriveled).

Mesoscutum with a slight tubercle. About 10 prealar setae. Leg ratios: ant 1.51, mid 0.52, hind 0.65.

Pupal exuviae: Cephalothorax blackish-brown, abdomen pale yellowish brown with darker markings, particularly muscle scars. Frontal tubercles short and conical, subterminal seta about 70 μ m long; no secondary tubercle or frontal warts. Length 10.8 (9.13-13.0) mm (Female: about 10.8 (9.5-13.0) mm; Male: about 10.8 (9.3-13.0) mm. Base of respiratory organ about 250 x 90 μ m, HR about 2.8. Recurved spines at apex of tergite of segment II about 62-100 in males and 72-93 in females; caudolateral spur of segment VIII with 9.1 (7-13) spines (female), 8.1 (6-11) spines (male), closely appressed. Swim fin with about 118-125 taeniae.



Pupal abdomen of C. staegeri (Sublette & Sublette, 1971)

Fourth instar larva a large plumosus-type larva, length 20 mm (fem. 13.5–28 mm; male 15.6-24.2 mm), anterior pair of VT usually shorter (Female: Ant. 3.12 mm (1.60-4.44), Post. 3.37 mm (1.60-4.52); Male: Ant. 2.51 mm (1.60-3.52), Post. 2.78 mm (1.48-3.68)). TLt about 340 (230-580) μ m. Anal tubules relatively long, abt 843 (785-960) μ m, at least 3x longer than wide, ventral pair may be shorter but narrower.

VHL about 413 micron (375-455); width of head capsule greater than 0.85mm (Hilsenhoff & Narf 1968). Gular region dark to very dark on posterior 1/2-2/3 with center of margin slightly lower and widest just above the posterior border, FC pale or slightly darkened.



Gula and frontoclypeus of C. staegeri (photos courtesy I. Proulx)

Salivary aperture relatively wide, 97.4 (83-127) μ m long and 3.0 (2.42-3.78) times longer than wide. Mentum (Fig. c, below) with sharp teeth, but which become rounded with wear; c1 tooth quite broad, c2 teeth well developed and very pointed (type III); fourth laterals slightly reduced (type I-II).

Central part of anterior margin of ventromental plates crenulate (Fig. b, below), a character not seen in Greenland larvae; width about 272 (268-276) μ m, w/d about 4.4 (3.9-4.9); VMR abt. 0.22-0.36, clearer than in *C. tigris*; about 90 (81-98) striae which are clear to about 2/3 to margin, but extend almost to margin. PE (Fig. a, below) with 15.4 (12-20) fine sharp teeth (type A).

Premandible (Fig. a, below) with relatively broad teeth, inner tooth 2.5-4.0 times the width of the outer tooth which narrows markedly along its length, both teeth coming to a sharp point

Antenna (Fig. e, below) with relatively long basal segment compared to total length of antenna, AR 2.1-2.4, and about 3.8 (3.3-4.5) times as long as wide; RO almost half way up from base of segment; ratio of segments (micron) 167 : 35 : 9.5 : 12.5 : 8.

Distance between the antennal bases less than that between the S4 setae, which are separated by about 90% of the FC width at that point.

Third inner tooth of mandible (Fig. d, below) lighter than other teeth (type IIIB) and may be worn or broken – probably reason for the note by Sublette & Sublette (1971) that there may be only 2 inner teeth; about 27.8 (16-31) furrows near the base, about 14.4 (13-16) taeniae in the PMa.



Mouthparts of C. staegeri (Photo 'a' courtesy of I. Proulx)

Identification: The larva of this species can be recognized by the combination of characters: large plumosus-type, dark gula but pale FC, greater than 80 striae on VM, which has a crenulated anterior margin. This does not separate it from *C. crassicaudatus* or *C. frommeri*, but does distinguish it from the larvae of *C. staegeri* in Greenland which is a melanotus-type larva and lacks crenulations on the VM.

Cytology: 3 polytene chromosomes with a modified thummi arm combination AB, CD, GEF.

Arm G region generally unpaired with a large nucleolus broken into 2 parts by a few bands; bands distal to nucleolus often not clear but may contain a BR. Arm B with a nucleolus just distal to the 4 characteristic bands. Inversion polymorphism in all arms except G.

- staA1: 1 2c, 10 12, 3 2d, 9 4, 13 19 i.e. as holomelas AI
- staA2: 1 2c, 10, <u>5e 9, 2d 3, 12 11</u>. 5d 4, 13 19
- staB1: possibly 1 8a, 21 16?, 8b 15, 22 25 i.e. as *longistylus* B1
- staB2: Puff with proximal dark bands (groups 7 8) near distal end of arm.
- staB3: Puff approximately as staB2.
- staB4: Puff with proximal dark bands (groups 7 8) about 1/3 from distal end of arm.
- staC1: 1 6c, 11 8, 15 11, 6gh, 17a 16, 7d-a, 6f-c, 17b 22 i.e. as *aberratus*, *pilicornis, tenuistylus*, etc.
- staC2: 1 5, <u>16 17a, 6hg, 14d 15, 8 11c, 6b 5f</u>, 7d-a, 6f-c, 17b 22
- staD1: 1a-c, <u>16 11, 3 1d</u>, 17 18d, 4 6d, 18e 20, 7 10, 21 24 i.e. derived
- staD2: 1 3, 11 18d, 4 6d, 18e 20, 7 10, 21 24

staE1: 1 - 3e, 5 - 10b, 4 - 3f, 10c - 13 i.e. as cingulatus, plumosus, etc. staE2: 4g-h, 10b - 5, 3e - 1, 4f - 3f, 10c - 13 1 - 6a, 11 - 17, 6c-b, 9 - 6d, 10, 18 - 23 staF1. from *obtusidens* and *frommeri* staF2: 1a-c, 9b-f, 6b-c, 17 - 11, 6a - 1d, 9a - 6d, 10, 18 - 23 1a-c, 9b-f, 6b-c, 17 - 14f, 6d - 9a, 1d - 6a, 11 - 14e, 10, 18 - 23 staF3: i.e. from staF2 1a-c, 9b-f, 6b-c, 17 - 14f, <u>1d - 6a, 11 - 1</u>4e, 9a - 6d, 10, 18 - 23 staF4: i.e. from staF2 staG1: Nucleolus near attachment to arm E.



Molecular data: There are sequences for *COI* (GenBank accession numbers KF278254-KF278268, KF278356) as well as in BOLD database; and *ITS-2* (GenBank accession number HQ656599).

Found: British Columbia - Royal Oak & Victoria (Townes 1945). Labrador - Hopedale (Townes 1945) Manitoba - Southern Indian Lake (Rosenberg *et al.* 1984); Aweme (Townes 1945); 4 km off Grand Rapids, Lake Winnipeg (Sæther 2012). Northwest Territories - Cameron Bay on Great Slave Lake (Townes 1945). Ontario - Copanspin Farm, Dunrobin (45.75°N, 75.87°W); Central Experimental Farm and Hogs Back, Ottawa; and South March, nr Mud Lake (44.88°N, 78.27°W), – all Carleton Co.; Lake Potier, w. Sudbury (47.534°N, 82.065°W); Clarke Lake (45.533°N, 78.271°W) and Costello Lake (45.58°N, 78.33°W), Algonquin Provincial Park, Nipissing Co.; Smokey Falls on Mattagami River (Townes 1945). Quebec - Lake D'Alembert (42.38°N, 79.02°W, Lake Duprat (48.33°N, 79.12°W), Lake Kinojévis (48.13°N, 78.90°W), Lake Opasatica (48.17°N, 79.33°W), all Rowyn-Noranda; Lake St. Joseph (46.88°N, 71.63°W), Quebec City (Proulx et al. 2015). Saskatchewan - Oungre Memorial Park Alabama - Auburn University pond. California - Lake Davis, Plumas Co.; Prosser Reservoir, Nevada Co. Colorado - Franklin Creek Watershed (38.93°N, 104.89°W).(Herrmann et al. 2016). District of Columbia - Washington (Townes 1945) Idaho - Moscow (Townes 1945) Illinois - Dubois (type locality of *C. fasciventris*), 3.5 ml n.e. Mahomet, Champaign Co.; Burlington (Townes 1945) Indiana - Myers Lake; Crooked Lake, Noble Co. Iowa - Davenport & Mt. Pleasant (Townes 1945) Kansas - Lawrence, Douglas Co. (Townes 1945) Louisiana - Many, Sabine Pa.; Mound (Townes 1945) Massachusetts - Lake Pleasant, Franklin Co.; Amherst & Cambridge (Townes 1945) Michigan - Detroit (Townes 1945) Minnesota - Anderson Lake, Clearwater Co.; Bad Axe Lake, Hubbard Co.; Saint Paul (Townes 1945) Missouri - Atherton, Charleston & St. Louis (Townes 1945) New Hampshire - Berlin (Townes 1945) New Jersey - Riverton (Townes 1945) New Mexico - Eagle Nest Lake, Colfax Co.; Hondo Valley and beaver dam on Rio Bonito 3 ml e. Bonito Lake, Lincoln Co. (33.45°N, 105.67°W) New York - Barge canal, nr Knowlesville, Orleans Co.; McLean, Sea Cliff & Syracuse (Townes 1945) North Carolina – Raleigh, Wake Co. (35.77°N, 78.63°W) (Townes 1945) North Dakota - McVille Dam, Nelson Co. Ohio - (Bolton 2012) Pennsylvania - Pottstown (Townes 1945) South Carolina - (Epler 2001) South Dakota - Yankton, Lake Francis Case & Gavins Point National Fish Hatchery, Yankton Co.; Ardmore (Townes 1945). Tennessee - White Oak Creek, Beaver Creek and Clinch River (Mile 20.8), Oak Ridge; 10-Mile Creek, Knox Co. Washington- Port Townsend & Seattle (Townes 1945) Wisconsin - Reeder Farm, Madison (43.08°N, 89,42°W), Dane Co.; Freibauer Lake, Bayfield Co.; East Horsehead Lake, Oneida Co.; Grand Portage Lake, Iron Co. (All of the above would be sites for *C. fasciventris*) Greenland - Egedesminde (now Aasiaat) (68.71°N, 52.87°W) (Type specimens) (Townes 1945) Often in lakes to depth of 20 m, but also present in shallow permanent pools. Martin and Wülker (1971) suggest these may represent separate species. Miller (1941)

noted that the life cycle took 2 years in the deep Costello Lake, Algonquin Provincial Park.

Morphology and cytology have been described by Wülker *et al.* (1971), with additional sequences for arms C and D by Kiknadze *et al.* (2004, 2010), the latter reference also having an updated photographic map. Some morphological characters show considerable variation, which may arise from the different habitats. The mt*COI* sequences do not indicate the

presence of more than one species, but sequence from a nuclear gene is needed to confirm this.

C. 'tigris' Butler and Kiknadze (Species r)

Now a nomen nudum as the name was published by Martin *et al.* (2008) and Kiknadze *et al.* (2016). *C.* sp. Am1 - Kiknadze *et al.* 1993. *C.* species r - Butler *et al.* 1995. This species is a member of the staegeri group erected by Wülker *et al.* (1971). In BOLD Bin No. <u>BOLD:AAP3004</u> i.e. the same as *C. frommeri* and *C. staegeri*.

Male: Derives its name from the yellowish base color and blackish stripes.

Adult:

No detailed description available.

Male hypopygium (left) and superior volsella (right) of C. 'tigris'.

About 3 setae at center of TIX, GS moderately swollen and tapering over posterior third, and SVo closest to D(e)-type of Strenzke (1959); IVo reaching beyond the end of the anal point to about mid-point of the GS.

Female: Wing length abt 5.57 mm, width abt 1.39-1.40 mm, VR abt 0.99, 3-4 SCf on brachiolum; abt 36-53 setae in the squamal fringe.

Antennal segments (µm): 220 (29%) ; 150 (52%) : 140 (50%) : 166 (53%) : 268. AR – 0.39 (0.38-0.400.38; A5/A1 – 1.21-1.22.

Frontal tubercles moderately developed, about 41.75 (35-46) μ m long and 2.3 (1.9-2.7) times longer than wide. Clypeal width about 2.2 times diameter of antennal pedicel; with about 58.5 (50-67) setae. Palpal segments (μ m) 98 : 69 : 267 : 284; 405; P5/P4 1.47, P5/P3 1.52.

Thoracic setae: Acrostichal – abt. 19-24; Humeral – 5-7 in an anterior group, then 3-8 roughly linear; Dorsocentral – 33-38 (Dorsocentral plus humeral 45-48); Prealar – 8.5 (8-9); Supraalar – 1-2; Scutellars with 2 to 3 anterior rows of 6-18 (5 & 13) and posterior row of 11-18 setae (total setae 17-36).

200	8 1911 81	is(pill)	and pro	portion	10.					
	Fe	Ti	Ta1	Ta2	Ta3	Ta4	Ta5	LR	F/T	Ta4/Ti
PI	1900	1820	2760	1360	1020	880	400	1.56	1.00-1.07	0.24
PII	2050	2030	1190	655	470	320	240	0.58-0.60	1.00-1.01	
PIII	2350	2520	1740	960	680	410	270	0.68- 0.70	0.93	

Leg lengths(µm) and proportions:





Setae on segment IX – abt 13-26; segment X - 2.9-3.1 times wider (at widest point) than long, with abt 15-23 setae. Cercus roughly rectangular with a slight basal dorsal lump and a rounded posterior margin.

Pupa: Caudolateral spur of segment VIII with about 5 or 6 spines.



Pupa of *C*. 'tigris' Cephalic tubules (above), spur (below)

Fourth instar larva a large plumosus-type (length, female: 17.6-20.4 mm) with anterior VT longer (female: ant. 1.5-2.4 mm; post. 1.3-2.1 mm). TLt about 130-210 μ m. Gular region completely dark, and FC usually darkened, and occasionally some darkening outside the FC. Salivary reservoir about 2.5-4 (usually less than 3.5) times longer than wide, higher values likely due to squashing during slide mounting.

Mentum with a relatively shallow curve, teeth pointed, 4th laterals reduced often nearly to level of 5th laterals (type I-II); c1 tooth broad with short parallel sides, c2 teeth well developed (type IIA or III). PE with about 11-19 sharp pointed teeth.

VM (d, below) separated by about 0.3-0.36 of mentum width, with about 34 - 46 striae which extend essentially to outer margin; VMR abt 0.06–0.27 depending whether a faint outer ridge can be seen or whether only the edge where the striae end is visible; anterior margin relatively smooth.

Premandible with broad inner tooth at least 3.7-4.4 times wider than the moderately broad outer tooth, teeth about equal in length,

Antenna (b, below) with a polymorphic segment 1, sometime short and broad at the base, other times longer and almost cylindrical, hence the relative length to width of this segment (about 2.6–3.43 times as long as wide) and AR (about 1.93-2.46) are variable; RO abt 0.4-0.6 up from base; antennal segments (μ m) 145 : 34 : 11: 16 : 8.

Distance between the antennal bases at least slightly greater than that between the S4 setae, which are about where the FC begins to widen.

Mandible (e, below) with third inner tooth separated but still relatively pale (type II-IIIB), about 14-18 grooves on outer margin at the base, 10-13 taeniae in Pecten mandibularis.



Identification: The larva can be most easily separated from the other members of the staegeri group by the smooth anterior margin of the ventromentum and by the lower number of striae 34-46 *cf*. 60-80, and the shorter TLt. The FC is also generally darker. The relatively shallow curve and sharp teeth of the mentum will also help to distinguish it from many species.

Cytology: 2 polytene long chromosomes, banding pattern often unclear. Arm combination modified thummi-group: GAB, FEDC. Nucleoli in the region of arm G and arm D, i.e. one in each chromosome. BRs distal to nucleolus in arm G. Centromere of arm E, along with adjacent proximal bands, translocated to arm D, where it forms the centromere of chromosome FEDC. The centromere of the GAB chromosome is at the fusion of arms G and A (Kiknadze *et al.* (2016).

(G) 1 – 2c, 10 - 12, 3 – 2d, 9-4, 13 – 19 (**B28**) 'tig'A1: as holA1 and staA1 'tig'B1: possibly 1 – 8a, 21 - 16?, 8b – 15, 22 - 25 – as longistylusB1 (Kiknadze) 'tig'C1: 1-6c, 11c - 8, 15 – 11d, 6gh, 17a - 16, 7d-a, 6f-c, 17b - 22 (D24g) as abe1 and staC1 'tig'D1: (E1a) 18?, 19-17f, 23ba - 20, E13fg, 1 - 3, 11 - 17c, 8c - 10, 4 - 8b, 23c - 24 'tig'E1: (D18) 1 - 3e, 5 - 10b, 4 - 3f, 10c - 13e, (F23f) i.e. Transposition of 13fg to arm D 'tig'F1: 1a-f, 5c -1g, 5d - 10, 17 - 11, 18 - 23 (E13e) from pigF1 similar to staG1, but attaches at nucleolar end (i.e. next to A1a) and with an extra 'tig'G1: BR.

(Band numbers in bold indicate the bands to which the ends of an arm attaches if not a telomeric band)



(after Kiknadze et al. 1993)

Molecular data: There is mt*COI* sequence in GenBank (KF278239-53), as well as specimens in BOLD, as *Chironomus* sp. Also sequence for gb2ß. This molecular data suggests that *C*. "tigris" should be included in the staegeri-group, although it lacks the crenulated margin of the VM seen in the other members of the group. The *COI* barcode sequence differs from that of *C. staegeri* by only 1-2% (Proulx *et al.* 2013).

Found: Ontario - Clarke Lake, Algonquin Provincial Park Quebec - Lake St. Joseph Minnesota - Lake Itasca, Clearwater Co., Spearhead Lake, Beltrami Co.; Turtle Lake, Becker Co. (46.78°N, 96.17°W) Wisconsin - Friebauer Lake, Bayfield Co.

In thick mud at depths around 6 m in lakes.

Proulx *et al.* (2013) give some key features of the larval morphology, notes on the cytology and relationships of the mt*COI* to that of other species. Cytology is also mentioned in Martin *et al.* (1974) and Martin (1979), described by Kiknadze *et al.* (1993) as *C.* sp. Am1, by Butler *et al.* (1995) as *C.* sp. r, and by Kiknadze *et al.* (2016) as *C. tigris* attributed to Butler & Kiknadze 2003 (which is only an abstract).

Chironomus species 5p.

Adult: No associated adult is known.

Claus Lindegaard (personal communication) has wondered if it could be the larva of the true *C. staegeri* Lundbeck 1898.

Pupa: Not known.

Fourth instar larva: A melanotus-type with a very small lateral tubule ($60\mu m$). Length abt 11.3 mm, VHL 346 μm ; anterior ventral tubule shorter ($0.88\mu m$) than posterior pair ($0.92\mu m$); no lateral projection; anal tubules with ventral pair longer (dorsal 380 μm , 3.5x longer than wide; ventral 420 μm , 3.5x longer than wide.



Gula and frontoclypeus both dark, gula color extending about half way and beyond the width of the mentum. Aperture of epipharynx not visible.

Mentum (Fig. b, below) width 233μ m, about 0.6 of VHL; c1 tooth wide (type IIB), 4th laterals reduced about to level of 5th laterals (ty. II), width c1 25μ m, between c2 teeth 38μ m; of central trifid tooth 56 μ m, and between 1st laterals 81μ m. Ventromentum width same as the mentum; about 3.17x wider than deep and separated from each other by about 0.27 of mentum width; VMR abt 0.26-0.29; about 51-52 striae.

Premandible of type D, with relatively broad teeth, inner tooth about 4x wider than outer tooth, both coming to a broad point. Pecten epipharyngis (Fig a, below) with about 15 broad

but very worn teeth.

Distance between S4 setae, about 74% of width of frontoclypeus, slightly larger than that between antennal bases; S5 setae about level with RO.

Antenna with A1 only about 0.37 of VHL, about 3.45x longer than wide, RO about 0.37-0.44 up from base; A2 about 0.23 length of A1; segment proportions (micron): 144 : 33 : 11 : rest missing.

Mandible (Fig. c below) with 3rd inner tooth relatively pale and at most only partly separated (type I-IIA), about 18-19 furrows on outer surface near the base; about 17 taeniae in PMa; MTR about 0.30.



Found: Greenland – Julianehåb (now Qaqortoq) (60.72°N, 46.03°W).

End staegeri -group

Chironomus tenuistylus-group

C. tenuistylus Brundin, 1949 (Species 3m)

European specimens are in BOLD Bin: <u>BOLD:AAW3994</u> Nearest neighbor BIN in BOLD database is BOLD:AAC0596, which is identified as *Chironomus hyperboreus* (but not Species x of this listing).

Adult:

(based on European specimens in Wülker 1991) Male:
Black, with a dark SVo. Fore tarsi without beard. Anal point may be more slender than that of *C. tenuistylus*. Brundin (1949) showed a slight constriction at the base of the anal point, but this was not seen at other localities.
Wing length 4.58 - 5.5.
AR 3.73 (3.65 - 3.81); anterior LR 1.36 - 1.60; BR 1.55 - 1.8 Frontal tubercle 30 - 40 μm in length. Setae: acrostichal 18; dorsocentral 26 - 30; prealar 5 - 6; scutellar 22. 5 - 6 setae on anal tergite.

Pupa: not known

Fourth instar larva of the plumosus-type, larger than larva of *C. longistylus*. Gular region completely dark, FC with dark stripe and antennal pedicel darkened as in *C. utahensis*, ring organ about 0.4 of the distance from the base of antennal segment 1.



Cytology: 4 polytene chromosomes with the thummi arm combination AB, CD, EF, G. Arm G frequently paired in the constricted region, nucleolus at this end with a large BR near the opposite end but separated from it by dark bands. No nucleoli in other chromosomes. Although polymorphism in arms A and B is known in Europe, no polymorphism has been recorded in North America.

- tstA1: 1-2c, 10-12, 3-2d, 9-4, 13-19 i.e. as holomelas, longistylus, etc.
- tstB1: about 1 3, 19 21, 8a 4, 17 18, 16, 8b 15, 22 25 (based on Wülker 1991a)
- tstC1: 1-6b, 11c-8, 15-11d, 6gh, 17a-16, 7d-a, 6f-c, 17b-22 i.e. as *aberratus, longistylus*, etc.
- tstD1: 1-3, 11a-c, 17-12, 18a-d, 7-4, 10-8, 18g-24.
- tstE1: 1 3e, 5 10b, 4 3f, 10c 13
- tstF1: 1 10, 17 11, 18 23

i.e. as *longistylus*, etc. i.e. as in *cucini*, magnus & tardus.

Found: Wisconsin - Crystal Bog (Lake 27-2), Vilas Co. (UWI.18.1) Type locality - Lake Grimsgöl, Vontjarn, Southern SWEDEN Also Finland and Norway. report from Netherlands needs checking.

Morphology and cytology described by Wülker (1991a).

Chironomus possibly near to C. tenuistylus (Species 4s)

Adult:

Male: At least one male specimen is in the J. E. Sublette collection in the Museum of the University of Minnesota, St. Paul, MN., and a reared male is currently in my collection. This latter male is the basis of the adult and pupal descriptions here. Wing length about 3.8 mm, width 0.84 mm, VR 1.05. AR about 3.7-3.9., LR 1.47. A brownish species.

Head: frontal tubercles small, about 26 x 10 μ m. Palpal proportions (micron): 48 : 51 : 190 : 228 : 300; P5/P4 1.32, P5/P3 1.58. Clypeus almost rectangular in appearance with about 25 setae.

Thorax with dark vittae. Setae: Acrostichal – 20; Dorsocentral – 15-16; Prealar – 5; Scutellar, anterior 9; posterior 13. Leg proportions (micron):

	Fe	Ti	Ta1	Ta2	Ta3	Ta4	Ta5	LR	F/T	BR
PI	1420	1380	2040	1000	820	720	320	1.47	1.02	2.2
PII	1420	1440	820	550	380	270	180	0.57	0.99	
PIII	1640	1780	1320	820	560	340	200	0.74	0.92	

No sensilla chaetica seen on mid Ta1, only 2 on hind Ta1 Note mid tibia is as long, or slightly longer than the femur Most setae on fore Ta1 about 2.2 times diameter of Ta1, with a few longer.



Hypopygium of C. sp. 4s.

Abdominal tergites with brownish black band covering at least the anterior 2/3 of segment, whitish in posterior region.

TIX with about 9 setae apparently in individual spots.

The anal point is relatively fine, gonostyles slender, tapering over posterior half, and the superior volsella is most similar to D(e)-type of Strenzke (1959), but is not dark as in *C. tenuistylus*. IVo reaching about to end of anal point and the middle of the gonostylus.

Pupa: - from a male exuvia: Length about 8.7 mm; inner margin of wing case about 1.8 mm. Cephalic tubercles about 105 μ m long, 78 μ m wide at base, with a subbasal seta about 38 μ m long.

Abdomen with a central patch of shagreen on segments II–V, grading from posterior 2/3 on seg. II, to whole length of segment on segment V; on segment VI the shagreen is restricted to the anterior third. Pedes spurii B and A are as usual for the genus. Numbers of L-setae on segments II-IV also as usual, the posterior two on segment IV arising very close to each

other.

Hook row on segment II with about 78 hooks, occupying about 40% of width of segment. Caudolateral spur of segment VIII with 1 or 2 spines.

About 72 taeniae on each side of anal lobe, in two rows posteriorly.

Fourth instar larva: A small plumosus-type larva, length about 8.8-11.2 mm (fem. 11.0-11.2 mm; males 8.8-9.7 mm); TLt (200-280µm), ventral tubules with anterior pair usually longer (ant. 0.84–1.16 mm; post. 0.80-1.16 mm (post.). Anal tubules relatively long (3.2- 4.6 times longer than wide), posterior pair usually longer and thinner (3.7–4.6).

Gula slightly darkened on posterior third, FC pale to dark, often with a stripe outside the margin.

Mentum (Fig. c) with sharp teeth; 4th lateral slightly reduced compared to the 3rd and 5th laterals (type I-II); central trifid teeth tall and linear in outline, with the c2 teeth well developed (type III).

Ventromental plates (Fig. e) separated by about 0.2-0.35 of mentum width, with about 39-40 striae which extend at least 2/3 to anterior margin; VMR 0.32-0.35.

PE (Fig. b) with about 11 or 12 sharp teeth. Premandible (Fig. a) of type B2 with inner tooth at least 4.5-5 x the width of the outer, coming to fine points.

Mandible (Fig. f) with teeth relatively pale, 3rd inner tooth sometimes pale and hardly developed, but in others partly to well developed and grading in coloration (type IA-IIIC). Antenna (Fig. d) with basal segment relatively long, and slightly curved, about 3.7-4.3 times longer than wide, and 3.3-3.7 times longer than segment A2; RO between 0.33 and 0.5 up from base of segment; AR about 1.8-1.65; segment proportions (micron): 129 : 36 : 10 : 13 : 8.

Distance between the antennal bases greater than the distance between the S4 setae on the frontoclypeus.



Distance between the antennal bases greater than the distance between the S4 setae on the frontoclypeus.

Cytology: 4 polytene chromosomes with the thummi-cytocomplex arm combination AB, CD, EF, G.

Nucleolus only in arm G, virtually terminal, but a heterochromatic cap visible in some specimens; two Balbiani rings, one distal, the other just distal of middle of the arm. Another BR, or a puff, proximal in arm B.

Centromeres not heterochromatic. Polymorphism at least in arm F, and F2 may carry the MD gene.

Arm A1: 1 - 2c; 10 - 12, 3 - 2d; 9 - 4; 13 - 19 i.e. as holomelas, tenuistylus, longistylus, etc.

Arm B1: BR ring or puff just distal to characteristic bands (25-27), which are in the usual position near the centromere.

Arm E1: 1 - 3e, 5 - 10b, 4 - 3f; 10c - 13

i.e. as *tenuistylus*, etc.

- Arm F1: possibly Standard, i.e. 1 23, if so, as *longistylus*, etc. Homozygous in females.
- Arm F2: simple inversion of at least middle half of the arm, possibly In17-11, i.e. as *tenuistylus*. Heterozygous in males.
- Arm G1: middle of arm rearranged compared with C. tenuistylus.



Found: Wisconsin - Why Not Bog Lake, County Road N, Vilas Co (46.02°N; -89.62°W).

All known specimens reared from two egg masses at the edge of a bog lake.

Although the adult appears similar to C. tenuistylus, the cytology makes it clear that this is a different, but probably related species. Arms A, E, and F have similar sequences, but arms B, C, D, and G show differences that cannot be explained by simple inversions.

End tenuistylus group -group

Ungrouped species

C. stigmaterus Say 1823 (Species n)

Tendipes (Tendipes) stigmaterus – Townes 1945

Texas, New Mexico populations are in BOLD Bin No. <u>BOLD:AEC0831</u> California, Arizona populations are in BOLD Bin No. <u>BOLD:AAW3998</u> The nearest neighbor Bin to both Bins is <u>BOLD:AAN5315</u> Species in the nearest neighbor Bin are identified only as *Chironomus*.

Some of the available data suggests that there are two species under this name – one in New Mexico and Texas (and Florida and the type locality??), the other in California and Arizona. However further data from other parts of the relatively broad distribution are needed to clarify the true situation.

Male: Wing 4.1 mm. long; leg ratio 1.45; antennal ratio 4.8; body of medium build; frontal tubercles large; clypeus small; middle portion of pronotum very strongly and abruptly widened; fore tarsus with a long dense beard. Light brown with a strong cinereous pruinosity. Flagellum and mouthparts brown; lighter portions of thorax tinged with green; wing veins stramineous; vein r-m dark brown; legs stramineous, the apices of the tibiae and basal tarsal segments brown; apical tarsal segments brown; abdomen brown, darker towards the apex and paler near the incisures.

Genitalia: Figure 135. Similar to those of *T. decorus* but with the superior appendage more slender and straighter.

Female: Similar to the male except for the usual sexual differences.

The pale brownish-cinereous color, the strongly produced central portion of the pronotum, and the long heavy beard on the fore tarsus of the male make this species easy to recognize.

Description from Townes 1945

Adult: (mostly from Sublette & Sublette 1974 – localities not specified) Male:

Wing length 4.06 (3.20-5.02) mm. VR 1.00-1.01. Wing veins stramineous, r-m crossvein dark brown.

Pale greyish brown; lighter portions of thorax with a greenish tint; abdomen brown, darker towards the apex and paler near the incisures.

AR - 4.3 (4.0-5.17); Frontal tubercles large, about 0.08 mm long and 1.7 times longer than width at base, clypeus small, about width of antennal pedicel with 22-38 setae; palp proportions (units) 10 : 8 : 16 : 24 : 40. P5/P4 1.67, P5/P3 2.5.

Central portion of pronotum strongly produced, no mesonotal tubercle. Thoracic setae - acrostichal in 2 staggered rows; dorsocentrals - about 18 in 1-3 rows; prealars - about 4; scutellar with posterior row of about 18 setae, anteriorly with about 12 smaller setae in a random pattern (i.e. total about 30).

Legs with apex of the tibiae and basal tarsal segments brown; fore tarsi with long heavy beard, BR - 6.0 (5-7). LR1 - 1.41 (1.33-1.49); LR2 - 0.58 (0.55-0.63); LR3 - 0.72 (0.68-0.75).



Hypopygium (left) and SVo (right) of a male from Austin, Texas.

Tergite IX with about 15-16 setae in individual pale areas. SVo long and slender, Etype, between h and i, of Strenzke (1959); IVo, with forked setae, reaching to about the tip of the anal point, which is broad at the base and narrows evenly to the tip. GS moderately swollen, narrowing gently from midway to distal 1/4 in the above Texas specimen, while the SEM in Sublette & Sublette (1974) is more swollen and narrows over post 1/3 to 1/4 and the IVo reaches to 3/4 along the gonostylus (origin not identified).

Female.

No detailed description available – basically described as similar to male except for sexual differences. From the figures in Sublette and Sublette (1974), the following characters can be adduced:

Antennal proportions (units, propn. neck in brackets); 15 (0.25): 10 (0.30): 10 (0.30): 9 (0.28): 22. AR abt 0.5, A5/A1 abt 1.5. Clypeal width about 1.2 times width of antennal pedicel; about 27 clypeal setae.

GcIX ovoid with about 6 setae. Seg. X about 0.4x wider at widest point than length of segment, with about 9 setae. Cercus longer than deep, with rounded posterior margin and a swelling at base of dorsal margin.



Pupa: Mean total exuvial length 9.67 mm (male 9.27 (8.21-11.01); female 10.22 (7.99-12.30). Cephalic tubercles of male about 1.1-1.4 times longer than the basal width, with a subapical seta; no secondary tubercle. The SEM photograph of the frontal apotome shows faint indications of structures which could be frontal warts., however it is not seen in other specimens. Integument blackish. Thorax finely papillose. Respiratory base 180 x 76 (HR 2.4) with the base of the thoracic horn markedly constricted in width at the midpoint. Recurved hooks simple, sometimes with a slight barb near the center of the row; 95 (80-116) in females, 79 (60-91) in males. Distinctive rugose stripes on abdominal segments IV-VI; shagreen more posterior on segments I-III.

PSB visible on segment II, and a large PSA at the posterior margins of segment IV, 225 μ m long and 135 μ m wide and extending about 25% of the segment length, PSA of segments V and VI comprised of spines. Posterolateral spurs with 5 (3-8) closely applied spines, often with a smaller spine at base of other spines. Anal fringe with a distinct sexual difference in number of taeniae: 103 (88-117) in males, 117 (94-139) in females; in at least 3 rows at posterior end.



Pupal cephalic tubercle and spur from Texas male

Fourth instar larva a medium to large plumosus-type. Length: female 15.5 (14.5-16.8) mm, male 14.9 (12.7-16.8) mm. VT long with posterior pair longer (Anterior: fem. 2.6-4.1 mm, male 2.0-3.6 mm; Post. fem. 3.6-5.5 mm, male 2.1-4.1 mm). TLt 330-730 μ m. Anal tubules (from a Portales male) dorsal 582 μ m long x 228 μ m wide; ventral 531 μ m long x 202 μ m wide (both 2.6 times longer than wide).

Gula darkened over posterior half, wider than the mentum and widest part way up from posterior margin; and FC (which may be darker in center) (Sublette and Sublette 1974 state that only the gula is darkened). Distance between the antennal bases, 188.1 (175-200) μ m, greater than that between the S4 setae, 175.8 (157-210) μ m, which occupy 0.86 (0.83-0.89) of the width of the frontoclypeus at that point. S5 setae about level with nearby RO. Salivary reservoir about 91.5 (78-134) μ m long by 24.8 (20-28) μ m deep, (3.09-4.82 times wider than deep).

Mentum of type I, with somewhat rounded teeth, which in trifid center tooth causes c1 tooth to narrow at base (type 1B).
Ventromentum about 242 (210-268) mm wide and 4.1 (3.8-4.2) times wider than deep; 1.2 (1.06-1.39) times wider than the mentum; with about 53.4 (47-60) striae, VMR 0.31-0.38; IPD about 0.32-0.41 of mentum width. PE with 16.2 (12-19) sharp teeth (type A or B). Premandible of type B2; with broad inner tooth with narrow point, about 3-4.5 times the width of the outer tooth which usually narrows to a point, but in a Californian specimen it narrowed sharply to a fine point (type B2).

Antenna with A1 about 3-3.94 times longer than wide; RO about 0.3 (0.25-0.40) up from base; AR – 2.28 (2.17–2.46); A2/A1 abt 0.18-0.25; segment lengths (micron) 139.5 : 31.5 : 8.5 : 14 : 7.

Mandible with 3rd inner tooth well developed and partially to fully dark (type IIIB-C); about 16.1 (14-19) furrows on outer surface near the base; 11-13 taeniae in the PMa; Mdt-Mat 40.1 (35-45); MTR 0.42-0.50.

Cytology: 4 polytene chromosomes with the thummi arm combination AB, CD, EF, G. Centromere are heterochromatic. Arm G closely paired except where heterozygous. Californian populations have 2 interstitial BRs in arm G, but some of the alternative sequences present in other areas appear to have only one BR because the other is inverted to a terminal position where it is difficult to see. Nucleolus is about the middle of arm D. Inversions are known in all arms, with some populations quite polymorphic (Hilburn 1979). Only a single specimen from Florida was available for the populations in eastern North America.

stiA1:	1a-2g, 8-9, 13a-f, 3f-2h, 6a-3g, 12c-10, 14-17d, 6b-7,	17e-19
	(all)	localities, highest Texas)
stiA2:	1a-2c, 15-14, 10-12, 3g-6a, 2h-3f, 13f-a, 9-8, 2g-d, 16-	.17d. 6b-7. 17e-19
		calities, highest California)
stiA3:	approx 1a-2c, 15-14, 10-12, 3g-6a, 2h-3f, 13f-a, 9-8, 2	g-d, del 16,17a-d, 6b-7,
	17e-19	(New Mexico)
stiA4:	approx 1a-2c, 15-14, 10-11, 3i-g, 12c-a, 4-6a, 2h-3f, 1	3f-a, 9-8, 2g-d, 16-17d, 6b-
	7, 17e-19	(Texas)
stiA5:	approx 1a-f, 11e-10, 14-15, 2c-1g, 12a-c, 3g-6a, 2h-3f	. 13f-a, 9-8, 2g-d, 16-17d,
	6b-7, 17e-19	(California)
stiB1:	Puff with distal dark bands (groups 7 & 8) near end of	arm, BR near
	characteristic 4 bands (24-26)	(in all localities)
stiB2:	Inversion of region around BR, about groups 21-23	(California)
stiC:	Four sequences are known, only C1 and C2 common	(all localities)
stiC2:	Inversion of proximal third of arm.	(New Mexico, Texas)
stiC3:	small proximal inversion within region of C2	(California)
stiC4:	small distal inversion	(California)
stiD:	Three sequences known, but only D1 common	(all localities)
stiD2:	Inversion of about distal third of arm	(Texas)
stiD3:	small inversion of region of nucleolus	(California)
stiE1:	1-3e, 8-5, 9-10b, 4-3f, 10c-13 i.e. as maturus (differ	rs from <i>aberratus</i> by In8-5)
		(all localities)
stiE2:	approx 1-3e, 8-7h, <u>6e-7g</u> , 6f-5, 9-10b, 4-3f, 10c-13	(Texas)
stiE3:	approx 1-3e, 8-5, 9-10b, 4hg, <u>4a-f</u> , 3f, 10c-13	(New Mexico)
stiE4:	approx 1-3e, 8-5, 9-10b, 4-3f, 10c-12b, <u>13f-12c</u> , 13g	(Texas)
stiE5:	approx 1-3e, 8-5c, <u>13a-10c</u> , <u>3f-4</u> , <u>10b-9</u> , <u>5ab</u> , 13b-g	(Texas)
stiE6:	approx 1-3e, 8-6f, <u>4a-h, 10b-9, 5-6e</u> , 3f, 10c-13	(Texas)
stiF1:	1-10, 17-16, 11-15, 18-23 i.e. derive	d from <i>aberratus</i> by In11-5
stiF2:	1-4, <u>6a-5</u> , 6b-10, 17-16, 11-15, 18-23	heterozygous in all males

stiF3:	1-4, ?-?, 6b-10, 17, <u>14h-11, 16d-a</u> , 15a-i, 18-23 (could	d be 1-10 or 1-4, 6a-5, 6b-
	10)	(California)
stiF4:	approx 1-8b, <u>14d-11, 16-17, 10-8c</u> , 14e-15, 18-23	(New Mexico)
Note:	limits of stiF4 shown in Hilburn are those of stiF3 of	Martin and Wülker, but he
	does not show limits for F3.	
stiF5:	approx 1-10, 17-16, 11a-c, <u>11i-d</u> , 15, 18-23	(Texas)
stiF6:	approx1-5, <u>7a-6a</u> , 6b-10, 17-16, 11a-15, 18-23	(New Mexico)
stiF7:	approx 1-10, 17-16, 11a-15i, 18-20g, <u>22d-20h</u> , 22e-2	3 (California)
stiG1:	Two BRs, one near each end of arm (throughout,	but highest California)
stiG2:	Inversion of about two thirds of the arm; takes one Bl	R to terminal position
		(New Mexico and Texas)
stiG3:	Inversion of the region around proximal BR of G1	(New Mexico and Texas)
stiG4:	Inversion of slightly more proximal region than in G3	3, but in G2
		(New Mexico and Texas)
stiG5:	Inversion of about half the arm from G1	(Texas)
stiG6:	Small inversion sharing distal break of G4 and within	the region of the G4
	inversion	-
stiG7:	Duplication of small proximal region of G2	(New Mexico)
stiG8	Small inversion of G1, proximal to, and sharing proxi	imal breakpoint of, G2
		(Texas)



Found: Numerous populations in: Arizona - Tucson (32.32°N, 110.82°W), Pima Co.; Douglas (Townes, 1945)

California - Hayward, Alameida Co.; Napa, and 1 mile s. Napa, Napa Co.; Riverside, Riverside Co.; Dolwig Lake, Vallejo, Solano Co.; Davis, Vacaville, Cutler, Oildale, Ontario, Cerritos, Palm Springs, Blythe (all Hilburn 1979) Florida- Lake Miccosukee (Alberta Lake), Leon Co.; Biscayne Bay and Ft. Lauderdale, Miami-Dade Co.; Charlotte Harbor, Charlotte Co.; Jacksonville, Duval Co.; Lake Worth and West Palm Beach, Palm Beach Co. (all Townes, 1945) Louisiana - Many, Sabine Co. Nebraska - Oak Creek at Lincoln (Townes 1945) New Mexico - Portales (33.42°N, 103.33°W), and 4 ml s. Portales, Roosevelt Co.; Santa Rosa, Guadalupe Co.; Roswell & Torrence Co. (Townes 1945); Lordsburg, 74. km w Quincey, Alamogordo (all Hilburn 1979) New York - Worlds fair Grounds, Flushing (Townes 1945) Ohio - Summit Co. (Townes 1945) Pennsylvania - one Say specimen (in Vienna Museum) South Dakota - (from P.L. Hudson?) Texas - Brackenridge Experimental Station, Austin, Travis Co.; Galveston & San Antonio (Townes 1945); Broncho, Fredericksburg, Van Horn, Davis Mountains, Marathon, Lubbock, Petersburg, Bronte, Junction, Nacogdoches (all Hilburn 1979) Mexico - Tlahualilo (Townes 1945) Cuba – (Bello Gonzalez 2010) Type locality United States

Often in sewage oxidation ponds, windmill tanks, etc.

Morphological description by Sublette and Sublette (1974). They note that pupa has distinctive rugose stripes on abdominal tergites. Morrow, Bath and Anderson (1968) described the egg mass as arcuate, with more than 900 eggs, and 50% of egg masses with an enlarged capitulum.

Cytology described by Martin and Wülker (1974). The work of Hilburn (1979,1980) suggests the Californian populations are a distinct species from those in New Mexico and Texas. The specimens in the BOLD bin from California or Arizona are in one Bin, while those from Texas are in the other.

A comparison of the sequences of 5 specimens from California, one from Arizona and 2 from Texas indicates 23 base differences in the Barcode region:

	base.																					
California/Arizona																						
С	А	А	А	А	Т	А	Т	С	А	С	С	С	G	Т	С	А	Т	С	С	Т	С	Α
Tez	kas	5																				
А	G	G	G	G	С	С	G	Т	G	Т	Т	Т	А	С	Т	G	С	Т	Т	С	Т	G

Note that, as appears common between *Chironomus* species, many of these differences are in the 3' half of the sequence, such that a short Barcode (550 bp) would be missing 6 of these differences.

Little morphological data on the possibility of two species is available, since Sublette & Sublette (1974) do not identify the source of illustrated material and only a few larvae are available for study. These latter suggest there may be differences in some characters, e.g.

antennal characters. Obviously more material from other regions is needed to determine whether the two groups are really distinct species or simply extremes of geographical clines.

Probably the New Mexico, Texas populations represent the true *C. stigmaterus*, so the available information from these populations is presented below:

In BOLD Bin No. BOLD:AEC0831

Male from Brackenridge, Austin Texas (UTX.1.2 male 2):

Wing length 3.44 mm, width 0.89 mm, VR 1.03, 3 SCf on brachiolum.

AR 3.82. Frontal tubercles 73 x 18 µm (l/w 4.14).

Palps: 54 : 46 : 140 : 153 : 185. P5/P4 1.21, P5/P3 1.32. Clypeus heart shaped, 0.88 of antennal pedicel diameter, with 38 setae.

Thoracic setae: acrostichal – not seen; dorsolateral – 17; prealar – 6; supra-alar 1; Scutellar abt 8 small anterior and 11 larger posterior (total, about 20).

Leg lengths (micron) and proportions:

		/	1 1							
	Fe	Ti	Ta1	Ta2	Ta3	Ta4	Ta5	LR	F/T	Ta5/Ti
PI	1202	1139	1645	949	683	645	305	1.44	1.05	0.24
PII	1315	1315	785	505	375	250	180	0.60	1.00	
PIII	1520	1545	1175	710	545	335	200	0.76	0.98	
BR 5.5										

TIX with about 7 setae in individual clear spots. SVo E type, IVo reaching about to end of the anal point, setae apparently simple (although the small branches shown by Sublette and Sublette may not be visible on a light microscope). Gonostylus markedly swollen and narrowing sharply over posterior third (see photograph above).

Pupa: (full data only for a single male exuvia) Length 8.78 mm, inner margin of wing case 1.77 mm. Cephalic tubercle 76 μ m high and 68 μ m diameter at the base. About 80 recurved hooks on segment II, those at the ends taller and narrower than those towards the midline. Hook row occupying about 60% of the segment width. Respiratory base 180 x 76 μ m (HR 2.4), with the base of the thoracic horn markedly constricted in width at the midpoint. PSB visible on segment II, and a large PSA at the posterior margins of segment IV, 225 μ m long and 135 μ m wide and extending about 25% of the segment length, PSB of segments V and VI comprised of spines. Postero-lateral spur as in photograph in previous description above, with 4-6 spines including a smaller one at the base of the longer spines. At least 86 taeniae on the anal fringe, in three rows distally.

Fourth instar larva: a medium to large plumosus-type. Length: male -16.8 mm. VT long with posterior pair longer (Anterior: 2.8 mm; Post. 3.2 mm). TLt 460 μ m. Anal tubules (from a Portales male) dorsal 582 μ m long x 228 μ m wide; ventral 531 μ m long x 202 μ m wide (both 2.6 times longer than wide).

Gula darkened over posterior half, wider than the mentum and widest part way up from posterior margin; and FC (which may be darker in center). Distance between the antennal bases, 188.1 (175-200) μ m, greater than that between the S4 setae, 175.8 (157-210) μ m, which occupy 0.86 (0.83-0.89) of the width of the frontoclypeus at that point. S5 setae about level with nearby RO. Salivary reservoir about 91.5 (78-134) μ m long by 24.8 (20-28) μ m deep, (3.09-4.82 times wider than deep).

Mentum of type I, with somewhat rounded teeth, trifid center tooth type 1B. Ventromentum about 242 (210-268) mm wide and 4.1 (3.8-4.2) times wider than deep; 1.2 (1.06-1.39) times wider than the mentum; with about 53.4 (47-60) striae, VMR 0.31-0.38; IPD about 0.32-0.41 of mentum width. PE with 16.2 (12-19) sharp teeth (type A or B). Premandible of type B2; with broad inner tooth with narrow point, about 3-4.5 times the width of the outer tooth.

Antenna with A1 about 3-3.91 times longer than wide; RO about 0.3 (0.25-0.40) up from base; AR - 2.28 (2.17- 2.46); A2/A1 abt 0.18-0.25; segment lengths (micron) 140 : 31.5 : 8.5 : 14 : 7.

Mandible with 3rd inner tooth well developed and partially to fully dark (type IIIB-C); about 16.1 (14-19) furrows on outer surface near the base; 11-13 taeniae in the PMa; Mdt-Mat 40.1 (35-45); MTR 0.42-0.50.



Cytology: 4 polytene chromosomes with the thummi arm combination AB, CD, EF, G. Centromere are heterochromatic. Arm G closely paired except where heterozygous; probably with 2 interstitial BRs, but some of the alternative sequences appear to have only one BR because the other is inverted to a terminal position where it is difficult to see. Nucleolus is about the middle of arm D. Inversions are known in all arms, with some populations quite polymorphic (Hilburn 1979).

stiA1:	1a-2g, 8-9, 13a-f, 3f-2h, 6a-3g, 12c-10, 14-17d, 6b-7, 17	7e-19
	(all lo	calities, highest Texas)
stiA2:	1a-2c, 15-14, 10-12, 3g-6a, 2h-3f, 13f-a, 9-8, 2g-d, 16-1	7d, 6b-7, 17e-19
	(all loca	lities, highest California)
stiA3:	approx 1a-2c, 15-14, 10-12, 3g-6a, 2h-3f, 13f-a, 9-8, 2g-	-d, <u>del 16</u> ,17a-d, 6b-7,
	17e-19	(New Mexico)
stiA4:	approx 1a-2c, 15-14, 10-11, <u>3i-g, 12c-a</u> , 4-6a, 2h-3f, 13:	f-a, 9-8, 2g-d, 16-17d, 6b-
	7, 17e-19	(Texas)
stiB1:	Puff with distal dark bands (groups 7 & 8) near end of a	rm, BR near
	characteristic 4 bands (24-26)	(in all localities)
stiC:	Four sequences are known, only C1 and C2 common	(all localities)
stiC2:	Inversion of proximal third of arm.	(New Mexico, Texas)
stiD:	Three sequences known, but only D1 common	(all localities)
stiD2:	Inversion of about distal third of arm	(Texas)
stiE1:	1-3e, 8-5, 9-10b, 4-3f, 10c-13 i.e. as maturus (differs	from <i>aberratus</i> by In8-5)
		(all localities)
stiE2:	approx 1-3e, 8-7h, <u>6e-7g</u> , 6f-5, 9-10b, 4-3f, 10c-13	(Texas)
stiE3:	approx 1-3e, 8-5, 9-10b, 4hg, <u>4a-f</u> , 3f, 10c-13	(New Mexico)

stiE4:	approx 1-3e, 8-5, 9-10b, 4-3f, 10c-12b, <u>13f-12c</u> , 13g	(Texas)
stiE5:	approx 1-3e, 8-5c, <u>13a-10c</u> , <u>3f-4</u> , <u>10b-9</u> , <u>5ab</u> , 13b-g	(Texas)
stiE6:	approx 1-3e, 8-6f, <u>4a-h, 10b-9, 5-6e</u> , 3f, 10c-13	(Texas)
stiF1:	1-10, 17-16, 11-15, 18-23 i.e. deriv	ed from <i>aberratus</i> by In11-5
stiF2:	1-4, <u>6a-5</u> , 6b-10, 17-16, 11-15, 18-23	heterozygous in all males
stiF4:	approx 1-8b, <u>14d-11, 16-17, 10-8c</u> , 14e-15, 18-23	(New Mexico)
Note:	limits of stiF4 shown in Hilburn are those of stiF3 of	Martin and Wülker, but he
	does not show limits for F3.	
stiF5:	approx 1-10, 17-16, 11a-c, <u>11i-d</u> , 15, 18-23	(Texas)
stiF6:	approx1-5, <u>7a-6a</u> , 6b-10, 17-16, 11a-15, 18-23	(New Mexico)
stiG1:	Two BRs, one near each end of arm (throughout,	but highest California)
stiG2:	Inversion of about two thirds of the arm; takes one B	R to terminal position
		(New Mexico and Texas)
stiG3:	Inversion of the region around proximal BR of G1	(New Mexico and Texas)
stiG4:	Inversion of slightly more proximal region than in G.	3, but in G2
		(New Mexico and Texas)
stiG5:	Inversion of about half the arm from G1	(Texas)
stiG6:	Small inversion sharing distal break of G4 and within	the region of the G4
	inversion	-
stiG7:	Duplication of small proximal region of G2	(New Mexico)
stiG8	Small inversion of G1, proximal to, and sharing prox	imal breakpoint of, G2
		(Texas)

C. nr. *stigmaterus* (species 5v)

In BOLD Bin: BOLD:AAW3998

Adult and Pupa: I have no information definitely relating to this form.

Fourth instar larva: A plumosus type larva, but larval bodies were discarded and only the head capsules retained. Data only available for two specimens and is largely similar to that of *C. stigmaterus*, although at the lower end of the range.

The gula is darkened, the frontoclypeus less so but with a darker stripe along the center. The salivary reservoir is 78 x 30 μ m (2.58 times wider than deep). Mentum width about 0.54 of the VHL; 4th laterals hardly reduced and center tooth type IB.

The ventromentum is about 220 μ m wide; 3.95 times wider than deep and 1.3 times the mentum width; with 45-60 striae; VMR 0.32-0.41. The number of PE teeth (18, of type B) is relatively high given the small size of many other measures. The premandible is type B2 with the inner tooth 3-3.8 times the width of the fine pointed outer tooth.

The relative lengths of the antennal segments and the AR (2.16-2.31) are similar, although again at the lower range of those of *C. stigmaterus*.

The distance between the S4 setae (157 μ m) is less than that between the antennal bases (185 μ m) and 0.85 of the FC width.

Mandible with 3rd inner tooth well developed and darkened (type IIIC); about 16-18 furrows on outer surface near the base; 10-11 taeniae in the PMa; Mdt-Mat 41; MTR 0.42-0.45.



Cytology: 4 polytene chromosomes with the thummi arm combination AB, CD, EF, G. Centromere are heterochromatic. Arm G closely paired with 2 interstitial BRs in arm G. Nucleolus is about the middle of arm D. Inversions are known in all arms except arm E, although with low polymorphism (Hilburn 1979).

nrstiA1:	1a-2g, 8-9, 13a-f, 3f-2h, 6a-3g, 12c-10, 14-17d, 6b-7, 17e-19	
	(all localitie	s, highest Texas)
nrstiA2:	1a-2c, <u>15-14</u> , <u>10-12</u> , <u>3g-6a</u> , <u>2h-3f</u> , <u>13f-a</u> , <u>9-8</u> , <u>2g-d</u> , 16-17d, 6b	-7, 17e-19
	(all localities,	highest California)
nrstiA5:	approx 1a-f, <u>11e-10, 14-15, 2c-1g</u> , 12a-c, 3g-6a, 2h-3f, 13f-a,	9-8, 2g-d, 16-17d,
	6b-7, 17e-19	(California)
nrstiB1:	Puff with distal dark bands (groups 7 & 8) near end of arm, Bl	R near
	characteristic 4 bands (24-26)	(in all localities)
nrstiB2:	Inversion of region around BR, about groups 21-23	(California)
nrstiC:	Four sequences are known, only C1 and C2 common	(all localities)
nrstiC3:	small proximal inversion within region of C2	(California)
nrstiC4:	small distal inversion	(California)
nrstiD:	Three sequences known, but only D1 common	(all localities)
nrstiD3:	small inversion of region of nucleolus	(California)
nrstiE1:	1-3e, 8-5, 9-10b, 4-3f, 10c-13 i.e. as <i>maturus</i> (differs from a	<i>aberratus</i> by In8-5)
		(all localities)
nrstiF1:	1-10, 17-16, 11-15, 18-23 i.e. derived from a	aberratus by In11-5
nrstiF2:	1-4, <u>6a-5</u> , 6b-10, 17-16, 11-15, 18-23 heterox	zygous in all males
nrstiF3:	1-4, ?-?, 6b-10, 17, <u>14h-11, 16d-a</u> , 15a-i, 18-23 (could be 1-10) or 1-4, 6a-5, 6b-
	10)	(California)
nrstiF7:	approx 1-10, 17-16, 11a-15i, 18-20g , <u>22d-20h</u> , 22e-23	(California)
nrstiG1:	Two BRs, one near each end of arm (throughout, but high	est California)

Chironomus nr stigmaterus

From the distribution of some other species that are shared between California and South America, it is possible that this is the form of *C. stigmaterus* reported from Mexico and Cuba. However there is no information on which to base such a conclusion – in fact the existence of a form of *C. stigmaterus* in Cuba is based entirely on a publication simply reporting the presence of the species (Bello Gonzalez 2010).

C. trabicola Shobanov, Wuelker & Kiknadze, 2002 (Species w)

Adult:

Based on Russian specimens from data of Shobanov et al. (2002).

Male: Dark species, thorax almost black, abdomen brown to dark brown, tergites uniformly colored. Sternite I with about 11-36 setae, sternite II with 2-6 lateral and about 70 medial setae. Ratio of 3^{rd} -5th maxillary palpomeres (µm) 416 : 386 : 411; P5/P4 1.06, P5/P3 0.89. AR 5.8-7.3.

Anterior basitarsus with beard, BR ave. 5.87. Anterior LR 1.03-1.08. Sensilla chaetica on LII 22-29. Legs unusual in that anterior femur is shorter than the anterior tibia. Leg proportions (micron):

	Fe	Ti	Ta1	Ta2	Ta3	Ta4	Ta5	LR	F/T	BR
PI	1613	1818	1896	1221	685	449	291	1.03-1.08	0.88-0.93	5.25-6.78
PII	1982	1846	1115	735	513	319	233	0.59-0.61	1.05-1.07	
PIII	2287	2448	1632	1052	780	411	266	0.66-0.71	0.93-0.98	

Sensilla chaetica L2 22-29



From Shobanov et al. 2002

Tergite IX with about 12 setae in individual pale areas in center of tergite. SVo closest to E(i)-type of Strenzke (1959), with thickening on the inner side. IVo conical, apparently with simple setae, reaching about halfway along the GS, which is broad, usually uniformly colored but sometimes with an obvious light area on external side; narrows sharply over posterior third. Anal point brown narrowing from base to top.

Female not described.

Pupa: Thorax dark brown, tergites of abdomen with grey spots, congruent with shagreen areas. Laterosternites dark brown. Abdomen length about 9.9-11.6 mm. Wing sheath length about 2.58-3.02 mm. Caudolateral spur of segment VIII almost black, usually with one spine, but sometimes with up to three, including a second tip.

Fourth instar larva a large salinarius-type, length up to 20 mm. Gular region, frontoclypeal and other dorsal areas of head darkened; in some specimens there may be a pale spot on the posterior half of the FC.



From Shobanov et al. (2002)

Mentum (c, below) of only specimen worn; c2 teeth well separated (type IIA or III), fourth laterals appear reduced, but in Russian specimens it appears only slightly reduced (Shobanov *et al.* 2002, below). PE (b, below) with 11-16 teeth, some broad and irregular (type C) – this variability appears common in arctic species. Ventromental plates with 47-58 striae. Premandible not described but from illustration in Shobanov *et al.*(2002), it appears the teeth are broad. Antenna (a, below), AR $1.91 \rightarrow 2.35$, RO almost half way (0.41-0.46) up from base; A1 about 2.7-3.0x longer than wide; A4 about same length as A5; approx proportions 106 : 22 : 6 : 9 : 5.5.

Mandible (d, below) with 3rd inner tooth pale (type IIA), 12-15 furrows on outer surface near the base; MTR about 0.39.



i.e. as *albimaculatus*

Cytology: 4 polytene chromosomes with the thummi arm combination AB, CD, EF, G. Arm G with heterochromatic cap; partly unpaired and relatively short (about 20 bands), with three BR.

Nucleolus in region 9 of arm D and small nucleolus in arm A near the centromere. The Ellesmere specimen has traB1 and traF1, the occurrence of the alternative sequences traB2 and traF2 in North America is unknown.

- traA1: 1-2c, 10-12, 3i-d, 13f-a, 4-9, 2d-3c, 14-19 i.e. as holomelas 2
- traA2: 1-2c, 10-12, 3ih, <u>8d-4, 13a-f, 3d-g</u>, 8e-9, 2d-3c, 14-19
- traB1: with the typical position of groups 8-9 in the middle of the arm, flanked distally by dark band groups.
- traC1: 1-3, 8-11c, 13-15, 4-6b, 12-11d, 6gh, 17a-16, 7d-a, 6f-c, 17b-22 i.e. as *heteropilicornis*
- traD1: 1-3, 11-18d, 9-10, 4-8, 18e-24g
- traE1: 1a-c, 2e-3e, 5a-8g, 2d-1d, 8h-10b, 4h-3f, 10c-13g
- traF1: 1-9f, 11-17, 10d-a, 11g-a, 18-2
- traG1: Three BRs spread along the length of the arm.



Wülker (pers. comm.) notes relationship to C. neocorax.

Found: British Columbia - Springhouse (51.97°N; -122.13°W) (R.A. Cannings) Nunavut (formerly Northwest Territories) - Skeleton Lake, Hazen, Ellesmere Island (Tarn 34 of Oliver & Corbet, 1966) (81.826°N; -71.483°W). Alaska - west shore of Prudhoe Bay, EWD1, tundra pond east of West Doc pad. Russia - Ice Sea coast: Lena Delta (Ust'Lena reserve, Danube Island, pool on polar station area, Type locality), Dikson, Archangelsk.

Cytology partially described by Wülker & Martin (2000). Full cytology and morphology described by Shobanov *et al.* (2002).

C. species 'algonquian' (Species g)

In BOLD Bin: BOLD:ACQ1765

Nearest neighbor Bin in BOLD database is <u>BOLD:ADC3157</u> An unidentified species from British Columbia

Adult: Some adults may be in the Sublette collection in the Museum of the University of Minnesota, St. Paul, MN.

Male: Only a photograph of the male hypopygium is available.

Anal point relatively broad and parallel sided. About 12 setae in a large pale circle on tergite IX. SVo of D-type(e) of Strenzke (1959), IVo extends to just beyond the tip of the anal point, GS narrowing relatively gently over posterior third.



Male terminalia (left) and pupal spur (right) of C. species algonquian

Pupa: caudolateral spur of segment VIII with about 3 spines (see figure above).

Fourth instar larva of the semi-reductus type, of medium size (fem. 12.9-14.0 mm; male 12.2-13 mm), lateral projections 170- 250 μ m). Posterior pair of VT longer (ant. 0.78–1.15 mm; post. 1.1–3.25 mm), anal tubules very long, 4-6 times longer than wide. Gular region dark, wider than mentum and highest at outer edges; FC also darkened. Salivary reservoir about 64 x 15 μ m and 4.2 times wider than deep.

Mentum (Fig. c, below) with pointed teeth, c1 tooth broad, c2 teeth moderately separated but tend to continue edge line of c1 tooth (type IB); 4th laterals markedly reduced (type III). Ventromentum abt 236 μ m wide and 3.32 times wider tha deep; VMR 0.29-0.36. PE (Fig. a, below) with about 14-15 sharp, variable teeth (a sharp variant of type D).

PreM (Fig. c, below) of type B1 with teeth relatively narrow coming to fine points, inner tooth about 3-4 times wider than the outer.

Distance between antennal bases greater than the distance between the S4 setae, which are separated by 73-77% of the FC width. S5 setae well anterior to RO.

Antenna (Fig. b, below) with RO about half–way up from base of A1, which is about 3.4 times longer than wide; AR about 1.8-2.3; antennal proportions about 122 : 32 : 7.5 : 13 : 7.5.

Mandible ((Fig. d, below) with 3rd inner tooth only partly separated and colored (type IIB), about 16 furrows on outer surface near the base and 13 taeniae in PecM; Mdt-Mat 23, MTR about 0.28.



Cytology: 4 relatively short polytene chromosomes with the thummi arm combination AB, CD, EF, G. Centromeres not noticeably heterochromatic.

Arm G relatively short, normally paired at ends, with a small median nucleolus that may be polymorphic and a BR near one end. Main nucleolus (N) in arm E. No inversion polymorphism in specimens seen.

 "alg"A1:
 1a - 2c, 10 - 12, 3i - 2d, 9 - 4, 13 - 19
 i.e. as holomelas

 "alg"E1:
 1-3e, 5-10b, 4-3f, 10c-13
 i.e. as aberratus
 Nucleolus at 3f.

 "alg"F1:
 "alg"F1:
 "alg"F1:
 Nucleolus at 3f.



Found: Ontario - Bat Lake (45.577°N, 78.523°W), Algonquin Provincial Park, Nipissing Co.; Frontenac Provincial Park, Sydenham (44.5178°N, 76.5394°W) (BOLD).

Amongst deep organic litter in highly eutrophic lake.

The manuscript name is one of the versions of the spelling for the Provincial Park in which it was found

Chironomus species Obatanga (Species 4z)

Adult and Pupa: Not known.

Fourth instar larva: Single known larva is a small (8.1 mm) halophilus-type. AT relatively long (3.1 or 4.7 longer than wide) with a constriction near the base, abt. 355 μ m long but ventral pair narrower. Gula and FC pale.

Mentum with 4th laterals slightly reduced (type I-II), central tooth with c2 teeth well separated (much more so than apparent in fig. c, below) and about ³/₄ height of c1 tooth (type IV).

VMs about 170 μ m long, about 3.05 times longer than deep and 1.22 times the width of the mentum; with smooth outer margin and 35-36 striae which reach about halfway to margin, VMR about 0.36; possibly separated by about 0.44 of mentum width.

PE with about 15 teeth, at least two small and fine (type D, but with sharp teeth).

Premandible with teeth relatively narrow, outer tooth longer, and inner tooth around 2-2.2 times wider than the outer.

Distance between antennal bases greater than that between S4 setae, which are separated by about 0.79 of the FC width.

Antenna with segment 1 about 3.8 times longer than wide, RO about 30-35% up from base of segment; AR about 2.16; segment proportions (μ m) 123 : 28 : 10 : 13 : 6.

Mandible with 3^{rd} inner tooth pale and only partly separated (type 1A), about 11-12 furrows on outer surface near base; 11-12 taeniae in PMa; distance from tip of dorsal tooth to apical tooth 24 μ m.



Mouthparts of *C*. sp. Obatanga. a. Pecten epipharyngis and premandibles, b. Antenna, c. Mentum, d. Mandible.

Cytology: Polytene chromosomes of only larva in poor condition. Arm G has a subterminal nucleolus. One chromosome appears to be EF.

In small pools amongst leaves on the bottom.

Found: Ontario – Obatanga Provincial Park (48.32; -85.09), Algoma Co.

It is possible that this is a species of Chaetolabis or Lobochironomus.

Chironomus species k

Adult not known for certain

Pupa: Caudolateral spur of segment VIII with a single long spine.



Fourth instar larva a small to medium plumosus-type (Fem. abt 11.4 mm (1)). VT moderately long. AT at least twice as long as wide. Gular region slightly darkened, FC pale to slightly dark.

Mentum (c, below) with pointed teeth; c1 tooth high and almost parallel sided, c2 teeth moderately developed (type III), 4th laterals hardly reduced (type I).

Ventromentum (e, below) with about 39-43 striae reaching about half way to margin. PE (a, below) with about 9-10 moderately long and fairly broad teeth (type C, sharp variant). PreM (b, below) with relatively broad, about equal length teeth, inner tooth about 1.9-2.3

PreM (b, below) with relatively broad, about equal length teeth, inner tooth about 1.9-2.3 times wider than outer tooth.

Antenna (d, below) with basal segment about 3.7-3.8 times longer than wide; RO about 40% up from base of segment; AR abt 1.69-1.75; segment proportions (microns): 140 : 38 : 11 : 18 : 8.

Mandible (f, below) with 3rd inner tooth only partly separated and pale (type 2A), about 15-16 furrows on outer surface near the base, PMa with about 13-15 taeniae.



Cytology: 4 polytene chromosomes with thummi arm combination AB, CD, EF, G. Centromeres only slightly heterochromatic.

Arm G generally unpaired, with a large terminal nucleolus and at least one BR near the other end; rather similar to arm G of *plumosus*. No nucleolus in any other arm. Arm E generally unpaired or may be a rearrangement. Polymorphism in B(?).

Arm A1: Olive (groups 4-6) about middle of arm.

Arm B1: Slight distal puff (group 7?).

Arm B2: Inversion in proximal half of arm.

Arm C1: typical groups 3-4 about 1/3 from centromere.

Arm D1:

Arm E: Lack of pairing may be a small inversion near distal end.

Arm F1:

Arm G1: Large terminal nucleolus, 1 or 2 BR near other end.



Found: Ontario - Costello Creek, Algonquin Provincial Park (45.58°N, 78.32°W) New Hampshire - Lancaster, Coos Co (44.49°N, 71.57°W).

Creeks and pools.

Chironomus sp. Greiner Lake (Species 5k)

Known from 2 larvae from Greiner Lake (#17), Victoria Island, Nunavut Available DNA sequence does not match anything in GenBank or the BOLD database, closest is *C. salinarius* with about 92% similarity.

Adult and Pupa not known.

Fourth instar larva: A halophilus-type larva, length about 14.2-15.8 mm; no LP; posterior VT about 0.48-0.56 μ m. AT a single lobe, dorsal (len. about 280 μ m and about 2-2.2X longer than wide), shorter than ventral (len. about 320-360 μ m and 2.3-3.2X longer than wide). Gula dark on posterior 1/3-1/2; FC sl.-dark with a streak, and outside a slightly dark line. Mentum quite wide compared to VHL (about 0.74 VHL length) with 4th laterals reduced to at least the level of the 5th laterals (type III), center tooth probably type III, with c1 quite wide on one larva.

Ventromental plates separated by about 0.34-0.38 of mentum width, with about 38-40 striae; VMR about 0.28-0.36. PE with about 13-14 somewhat irregular teeth and smaller teeth at the outside edges (type C).

Premandible with teeth only moderately wide, possibly with outer tooth longer and inner tooth about 3 times wider.

Distance between the antennal bases larger than that between the S4 setae, which are separated from each other by 80% of FC width.

Antenna has A1 about 3.5 times longer than wide and equivalent to about 45% of VHL, DRO about 0.4-0.5 up from base of segment; AR abt 2.14-2.33; antennal proportions (μ m) 154-158; 37-40: 8: 15-17: 7-8.

Mandible with third inner tooth partly or completely separated and partly colored (II-IIIB); 15-19 furrows on the outer surface at the base; 11 taeniae in PMa.



Cytology: Cytology of available specimen relatively poor, but some information was obtained:

4 polytene chromosomes with the thummi-cytocomplex arm combination AB, CD, EF, G. Nucleolus terminal or subterminal on arm G; there could be a BR about a third from the other end. A second nucleolus is between the centromere and the "olive" of arm A.

End of arm B unpaired, possibly due to inversion heterozygosity but the sequence is not clear enough to be certain.



Found: Nunavut - Greiner Lake, Victoria Island (69.19°N, 104.93°W).

C. species Anchorage (Species 3p)

This species is in BOLD Bin: BOLD:AAI4309

Adult:

From photographs on BOLD database:

Male – relatively dark; thorax light brown, with dark vittae, scutellum and postnotum, abdomen with dark brown basal dark band, occupying about half of the segment, until about 3/4 on segments VI and VII. Legs yellowish-brown with dark knees, becoming darker from base of Ta1 to Ta5. Crossvein probably darkened.

Female – dark, thorax brown with vittae, scutellum and postnotum black, abdomen with basal dark brown patches extending from half of segment I to virtually all of segments VII and VIII. Femur and tibia pale, with darkened knees. Wing length about 5 mm,, anterior veins and crossvein dark.

Fourth instar larva: Length about 10 mm. A thummi-type larva, VT relatively long. Dark gula and probably most of head capsule relatively dark. No other information available.

Cytology: 4 relatively short polytene chromosomes with the thummi arm combination AB, CD, EF, G.

Arm G with a large subterminal nucleolus; nucleolus also in arm C, in region 15-11; homologs often partially unpaired. Polymorphism in at least arms B and D. Preliminary mapping of banding patterns by the late Prof. Iya Kiknadze.

Arm A1:1 - 2c, 10 - 12, 13 - 14e. 4a - 9 2d - 3i, 14f - 19 (unclear dividing region 14)Arm B1:Puff (group 7) may be about middle of the arm, just proximal to inverted region

Arm B2: Small inversion about a third from distal end (see figure).

- Arm C1: 1 6b, 11c 8a, 15e 11d, 6gh, 17a 16a, 7d-e, 6f-c, 17b 22
- Arm D1:
- Arm D2: Large inversion of most of the arm (see figure).
- Arm E1: 1 3e, 10b 3f, 10c 13 as pluE1, *aprilinus*, etc.
- Arm F1: 1a-I, 15a 17d, 10d 2a, 14a 11a, 18a 23
- Arm G1: paired only at nucleolus



Photo courtesy of Prof. Iya Kiknadze

Found: Manitoba - Churchill (58.74°N, -93.82°W)(T. Ekrem and BOLD) Newfoundland and Labrador - Malady Head (48.649°N, -53.917°W), Terra Nova N.P.; Saglek (58.45°N, -62.8°W) Torngat Mountains N.P. (both BOLD) Northwest Territories - Nailicho (61.606°N, -125.758°W), Nahanni N.P.Reserve (BOLD) Yukon Territory - Lake Laberge (60.958°N, -135.184°W); Wolf Creek, Whitehorse (60.5954°N, -134.953°W) (both BOLD) Alaska - Potter Marsh (61.054°N, -149.792°W), Anchorage Co.

C. sp. Le1 of Kiknadze et al. (1996) (Species 3x)

Close to C. riihimakiensis - Kiknadze et al. (2016)

Adult and Pupa not known.

Fourth instar larva possibly a salinarius-type, but Kiknadze *et al.* (1996b) describe the Russian larvae as bathophilus-type. Dark gular (and FC?).

Mentum with rounded teeth, c1 tooth relatively broad and c2 teeth well separated (type IIA), fourth laterals only slightly reduced (type I-II).

Cytology: 4 relatively short polytene chromosomes with the thummi arm combination AB, CD, EF, G. Centromeres recognizably heterochromatic.

Arm G with nucleolus near the centromere, followed by a constriction and a large median BR; superficially similar to that of *C. cucini*. Nucleoli in the long arms in both C and D; homologues often partially unpaired. All sequences, other than LE1A2 and LE1F2, have a Holarctic distribution.

Le1A1:	1-2c, 10-12, 3-2d, 9-4, 13-19	i.e. as holomelas2
Le1A2:	1-2c, 10, 8d-9, 2d-3, 12-11, 8c-4,	13-19
Le1B1:	Puff about 1/3 from distal end.	
Le1C1:	1-6b, 11c-8, 15-11d, 11h-d, 6gh, 1	7a-16, 7d-a, 6f-c, 17b-22 (Kiknadze <i>et al</i> .
2016)		
Le1D1:	1-3, 11-18f, 7-4, 10-9, 18g-24	(Kiknadze et al. 2016)
Le1E1:	1-3e, 5-10b, 4-3f, 10c-13	i.e. as aberratus, cucini, etc.
Le1F1:	1-10, 17-11, 18-23	i.e. as <i>aberratus</i> ,
	riihimäkiensisF1, etc.	
Le1F2:	1-8c, 12-17, 10-8d, 11i-a, 18-23	i.e. as <i>riihimakiensis</i> F2
Le1G1:	Subterminal N, median BR.	



- Found: Nunavut (formerly Northwest Territories) Permanent Pond #78, Hazen, Ellesmere Island (81.82°N; -71.17°W) (Oliver & Corbet, 1966). Alaska - Point Barrow, Pond B.R. Russia - Lena delta.
- Karyotype described by Kiknadze *et al.* (1996 and 2016) and redescribed by Wülker & Martin (2000).

Chironomus riihimakiensis Wülker 1973

In BOLD Bin: BOLD:ADA8845

Specimens in this Bin are also called *C*. sp. 8TE, or *C*. nr. *longistylus* with an overlapping sequence distribution.

Cytology: 4 polytene chromosomes with the thummi arm combination AB, CD, EF, G.

riiF1: 1-10, 17-11, 18-23 riiF2: 1-8c, 12-17, 10-8d, 11i-a, 18-23 i.e. as aberratus, etc.

Found: Type locality – Riihimäki, Finland. Greenland

Chironomus sp. NAII of Proulx *et al.* (2013) (Species 4t) *Chironomus* sp. NAII of Proulx *et al.* (2013)

BOLD:AAG5454

Adult: Some data for a likely male of this species from BOLD specimens.

Male:



Adult male 08bbdip-1839+1234984344 from BOLD Database

A dark species: thorax dark brown, vittae and postnotum black. Abdomen dark brown, with narrow anterior pale region on segment I, and a posterior pale band on segments II-VII, becoming wider on segment VII. Anterior tibia paler basally, but becoming darker and femur and tarsi all darkened, no obvious beard; other legs all darkened. Wings pale, but anterior veins slightly darkened, particularly at crossvein. Wing length about 4.75 mm, width abt. 1.15 mm, VR about 0.98. Approximate leg measurements and proportions (micron):

	Fe	Ti	Ta1	Ta2	Ta3	Ta4	Ta5	LR	F/T	BR
PI	1200	1200	1800	1000	800	800	280	1.5	abt 1	low
PII	1520	1440	950	400	240	200	180	0.7-0.75	1.05	
PIII	1400	1550	1210	790	550	400	220	0.75-0.78	abt 0.9	

Female: Not known

Pupa: Not known.

Fourth instar larva: A medium sized salinarius-type larva; length about 11–13 mm. The frontoclypeus has a dark stripe and the gular region is darkened on posterior half. Salivary reservoir about 2.7 times wider than deep with the flanges down-turned. The c2 teeth of the mentum (Fig. c, below) trifid tooth are almost completely separated from the c1 tooth (type III); 4th laterals reduced to about the same height as the 5th lateral teeth (type II).

Anterior margin of the ventromental plate (Fig. d, below) is smooth, with about 51-52 striae reaching just beyond middle of the plate, and with faint striae reaching at least half the remaining distance to the anterior margin, VMR about 0.25.

PE (Fig. a, below) with 12-14 pointed and rather uniform teeth. Premandible (Fig. a, below) with broad inner tooth about 2.7-4 times wider, and slightly longer than, the outer tooth. Antenna (Fig. b, below) with A1 about 3.41 (3.25-3.50) times longer than wide; AR about 2.23 (2.06–2.38); ratio of segments (μ m) 141 : 34 : 12 : 15 : 7.

The 3rd inner tooth of the mandibles (Fig. e, below) is partially colored and fused to the lower margin (type IIB), at least 11 furrows on the outer surface near the base.



Photos courtesy of Isabelle Proulx

Cytology: Cytology of available specimens was not good. 4 polytene chromosomes, probably with the thummi arm combination AB, CD, EF, G. Arm G often unpaired and with a virtually terminal nucleolus.

Found in an oligotrophic lake at a depth of 4 m.

- Found: Alberta Johnson Lake, Banff N.P. (51.198°N, -115.479°W)(BOLD).
 Northwest Territories Pontoon Lake, Yellowknife (62.535°N, -113.976°W)(BOLD)
 Ontario G.Hall Public School, Little Britain (44.2817°N, -78.858°W)(BOLD);
 Silver Lake (46.43°N, -81.03°W), Sudbury area. (Proulx *et al.* 2013)
 Saskatchewan La Loche Community School (56.482°N, -109.435°W)(BOLD)
- This larva does not correspond to any of the other currently known salinarius-type from North America or the Palearctic.

Chironomus species TE11, from BOLD database. (Species 4y)

In BOLD Bin: <u>BOLD:AAB4581</u>

Some specimens identified as *C. lugubris*. The nearest neighbor Bin in the BOLD database is <u>BOLD:ADL5630</u> which is also identified as *C.* species TE11

Adult:

Male

Some specimens in the BOLD database are almost completely dark brown-black, while others are much lighter, as indicated below.

AR about 3.5-4

Wing – about 22 setae in squamal fringe.

Legs – LR about 1.52-1.57; femurs yellowish, knees darkened; Anterior femur and tarsal segments dark; other femurs yellowish, with darkened tarsi.

Abdominal tergites largely dark with a narrow yellowish band at posterior margin. About 6-11 setae near center of tergite IX in 3-5 discrete patches.



SVo closest to Strenzke's S(c), but end quite pointed with a ridge making it appear like the bill of a bird. IVo reaching about to end of anal point or about 1/3 of gonostylus. Anal point broad, parallel sided. Gonostylus slender, tapering over posterior third.

Female:

Antennal proportions (percentage of neck in brackets): 26 : 14 (57) : 14 (57) : 13 (54) : 28. AR about 0.42, A5/A1 about 1.08 Palpal proportions (segs. 2-5): 8 : 30 : 30 : 52 ; P5/P4 about 1.73. Cercus with anterior margin longer, ventral margin essentially straight with rounded

ends, no evident bulge on anterior margin.

(From BOLD specimen MIDGE245-06 identified as C. lugubris).

Pupa not known.

Fourth instar larva: (from single larva) Medium sized thummi-type larva (fem. abt. 14mm), anterior VT longer than posterior pair (1.24 cf. 1.18). Gula dark on posterior 1/3 for abt width of mentum, FC very slightly dark, with slight darkening elsewhere. AT abt 410 x 180 μ m (2.3 times longer than wide). Salivary reservoir 63 x 23 μ m (2.8 times longer than wide). VHL 331 μ m; mentum width 170 μ m, 0.51 of VHL. Mentum with 4th laterals slightly reduced (Ty. I-II); center tooth either type IIA or worn III.

Ventromental plates about 197 μ m long and 3.5 times wider than deep; 1.16 times the MW; with about 53 striae; IPD 0.49; VMR 0.30-0.32. PE not seen.

Premandible with broad teeth, inner tooth about 4.3 times wider than outer tooth.

Antenna with A1 about 0.41 of VHL; 4.1 times longer than wide; RO about 0.37-0.39 up from base; AR 1.96; A5 0.67 the length of A3; approximate segment lengths (micron) 137 : 38 : 11 : 12.5 : 7.5. Distance between the antennal bases (159 µm) greater than that between the S4 setae (147 µm) which are separated by O. 77 of the FC width.

Mandibles about 255 μ m long; third inner tooth partly separated and colored (type IIB); 16-17 furrows on outer surface near the base; about 11 taeniae in PecM; Mdt-Mat abt 30 μ m, MTR 0.36.

Cytology: 4 polytene chromosomes with the thummi-arm combination AB, CD, EF, G. Arm G with a virtually terminal nucleolus and a prominent BR about a quarter from the other end. Paired only at nucleolus. No nucleoli in the other chromosomes. Centromeres obvious. Arm A1: 1-2c, 10-12, 3-2d?, 9-4, 13-19? i.e. perhaps as holomelas

- Arm B1: Puff (group 7) near distal end of the arm.
- Arm C1: Constriction (group 4) about one third from centromere.
- Arm D1: Not clear

Arm E1: possibly 1 - 3e, 10b - 3f, 10c-g, 11 - 13.

- Arm F1: 1 4?, ?, 9 8, 13-11, 16, ?, 20 23.
- Arm G1: Virtually terminal N, two BRs about a quarter from other end with only a few bands between them.



Chromosome complement of Ontario specimen/

Ontario sample from shallow pool with leaves and debris.

Found: Alberta – Banff Natl. Pk. (51.436°N, -116.188°W); Wood Bison Trail, Elk Island Natl. Pk. (53.567°N, -112.841°W); Jasper Natl. Pk. (53.1925°N, -117.954°W)(all BOLD).
Ontario – about 4.5 km S. & W. Clarence Creek, Russell Co. (45.50°N, -75.22°W); Kawartha Lakes (44.366°N, -78.478°W) (BOLD).
Manitoba – Churchill (58.772°N, -93.843°W) (Ekrem & Stur, BOLD).
Saskatchewan - Prince Albert Natl. Pk. (53.907°N, -106.075°W)(BOLD).
Yukon Territory - Ivvavik National Park (69.162°N, 140.155°W) (BIOUG17183-B09); Kluane Natl. Pk. & Reserve (60.748°N, -137.513°W) (both BOLD) Also Norway

Chironomus sp. 5h

Only a single larva is known for this species. It is quite similar to that of species 5c (sp. ITE).

Fourth instar larva GP8.2.1 21F: A halophilus-type larva, very similar to those of Sp. 1TE. Length about 10.8 mm, VT shorter than those of other specimens from the same site: anterior 0.18 mm. posterior 0.24 mm; anal tubules much shorter 300-400 μ m (ventral pair longer) and about 1.9-1.8 times longer than wide. Head capsule generally dark, gula darkened over posterior 2/3. Distance between the S4 setae about the same as that between the antennal bases, but the setae are closer to the margin (80% of width of FC between them). Mentum with 4th laterals reduced to about the level of the 5th laterals (type II) and central tooth probably type IB. Ventromentum (about 45 striae) and PE (12 teeth of type C) as in other larvae; Premandible not clear. Antenna also essentially as in the other larvae, but A1 is half the VHL; A5 is the same length as A3: AR 2.3. Antennal proportions (micron) 157 : 35 : 11: 13 : 11.

Mandible of type IIB, and 11-12 PMa taeniae; with 14-15 furrows on the outer surface near the base.

Cytology: 4 polytene chromosomes with the thummi-cytocomplex arm combination AB, CD, EF, G. Nucleolus essentially terminal on arm G.



Found: Greenland - Nedre Midsomer Sö, Peary Land, sample GP8 (82.63°N, 32.50°W).

Chironomus riihimakiensis Wülker 1973

Possibly in BOLD Bin: <u>BOLD:ADA8845</u> (see under *C. laetus*, below)

Adult:

Male: Almost entirely black. Anal tergite with reticulated pattern laterally. SVo closest to E(h) type of Strenzke (1959

Fourth instar larva: Larva a thummi-type (i.e. no lateral tubules, posterior ventral tubules coiled).

Cytology: 4 polytene chromosomes with the thummi-cytocomplex arm combination AB, CD, EF, G. Arm G a flask or amphora-type, typical of species of the riihimakiensis-complex.

laetus-cytocomplex (Bolshakov et al. 2022)

Chironomus laetus Belyanina & Filinkova 1996 (Species 5i) (This is technically a junior homonym of *laetus* Meigen 1818 which is now in *Polypedilum*)

Previously C. sp. 8TE from BOLD Database

In BOLD Bin: BOLD:AAC0595

The nearest neighbour Bin is <u>BOLD:ADA8845</u> Specimens in this Bin are also called *C*. sp. 8TE, or *C*. nr. *longistylus* or *C*. *riihimakiensis,* with an overlapping sequence distribution.

Further data are required to determine the nature of the differences between the species in these two Bins. However it is most likely that the species in both bins are members of the *C. riihimakiensis* complex. The cytology of *C. laetus* indicates close relationship members of this complex (see below).

Adult:

From original description of Belyanina and Filinkova (1996) with some additional male data from specimen of sp. 8TE from BOLD database:

Male: Color dark blackish-brown, scutellum brown, halteres pale; abdomen with a thin pale stripe at the posterior margin of segments II-VII (not recorded in type material); legs apparently paler, knees slightly darkened.

Body length 8.5-9.5 mm; wing length 4-5.5 mm; width about 1.1 mm, VR about 1.0; 2-4 SCf on brachiolum; anterior veins slightly darkened.



Adult male of *C. laetus* from Greenland (BOLD Database as sp. 8TE)

AR 4.2-5.4. Palps pale, segment lengths (micron) 71 : 80 : 307 : 288 : 371; P5/P4 1.29, P5/P3 1.21. FT 49 (43.8-52.5) µm long, 12.5 µm wide (4.1 times longer than wide).

Thoracic setae: 6-12 acrostichal; 36-64 dorsocentral; 6-14 prealar; 2 supra-alar; 44-50 scutellar.

_	Legs with a weak beard, DK 1-1.5, EK1.5-1.4. Approximate proportions (interon).											
	Fe	Ti	Ta1	Ta2	Ta3	Ta4	Ta5	LR	F/T	BR		
PI	1665	1765	2600	1170	750	645	265	1.3-1.4	1.07	1.0-1.5		
PII	1715	1590	950	570	540	345	205	0.60	1.05	-		
PIII	2140	2460	1715	945	600	490	245	0.70	abt 0.8	_		

Legs with a weak beard, BR 1-1.5; LR1.3-1.4. Approximate proportions (micron):

Setae of TIX not specified but shown as in a small patch. SVo of c-type of Strenzke (1959); IVo slightly longer than anal point, to about a third of gonostylus, which is

relatively narrow and narrows evenly along its length. Anal point short and broad (about twice as long as wide, narrowing slightly along its length.



Male (left) and female (right) hypopygia

Female: Coloration as in male but both scutellum and postnotum brown; sternite VIII the same color as sternite VII.

Body length 7-10 mm; wing length 5.5-6.0 mm; FT 27.1 (18.8-31.3) long and 12.5 μm wide. LR 1.45; BR 0.85-1.

Wing length 5.5-6 mm; 2-4 SCf on brachiolum.

Thoracic setae: 8-14 acrostichal; 55-61 dorsocentral (incl. humeral); 7-12 prealar; 2 supra-alar; 63-76 scutellar.

GcIX with 6-10 setae; TX with dark anterior margin and 12-14 setae, about 3 times longer than greatest width. Cerci described as lobe-like (as in ear?) with a large swelling at base of ventral margin.

Pupa: Exuvia with grey pattern. Cephalothorax, lateral margins of tergites V-VIII, scalar pattern on tergites I-VII, and anal lobe brown. Tergites, except first one, covered with small spinelets to varied extent. Spur of segment VIII broad with about 8 open spines



Pupa abdomen (left) and larval head (right)

Fourth instar larva: The larva is a thummi-type larva, with LP noted as longer or equal to length of posterior parapods. Length 15-18 mm. Head capsule width 754.2 μ m; with very dark posterior 2/3 gula, wider than the mentum, which is 216.3 (212.5-225) μ m wide. Mentum of type I and central tooth possibly type IIA. Ventromentum with smooth anterior margin, about

 $225.5 \ \mu m$ wide; about 1-1.06 times the mentum width; with 44-56 striae. PE with 13-15 teeth. Premandible with usual two teeth, but no other details.

Antenna with basal segment 37.5-43.6 μm wide at base, RO about a third up from base, AR 1.7-1.8.



Larva of C. laetus from Manitoba, Canada (BOLD Database)

AT appear to be relatively long and possibly with a constriction in the middle.

Cytology: (based on Russian, i.e. type, material) 4 polytene chromosomes. The original photograph of the polytene chromosomes in Belyanina and Filinkova (1996) was not clear (see below) and the arm combination was misidentified as pseudothummi-complex. Subsequent analysis by Bolshakov et al. (2022) clarified the new laetus arm combination AE, BC, DF, G. This combination is derived from the pseudothummi-complex by translocation CD, BF to BC, DF probably from a member of the C. riihimakiensis-related species. This is indicated by the banding sequences and by the 'amphora-shaped' arm G typical of that group. laeA1 1a - 2c, 10a - 12c, 3i - 2d, 9e - 4a, 13a - 19f as spAl1; spLe1A1 1a - 2d, 22d - 21a, 7a-8a, 3c - 2e, 20g - 18a, 6f-a, 4a - 5e, 13g - 8a, 17d - 14a, 23 laeB1 28a as spAl1B2 laeC1 1a - 6b, 11c - 8a, 15e - 11d, 6gh, 17a - 16a, 7d-a6f-c, 17b - 22g as spAl1C1, sorC2, abeC1 laeD1 1a - 3g, 11a - 18f, 7g - 4a, 10e - 8e, 18g - 24g as spLe1D1 laeE1 1a - 3e, 5a - 10b, 4h - 3f, 10c - 13g as spLe1D1, plumosus, etc. laeF1 1a -8c, 12a - 17d, 10d - 8d, 11i-a, 18a - 23f as spLe1F1 laeG1 subterminal nucleolus, narrow neck, then broadens with a BR, narrowing again before broadening with another BR – giving the typical 'amphora' appearance of the *C. riihimakiensis*-gp.

Note that Wülker (1999) suggested that spAll was probably identical to *C. frequentatus* Belyanina and Filinkova 1996, another member of the *C. riihimakiensis*-group.



Polytene chromosomes of C. laetus, modified to show the correct arm combination

Molecular sequence:

mt*COI*: There is sequence in GenBank (e.g. MN676646) and in the BOLD Database as either sp. 8TE or *C. laetus*.

The close relationship between *C. laetus* and other members of the "riihimakiensis-complex" suggests that the whole arm translocation has occurred relatively recently (evidenced by the finding of a hybrid with *C.* spLe1 – which would probably have been infertile due to meiotic mis-division) so that there would be no selection for genetic isolation, and little mutation has occurred since the occurrence of the translocation.

Found: Manitoba - nr. Churchill (58.738°N, -93.819°W) (BOLD) Nunavut – Kitikmeot, Cambridge Bay (69.2194°N, -104.92°W); Greiner Lake, Victoria Island (69.19°N, -104.93°W)(GenBank). Yukon Territory - (BOLD) Greenland - Lake Tasersuaq, Kitaa, Kujalleq (60.2643°N -44.5389°W); Narsarsuaq, Kujalleq (61.1556°N, -45.3785°W) (both BOLD) Russia – 38 Km. nw. Labytnangii (abt 69.41°N, 66.23°E), Polar Urals (Type locality); Yamal Peninsular (abt.71.25°N, 71.64°W(Bolshakov *et al.* 2022).

Chironomus vitellinus Freeman 1961 (Species 5y)

Synonyms:

Placed as a synonym of *C. javanus* by Chaudhuri *et al.* 1992, but this is incorrect as the anal point of the adult males is expanded at the end; there is usually a difference in tooth number of the larval premandible and there are significant differences in the BARCODE sequences.

In BOLD Bin: BOLD:AAG6924

as C. javanus, but most specimens are actually C. vitellinus.

North American specimens were originally thought to be the adult of *C*. sp. Florida (see Pt. 2), but now recognized to be the widespread and rather variable *C*. *vitellinus*, originally described from Australia but now known to extend through essentially the whole tropical region.

Freeman's original description:

Thorax of a yolky colour, dull with practically no pruinosity; legs whitish, especially on tibiae, tarsal segments black at joints; abdomen without dark markings but quite strongly pruinose at incisures and on segments 5 and 7; anal point of male narrow in side view. The white legs with dark marked tarsi and the pale abdomen make this species easily recognized: the hypopygium is also characteristic.

Wing length. -2.5-3.0 mm.

Male. – Head., mouthparts, and antennae yellowish brown, plumes paler, FT present, A.R. about 4.5. *Thorax* a dull reddish yellow, yolky colour, very slightly pruinose near the front; shoulders and immediate areas slightly tinged with greenish; dorsocentral bristles only present in in posterior half of thorax. *Legs* with femora very pale green, tibiae and tarsi whitish, the tarsi have definite black markings across the joints between segments; anterior tarsi not bearded, LR about 1.8. *Wings* pale, crossvein darkened. *Abdomen* yellowish green, lacking definite dark markings; incisures and segments 5 and 7 with quite strong pruinosity; hypopygium with anal point narrow at base, curved and finger-like in lateral aspect; appendage 2 (IVo) short and stout, styles broad at base and rather sharply narrowed at apex.

Female. – Resembles male; sensory hairs on apical antennal segment longer than usual.

Further information from a Paratype male from Mafulu, Papua New Guinea:

Cephalic tubercles about 30 µm; 14 clypeal setae.

Palpal proportions (segs 2-5 (μ m)) 60 : 170 : 200 : 140 (shrivelled).

Thoracic setae: 6-7 dorsocentral; prealar 4; others not evident.

Male	Fe	Ti	Ta1	Ta2	Ta3	Ta4	Ta5	LR	F/T	BR
PI	1220	1050	1120	955	840	770	350	1.60	1.16	no beard
PII	1305	1130	760	395	280-	190	140	0.67	1.15	-
PIII	1435	1445	1130	610	465	290	160	0.78	0.99	-

Leg lengths (micron) and proportions:

9 setae in individual pale spots on TIX. Setae on IVo simple.



SVo and IVo (left); TIX setae and lateral view of anal point of paratype male of C. vitellinus

Limited information is currently available for North American populations, so descriptions are based on specimens from other regions:

Male

A yellowish-green species with dark bands on the tarsi and darkening of the cross veins of the wings. Thorax of a yolky colour, dull with practically no pruinosity; legs whitish especially on tibiae, tarsal segments black at joints; abdomen without dark markings but quite strongly pruinose at incisures and on segments 5 and 7, anal point of male narrow at base in side view:

Wing length 2.64 (2.07-3.00) mm, width 0.64 (0.56-0.73) mm; VR 1.05 (1.02-1.08); 2 Scf on brachiolum, 19.5 (17-22) setae on squamal fringe.

Freeman (1961) quotes AR about 4.5, but in other populations the AR is lower 3.34 (2.89-4.5).

Head: Frontal tubercles 43.7 (30-55) μ m long and 2.4-3.7 times longer than wide. Palpal proportions (micron): 49 : 46 : 157.5 : 191 : 272 : P5/P4 1.45 (1.25-1.74); P5/P3 1.79 (1.40-2.17). Clypeus about 0.60 (0.47-0.75) of antennal pedicel, with about 18.2 (11-23) setae.

Thoracic setae: acrostichal from about 4-11; dorsocentrals 9.7 (5-14); Prealar 4.25 (3-5); Supraalar 1; Scutellar 10.75 (13-17), 2-6 in anterior row, 5-11 in posterior row. Leg lengths (microns) and proportions as follows:

Male	Fe	Ti	Ta1	Ta2	Ta3	Ta4	Ta5	LR	F/T	BR
PI	1265	975	1320	930	825	755	355	1.60-1.93	1.16-1.27	2.1-2.5
PII	1210	1060	705	365	270	180	130	0.63-0.68	1.11-1.17	
PIII	1325	1330	1065	575	430	265	150	0.77-0.83	0.90-1.00	

Tergite IX with 9.3 (5-17) setae, in individual pale patches.

Hypopygium with narrow anal point expanded at distal end, strongly turned down and narrow in lateral aspect. Superior volsella well developed and curved, not like any of Strenzke's types perhaps closest to E-type(g); Inferior volsella reaching about to 1/3-1/2 length of gonostylus, with 12-14 incurved simple setae (although Tokunaga's figure appears to show them as forked). Gonostylus may not be as swollen as shown in Tokunaga's figure (below) but narrows conspicuously over posterior third to half, with 5+1 setae at the tip.



Male hypopygium of C. vitellinus from Tokunaga (1945).

Female

Freeman's (1961) description for *C. vitellinus*: Resembles male; sensory hairs on apical antennal segment longer than usual.

Other:

Wing length 2.91 (2.58-3.16) mm, width 0.79 (0.75-0.91) mm; VR 1.09 (1.07-1.11); 2 Scf on brachiolum; 15.5 (13-16) setae in squamal fringe.

Coloration essentially as in male.

Head: FT present 28.1 (7.5-35) long and 1.96 (1.0-2.8) times longer than wide. Antennal segments (micron) with percentage neck in brackets: 171 (28) : 126 (46) :

130 (49) : 129 (49) : 240; AR 0.37 (0.33-0.43); A5/A1 1.23 (1.0-1.46).

Palpal segments (micron): 55:49:180:220:345; P5/P4 1.60; P5/P3 1.85. Clypeus heart-shaped, about 1.28-1.55 wider than antennal pedicel; abt 23 (16-39) setae. Thoracic setae: Acrostichals – 11.5 (9-16); Humerals – 3.6 (3-5), mostly linear but may be grouped (e.g. as a triangle); Dorsocentrals – 15.2 (9-26); 18.7 (13-30) including the Humerals (lower in Pacific Islands); Prealars – 5 (4-8); Scutellars in two rows – 2.2 (0-6) in anterior row and 10 (8-13) in posterior row, total 12.5 (9-19).

Fe	Ti	Ta1	Ta2	Ta3	Ta4	Ta5	LR	F/T	Ta4/Ti
1345	950	1715	975	825	875	390	1.69-1.92	1.16-1.65	0.80-0.99
1240	1160	690	335	245	180	125	0.58-0.65	1.04-1.13	
1365	1460	980	505	415	270	155	0.63-0.77	0.90-0.99	
	Fe 1345 1240 1365	Fe Ti 1345 950 1240 1160 1365 1460	Fe Ti Ta1 1345 950 1715 1240 1160 690 1365 1460 980	Fe Ti Ta1 Ta2 1345 950 1715 975 1240 1160 690 335 1365 1460 980 505	Fe Ti Ta1 Ta2 Ta3 1345 950 1715 975 825 1240 1160 690 335 245 1365 1460 980 505 415	Fe Ti Ta1 Ta2 Ta3 Ta4 1345 950 1715 975 825 875 1240 1160 690 335 245 180 1365 1460 980 505 415 270	Fe Ti Ta1 Ta2 Ta3 Ta4 Ta5 1345 950 1715 975 825 875 390 1240 1160 690 335 245 180 125 1365 1460 980 505 415 270 155	Fe Ti Ta1 Ta2 Ta3 Ta4 Ta5 LR 1345 950 1715 975 825 875 390 1.69-1.92 1240 1160 690 335 245 180 125 0.58-0.65 1365 1460 980 505 415 270 155 0.63-0.77	Fe Ti Ta1 Ta2 Ta3 Ta4 Ta5 LR F/T 1345 950 1715 975 825 875 390 1.69-1.92 1.16-1.65 1240 1160 690 335 245 180 125 0.58-0.65 1.04-1.13 1365 1460 980 505 415 270 155 0.63-0.77 0.90-0.99

Leg lengths (microns) and proportions as follows:

Anterior Ta4 longer than Ta3.

GcIX with 3.7 (2-6) setae; segment X usually a half-oval 91-177 μ m wide and 2.99 (2.1-5.36) times longer than greatest width, with about 11.4 (10-13) setae. Sasa & Hasagawa (1983) note that the cercus is roughly rhombic, 112x152 μ m; usually with a ventral basal bulge.

Pupa has been illustrated by P.S. Cranston in his Electronic Guide to Chironomidae of Australia (reproduced below with permission).

Length: Male 6.40 (6.38–6.70) mm; female 7.01 (6.90–7.14) mm. Exuviae grey. Cephalic tubercles 86.3 (81-110) μ m long and 56.5 (51-70) μ m in diameter, subapical seta about 56 (38-90) μ m long, i.e. about as long as the tubercles. There is slight development of a frontal wart (see Cranston figure g below) – abt 38 x 6 μ m. Respiratory base about 132.7 (119-157) x 62.25 (51-81) μ m wide; HR 2.16 (1.94-2.35). 2 pairs of precorneal setae.

Abdomen with PSA caudolateral on segments IV-VI, that on segment IV about 145 (116-157) x 89 (71-111) μ m wide and about 22 (18-24)% of the segment length; PSB basolateral on segment I and small caudolateral on segment II, which also bears a caudal row of about 66.2 (54-81) hooks which occupy 58-68% of the segment width. Caudolateral spur of segment VIII usually with 1+2sm (1-4) spines, although commonly only 1 is long. Swim fin with about 70.3 (61-78) taeniae in two rough rows (particularly distally).



Reproduced from Cranston's Electronic Guide to Chironomidae of Australia, (with permission)

Fourth instar larva: a medium sized, essentially plumosus-type larva, TLt (656 (520-800) μ m long) are more ventrally placed than in other species. Length 11.5 (10.5-12.5)(female) 9.75 (9-10.5)(male) mm and VT long, anterior pair 1.84 (1.32-1.84) μ m generally longer than posterior pair 1.62 (0.96-2.16) μ m. AT with median constriction, dorsal about 550 (500-600) μ m long and 3.57 times longer than wide; ventral 430 (420-440) μ m long and 3.67 times longer than wide; ventral 430 (420-440) μ m long and 3.67 times longer than wide; ventral 430 (420-440) μ m long and 3.67 times longer than wide; ventral 430 (420-440) μ m long and 3.67 times longer than wide; ventral 430 (420-440) μ m long and 3.67 times longer than wide; ventral 430 (420-440) μ m long and 3.67 times longer than wide; ventral 430 (420-440) μ m long and 3.67 times longer than wide; ventral 430 (420-440) μ m long and 3.67 times longer than wide; ventral 430 (420-440) μ m long and 3.67 times longer than wide; ventral 430 (420-440) μ m long and 3.67 times longer than wide; ventral 430 (420-440) μ m long and 3.67 times longer than wide; ventral 430 (420-440) μ m long and 3.67 times longer than wide. Salivary reservoir (1 specimen) 86 x 15 μ m (5.4 times wider than deep. Gula pale or slightly darkened on posterior third, slightly wider than mentum width and widest at the posterior margin; FC pale.
Mentum (c, below) with the central trifid tooth set below the 1st laterals (although not always obvious if the 1st laterals are worn), and the c2 teeth markedly separated from c1 tooth (type IIA) and pointed towards it; 4th laterals at most slightly reduced (type I). PE (a, below) with about 14 (12-16) often irregular teeth (Type D). Ventromental plates (d, below) about 171.4 (159-188) μ m wide and 3.77 (3.6-3.9) times wider than deep and 1.11 (1.06-1.15) times the mentum width; separated by about 0.39 of the mentum width, with about 31 (27-32) striae; VMR 0.30 (0.22-0.33).

Antenna (b, below) with the basal segment about 3.4 (2.9-3.4) times as long as wide and about a third of the ventral head length; AR about 2.65 (2.47-2.83); ratio of segments 116.5 : 25 : 5.5 : 8 : 5. Distance between antennal bases, 142 (139-146) µm, larger than width between S4 setae, 121 (118-124) µm. S5 setae about level with nearby RO. Mandible (e, below) about 207 (199-215) µm long, with third inner tooth darkened and completely separated (type IIIC), with three spines on inner margin, and about 11-12 furrows on the outer surface at the base; 11.13 (10-13) taeniae in PecM; Mdt/Mat about 24 (23-26.5), MTR 0.38 (0.32-0.48).



The larva is most readily recognised by the unusual premandible, which has 7 teeth although the Florida population may have only 6 teeth, i.e. there is polymorphism for tooth number (6 teeth have also been reported for Japanese specimens).



Premandible of C. vitellinus larva with 7 teeth.

Cytology: The cytology of North American specimens is not known, but the polytene chromosomes are generally of rather poor quality. The arm combination appears to be the thummi-group. i.e. AB, CD. EF and G. Arm G has a subterminal nucleolus and a BR about 1/3 from other end.



Arm G of C. vitellinus

Found: Type locality - Darwin, Northern Territory, Australia.

Florida – specimens in collection of John Epler.

Broadly distributed through Indonesia, Thailand (Hashimoto et al. 1981 as *C. javanus*), Japan and Pacific Islands such as Papua New Guinea, Fiji, Caroline Islands and Marshall Islands (Tokunaga 1964 as *C. javanus*). Also recorded at Blantyre, Malawi.

Species		
Benthalia brunn	eipennis (Johannsen) (sp.
3k),	-	Pt 2
Benthalia natch	itocheae (Sublette) (sp.	4n),
		Pt 2
C (Chaetolabis) atroviridis Townes (si	n
2i)		Pt 2
C (Chaetolahis)) hitumineus I & V (si	n 112
2h)) often minet $\Delta \Delta \mathbf{v}$. (5)	р. Dt 7
C (Chaptalabia)) ophraptus Townes (sn	112
C. (Cliaetolauis) ochieatus Townes (sp	D+ 2
C an abamatus	of Wällson (an. 2h)	ГІ <u>2</u> D4 1
C. nr. aberratus	of wulker (sp. $3b$),	
C. acerbiphilus	Tokunaga (sp. 4j)	Pt 2
C. acidophilus I	(sp. 4f)	Pt 2
C. alchichica A	costa & Prat (sp.5w)	Pt 1
C. annularius se	ensu Strenzke	Pt 1
C. anonymus W	'illiston (sp. 20),	Pt 2
C. anthracinus Z	Zetterstedt (sp. q),	Pt 1
C. annularius (s	p. 3d),	Pt 1
C. nr. anthracin	us (sp. 3b),	Pt 1
C. nr. anthracin	us (sp. 3c),	Pt 1
C. athalassicus	Cannings (sp. 2v),	Pt 1
C. atrella Town	es (sp. i),	Pt 1
C. nr. atrella Ar	derson & Hitchcock (s	p.
2n), C. ?nr. atre	lla (sp. 2r),	Pt 1
C. atritibia Mall	loch (sp. 4c)	Pt 1
C. (Lobo.) austi	ni (Beck & Beck) (sp.	4h)
		Pt 2
C (Lobo)?nr	austini (sp. 2e)	Pt 2
C balatonicus (misident) (sp $3a$)	Pt 1
C bifurcatus (si	n a)	Pt 1
C biseta (sp. 4r). ()	Pt 2
C. blavlocki (sp. 4p	<i>''</i>	D+ 1
C. butleri' (sp	. 1), 2g)	Π D+ 1
C. colligraphus	2g), Cooldi (an Juu)	D_{\pm}
C. calligraphus	Ty 2(howaiiangia)	ГІ <u>2</u> D+ 2
C. cariigraphus	1 y 2(liawalielisis)	Pt Z
C. calumbiansis	Wueller et al (ap. 4a)	
C. columbiensis	wuchker et al.(sp. 4a)	Dt 🤈
C conformia (and C. constinuuli)	Tt∠ D+
	see C. sancupaun)	Γl
l C. amagiagu data	a Mallach (an D i)	D4 1
C. crassicaudatt	(sp. 2j),	
C. cucini webb	(sp. 0),	Γt I D4 1
C. decorus Joha	nnsen (sp. 3a),	
C. decorus grou	р (B=sp. 1),	Pt I
C. decorus grou	p (sp. b),	Pt 1
C. decorus grou	p (sp. c),	Pt 1
C. decorus grou	p (sp. j),	Pt 1

C. decorus group (sp. z),	Pt 2
C. decorus group (sp. 2a),	Pt 1
C. decorus group (sp. 2t),	Pt 1
C.decorus R.&F (sp.3f) - see also deco	rus
Joh.	Pt 1
C. decorus group (sp. 3h),	Pt 1
C. decorus group (sp. 3i),	Pt 1
C. decorus group (sp. 3j),	Pt 1
C. decorus group? (sp. 41)	Pt 1
C. decorus group sp. 1- see bifurcatus	
C. decorus group sp. 2 - see 'butleri'	
C. decorus or riparius group (sp. 31),	Pt 2
C. ?decumbens (sp. 2x)	Pt 1
C. nr. decumbens (sp. h),	Pt 1
C. dilutus Shobanov et al. (sp. t),	Pt 2
C. (Lobo.) dorsalis Meigen (sp. 4g)	Pt 2
C. entis Shobanov&Djomin (sp. 30),	Pt 1
C. flaviventris - see C. staegeri	
C. frommeri Sub. & Subl. (sp. 2d),	Pt 1
C. fulvipilus - see Goeldichironomus	
holoprasinus	
C. grodhausi (sp. 2r),	Pt 2
C. harpi Sublette (sp. 2z),	Pt 1
C. hawaiiensis (sp. 50)	Pt 2
C. hyperboreus (sp. x),	Pt 1
C. islandicus (sp. 5s)	
C. jonmartini Lindeberg & Wiederholm	n
(sp. 4e)	Pt 1
C. karensis - see C. anonymus	
C. longistylus Goetghebuer (sp. 3n),	Pt 1
C. lugubris (sp. 5n)	Pt 2
C.magnus White & Ramsey (sp. 2q),	Pt 1
C. major Wülker et al see C. magnus	
C. maturus Johannsen (sp. d),	Pt 2
C. melanescens (sp. e)	Pt 2
C. (L.) montuosus Ryser et al. 1985 (sp	5. 5f)
	Pt 2
C. mozleyi Wülker (sp. 3u),	Pt 1
C. muratensis (misident) (sp.3t)	Pt 1
C. pallidivittatus sensu Beermann (195	5)
(sp. 21),	Pt 2
C. pilicornis (Fabricius) (sp. m),	Pt 1
C. plumosus (Linn.) (sp. p),	Pt 1
C. prior Butler (sp. 3z)	Pt 1
C. 'proulxi' (sp. 2b),	Pt 1
C. (L.) pseudomendax(?) (sp. 4i)	Pt 2
C. pungens - see Kiefferulus pungens	
C. quinnitukqut (sp. 2n)	Pt 1

C. rempelii Thienemann (sp. 2m),	Pt 1
C. riparius group (sp. f),	Pt 1
C. riparius Meigen (sp. y),	Pt 1
C. sanctipauli (sp. 5g),	Pt 1
C. staegeri sensu Townes (sp. s),	Pt 1
C. stigmaterus Say (sp. n),	Pt 1
C. nr. stiigmaterus (sp. 5v)	Pt 1
C. (L.) storai (sp. 5a)	Pt 2
C. striatipennis Kieffer (sp. 4b)	Pt 2
C. tardus Butler (sp. 3s),	Pt 1
C. tentans Fabricius (sp. 3y)	Pt 2
C. tenuistylus Brundin (sp. 3m),	Pt 1
C. nr. tenuistylus (sp. 4s)	Pt 1
C. 'tigris' Butler & Kiknadze (sp. r).	Pt 1
C. trabicola Shobanov <i>et al.</i> (sp. w).	Pt 1
C. tuberculatus Townes (sp. 4d)	Pt 2
C. tuxis Curran (sp. 40)	Pt 1
C nr tuxis (sp u)	Pt 1
C utahensis Malloch (sp. 2p)	Pt 1
C vancouveri (sp. 5m)	Pt 2
C c f venustus (sp NAIII)	Pt 1
C vitellinus Freeman (sp. 5v)	Pt 1
C vockerothi Rasmussen (sp. 3w)	Pt 2
C whitseli Subl & Subl (sp. 24),	Pt 2
C winnelli Wülker (sp. 3v)	Pt 1
C sn k	Pt 1
C sp μ (nr hyperboreus aberratus	111
sororius)	Pt 1
C = sn = nr + sn = u	Pt 1
$C \sin 1 (\sin 2\alpha)$	Df 1
C sp. 1 (sp. 20),	Pt 2
C sp. 2t (calligraphus gp. ?)	Pt 2
C sp. 2g (callgraphus gp. 1)	Pt 1
C sp. Jg,	\mathbf{P}_{t}
C sp. Florida	Dt 1
C. sp. Γ on dt	FLI Dt 1
C. sp. NAII (sp. 4t) C. sp. NAII (a f. vanustus) (sp. $4u$)	Ft 1
C. sp. NAII (c.i. venusius) (sp. 4u) C. sp. NAI (cn. $4u$) (nr. anthrasinus)	Ft 1
C. sp. $NAI (sp. 4x) (m. anumacinus)$	Pt I
C. (L_{aba}) and (M_{aba})	Pt 1
C. (Lobo.) sp. 4v,	Pt 2
C. sp. 4w, $C_{\rm eff} = 5 \circ (1 \text{TE}) (\text{POLD DD})$	Pt 2
C. sp. 5c (TTE) (BOLD DB)	Pt 2
C. sp. 5d,	Pt 2
U. sp. 5e,	Pt I
$C = \frac{1}{2} \left(\frac{1}{2} \left(\frac{1}{2} \right) - \frac{1}{2} \left($	Pt 2
C. sp. 51 (δ 1 E) (BOLD DB),	Pt 2
U. sp. 5j,	Pt 2
C. sp. 5p (?staegeri Lundbeck),	Pt I

C. sp. 5t	Pt 1
C. sp. 5x	Pt 2
Einfeldia chelonia (sp. 5q)	Pt 2
Einfeldia pagana (Meigen) (sp. 2k),	Pt 2
Einfeldia synchrona - see E. pagana	
Einfeldia sp. (sp. 2y),	Pt 2
Einfeldia sp. A (sp. 5b)	Pt 2
Goeldichironomus carus (Townes) (sp.	2s),
	Pt 2
Goeldichironomus holoprasinus (Remp	el)
(sp. 4r)	Pt 2
Kiefferulus dux (Johannsen) (sp.5r)	Pt 2
Kiefferulus pungens (Townes) (sp.4q)	Pt 2
Species 1 of Poulson & Metz (1938) (C	2.
riparius)	Pt 1
Species 2 of Poulson & Metz (C. decor	us
group, probably sp. j)	Pt 1
Sp. 9 of Wülker et al. (1989) (sp. 3d)	Pt 1
Sp. 51 of Frommer (sp. 20),	Pt 2
Sp. 52 (sp. 2w),	Pt 2
Species algonquian (sp. g)	Pt 1
species Anchorage (sp. 3p),	Pt 1
species Apple Valley (sp. 3b),	Pt 1
species Cape Cod (sp. 4k)	Pt 1
species Coyote Creek (sp. 2w),	Pt 2
species Florida of Epler (sp. 4w)	Pt 2
species Greiner Lake (sp. 5k)	Pt 1
species B of Hilsenhoff & Narf (poss. s	sp.
o)	Pt 1
species C of Hilsenhoff & Narf (sp. 3c))
	Pt 1
species D of Hilsenhoff & Narf (sp. 3d)
	Pt 1
species Julianehåb (sp. 51)	Pt 1
species Le1 of Kiknadze et al. (sp. 3x),	
	Pt 1
species Obatanga (sp. 4z)	Pt 1
species parariparius (sp. 2c),	Pt 2
species WOC of Wülker & Morath (sp.	. 3r),
	Pt 2

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