

NEW ZEALAND CHIRONOMUS SPECIES

Integration of data of Jon Martin¹ and Don Forsyth²

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When Freeman (1959) revised the New Zealand Chironomidae he recognized two species of the genus Chironomus (s.s.): C. zealandicus Hudson 1892 and his newly identified C. analis. However, even at this stage it was known that adults of C. zealandicus could be produced from two distinct larval types, a salinarius-type (lacking ventral tubules) or a so-called thummi-type (with ventral tubules) (Forsyth, 1971), although in fact the latter are a bathophilus-type. (see below for current classification scheme).

Analysis of the polytene chromosomes further indicated that there were different chromosome number forms of both the salinarius- and the bathophilus-type larvae (Lentzios et al., 1980), which appeared to be distinct species. With the integration of the morphological and ecological analyses of Don Forsyth, the karyotypic studies of Jon Martin, and DNA studies with associated larvae from Ian Hogg and Sofia Ibarrarán, we have concluded that there are at least fourteen species of *Chironomus* in New Zealand (Note that this only includes one of the two species described by Sublette & Wirth (1980) from the subantarctic islands) and includes an Australian species that may be a recent migrant. DNA studies are suggesting some of these species are groups of closely related species as is common in other parts of the world. Where possible a link to the relevant Bin in the BOLD Database (http://www.boldsystems.org/index.php) is given.

Seven of the currently recognised species have a salinarius-type larva, and seven others are bathophilus-type, although two are polymorphic for ventral tubule development with some having only slight development of the posterior pair of ventral tubules (halophilus-type). These differences may reflect relationship, but in other cases it is a response to the environmental conditions in which the larvae develop, such that closely related species may have a different larval type. The larva of the fourteenth species is unknown.

For convenience, some Pacific island species are also included (see below).

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

Abbreviations

AR – Antennal ratio. In larvae it is A1/A2-A5, measured only from the sclerotized parts of each segment as the soft tissue between each segment can stretch to different extents during slide mounting.

ASA - distance between antennal bases

AT – Anal tubules

BOLD - Barcoding of Life Database (http://www.boldsystems.org/index.php)

BR - Balbiani ring

COI - Cytochrome oxidase subunit I

CS – sensilla chaetica

Cyt B - Cytochrome b

FC - Frontoclypeus

FT – Frontal tubercles

Gb2B - Globin II Beta

Gb9 - Globin IX

GC - Gonocoxite IX

GP – Gonopophysis VIII

GS – Gonostylus

H setae – Found only in the adult female. Usually included in the dorsocentrals (sometimes with a note that these begin more anteriorly in females than males), but since they are anterior to the parapsidal suture they are humerals and their arrangement has some taxonomic usefulness (e.g. linear, in a cluster, or a mixture).

HR – ratio of length to width of pupal respiratory base

IPD - see VPA

IMW – Inner margin of wing sheath

IVo – Inferior volsella

LR – Leg Ratio (Ta1/Ti, usually of fore leg)

MD – male determining (gene)

Mdt-Mat – distance from the tip of dorsal tooth to the tip of apical tooth of the mandible.

Mt – Mitochondrial

MTR – Mdt-Mat divided by mandible length

MW - Mentum width

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

TLt – posterolateral tubules

PMa – Pecten mandibularis

- PreM Premandible
- PSA Pedes spurii A
- PSB Pedes spurii B
- RO Ring Organ of larval antenna and dorsal head
- S4A distance between larval S4 setae
- SAL Salivary reservoir outlet
- SCf Sensilla campaniformia (on brachiolum)
- SCh Sensilla Chaetica
- SVo Superior volsella
- VHL Ventral head length
- VM Ventromentum
- VML length of VM
- 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. It should be noted that there are two ways in which this may be calculated, one usually giving a value above 1, the other a value below 1 these are reciprocals of each other. The value used here is that given in Sæther (1981): length of M-vein to length of crossvein.
- VT Ventral tubules
- i presence at locality not confirmed.

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.

Provisional Key to Adult Males

(based on supplemented data of D.J. Forsyth)

1. An	al point stout	. C. analis
An	al point narrower	2
2.(1b)	Abdominal tergites with dark saddle spots	5
Ab	dominal tergites with bands or patches covering most of the segment	3
. ,	Anterior tergites with a band over only about two thirds of the segment	
4.(3a)	Pigmentation of the anterior tergites narrowing to the posterior edge C. 'c	astaneum

nt /
C. 'paracastaneum'
C. zealandicus
<3) C. forsythi
R 3-7)
e in single patch eelandiae (Ty.1 & Ty.2)
aezelandiae since Don ew Zealand on the basis that of Australian
wing species are known:

In the adult descriptions reference is made to the types of superior volsella 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 SV).



Abb. 4. Grundformen der Claspette des Chironomus-Hypopygs (3). a—c S-Typ (a halophilus, b thummi thummi, c luridus), d—f D-Typ (d, e dorsalis, f striatus), g—i E-Typ (g cingulatus, h salinarius, i annularius).

In the following key and 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), who recognize 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

bathophilus – moderate to long, essentially straight VT.

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

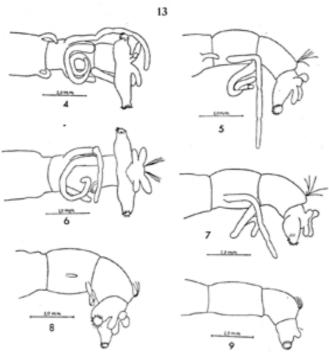
Possessing TLt:

reductus – lacking ventral tubules.

semireductus – short straight or slightly curved VT.

melanotus – moderate to long, essentially straight VT.

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



Figs. 4-9. Hind parts of larvac.

- plumosus type (total length 15 cm; loc. 12; 13.VII.1943); ventral view; right tubuli
- 5. as 4, but seen from the left; left tubuli only drawn.
- 6. thummi type (total length 17 cm; loc. 1; 5.VII.1944) ventral view; right tubuli cut off.
 7. as 6, but seen from the left; left tubuli only drawn.
- as 6, but seen from the sert; sert tubuli only drawn.
 halophilus type (total length 12 cm; the fjord; 27.IV.1942); seen from the left, slightly
- from the ventral side.

 9. salinarius type (total length 15 cm; the fjord; 27.IV.1942); seen from the left.

Note that only about three of these categories, lacking lateral tubules, have been found in New Zealand.

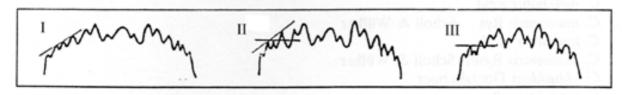
Reference is also made to a number of larval characters including the mentum and mandible types originally devised by Webb & Scholl (1985), Vallenduuk & Moller Pillot (1997) and Proulx *et al.* (2013). These classifications were made for relatively small numbers of species, but with the much larger number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

The mentum type 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

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)

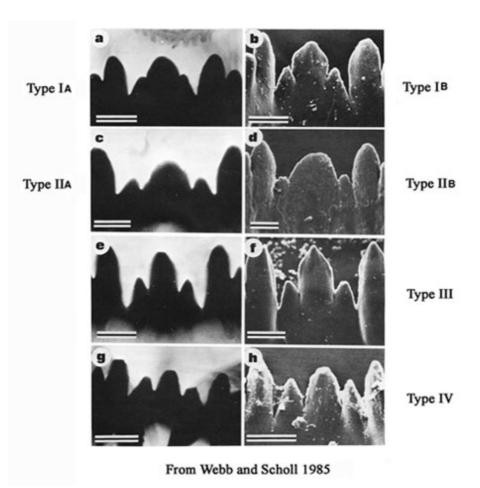
Type IB - c2 teeth slightly more separated (figure b)

Type IIA - c1 broad, c2 teeth distinctly separated (figure c) (there is also a variant with a narrower c1).

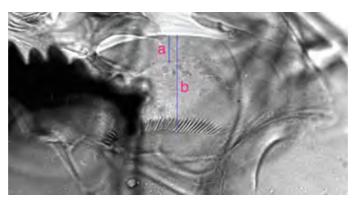
Type IIB – c1 very broad, c2 less separated (figure d).

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)

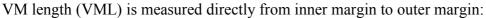


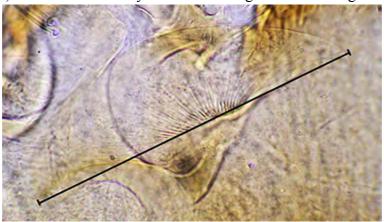
Ventromentum



VMR = a/b

b also serves as a measure of the depth of the Vm for the ratio of length to depth of the VM plates.

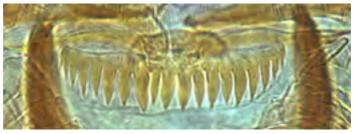




Pecten epipharyngis

Proulx *et al.* (2013) recognised 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 D does not occur in any known New Zealand species.

Type A – fine sharp rather uniform teeth.



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



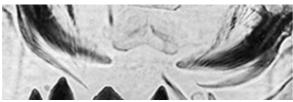
Type C - rounded and usually short. Worn type B teeth may be mistaken for this type.



Type D - rounded teeth with smaller teeth interspersed (generally found in the subgenera *Lobochironomus* or *Chaetolabis* which have not been found in New Zealand).

Premandible

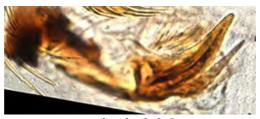
Type A: Both teeth narrow and coming to a fine point.



ratio at least 4

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.



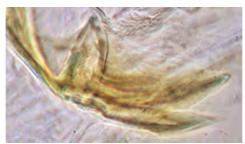
ratio abt 3-3.5

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



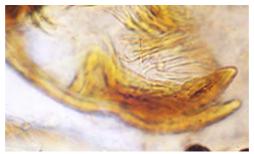
Ratio abt 3.5-4

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



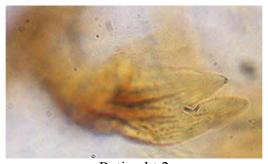
Ratio abt 3.5

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



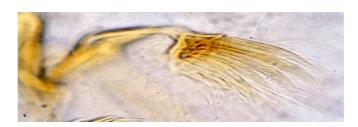
Ratio abt 2.5

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

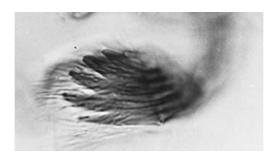


Ratio abt 2

Type F: A six or seven toothed premandible (*C. vitellinus* has seven teeth).



Species of *Kiefferulus* also have a five-toothed premandible, but it is broader than that of *C. javanus*.



The mandible type is defined by the degree of darkening and separation of the 3rd inner tooth.

It appears preferable to consider these as separate characters:

Separation

Type I - almost completely fused on lower margin;

Type II - tooth partly free on lower margin;

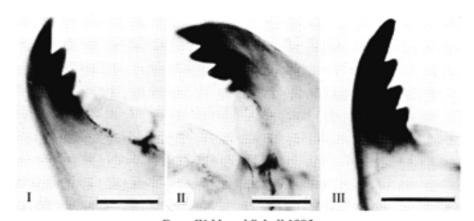
Type III - 3rd tooth completely separate.

Colour

Type A – pale

Type B – slightly darkened

Type C – as dark as other teeth



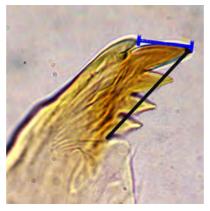
From Webb and Scholl 1985.
These would represent IA, IIB, and IIIC respectively

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 mandible (black line in figure above) to obtain the MTR.

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 at the anterior end near the antennal bases, and where the S4 setae are in relation to the broadening of the clypeus (see figure below). This relationship can be further characterized by the distance of the S4 setae from the edge of the FC – most easily expressed by the fraction of the FC width between the two S4 setae. This has two components: how far the setae are from the FC margin, and how close they are to the widest point of the FC.



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 a characteristic of species of *Chironomus*.

Salivarium (or Salivary outlet 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.

Provisional Key to Fourth Instar Larvae

1.	. Larva a salinarius-type	2
	Larva with at least some development of VTs	. 8
	. More than 60 striations on VM	ĺ
	. Anal tubules rel. long sometimes with sl. constriction near middle <i>C. forsythi</i> (in part)	

Anal tubules relatively short, pointed or rounded
4. FC region of head pale or only slightly darkened
5. c1 tooth of mentum broad (IIA)
c1 tooth of mentum generally narrower (IIA)
6. c1 tooth of mentum broad (type IIA)
c1 tooth of mentum generally narrower.(narrow type IIA)
7. Mandible of ty. IIB or C (occasionally IIIC), with about 15- 25 furrows
Mandible of ty IIIC, with about 26 furrows
8. Slight development of posterior ventral tubules only
Two pairs of ventral tubules present
9. Anal tubules relatively long, as in <i>C. forsythi</i>
Anal tubules shorter and rounded
10. Anal tubules all about equal & 2.5x longer than wide (3 polytene chromosomes) . C. sp.8
Anal tubules about 2.5-4.2 times longer than wide, dorsal pair often longer
11. Head somewhat narrower, ratio mentum width/VHL less than 0.65, ventral tubules sometimes longer than 1.0 mm, post pair longer <i>C. novaezealandiae</i> group 12 (Note: with frontoclypeus generally darkened but sometimes pale or only slightly darkened (possibly Ty. 2))
Head broader, ratio mentum/VHL above 0.65 with darkened frontoclypeus <i>C.</i> sp. Not in North Island.

Chironomus zealandicus Hudson, 1892

There have been several attempts to relate *C. zealandicus* to one of the cytologically known species, and it has been suggested at different times that it was either species 1 (Forsyth, 1978 and Martin, 1998) or C. species a (Forsyth 1996; Martin, 1996).

However, species 1 can be ruled out because there is an entry in Hudson's journal that he reared the specimens of *C. zealandicus* from larvae without ventral tubules. Forsyth therefore considered that *C.* species a was the species that corresponds to Hudson's *C. zealandicus*. This is supported further by the fact that *C.* species a is the largest of the reared species, about the size of Hudson's *C. zealandicus* specimens.

The name is therefore used in this sense in this work.

The chromosomal banding patterns were originally defined using the Australian standard of Martin (1969) but are now in the process of being transferred to the more universally used (although more difficult for defining a specific band) standards of Keyl (1962) and Devai *et al.* (1989).

Species descriptions:

1. Chironomus novaezelandiae Kieffer, 1921

Although originally allocated the name *Chironomus novae-zelandiae*, such names are no longer acceptable under the Code, and it should now be *Chironomus novaezelandiae*. Freeman (1959) named a male from Wellington as lectotype, although erroneously placing the species as a synonym of *C. zealandicus*.

The identification of this material as *C. novaezelandiae* is based on the similarity to the specimens in the type series, plus the broad distribution of the species. However, it is quite likely that the type series is also a mixture of members of the *C. novaezelandiae*-species group.

In BOLD Bin: BOLD AAJ0168 and BOLD ABZ5458

BOLD ABZ5458 is the nearest neighbor-Bin to BOLD AAJ0168 (Bin for *C.* spNZ12) and the reasons for the separation are possibly the presence of two very closely related species in *C. novaezelandiae* as well as the presence of polymorphic sites in which one base is identical to the fixed base in *C.* spNZ12 (see below).

C. 'thermarum' is also a member of this species group and is also in Bin BOLD AAJ0168.

CHIRONOMUS NOVÆ-ZELANDIÆ ~ n sp. \circlearrowleft Yellowish. Frontal lobes very small. Antennae and plumes brown, black scape, twelfth segment very long, four times as long as 2-11 combined, 3-11 very broad. Metanotum, three

shortened bands of mesonotum and mesosternum brownish red and dull. Halteres white. Wing hyaline, lobe at right angles, transversal black, cubitus a half longer than the radius, and nearer to the tip of the wing than the discoidal; cross-vein under the transversal. Legs yellowish, fourth and fifth segments of the tarsi a dark brown, anterior metatarsus a third longer than tibia, its distal third and the second segment setae long, 2-4 gradually shorter, pulvilli large, the two spurs of the posterior tibia short. Abdomen brown black, hind margin of tergites whitish. Claspers brown black; terminal parts very long, nearly twice as long as the basal, slightly curved and slightly thinned in their distal half, median part bearing, before the extremity, a row of six rigid bristles, almost twice as long as the width of the segment; superior appendage red, exceeding the basal segment, strongly curved, equally wide to the extremity, which is rounded; inferior appendage twice as long as the superior, exceeding the basal third of the terminal segment, with numerous long, curved, dorsal hairs; anal point moderately long. L. 8 m/m.

(i.e. AR about 4; LR about 1.3)

 \bigcirc . Antennae brown, second segment has neck a little longer then wide; 3-5 a neck shorter than the base, the sixth half longer than the fifth. L. 7 m/m. New Zealand, 7 ? 1 ? 1

Translation of Kieffer's original 1921 description

Adult:

There are 5 males, including the lectotype from Wellington, and two females (so Kieffer may have only examined one of them) of the type series, collected by Osten-Sacken, in the DEI near Berlin, Germany. These specimens are lacking many leg segments, and the abdomen in one case. They were examined in 1997.

Males:

AR about 3.23-3.6 (Kieffer gives 4.0); Clypeal setae about 22-37. Thoracic setae could not be clarified.

Wing length could not be measured, but VR about 1, as noted by Kieffer.

Legs: Anterior tarsi with a beard of dense shorter setae (BR 1.6-4.3) and sparse longer setae (BR about 3-7) i.e. about twice the length of the shorter setae.

Leg segments (relative lengths only):

	Fe	Ti	Ta1	Ta2	Ta3	Ta4	Ta5	LR	F/T	BR
PI	285	265	377	200	140	-	-	1.34-1.45	0.96-1.09	2-4, 3-7
PII	290	275	160	100	-	-	-	0.58-0.60	1.02-1.08	
PIII	370	390	254	154	-	-	-	0.60-0.69	0.89-1.0	

BR – there is a double beard, mainly short (2.1-4.3) and sparse longer (3.15-7.15)

Abdominal segments generally with a dark band and a narrow pale posterior stripe. One specimen (labelled only 'New Zealand') has a narrower dark band and has green on the abdomen as well - since this specimen has only a shorter beard (BR 3.15), it may be a different species.

Data from other specimens: The identification of the current material as *C. novaezelandiae* is based on the similarity to the specimens in the type series, plus the broad distribution of the

species. There is considerable variability probably due to the broad distribution from north to south. However, some of the variability arises from the presence of closely related species, mainly recognizable from banding sequence of the polytene chromosomes and the base sequence of the BARCODE region of the mitochondrial DNA (see below). It is also quite likely that the type series is also a mixture of members of the *C. novaezelandiae* species group.



Male of *C. novaezelandiae* from BOLD database (NZINS-83)

Wing length about 4.06-5.93mm; width about 0.88-0.96 mm, VR about 1.02; 3 setae on brachiolum, 27-32 setae in squamal fringe. AR about 3.2-3.6.

Head: Small FT about 10-15 μm long and 7.5 μm wide; 15-23 setae on clypeus. Palpal segments (micron): 58 : 60 : 250 : 249 : 354; P5/P4 1.01-1.42; P5/P3 1.39-1.62.

Thoracic setae: Acrostichal - not seen; Dorsocentral - 12-17; Prealar - 5-6; Scutellar in roughly two rows, 4-10 anterior and 11-12 posterior (total 15-22).

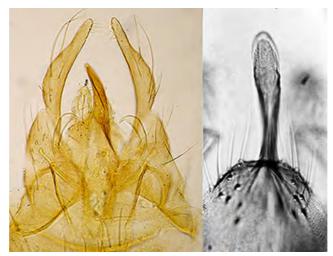
Anterior tibiae and tarsus bearded, with mainly shorter setae (BR about 3) and sparse longer setae (i.e. essentially a sparse long beard).

Leg lengths (micron) and proportions:

	Fe	Ti	Ta1	Ta2	Ta3	Ta4	Ta5	LR	F/T	BR
PI	1560	1495	1925	1205	845	665	340	1.23-1.36	1.03-1.05	3.3-6.5
PII	1680	1685	945	625	460	315	225	0.55-0.57	0.96-1.02	
PIII	2050	2147	1385	910	675	440	260	0.63-0.65	0.94-0.98	

BR – double beard only measured from one specimen: short 3.3, sparse long 4.5

Abdominal tergites with brown bands covering the basal 2/3 of TII-VI, wider in midline than at edges, and about 4/5 of tergites VII and VIII. About 10.3 (4-15) setae in a pale patch on tergite IX.



Male terminalia (left), and Anal point (right)

Hypopygium: SVo hooked, E(h)-type of Strenzke (1959); IVo reaching almost to the end of the long anal point which is narrow at the base and expanded at the end (about midpoint of gonostyle) with more-basal setae of IVo ramose. Gonostylus only moderately swollen and reduces relatively sharply over distal third.

Females:

Osten-Sacken female – just labelled 'New Zealand'

Few metrics able to be ascertained;

Antenna (relative lengths): 100: 200: 175: 180: 265. AR 0.41-0.42;

Most leg segments missing:

Leg segments (relative lengths only):

	Fe	Ti	Ta1	Ta2	Ta3	Ta4	Ta5	LR	F/T
PI	225	235	-	-	-	-	-	-	0.94
PII	205	215	-	-	-	-	-	=	0.95
PIII	370	390	255	-	-	-	-	0.63	1.0

Other females:

Wing length 4.43 (3.96-4.88) mm, width 1.20 (0.96-1.40) mm; VR 0.89 (0.81-0.98), about 3-4 Scf on brachiolum, and about 22-25 setae in squamal fringe.

Head: Antennal proportions (micron)(propn of neck in brackets): 197 (0.31); 139 (0.42); 145 (0.33): 143 (0.43): 241. AR 0.40 (0.37-0.44); A5/A1 1.16 (1.14-1.30).

FT about 23-35 μ m and 3-3.6 times longer than wide; palpal segments (μ m) 100 : 68 : 230 : 260 : 387: P5/P4 1.34-1.53; P5/P3 1.41-1.91.

Clypeal width about 1.6 times the diameter of the antennal pedicel; 44.3 (41-51) setae. Thoracic setae: Acrostichal – 14.25 (12-19); Humeral about 3-5 linear sometimes incl. perhaps 3 small setae, Dorsocentral – 16-30 (Humeral+ Dorsocentral 26.8 (19-34)); Prealar – 6.8 (5-9); Supra alar - 1; Scutellar – anterior row 10-27, posterior row 12-20 (total 22-47).

Leg lengths (micron) and proportions:

	Fe	Ti	Ta1	Ta2	Ta3	Ta4	Ta5	LR	F/T	Ta4/Ti
PI	1467	1320	1860	1027	767	620	327	1.38-1.44	1.11	0.45-0.50
PII	1520	1480	833	493	360	333	207	0.56-0.57	0.95-1.06	
PIII	1787	1853	1240	760	580	353	233	0.63-0.69	0.94-1.00	

BR 1.63-2.21

Abdominal tergites with darkened bands, at anterior of TII to TIV then more extensive.



Terminal region of female showing the narrow semicircular segment X, and the rounded posterior margin of the cercus

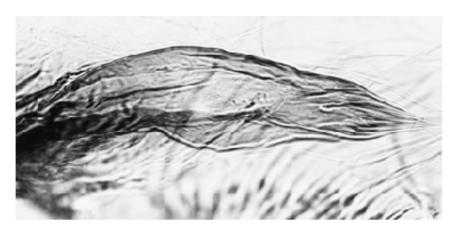
Segment X a narrow semicircle about $210 \times 34 \mu m$ (6 times longer than widest point) with 11-14 setae; cerci also appear to be essentially semicircular.

Pupa: about 9.8-12.8 mm long (South Island females), males 11.7 (10.6-12.5) mm; cephalic tubercles about 135 (100-166) (male 106-134) μ m long and 122 (94-151)(male 75) μ m wide at base, subapical seta about 77 (65-90) μ m long. Thorax and muscle scars pale yellow brown, abdomen relatively pale; shagreen largely in mid-line - on post half of segment II, post 2/3 of segment III and wider on segments IV-VI; none apparent on segments VII-IX. Basal respiratory ring about 181 (158-205) μ m long x71 μ m wide, HR about 2.4-2.6, with the respiratory bases markedly narrowed in the middle.

About 85 (65-100) recurved hooks at posterior of segment II, covering about 63 (59-67)% of the segment width.

Pedes spurii B at posterior of segments II and III; a large pedes spurii A on segment IV, about $230-260 \mu m$ long and $95-109 \mu m$ wide, about 0.27-0.32 of the segment length.

Caudolateral spurs of segment VIII sometimes with numerous appressed spines (c.f. *C. zealandicus*); about 6.3 (3-9) longer spines and possibly a small one near the base. In some populations with paler larval heads there are only two or three spines but it is probable that some of these would be *C.* 'thermarum'.



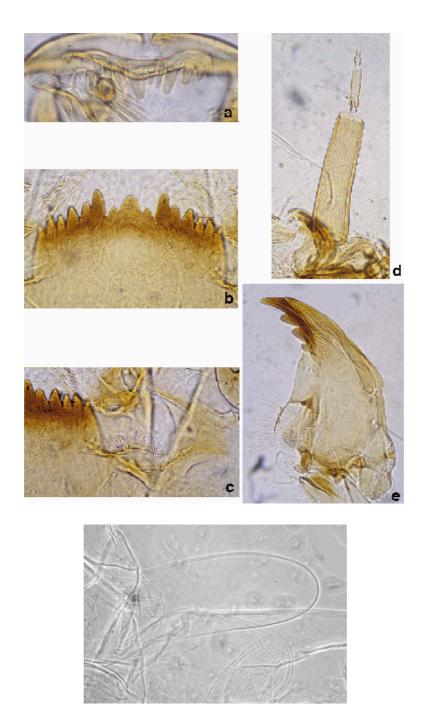
About 132 taeniae on the swim fin, in up to three rows in some places.

Fourth instar larva: Usually a bathophilus-type larva, but variable with habitat. Size also variable depending upon habitat and South Island specimens tend to be larger. Length from 9.3-20.8 (fem 12.3-20.8; male 12.3-16.2) mm. Ventral tubules also variable, from 0.4-1.5 mm., anterior and posterior pair essentially equal size but varying between individuals. Anal tubules again variable in length but dorsal pair generally slightly shorter (165-500 vs 195-555 μ m) but often slightly wider than the ventral pair (90-320 vs 100-240 μ m), length/width about the same (1.4-3.8).

Head capsule normally very dark in gular and frontoclypeal regions. However some individuals have the gular region only slightly darkened and almost no darkening of the frontoclypeus - may be polymorphic in some North Island populations and fixed in some South Island populations (Martin, 1998). These latter may include other species (including C. 'thermarum' or *C*. sp. 10) but many seem to have very similar cytological features, except for the sequence of arm G, which is G3. These are now classed as 'Type 2' (see below). Head relatively narrow, mentum width less than 0.65 of the VHL. Width between antennal bases (147-218 μm) usually greater than that between the S4 setae (126-213 μm). Mentum (b, below) of type II, i.e. 4th laterals reduced almost to level of 5th laterals, with centre teeth of normal type IIA (c2 teeth relatively more distinct than those of *C*. 'thermarum'). VM (c, below) about 194-240 μm wide and 3.2-3.9 times wider than deep; with about 35-56 striae (lower in smaller specimens); IPD 0.27-0.44 of MW; VMR 0.30-0.31. PE (a, below) with about 9-18 often irregular teeth (type D). Premandible with inner tooth about 5 times width of outer tooth (Ty. D).

Antenna (d, below) with basal segment relatively long and narrow, about 3 to 4 times longer than wide; AR about 1.9-2.6; antennal segment proportions (micron) 157: 33: 9: 13: 7. Distance between antennal bases overall about the same as that between the S4 setae, i.e. S4 setae, which occupy 0.76-0.94 of the FC width, as likely to be more separated as to be less separated than the antennal bases.

Mandible (e, below) mostly of type IIB, but may be only IB; 11 to 27 furrows on outer surface near base; 10-15 taeniae in PecM (again reflecting variation in size); Mdt-Mat abt 34, MTR about 0.32.



Third instar larva: A single larva was available, length unknown; anterior VT 0.64 μ m, posterior 0.68 μ m, ventral head length (281 μ m) within the range of the 4th instars, but mentum width only 185 μ m. Dorsal AT 165 μ m and ventral AT 195 μ m in length and both about 1.7-1.8 times longer than wide. Gula darkened over posterior 2/3; FC also darkened. Ventromental plates separated by 0.37 of the mentum width; with 28-30 striae; VMR 0.31-0.38. PE with 13 teeth.

Distance between antennal bases (about 138 μ m) about the same as that between the S4 setae (about 137 μ m). The relationships of A1 to the VHL about the same as in the smallest 4th instars; distance of RO up from base of segment very different (0.32 & 0.47), A1 2.5 times longer than wide; AR 2.31; relative length of segments (μ m) 95 : 19 : 6 : 11: 5. Mandible about 240 μ m long, of type IIIC; with 13-14 furrows and 10 taeniae in the PecM.

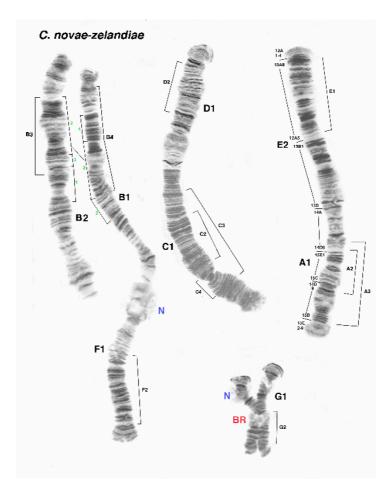
Cytology: 4 polytene chromosomes with the pseudothummi-cytocomplex arm combination (BF, CD, AE, G). Centromeres of metacentric chromosomes thin but distinctly heterochromatic, most strongly developed on the CD chromosome. Main nucleolus proximal in arm F (Lentzios & Stocker 1979 (see below)).

Arm G often with a small nucleolus (see figure), although this may be hard to distinguish from a BR unless the nuclear envelope can be seen, and some individuals have been heterozygous for a BR and a nucleolus. Usually another obvious BR, which may be either terminal or medial as the result of an inversion. Specimens without the nucleolus and with a large BR in this region, may be a different species (perhaps *C.* sp.NZ12).

Arm A with sequence oppA4 of Australian species and two other sequences; arm C with one sequence apparently as C. tepperi C1; arm D with one sequence as C. australis; arm E with one sequence as oppE1 of Australian species, as well as an additional sequence. One of the sequences of arm F appears identical to oppF3 and ausF1.

Polymorphic in all arms. Sequences A3 and C4 known only as homozygotes in sample C from Waikato area. Analysis of an egg mass from Auckland indicates that the MD is not on arm A.

Lentzios et al. (1980) give some information on the number and location of C-bands.



nzlA1: 1a-e, 11 - 10, 2c - 1f, 3e - 2d, 8 - 9, 3f-i, 12c-a, 4 - 7, 13 - 19 as oppA4, anlA1, forA1

nzlA2: 1a-e, 11 - 10, 2c - 1f, 3e - 2e, <u>7 - 4</u>, <u>12a-c</u>, <u>3i-f</u>, <u>9 - 8</u>, <u>2d</u>, 13 - 19

nzlA3: 1a-e, <u>13a-f</u>, <u>2d</u>, <u>8 - 9</u>, <u>3f-i</u>, <u>12c-a</u>, <u>4 - 7</u>, <u>2e - 3e</u>, <u>1f - 2c</u>, <u>10 - 11</u>, 14 - 19

nzlB1: Large puff (group 7) with distal dark bands, near middle of arm.

nzlB2: Possibly the result of overlapping inversions. Puff with reduced dark bands

nearer distal end of arm.

nzlB3: Derived from B2 by a short inversion of the region with the puff, so that the

reduced dark bands are now proximal. may be only in sp. NZ12.

nzlC1: Characteristic band groups 3-4, with 5 distal, near distal end as tepC1

nzlC2: small median inversion, just proximal to groups 3-4.

nzlC3: inversion of most of arm

nzlC4T: Small inversion at distal end of the arm, from group 3-4.

nzlD1: 1 - 2, 16 - 10d, 3a-d, 9 - 3e, 10a-c, 17 - 24 as australis D1

nzlD2: 1 - 2, 16c-a, <u>17e-a</u>, <u>10c-a</u>, <u>3e - 9</u>, <u>3d-a</u>, <u>10d -15</u>, 18 - 24

nzlD3 1 -2, 16 - 11, 4c - 9, 3d-a, 4ba - 3e, 10a-c, 17 - 24

nzlE1: 1 - 3e, 10b - 3f, 10c - 13 as oppositus E1, analis, forsythi

nzlE2: 1a-c, 5 - 10b, 3e - 1d, 4 - 3f, 10c - 13

nzlE3: 1a-c, 5 - 7c, <u>10g-c</u>, <u>3f - 4</u>, <u>1d - 3e</u>, <u>10b - 7d</u>, 11 – 13 may be only in sp. NZ12

nzlF1: 1 - 2a, 10 - 2c, 15c - 11a, 2b, 15d - 23 i.e. as oppositus F3, analis, forsythi

nzlF2: 1a-e, <u>12 - 15c, 2c - 10, 2a,</u> 11i-a, 2b, 15d - 23

nzlG1: Subterminal BR and median BR (often just a pale space).

nzlG2: Inversion of region from median BR to near distal end of arm.

nzlG3: Subterminal nucleolus and adjacent BR, or no visible BR. (Ty. 2)

It is still likely that this is a mixture of at least two species, as there seem to be two types of arm G: one with no apparent nucleolus and with a large subterminal and a medial BR (G1) (the latter moved distally in G2), and another with a subterminal nucleolus and often without an obvious BR (G3), or with a BR very close to the nucleolus – but other than the presence or absence of the nucleolus or BR, the sequence appears identical.

Mt*COI* data suggests a common variable species and a possible second less common form. This seems to represent the difference between the sequence of *C. novaezealandiae* (common & variable) and *C.* spNZ12. These two species differ at a number of sites in the barcode sequence, with somewhat more differences from *C.* 'thermarum' (see below).

However, even the common species seems to be separable into two groups that differ at a number of sites. The least common of these is also associated with the banding sequence G3, and has been called *C. zealandicus* Ty. 2 (see below).

The pupa differs from that of *C. zealandicus* in that the spines of the spur are appressed (above), while those of *C. zealandicus* are splayed (see below).

DNA analysis:

Mt *COI* – a number of sequences in the BOLD database. These sequences are for 3 or 4 species spread across 2 BOLD Bins. There are however distinct differences at a number of sites, as shown below. Type 1 is designated on the basis that this is the form found at Wellington – the designated type locality.

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	C. novaezelandiae Ty. 2																														
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Mt cytB - GenBank (AF192178.1)

Occurs in a wide variety of habitats, from lakes to pools, including thermal waters, and fresh to brackish. While there may be more than one species still included, the available DNA data is consistent with this wide habitat tolerance.

Localities

North Island:

Auckland (-36.92°S, 174.78°E), North Auckland (D.J.Forsyth) (NZ.8.2) 25-i-1965; & (A. & R. Mesa) (NZ.8.1) 25-i-1969

Kerosene Creek (-37.94°S, 175.56°E), South Auckland (Sofia Ibarrarán) (NZ.78.1) 5.ii.2007 (rare)

Lake Ngahewa (-38.31°S, 176.37°E), South Auckland (Sofia Ibarrarán) (NZ.76.1) 29.viii.2007

Lake Ngapouri (-38.00°S, 176.50°E), Waiotapu, South Auckland (D.J. Forsyth) (NZ.9.1) 15-ii-1972; and Jon Martin and D.J. Forsyth) (NZ.9.3) 5-xii-1973.

Lake Okaro (-38.30°S, 176.40°E), 10 Km s. Rotorua (NZ.10.9) (Sofia Ibarrarán) (NZ.10.9), 5-ii-2007

Lake Rotoiti (-37.28°S, 174.67°E), about 16 Km north east of Rotorua, South Auckland (Sofia Ibarrarán) (NZ.19.3) 29-viii-2007

Lake Rotowhero area (-38.30°S, 176.40°E), South Auckland (Sofia Ibarrarán) (NZ.11.6) 5.ii.2007

Ornamental pool, Rotorua (-38.156°S, 178.58°E) (D.J.Forsyth & Jon Martin) (NZ.26.3) 12-xii-1973

Oruanui Link Road, north Taupo (-38.36°S, 176.08°E) (D.J. Forsyth) (NZ.71.1) 18-ix-1990 – "G3"

Potting shed pond, Taita (-41.18°S, 174.95°E) (D.J.Forsyth) (NZ.14.1) 13-x-1972; and (NZ.14.2) (D.J.Forsyth) 26-i-1973 (rare)

Potting shed pond, Taita Soil Bureau, Wellington Taita (-41.18°S, 174.95°E) (Jon Martin & D.J.Forsyth) (NZ.14.3) 22-xii-1973

Queen Elizabeth Park (-37.94°S, 175.56°E) South Auckland (Sofia Ibarrarán) (NZ.80.1) 5.ii.2007

Artist's Palette, Waitapu thermal area (-38.21°S, 176.22°E) (D.J.Forsyth & Jon Martin) (NZ.21.1) 9-xii-1973

Sulphur Point, South Auckland (-37.94°S, 175.56°E) (Sofia Ibarrarán) (NZ.79.1) 5.ii.2007

Trentham Sewage Plant, Upper Hutt (-41.14°S, 175.05°E) Tank G (D.J.Forsyth) (NZ.13-1) 5-xii-1972.

Waikato area (-37.50°S, 175.33°E)(Sample C) (J. Kanapathipillai) (NZ.73.3) 26-iv-1996. (both with & without nucleolus)

Waikato (-39.79°S, 175.32°E), South Auckland (Melissa Hill) 26-ix-2012.

Waikato area (-37.802°S, 175.334°E) (I. Hogg) 16.vii.2012 and (E. Doyle & N. Binks) 12.xi.2013.

Wellington (Osten-Sacken) Lectotype male

South Island:

Belfast, c. 10 km n. Christchurch (-43.45°S, 172.62 °E) (D. Matthews) 7-1-1974 Bromley, Christchurch (-43.54°S, 172.69°E), Canterbury (D.J. Forsyth) (NZ.15.1) 7-xii-1973

Bromley Sewage Works (-43.45°S, 172.62°E), Christchurch, Canterbury (Jon Martin) 28-xii-1973 (Region 4-6 of Robb's samples); and (Jon Martin) (NZ.07.6) 11-i-1974 Christchurch (-43.45°S, 172.62°E), Canterbury (A.E. Lambden) (NZ.07.1) Jan-Feb.1969

Glen Lake (-43.02°S, 172°78°E), Canterbury (C.A. Woodward) (NZ.72.1) 7-i-2004 Fairfield Sewage Works (-45.897°S, 170.388°E), Taieri County Council. Otago (Jon & C.J. Martin, T. Dodgshun) (NZ.37.1) 3-i-1974

Haast junction (-43.88°S, 169.05°E), Westland, Egg masses (Jon Martin) (NZ.67.1); and (Jon Martin & C.J. Martin) (NZ.67.2) 25 & 26-i-1978.

Kinloch Marina, Lake Taupo (-73.36°S, 176.00°E), South Auckland (D.J. Forsyth) (NZ.12.1) 18-xi-1972.

Lake Fergus, abt. 80 Km n.w. Te Anau (-44.845°S, 168.111°E) on road to Milford Sound, Southland (Jon Martin) (NZ.60.1) 19-i-1978.

Lake Hayes, c.13 Km n.e. Queenstown (-44.98°S, 168.80°E), Westland (Jon Martin) (NZ.47.1) 7-i-1974.

Lake Horseshoe (-42.358°S, 172.52°E), Waiau River Valley, Canterbury (B.V. Timms) (NZ.68.1) 9-vii-1979; & (C. Woodward) (NZ,68.2) 15-i-2004.

Lake Ianthe (-43.04°S, 170.623°E), c. 18 km n. Harihari, Westland (Jon Martin) (NZ.51.1) 9-i-1974.

Lake Lochie, on Te Anau-Milford Road (-44.83°S, 168.12°E), Southland (Jon Martin) (NZ.45.1 & 2) 6-i-1974; and 19-i-1978.

Lake Pukaki (-43.965°S, 170.165°E), Canterbury ((Jon Martin) (NZ.33.1) 31-xii-1973 Lake Rotoiti, Canterbury (B.V.Timms) 1978/79.

Lake Te Anau (-45.41°S, 167.50°E), Southland (Jon Martin) (NZ.46.2) (1 larva), 7-i-1974.

Long Point (-46.52°S, 169.38°E), nr. Tahakopa, South Otago (J.S. Pillai) (NZ.5.3) 25-iv-1968

Long Point (-46.52°S, 169.38°E), nr. Tahakopa, South Otago (Jon Martin) (NZ.5.4) 4-i-1974 (in different BOLD Bin)

Mirror Lake (-44.82°S, 167.78°E), 58 km n.w. Te Anau, Southland (Jon & C.J. Martin) (NZ.45.1) 6-i-1974

12.5 km Hermitage (-43.50°S, 170.06°E), Mount Cook, Canterbury (Jon Martin) (NZ.34.1) 31-xii-1973

Otago (Osten-Sacken) in type series.

Owaka River (-46.42°S, 169.60°E) , South Otago (D. Dodgshun & J.S. Pillai) (NZ.56.1) 14-iii-1974

Purakaunui Bay (-46.55°S, 169.62°E), Southland (T. Dodgshun & C.J. Martin) (NZ.42.1) 4-i-1974 (no nucleolus)

Tahakopa (-46.52°S, 169.38°E), South Otago (J.S. Pillai) (NZ.05.2) 25-iii-1968. Waikouaiti River (-45.64°S, 170.66°E), Otago (Jon Martin) (NZ.35.1) 31-xii-1973. Winton (-46.17°S, 168.33°E) Westland (NZ.43.1) (Jon Martin) (NZ.43.1) 4-i-1974.

1b. Chironomus novaezelandiae Type 2

No morphological data is available for adults or pupae as there is no associated mtCOI data.

Fourth instar larva: A bathophilus-type larva, very similar to that of type 1. Length from 9.3-20.8 (fem 13.7-20.8; male 12.2-15.2) mm. Ventral tubules also variable, from 0.5-1.6 mm., anterior and posterior pair essentially equal size but varying between individuals. Anal tubules again variable in length but dorsal pair sometimes slightly longer (240-430 vs 240-380 μm) with width variable (90-180 vs 120-180 μm), length/width about the same (1.7-2.4). Head capsule normally slightly to very dark over post 1/3 to almost whole gular (mostly 1/2 to 2/3) and frontoclypeal region pale to very dark, generally mirroring the extent of gular darkening. Head relatively narrow, mentum width less than 0.66 of the VHL. Mentum (b, below) of type II, i.e. 4th laterals reduced almost to level of 5th laterals, with centre teeth of normal type IIA (c2 teeth relatively more distinct than those of *C*. 'thermarum').

Width between antennal bases (164-195 μ m) slightly greater on average than that between the S4 setae (161-205 μ m). Mentum of type II, i.e. 4th laterals reduced almost to level of 5th laterals, with centre teeth of normal type IIA.

Cytology: 4 polytene chromosomes with the pseudothummi-cytocomplex arm combination (BF, CD, AE, G).

nzlG3: Subterminal nucleolus and adjacent BR, or no visible BR. (Ty. 2)

Localities

North Island:

Hamilton (-37.50°S. 175.33°E), South Auckland (J. Kanapathapillai) (NZ.73.3) 24-iv-1996.

Puarenga Stream, Whakarewarewa (-38,17°S, 176.25°E), South Auckland (Jon Martin & D.J. Forsyth) (NZ.20.1) 8-xii-1973 – "G3"

Sulphur Point (-37.94°S, 175.56°E), South Auckland (S. Ibarraran) (NZ.79.1 & 2) 5-ii-2007.

South Island:

City Reservoir, Dunedin (-45.80 °S, 170.87 °E), South Otago (J.S.Pillai) (NZ.8.2) 12-iii-1968.

Horseshoe Lake (-43.02°S, 172.78°E), Canterbury (C.A. Woodward) (NZ.68.2) 15-i-2004.

Haast junction (-43.88°S, 169.05°E):, Westland, Egg masses: (Jon Martin) 25-i-1978, and (Jon Martin & C.J. Martin) (NZ.67.1 & 2) 26-i-1978.

2. Chironomus zealandicus, Hudson 1892

Redescribed by Hutton 1902.

Freeman 1959 reviewed specimens of both *C. zealandicus* and *C. novaezelandiae* and erroneously, but not surprisingly in the absence of any information on the immature stages, decided that *C. novaezealandiae* was a junior synonym of *C. zealandicus*. Described, along with *C. novaezealandiae*, by Forsyth (1971) who considered the existence of two larval types as simply a polymorphism. Consequently, in our earlier work the name *C. zealandicus* was associated with larvae with ventral tubules (i.e. *C. novaezealandiae*) until Don Forsyth noted the reference in Hudson's notes that he reared the adults from larvae without ventral tubules. Hence there is considerable confusion in earlier publications.

C. species a (Forsyth 1978; Martin 1996) is now considered to refer to C. zealandicus.

In BOLD Bin: BOLD AAS1265

Adult:

Male:

Hutton's description, aside from a detailed colour description, provides the information that length is 6-8 mm, wing length about 5.5 mm, and LR about 1.3. Forsyth (1971) notes that an adult reared from a salinarius-type larva did not differ significantly from those reared from a thummi-type larva.

Additional specimens:

AR about 3.7 (3.0-4.1).

Wing length 5.14 (4.63-5.53) mm; width 1.06 (0.96-1.16) mm.; VR 1.03 (0.97-1.06); abt 4 (3-5) SCf on brachiolum; 9 (5-12) setae in squamal fringe; crossvein darkened. FT present, 29.6 (17.5-35) micron, abt. 2.8 (2.6-2.9) times longer than wide. Clypeus about 0.8-0.95x width of antennal pedicel with 26 (16-34) setae; palp proportions (μ m) 77 : 70 : 283 : 277 : 374; P5/P4 1.41, P5/P3 1.39.

Thoracic setae: abt. 16 Acrostichal; 18.5 (16-22) Dorsocentrals; 6 (5-7) Prealars; 1-2 Supra-alars; 23 (17-29) Scutellar, in two rows (posterior setae larger) sometimes in multiple rows, in which case there are more numerous anterior rows (5-11 setae) than single posterior row (12-18) setae, total 24 (17-29).

LR abt 1.29 (1.23-1.35); Anterior tarsi with a short dense beard (BR about 3-4) and sparse longer hairs (BR about 5.9-7).

T 1 41	(:)		
Leg lengths	microns) and	proportions:

	Fe	Ti	Ta1	Ta2	Ta3	Ta4	Ta5	LR	F/T	BR
PI	1600	1535	1975	1234	864	687	347	1.23-1.35	1.03-1.05	3-4; 6-7
PII	1722	1702	958	637	470	325	200	0.55-0.57	0.96-1.02	
PIII	2110	2194	1421	936	696	451	268	0.63-0.66	0.94-0.98	

Abdominal tergites with a broad dark band covering most of the surface, leaving only a narrow pale band at the posterior edge. About 11.5 (8-15) setae, probably in barely visible individual spots in a large pale triangular patch on TIX.



Anal point narrow at base, slightly expanded at distal end, reaching about 2/3 along the length of the GS. SVo of E-type (between h and i) of Strenzke (1959); IVo reaching almost to end of anal point, with simple setae.

GS moderately swollen, narrowing over posterior third to quarter, sometimes relatively sharply.

Female

Based on one specimen (with LR from a second specimen).

Wing length 4.4 mm; width 1.2 mm; VR 1.06; 4 SCf on brachiolum; 17-20 setae in squamal fringe.

Head: FT about 38 x 10 µm (3.75 times longer than wide).

Antennal proportions (micron) 211(0.41) : 136 (0.56) : 141(0.45) : 146 (0.53) : 221.: AR about 0.35; A5/A1 1.05.

About 41 clypeal setae. Palpal proportions (micron) 65.5 : 80 : 231 : 291 : 432; P5/P4 1.48; P5/P3 1.87.

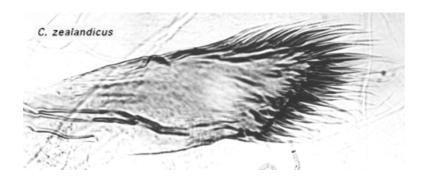
Setae: 18-19 Acrostichal; 22 Humerals+Dorsocentrals; 6 Prealars; 1 Supra-alar; 33 Scutellar in two rows (18 in anterior row, 15 larger setae in posterior row).

BR 1.35. Leg lengths (micron) and proportions:

	Fe	Ti	Ta1	Ta2	Ta3	Ta4	Ta5	LR	F/T	Ta4/Ti
PI	1635	1445	1960	1040	785	610	315	1.36-1.38	1.13	0.31
PII	1645	1570	885	495	365	255	190	0.56	1.05	
PIII	1875	1875	1230	735	570	470	230	0.66	1.00	

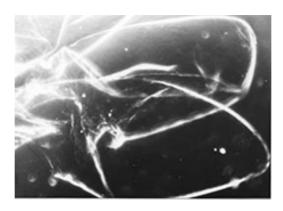
Abdomen generally brown.

Pupa: Length about 11.8 (10.2–12.5) mm (male 12.0 & 12.5 mm), IMW about 2.29 (2-2.45) mm (males). Cephalothorax and abdomen yellow-brown. Cephalic tubercle about 133.5 (106-160) (106-134 x 75 μm, male); 134-160 μm (female), with sub-apical seta about 65-78 μm (male), 78-88 μm (female); and slight indication of a frontal wart. Respiratory base about 181.5 (158-205) μm long 71 (62-80) μm wide, HR 2.46-2.56, base of respiratory filaments almost divided in middle. Muscle scars on abdominal tergites slightly darkened; about 85.25 (65-100) recurved hooks on abdominal segment II, occupying about 63 (58-67)% of segment width. Pedes spurii A of segment IV about 258 (215-301) x 201.5 (180-223) μm, and 0.23 (0.20-0.26) of the length of segment IV. Caudolateral spur of segment VIII with 24.1 (16-37) splayed spines, (below), sometimes with small spines at the outer edge. About 111.75 (107-115) taeniae on each side of the anal lobe, somewhat irregular anteriorly, then mostly in a single row. Shagreen thickest in the centre third of the posterior 2/3 of segments II-IV and spreading laterally at the posterior margin; wider and further anterior on seg. V and essentially reduced to 2 small patches around the posterior setae of segment VI; little on VII-IX.



Fourth instar larva: salinarius-type larva. Largest NZ species, length about 17.6 (15-21.5 mm (female), 16.8 (15.3-18.7) mm (male). Head capsule with frontoclypeus and most of the

gular region very dark. Anal tubules short and pointed: dorsal 435.8 (266-607) μ m; ventral 347.8 (230-430) μ m, both about 1.6 times longer than wide. SAL 90.6 (66-109) wide and 32.2 (28-40) deep 2.86 (2.13-3.82) times wider than deep.



Mentum (Fig. c, below) of type II (i.e. 4th laterals reduced at least to the level of 5th laterals), with central trifid tooth of Type IB, although sometimes the c2 teeth are less separated from the c1 tooth.

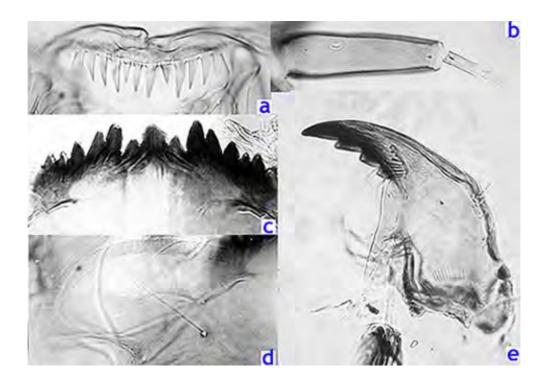
Ventromentum (Fig. d, below) about 285 (271-293) µm wide and about 4.8 times wider than deep; and 1.01-1.04 times wider than the mentum; with about 86.2 (75-99) striae; VMR 0.29 (0.25-0.33).

PE (Fig. a, below) with about 13.8 (12-16) relatively sharp teeth, some reduced.

Premandible with the usual two teeth, inner tooth about 3.66 (2.5-4.8) the width of the outer and coming to a broad point (i.e. type C).

Antennal segments (Fig. b, below) in proportions (micron) 159 : 37.5 : 8 : 15 : 8 ; AR 2.38 (2.15-2.58); basal segment of antenna relatively long and narrow, about 3.36 (3.1-3.6) times longer than wide; RO 0.35 (0.27-0.43) up from the base of A1; A5 abt as long as A3 (1.02 (0.89-1.14).

Mandible (Fig. e, below) with 3rd inner tooth darkened and often fully separate (i.e. Type II-IIIC); about 20.4 (17-23) furrows on the outer surface near the base; 13.6 (12-17) taeniae in the PecMand; Mdt-Mat about 38; MTR 0.33-0.38.



Aside from the salinarius type larva, the most distinctive feature is the high number of striae (75-99) on the ventromental plates.

Cytology: 3 polytene chromosomes, modified pseudothummi-complex arm combination (BF, CD, GEA). Three nucleoli, one in arm G, one proximal and another medial in arm F. The medial nucleolus may not always be developed, as it was not detected by Lentzios & Stocker (1979). zeaA1 differs from *oppositus* A4 sequence by a simple inversion11-2e; arm E pattern not typical but zeaE1 appears to differ by a small inversion 5c-7 from *oppositus* E1. Arm F differs from that of *novaezelandiae*, etc., by inversion of the region 11-7. Considerable polymorphism in arms A, B,C, E, and G(?); sex determiner (MD) in arm C, probably near the centromere (Martin & Lee, 1984). A spontaneous whole arm translocation (GEA/BF to GEB/AF) was found in a male larva from a laboratory stock.

zeaA1: 1a-e, 2e - 3e, 1f - 2c, 10 - 11, 2d, 8 - 9, 3f-i, 12c-a, 4 - 7, 13 - 19

hypothetical 1a-e, 2g - 3a, 4a, 12a-c, 3i-f, 9 - 8, 2d-f, 11 - 10, 2e - 1f, 3e-b, 4b - 7, 13 - 19

zeaA2: 1a-e, 10 - 11, 2f-d, 8 - 9, 3f-i, 12c-a, 4a, 3a - 2g, 1e-d, 2e - 1f, 3e-b, 4b - 7, 13 - 19

zeaB1: Puff, with proximal dark bands (groups 7 and 8), in proximal third of arm.

zeaB2: Inversion of distal end of arm, distal to large puff.

zeaC1:

zeaC2: Inversion of about 2/3 of arm.

zeaD1: about 1 - 2, 12 - 16, 11 - 12d, 3 - 9, 10a-c; 17 - 24

zeaD2: possibly as duplex D1 (i.e. Inversion of about 16-12 from zeaD1)

zeaE1: (approx) 1?- 3e, 10b - 8, 5c - 7, 5b - 3f, 10c - 13

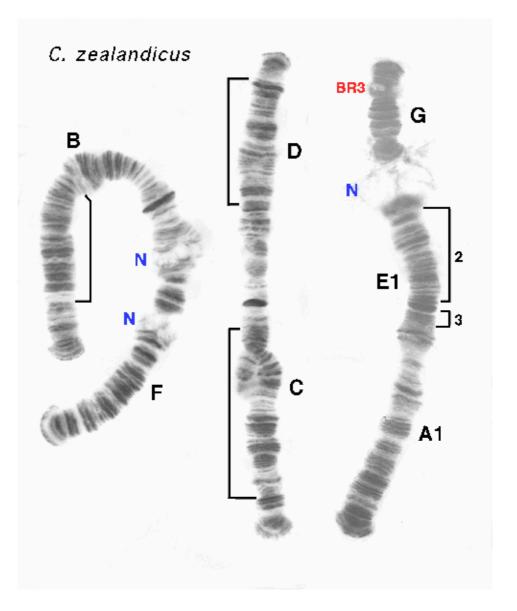
zeaE2: (approx) 1?-f, 4b - 5b, 7 - 5c, 8 - 10b, 3e - 2, 4a - 3f, 10c - 13

zeaE3: (approx) 1?- 3e, 10b - 8, 5c - 7, 5b - 3f, 10c-g, 12 -11, 13

zeaF1: 1 - 2a, 10 - 8, 11 - 15c, 2c - 7, 2b, 15d - 23 (In7 - 11 from oppositus F3)

zeaG1: fused to arm E.

zeaG2: possible inversion



Molecular:

Mt COI - GenBank (AF192209.1), also BOLD

Gb2B - GenBank (AJ003813)

Gb9 - GenBank (AJ003814.1)

Localities:

North Island:

Tank I & N, Lake Okaro, Rotorua area (-38.30°S, 176.40°E) (D.J. Forsyth) (NZ.10.2, NZ.10.3 and NZ.10.7) 8-xi-1972

Lake Ngapouri, Waiotapu (nr. Rotorua) (-38.00°S, 176.56°E) (Jon Martin & D.J. Forsyth) 5-xii-1973

Lake Okaro, (NZ.9.3) Rotorua area (-38.30°S, 178.40°E) (D.J.Forsyth) (NZ.10.7) 14-ix-1982

Ornamental pool, (Te Rotorua-nui-a-Kahumatamomoe) Rotorua (-38.156°S, 176.58°E) (Jon Martin & D.J. Forsyth) E. mass 2S. (NZ.26.3) 12-xii-1973 Lake Ngaroto, South Auckland (-37.96°S, 175.29°E) (Sofia Ibarraran) (NZ.75.1) 29-viii-2007

Waikato area, South Auckland (-37.50°S, 175.33°E) (J. Kanapathipillai) (Sample B) 26-iv-1996

1km west Waimangu (-38.17°S, 176.23°E) (D.J. Forsyth & Jon Martin) (NZ.27.1) Egg mass 1S. Coll:13-xii-1973

South Island:

Avon River, Christchurch (-43.28°S, 172.62°E) (Jon Martin) (NZ.55.1) 12-i-1974 Belfast, Canterbury (-43.45°S, 172.62°E) (D. Matthews) (NZ.54.1) 27-1-1974 (Oxidation ponds).

Bromley Sewage Works, Christchurch, Canterbury (-43.45°S, 172.62°E) (A.E. Lambden) (NZ.7.2)Jan-Feb.1969 (Oxidation Ponds)

Glen Lake (-43.02°S, 172.787°E), Canterbury (C.A. Woodward) (NZ.73.1) 15-i-2004 Twizel, Canterbury (Jon & C.J. Martin) (NZ.32.1) 30-xii-1973.

Lake Ellesmere -43.79°S, 172.49°E), Kaituna, Canterbury (Jon & C.J. Martin) (NZ.30.1 & 2) 29-xii-1973 & 12-i-1974.

Lake Te Anau (-45.17°S, 167.50°E), Fiordland (Jon & H.I. Martin) (NZ.46.5) 6-i-1974 & (NZ.46.6) 23 -i-1978.

The species is easily maintained in the laboratory and produced viable offspring with the Australian species *C. duplex* Walker and *C. occidentalis* Skuse.

3. Chironomus analis Freeman, 1959.

Likely to be in BOLD Bin <u>BOLD:AAL7011</u> and <u>BOLD ACG0887</u> (these are nearest neighbour bins and at one locality both are found)

Chironomus (Chironomus) analis sp. n.

This species is very similar to zealandicus in general appearance, but the male anal point is much heavier and stouter (Text-fig. 3, c). In colour it tends to be rather paler and the male abdomen is green with a saddle-shaped dark mark placed in the basal two-thirds of each of segments 2-5. In other structural features the two species are identical.

Holotype male Nelson: Blackball, v.1920 (J. W. Campbell). Wellington: Ohakune, I $3, 3 \supseteq (J. W. Campbell \text{ and } T. R. Harris)$. Westland: W. Coast, I 3, 1925 (T. R. Harris). Otago: Queenstown, I 3, 1919 (T. R. Harris). All specimens are in the British Museum.



Description of C. analis from Freeman 1959

Adult:

Male: (From two available specimens):

Wing length 4.08-5.28 mm.; width 0.89-1.22 mm.; VR abt. 1.00-1.03.

AR about 3.3-3.4. FT about 26-38 µm long and 15-16 µm wide. Palpal proportions

(μm): 63 : 63 : 268 : 273 : 245+ (broken). About 25-26 clypeal setae.

Thoracic setae: acrostichal - at least 9-15; dorsocentrals - 17-18; prealars 6-7;

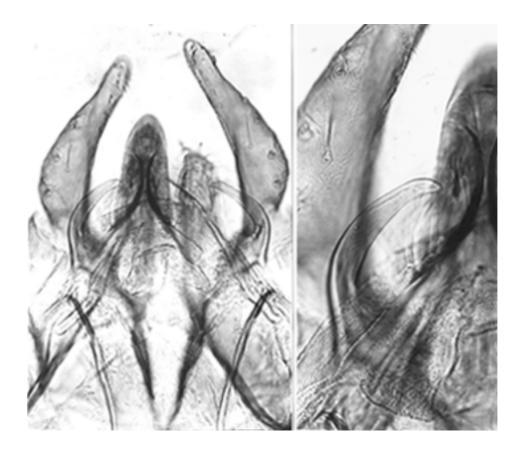
scutellars in two rows - about 9 anterior, about 13 posterior.

Leg lengths (microns) and proportions as follows:

	Fe	Ti	Ta1	Ta2	Ta3	Ta4	Ta5	LR	F/T	BR
PI	1630	1540	2210	1260	920	780	370	1.39-1.50	1.02-1.10	4.8-5.6
PII	1740	1660	1020	635	460	310	213	0.61-0.62	1.04-1.06	
PIII	2025	2105	1465	900	645	425	245	0.68-0.72	0.96-0.97	

Ant Ta5/Ti about 0.23-0.25. Abt 34 sens.chaet on midTa1, abt 30 on hindTa1.

Abdomen: Segments II-V greenish with dark saddle spots in the basal region of the segment. 9-11 setae in individual(?) pale areas on tergite IX.



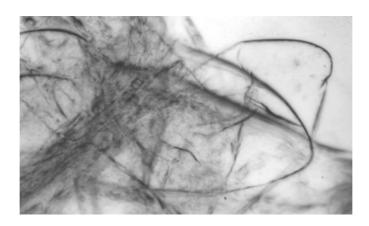
Anal point broad and stout, wider at base, SVo curved, D(g)-E(h)-type of Strenzke (1959). IVo with simple setae, not reaching the end of the anal point and to about the middle of the GS which is markedly swollen and reducing over posterior 1/3.

Female: No confirmed specimens available.

Pupa: From notes by Don Forsyth, the pupa has a dark exuvia, with dark muscle scars, and a very dark lateral spur on segment VIII with multiple appressed spines (3 in figure below).



Fourth instar larva: A salinarius-type larva. Head capsule coloration similar to that of C. *zealandicus*, but a smaller species, length about 11.4-19.0 mm (female), 10.7-16.0 (male). Anal tubules (below) short and rounded or pointed, length 200-375 μ m, less than 3 times longer than wide. SAL about 85x30 μ m (abt 2.8 times wider than deep).



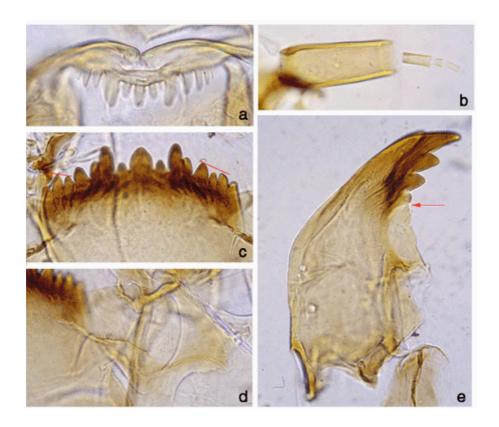
Mentum (c, below) generally type II; centre tooth with c2 teeth well separated (i.e. type IIA); 6th laterals slightly turned out.

Ventromental plates (d, below) separated by between a third and a half (ave. 0.44) of the mentum width, with only about 30-42 striae; VMR 0.28-0.31. PE (a, below) with about 8-16 teeth, some reduced (type C, but teeth sharp).

Premandible with inner tooth about 2-3.3 times wider than outer tooth and coming to a broad point (type C).

Basal segment of antenna (b, below) not as long and narrow as in other NZ species such as *C. zealandicus*, and about 2.8-3.5 times longer than wide (similar to *C.* species 6); Ring organ between a quarter and half way up from base. Antennal proportions (microns) 132:32:7:15:7; A3 shorter than A4, but only slightly shorter or same length as A5; AR 1.88–2.3. Distance between the S4 setae greater than that between the antennal bases.

Mandible (e, below) with 3rd inner tooth only slightly darkened and hardly separated (type IIB), with about 22 (19-27) furrows on outer surface near the base; 12.7 (10-14) taeniae in PecM; MTR about 0.36.



Cytology: 4 polytene chromosomes, pseudothummi-complex arm combination (BF, CD, AE, G). Two nucleoli; a large one in arm F and a small one in arm G, often difficult to distinguish from a BR. Two BRs towards the other end of the arm. Arm G usually only partially paired, sometimes more so than others. Arm A as sequence A4 in Australian species; arm E with sequence E1 as Australian species; anlF1 as F3 in Australian species. Polymorphism at least in arms B, C and F.

anlA1: 1a-e, 11 - 10, 2c - 1f, 3e - 2d, 8 - 9, 3f-i, 12c-a, 4 - 7, 13 - 19 i.e. as oppA4, forA1, nzlA1.

anlB1: Puff (group 7) in proximal third of arm, separated from usual associated dark bands. as *forsythi* B1

anlB2: inversion of distal half of arm, seen as heterozygote at Lake Ngapouri.

anlC1: as oppositus C4?

anlC2: as oppositus C3? seen at Lake Ngapouri.

anlD1: 1-2, 16 - 13, 8- 3e, 9, 3d-a, 10d - 12, 10a-c, 17 - 24

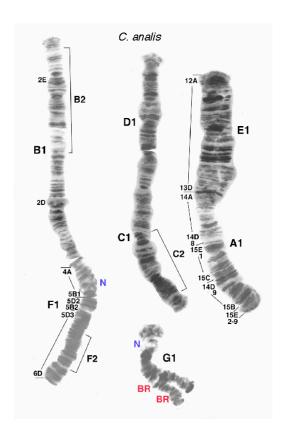
anlE1: 1 - 3e, 10b - 3f, 10c - 13

i.e. as *forsythi* E1, etc.

anlF1: (approx.) 1 - 2a, 10 - 2c, 15c - 11a, 2b, 15d - 23 i.e. as oppositus F3, forsythi, novaezelandiae

 $anlF2: \ 1-2a, \ 10-9c, \underline{2c-9b}, \ 15c-11, \ 2b, \ 15d-23.$

anlG1: essentially as C. novaezelandiae?



Found in lakes

Localities:

North Island:

Lake Karapiro, South Auckland (-37.94°S, 175.56°E) (Sofia Ibarrarán) (NZ.77.1) 15-ii-2007

Lake Karapiro, South Auckland (-37.94°S, 176.56°E) (Sofia Ibarrarán) (NZ.77.2) 29-viii-2007

Lake Ngapouri, Waiotapu (-38.00°S, 176.50°E) (D.J. Forsyth) (NZ.9.1 & 2) 15-ii-and 8-xi-1972; 5-xii-1973

Lake Okaro, Rotorua area (-38.30°S, 176.40°E) (D.J. Forsyth) (NZ.10.1 & 8), 19-ix-1972 and 14-ix-1982 also (Sofia Ibarrarán) (NZ.10.9) 14-ii-2007)

Ngapuna, Rotorua area (-38.15°S, 176.27°E) (Jon Martin & D.J. Forsyth) (NZ.17.1) 7-xii-1973

Ohakune, Wellington (Freeman 1959)

Waikato area (-37.202°S, 175.502°E) (Doyle & Binks)(BOLD database BOLD ACG0887)

South Island:

Blackball, Nelson (Freeman 1959) (Holotype)

Lake Brunner, Mitchell (-42.62°S, 171.43°E), Westland (Jon Martin) (NZ.52.1 and NZ.52.2) 9 & 10-i-1974

East Cove, Lake Te Anau (-45.17°S, 167.83°E), Southland (Jon & H.I. Martin) (NZ.46.5) 23-i-1978

Owaka River (-46.42°S, 169.60°E), South Otago (D. Dodgshun & J.S. Pillai) (NZ.56.1) 14-iii-1974

Queenstown, Otago (Freeman (1959)

West Coast, Westland (Freeman 1959)

(These require further checking, as some may be *C. forsythi*. Many collections assumed to be *C. zealandicus*, e.g. material studied by Robb, seem to be identical with the larval material identified as *C. analis* by Forsyth.)

Some information on larval morphology and cytology given by Martin (1998). Stark (1981) in his key to larvae, has *C. analis* as a thummi-type larva, but the basis for this conclusion is not known.

NZ3a.

Adult female (reared) (perhaps *C. analis*? but perhaps more likely *C.* sp. nr. *antipodensis*). This is a large specimen:

Wing length 6.15 mm, width 1.67 mm, VR 1.05. 4 Scf on brachiolum, about 40, 43 setae in squamal fringe.

FT a wrinkled cylinder 55 x 18 µm (3.14 times longer than wide).

Antenna (micron)(fraction of neck in brackets): 234 (0.22) : 145 (0.47) : 163 (0.58) : 160 (0.56) : 270; A5/A1 1.15; AR 0.39.

Palp proportions (micron): 91: 89: 250: 280: 335; P5/P4 1.2-1.6; P5/P3 1.25-1.31.

Clypeus 2.43 times the diameter of antennal pedicel; 35 clypeal setae.

Thoracic setae: at least 17 Acrostichal; Humeral 5 linear & 5 linear+sm. gp. of 2;

Dorsocentral 18-19 (Humeral+dorsocentral 23-26); Prealar 7-9; supra-alar 1;

Scutellum 6+17 in 2 anterior rows, 9 in posterior row (Total 32 setae).

Leg lengths (micron) and proportions:

	Fe	Ti	Ta1	Ta2	Ta3	Ta4	Ta5	LR	F/T
PI	1760	1700	-	-	-	-	-	-	1.04
PII	1925	1975	1010	710	460	300	240	0.51	0.97
PIII	2330	2535	1520	1000	850	420	265	0.60	0.92

Abdomen with an anterior dark stripe which gradually expands from being anterior on segments II-V (with slight posterior extension in midline of II), to virtually all of tergite from VI-VIII.

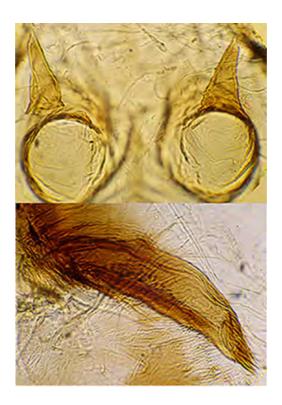


Segment X with 10-11 setae, essentially a semicircle about $126.5 \times 58 \mu m$ (2.2 times longer than wide); cerci with no obvious bulges, ventral and posterior margins curved, and with posterior end rounded.

Pupa: Yellow brown with brown muscle scars. Length of female exuvium 12.8 mm; length antennal sheath 1.27 mm and length IMW 2.64 mm. Slight indication of frontal wart. Cephalic tubercles 166 x 151 μ m with a seta about 75 μ m long. Respiratory base 166 μ m long and 80 μ m wide (HR 2.44).

About 81 hooks at posterior of segment II in row covering about 0.54 of segment width. PSB marginal on this segment.

PSA on segment IV large – about 330 μ m long and 175 μ m wide; about 0.25 of the length of the segment; that of seg V about 145 μ m of spines and of seg VI abt 125 μ m of spines. Posterolateral spur of segment VIII with 8 lge & 2 sm and 9 lge & 1 sm spines, closely appressed. About 139 taeniae on anal lobe in 2 rows anteriorly and 3 rows distally. Shagreen over most of the segment with stronger band towards posterior; weak anterior on seg. VII and weak posterior on seg. VIII.



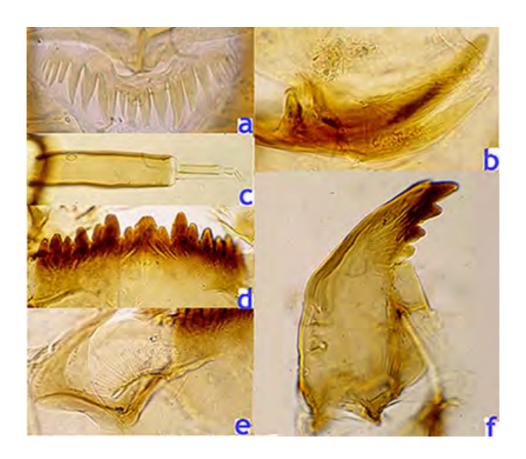
Fourth instar larva: No information on larval size or type. Ventral head length 411 μ m. Gula darkened over posterior half, wider than the mentum and widest part-way up from posterior margin.

Mentum of type III (i.e. 4th laterals reduced lower than the 5th laterals), centre tooth possibly of type IIB if not worn; width about 0.65 of VHL.

Ventromental plates separated by $105 \mu m$ (0.40 of the mentum width), width $250 \mu m$, $3.65 \mu m$ times wider than deep and about 0.95 the width of the mentum; about 47-52 striae; VMR about 0.3.

PE with 20 teeth including 6 small teeth (as in *C. analis*). Premandible with inner tooth about 3.75 times wider than the outer tooth and coming to a slightly broader point (type B2). Antenna with segment 1 quite long (3.8 times longer than wide) and about half of the VHL, RO about a quarter up from base of segment; segment lengths (micron) 170:52:15:17:7.5, i.e. segment A3 about 90% length of A4 and twice the length of A5; AR about 2.75. Distance between the antennal bases (276 μ m) greater than that between the S4 setae (205 μ m) which is about 80% of the FC width.

Mandible of type IIB with about 20-21 furrows on outer surface near the base; 15-17 taeniae in PecM; Mdt-Mat 38 μ m; MTR about 0.30.



This female is most notable for the large size (wing len. 6.15 mm), larger even than *C. zealandicus* (5.5 mm) or *C.* nr. *antipodensis* (5.1 mm).

Localities:

South Island

Moke Lake, abt. 10 Km Queenstown, South Island, (-45.00°S, 168.57°E) Jon and C.J. Martin NZ.63.1 (7-i-1974).

4. Chironomus forsythi Martin, 1998.

In BOLD Bin:

Adult:

Male:

Wing length: 3.6-5.3 mm; width 1.1-1.3 mm; VR 0.98-1.04.

AR: 3.6-4.4

Palpal segments 2 -5 (micron): 80 : 280 : 260 : 330. Clypeal setae about 28–38.

At least 12–18 acrostichal setae; 16-24 dorsocentrals; 5-7 prealars; scutellars in two approximate rows - anterior row about 6-23, posterior row about 15-18 setae.

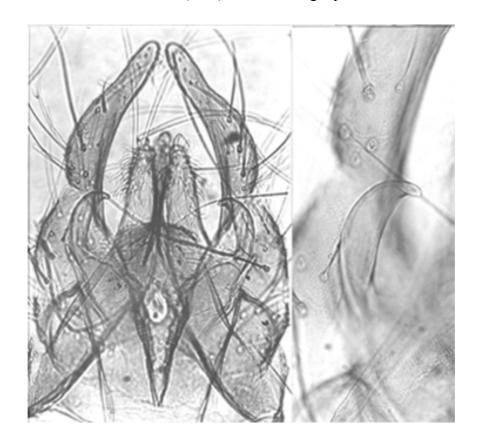
Fore LR: 1.52-1.56 may be diagnostic. Legs pale, unbanded, with only a short sparse beard.

Leg proportions (micron):

	Fe	Ti	Ta1	Ta2	Ta3	Ta4	Ta5	LR	F/T	BR	١
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PI	1660	1510	2240	1280	890	700	330	1.52-1.56	1.06-1.11	1.7-2.09
PII	1700	1620	940	620	430	285	215	0.57-0.59	1.05	
PIII	1980	2040	1410	880	620	390	245	0.68-0.73	0.96-0.98	

Abdominal tergites 2-5 with dark brown patches across about two thirds of the segment, although sometimes reducing towards the basal margin to a more triangular appearance. TIX with about 7.6 (3-11) setae in a single pale area.



Anal point relatively narrow, wider at tip; SVo curved, essentially D(e)-type of Strenzke 1959); IVo reaching about to end of the anal point or middle of gonostylus with forked setae. GS relatively swollen, reducing significantly on distal third.

Female:

Wing length: 3.4-5.9 mm; wing width: 0.9-1.7 mm; VR 1.03-1.07

AR: 0 32-0.49; flagella length (micron): 190 : 140 : 135 : 130 : 210

Palpal segments 2-5 (micron): 70 : 230 : 250 : 385. Clypeal setae about 26–56.

About 15 acrostichal setae; 18-38 dorsocentrals (incl. humerals); 6-8 prealars; scutellars in two approximate rows - anterior row about 5-23, posterior row about 6-30 setae.

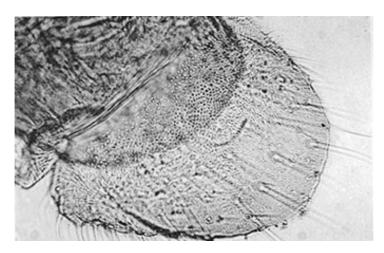
LR slightly lower than that of males, about 1.47–1.48 (2).

Leg proportions (micron):

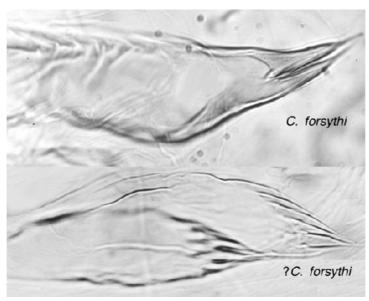
	Fe	Ti	Ta1	Ta2	Ta3	Ta4	Ta5	LR	F/T	Ta4/Ti
PI	1550	1410	1990	940	785	650	270	1.47-1.48	1.08-1.11	0.46
PII	1580	1595	830	470	350	245	195	0.55-0.57	0.97-1.00	

PIII 1860 1940 1300 780 600 320 220 0.63-0.72 0.96-0.97	
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Cercus (below) with rounded outline but with a relatively large bulge on the ventral base.



Pupa: Length of exuvia about 11.3-11.8 mm (female) and 6.8-10.5 mm (male); IMW about 2.15 mm. Cephalic tubercles about 110-160 micron, with sub-apical seta about 45-65 micron. About 76-112 recurved hooks on segment II; muscle scars on tergites pale or slightly pigmented; caudolateral spurs of segment VIII with only two to three appressed spines at the type locality (Haast) and in most North Island populations, but about 6-11 spines are found in some, mostly South Island, specimens tentatively assigned to this species. About 135-157 (female) and 56-131 (male) multiple ranked taeniae on each side of the anal lobe.



Pupal spurs of *C. forsythi*Both islands (above, from Haast egg mass #6) and South Island only (below)

Fourth instar larva: A salinarius-type larva. Length about 12.0-20.5 mm (female), 12.2 - 20.0 (male) (larger specimens may be a further new species). Anal tubules elongated and

rounded, from 2-3 times as long as wide and often constricted in the middle, in some populations, but short with no constriction in others.



Anal tubules of a larva from nr. Haast, egg mass #6

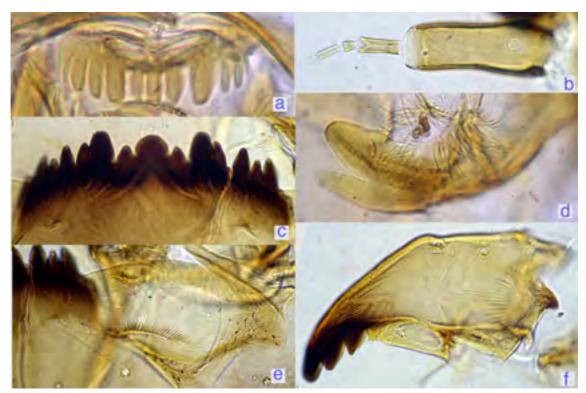
Head capsule with posterior third of gula dark, FC slightly dark to dark, and in some larvae dark triangular marks on the posterior margin either side of the FC.

Mentum (Fig. c, below) with 4th laterals reduced almost or beyond the level of the 5th laterals (type II); central trifid tooth with c1 tooth relatively broad and c2 teeth separated (type IIA). Ventromental plate (Fig. e, below) with about 30-43 striae; VMR about 0.30. PE with about 8-16 usually relatively broad even teeth.

Premandible (Fig. d, below) of type D, with inner tooth about 3.7 times the width of the outer tooth and coming to blunt points (unless due to wear).

Antenna with a moderately broad basal segment about 2.6-3.4 times longer than wide; RO about 1/3 to $\frac{1}{2}$ up from base of segment; AR 1.8-2.5; antennal proportions (microns) 133 : 29 : 8 : 14 : 7.

Mandible (Fig. f, below) generally type IIA, although 3rd inner tooth somewhat variable in pigmentation and degree of separation; about 13-23 furrows on outer surface near base.



Mouthparts of a larva from the same egg mass as the Holotype.

Cytology: 4 polytene chromosomes, pseudothummi-complex arm combination (BF, CD, AE, G). Probably only one nucleolus, proximal in arm F, another subterminal in arm G, is not always apparent and may not be present in true *C. forsythi* (see below). Arm G closely paired in some populations, paired only in the middle in others, with a median BR and a smaller one towards the opposite end from the nucleolus.

Arm A of some individuals with sequence oppA4 of Australian species; forD1 as nzlD2; forE1 as oppE1 of Australian species.

Polymorphism in arms A to F; some South Island populations polymorphic in all these arms, including at least 3 sequences each in arms A, B, and F, others with limited polymorphism in arms A and B. This may indicate that 2 species are present in these populations.

forA1: 1a-e, 11-10, 2c-1f, 3e-2d, 8-9, 3f-i, 12c-a, 4-7, 13-19 i.e. as oppA4, anlA1, nzlA1. (type 1 & 2)

for A2: 1a-e, <u>2e - 3e, 1f - 2c, 10 - 11</u>, 2d, 8 - 9, 3f-i, 12c-a, 4 - 7, 13 - 19 (from for A1) (type 2 and 3 & rare type 1)

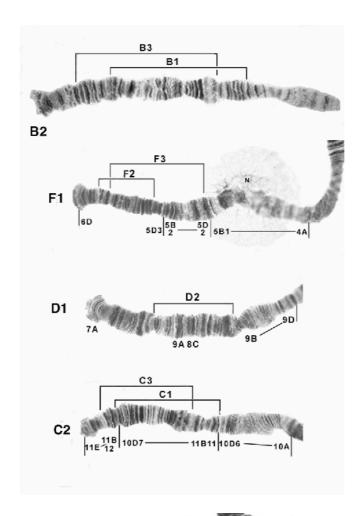
for A3: 1a-e, 10d-a, 2c - 1f, 3 - 2e, 10e-11e, 2d, 8 - 9, 3f-i, 12c-a, 4 - 7, 13 - 19 (from for A2) (holotype)(type 1 and 3(few))

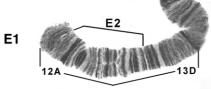
forA4: large inversion of forA1?

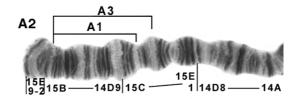
forB1: Puff (group 7) with proximal dark bands (group 8) about middle of arm.(all types)

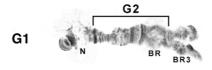
forB2 Inversion of centre half of arm, reversed puff and dark bands (groups 7 & 8).(holotype)(all types)

- forB3: From B2 by inversion which reverses puff (group 7) again and moves it near the distal end. (type 1 and 3)
- for C1: as C1 of oppositus and. australis. (holotype)(all types but rare type 3?)
- forC2: large inversion taking typical groups 3 4 to near the centromere as *analis* C1 (all types but common ty.2)
- forC3: a smaller inversion of forC2, not including the typical groups 3 4.(rare ty. 2)
- forD1: 1 2, 16c-a,17e-a, 10c-a, 3e 9, 3d-a, 10d -15, 18 24 as nzlD2 (holotype) (ty.1 and ty.2)
- forD2: 1 2, 16c-a, 17e-a, 10c-a, 3e-g, 18, 15 10d, 3a-d, 9 4, 19 24 (from forD1) (rare ty. 1 & 2, common ty. 3)
- forD3: approx. 1 2, 16c-a, 10c-a, 3e 5, <u>19 18, 15 10d, 3a-d, 9 6, 20 24</u> (from forD1) (rare ty. 2)
- forE1: 1 3e, 10b 3f, 10c 13 i.e. as *oppositus* E1, *analis, novaezelandiae* (holotype) (common ty. & 2.)
- forE2: 1a-i, <u>3f 10b, 3e 2a</u>, 10c 13 (ty 2 (rare) & ty. 3)
- forF1: 1 2a, 10 2c, 15c 11a, 2b, 15d 23 i.e. as *oppositus* F3, *australis*, *analis*, *novaezelandiae* (holotype) (comm. ty. 1, rare ty. 2 & 3)
- for F2: (approx.) 1-2a, 10-9c, 4-9b, 3-2c, 15c-11, 2b, 15d-23 (found on both islands) (rare ty. 1 & 3, common ty. 2)
- forF3: (approx.) 1 2a, 10 7d, <u>11 15c, 2c 7c</u>, 2b, 15d 23 in Sth Is. specimens (ty. 3)
- forG1: no BR near the subterminal nucleolus. (all types)
- for G2: (rare ty. 2)









DNA analyses:

Mt COI - see BOLD

COI data for larvae from the same egg mass (NZ.67.1 E.mass #6) as the holotype larva, are different from that of other specimens. One possibility is that the female parent was of hybrid descent from a female of a different species; alternatively it reflects the presence of multiple species under this name. On the other hand, the data suggests the presence of three species under this name (see similar note for arm F sequences, below)

Morphology and cytology given by Martin (1998), but some details were incorrect. Both arms A and F show a different sequence to that stated, and the limits of some inversions on these arms were incorrect. (corrected above).

Based on the banding sequence of arm F, there could be three species included under this name, because the three sequences identified usually occur only as homozygotes (see below). If this proves to be true, the type specimen of *C. forsythi* is associated with sequence F1. Larvae with F2 are more common in the South Island, but a single larva from Lake Ngaroto, Sth Auckland, was heterozygous for F1.2. Larvae with F3 have been found at only three localities in the South Island: Bealey, Ross Creek Dam and Lake Wakitipu, although a larva from Sullivans Dam was heterozygous F1.3.

Material associated with the Holotype and Allotype:

To help resolve this matter, the description of material from NZ.67.1 egg mass#6 is given: The holotype is a polytene chromosome squash with associated larval body mounted on the same slide.

Adult:

No adult male associated with the Holotype larva or Allotype female is known. Allotype female:

Wing length: 4.23 mm; wing width: 1.18 mm; VR 1.08

AR: 0.49; flagella length (micron): 181: 126: 141: 131: 251

Palpal segments 2 - 5 (micron): 60 : 201 : 201 : 417. Clypeal setae about 37.

About 15 acrostichal setae; 27 dorsocentrals; 6 prealars; scutellars in two approximate

rows - anterior row about 23, posterior row about 17 setae.

Leg proportions (micron):

	Fe	Ti	Ta1	Ta2	Ta3	Ta4	Ta5	LR	F/T	Ta4/Ti
PI	1420	1220	1820	1040	740	600	300	1.47	1.15	0.49
PII	1500	1400	800	440	320	220	180	0.57	1.07	0.13
PIII	1680	1740	1160	680	520	300	200	0.67	0.97	0.11

Pupa: (based on male exuvia and a female prepupa) Length of exuvia about 6.8 mm. Cephalic tubercles about 120 micron, with sub-apical seta about 46 micron. About 76-78 recurved hooks on segment II, occupying about 60% of width of segment; muscle scars on tergites pale or slightly pigmented; line of fine spines on conjunctive V/VI, and patch on conjunctive VI/VII; caudolateral spurs of segment VIII with three or four appressed spines (in case of four spines there were three large and one small). About 56-58 multiple ranked taeniae on each side of the anal lobe.

Fourth instar larva: Length about 12 mm (probably small because laboratory reared); anal tubules about 3 times as long as wide (see figure above). Gula dark to very dark over posterior 2/3, FC darkened with a triangular dark patch on each side at the posterior margin of the head.

Mentum with rounded teeth, 4th laterals reduced below level of 5th laterals (type III); central trifid tooth with c1 tooth relatively broad and c2 teeth separated (type II).

Ventromental plates separated by about 1/3 of mentum width, about 42 - 43 striae. PE with about 12 broad teeth, narrowing towards the edges.

Premandible probably with outer tooth longer than inner tooth, which is about twice as wide as the outer tooth.

Antenna with basal segment about three times longer than wide, ring organ about a third of the way up from the base of the segment; AR about 1.74; proportions of segments (μ m) 110 : 26 : 6 : 14 : 5.

Mandible with third inner tooth relatively dark, but only partly separated (type II); about 12 - 13 bristles in the PMan, 15-16 furrows on the outer surface at the base. (see figures of mouthparts above)

Cytology at type locality: Sequences recorded are: forA3, forB2, forC1, forD1, forE1, forF1, and forG1 (apparently without a nucleolus).

Localities:

North Island:

Lake Ngaroto, South Auckland (-37.96°S, 175.29°E) (S. Ibarraran) (NZ-75-1) 27-viii-2007– probable hybrid (het 5 arms)

Lake Karapiro, South Auckland (-37.96°S, 175.29°E) (Sofia Ibarrarán) (NZ.77.1) 15-ii-2007

Lake Ngaroto, South Auckland (-37.96°S, 175.29°E) (Sofia Ibarrarán) (NZ.75.1) 29-viii-2007

Potting shed pond, Taita Soil Bureau, Wellington (-41.18°S, 174.95°E) (D.J. Forsyth) (NZ.14.1) 13-x-1972

Potting shed pond, Taita Soil Bureau, Wellington (-41.18°S, 174.95°E) (D.J. Forsyth) (NZ.14.2) 26-i-1973

Potting shed pond, Taita Soil Bureau, Wellington (-41.18°S, 174.95°E) (Jon Martin & D.J. Forsyth) (NZ.14.3) 23-xii-1973

South Island

Bealey, 11 Km east of Arthurs Pass, (-43.04°S, 171.64°E) (J.A. & M.M. Thomson) (NZ.3.1) 1-ii-1968 – could be F3.3

Belfast (abt 10 Km north of Christchurch), Canterbury (-43.45°S, 172.62°E) (D. Matthews) (NZ.54.1) 7-i-1974

Botanic Gardens, Christchurch (-43.55°S, 172.67°E), Canterbury (J.A. & M.M. Thomson) (NZ.2.1) 31-i-1968

Dunedin, Otago (-45.80°S, 170.87°E) (shorter anal tubules) (J.S. Pillai) (NZ-6-1 & 2) 12-iii-1968

Haast junction, Westland (-43.88°S, 169.05°E) (Jon Martin) (NZ.67.1) 26-i-1978 Egg masses #6, #10 (long anal tubules) (Type locality)

Haast junction, Westland (-43.88°S, 169.05°E) (Jon Martin) (NZ.67.2) 26-i-1978

Lake Hawea, Otago (-44.50°S, 169.28°E) (Jon & C.J. Martin) (NZ.48.1) 7-i-1974

Lake Lochie, (-44.83°S, 170.165°E) 87 km n.w. Te Anau, Fiordland (Jon Martin) (NZ.45.1) 6-i-1974

Lake Pukaki, Canterbury (-43.965°S, 168.12°E) (Jon Martin) (NZ.33.1) 31-xii-1973 c.1 m in Lake Te Anau, Te Anau, Fiordland (-45.41°S, 167.50°E) (Jon Martin) (NZ.46.2) 7-i-1974

Lake Wakatipu, Kingston, Southland (-45.33°S, 168.72°E) (Jon & C.J. Martin) (NZ.62.1) 21-i-1978 – could be F3.3 (shorter anal tubules)

Mirror Lake, 6 Km Knob Flat on Milford Road, Southland (-44.82°S, 167.78°E) (Jon & C.J. Martin) (NZ.44.1) 6-i-1974

Owaka River, South Otago (-46.42°S, 169.60°E) (T. Dodgshun & J.S. Pillai) (NZ.56.1) 14-iii-1974

Sullivans Dam, Dunedin City Council Water Dept., Otago (-45.808°S, 170.524°E) (Jon & C.J. Martin & T. Dodgshun) (NZ.36.1) 3-i-1974

Possible new species ('F2-type':

Bromley Sewage Works, Christchurch, Canterbury (-43.45°S, 172.62°E) (Jon Martin) (NZ.7.3) 28-xii-1973 (Region 4-6 of Robb's samples) – homozygous F2.2 (shorter anal tubules)

Pond 3, Bromley Sewage Works, Christchurch, Canterbury (-43.45°S, 172.62°E) (Jon Martin) (NZ.7.6) 11-i-1974 – homozygous F2.2

Bromley, Christchurch, Canterbury (-43.54°S, 172.69°E) (D.J. Forsyth) (NZ.15.1) 22-ii-1973 – homozygous F2.2

Lake Brunner, Mitchell, Westland (-42.62°S, 171.43°E) (Jon Martin) (NZ.52.2) 10-i-1974 – homozygous F2.2

Long Point, nr. Tahakopa, Catlins District, Otago (-46.52°S, 169.38°E) (Jon Martin) (NZ.5.4) 4-i-1974 – homozygous F2.2

Ross Creek Dam, Dunedin City Council Water Dept., Otago (-45.847°S, 170.499°E) (Jon Martin & T. Dodgshun) (NZ.39.1) 3-i-1974 – homozygous F3.3

5. Chironomus 'thermarum' (ms name of D.J. Forsyth).

This is a morphologically variable species in regards to larval type.

In Bold Bin: BOLD: AAJ0168 or BOLD: ABZ5458

Adult:

Male: Forsyth describes the abdominal tergites as largely dark with only a narrow pale posterior margin of segments II to VI and on four fifths of segments VII and VIII.

A laboratory-reared male and a pupa with a pharate male is available, from which the following characters could be determined:

Male:

Wing length: 5.0 mm; width 0.96 mm; VR 0.98.

AR: 2.6.

Palpal segments (micron): 90 : 78 : 265 : 275 : 376: P5/P4 & P5/P3 1.42. Clypeal setae about 32.

At least 9 acrostichal setae; 13 dorsocentrals; 1 supraalar; scutellars in three approximate rows – 10 in partial double anterior row, 14 in posterior row, total about 24 setae.

Fore LR: 1.35. Legs pale, unbanded, with a short beard (BR=2.82) and a sparse longer beard (BR=4.09).

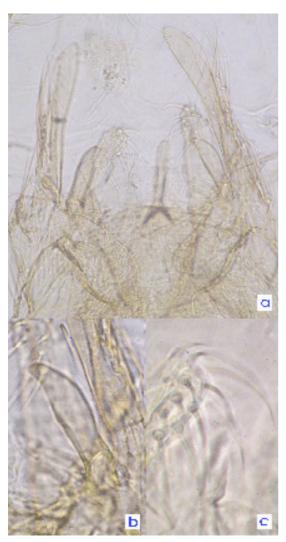
Leg proportions (micron):

	Fe	Ti	Ta1	Ta2	Ta3	Ta4	Ta5	LR	F/T	Ta5/Ti
PI	1655	1520	2060	1190	855	690	355	1.35	1.09	0.23
PII	1785	1745	985	605	430	290	220	0.57	1.02	
PIII	2050	2150	1465	910	695	430	245	0.68	0.95	

(Note: These details may refer to Species 10)

Tergite IX with 5 setae in individual pale areas. Anal point narrow at base, wider at tip. SVo essentially E(h)-type of Strenzke (1959); IVo reaching about to the end of the anal point with simple setae. Gonostylus GS quite swollen, reducing significantly on distal third.

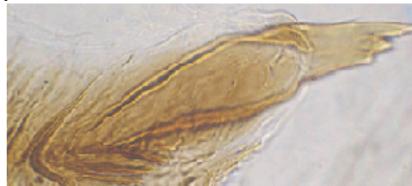
SVo (b) with just a small hook at the tip (D(e)-type of Strenzke 1959); IVo (a, above)



extending to about middle of gonostylus with setae simple (c). Gonostylus (a) moderately broad but narrowing over distal third, about 4+1 setae at tip.

Pupa: about 6.9-10.2 mm (2 males) - 8.3 mm (1 female) long; IMW about 1.44-2.34 mm. Cephalic tubercle about 85-150 x 80 μ m long. Basal ring about 115-170 μ m long and 57-65 μ m wide, HR 1.77-2.56. Thorax and muscle scars yellow-brown, otherwise pale.

Fine shagreen on posterior half of segment II, increasing to whole of segments IV-VI, little on segments VII & VIII. Hook row with 70-92 hooks, occupying about 0.6-0.8 of segment width. PSB on segs II and III; PSA of segment IV large (202-218 µm long, 101-145 µm wide) and about 0.21-0.28 length of segment; PSA of segment V of spines. Caudolateral spur of segment VIII with about 2 or 3 spines, at least 1 small. 68-93 taeniae on each side of anal lobe, mostly in a single row, but some double at posterior end.



Fourth instar larva: A bathophilus, with both short anterior and posterior VT (over 0.32 mm in length) to halophilus with only poster pair up to about 0.12 mm) to salinarius-type. Generally smaller due to growing at higher temperature (but larger when reared in the lab at

lower temperatures), length about 10-14 mm (fem. 10.3-14.0; male 9.2-14.0 mm). Where present ventral tubules approximately equal in length, anterior 0.63 (0.44-0.96) mm and posterior 0.64 (0.32-1.01) mm. Anal tubules from 1.5-2.1 times longer than wide. Head capsule with FC generally pale or slightly darkened, gula darkened and can be broader than the mentum width. Head relatively narrow, mentum width about 0.54-0.60 of ventral head length

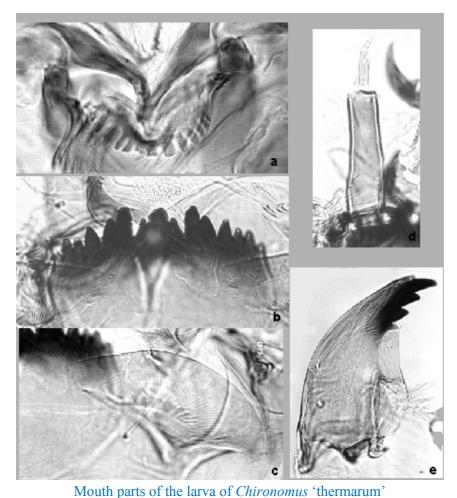
Mentum (Fig. b, below) with 4th laterals reduced about to level of 5th lateral, or sometimes lower (type II-III); centre tooth relatively narrow, with c2 teeth only partly separated on its shoulders (i.e. type IB or occasionally III); 6th laterals arising from a lower level. VM (Fig. c, below) about 182-205 μm wide and 3.04-3.86 times wider than deep, and 1.0-1.1 time wider than the mentum, with about 37.9 (33-41) striae; IPD 0.28-0.35; VMR 0.24-0.35. Pecten epipharyngis (Fig. a, below) with about 11-16 sometimes uneven teeth (type B), with about 45-55 striae; VMR about 0.29-0.32. PE with about 11-16 sometimes irregular teeth.

Premandible with teeth usually about equal length, inner tooth about 2+-5 times the width of the outer tooth, both coming to a broad, or occasionally a narrow, point.

Antenna (Fig. d, below) with basal segment about 3-4 times longer than wide; RO about 0.29 (0.24-0.38) up from base of segment; A4 relatively short, only about 20% longer than segment 3; A5 about as long as A3 (0.73-1.08 times as long); AR 2.27 (2.00-2.44); segment proportions (micron) 119; 27: 7.5: 10: 6.5.

Distance between the S4 setae (150.7 (126-162) μ m) basically similar to that between the antennal bases (148.2 (129-167) μ m).

Mandible (Fig. e, below) about 220-252 μ m long (heel to Mdt) of type II, with about 13-15 furrows on outer surface near base; 11-13 taeniae in Pecten mandibularis; Mdt-Mat 22-30 μ m; MTR 0.30-0.43.



a. Pecten epipharyngis; b. Mentum; c. Ventromentum; d. Antenna; e. Mandible.

Cytology: 4 polytene chromosomes, pseudothummi-complex arm combination (BF,CD,EA,G). Arm G normally closely paired with a large subterminal BR, and a smaller one medially. Nucleolus on arm F at about group 19. Arm A with sequence A4 as found in Australian species; arm E with sequence E1 of Australian species.

thmA1: 1a-e, 11 - 10, 2c - 1f, 3e - 2d, 8 - 9, 3f-i, 12c-a, 4 - 7, 13 - 19 i.e. as oppA4, anlA1, forA1, nzlA1.

thmB1: Puff (group 7) often hardly developed, with proximal dark bands (group 8) near distal end as nzlB4.

thmB2: possibly as *oppositus* B1- possibly in non-thermal habitats (or Sp.10?)

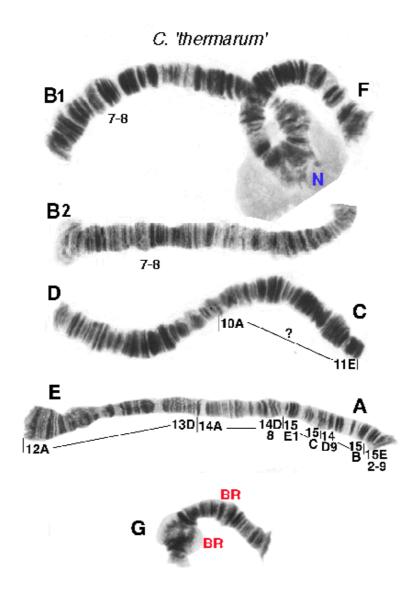
thmC1: As nzlC1, forC1, and oppositus?

thmD1: 1 - 2, 16c-a, 17e-a, 10c-a, 3e - 9, 3d-a, 10d - 15, 18 - 24 as for D1

thmE1: 1 - 3e, 10b - 3f, 10c - 13 i.e. as *oppositus* E1, *analis*, *forsythi*.

thmF1: appears to be 1 - 2a, 10 - 2c, 15c - 11a, 2b, 15d - 23 i.e. as oppF3, nzlF1, etc.

thmG1: large subterminal BR and median BR.



Molecular:

Mt *COI*: *COI* sequence suggests that *C*. 'thermarum' may be an ecologically plastic species that can live in thermal habitats as well as habitats with lower water temperatures. At normal temperatures the larvae are a bathophilus type, with the VT, particularly the anterior pair, reducing in size with higher temperatures (see below).

The cytology shows close relationship to sequences of the Australian species *Chironomus oppositus*, suggesting that perhaps an ancestor was blown across the Tasman from Australia.

Localities:

North Island:

Kerosene Creek (thermal), South Auckland (-39.94°S, 176.56°E) (S. Ibarraran) (NZ.78.1) 27-viii-2007

Lake Ngahewa (-38.31°S, 176.37°E), South Auckland (S. Ibarraran) (NZ.76.1) 28-viii-2007.

Lake Rotowhero, South Auckland (-38.30°S, 176.40°E) (D.J. Forsyth and Jon Martin) (NZ.11.3) 23-xii-1973; (S. Ibarrarán) (NZ.11.6) abt 15-ii-2007

Queen Elizabeth Park (-37.94°S,175.56°E), South Auckland (S. Ibarraran) (NZ.80.1) abt 15-ii-2007.

Waiotapu Stream (thermal) South Auckland (-38.38°S, 176.35°E) (D.J.Forsyth) (NZ.69.1) 14-vi-1983

Waiotapu Stream (thermal) South Auckland (-38.38°S, 176.35°E) (D.J.Forsyth) (NZ.69.2) 25-iii-1998

South Island

Lake Te Anau, nr. Te Anau (-45.10°S, 167.50°E). Fiordland (J. & H.I. Martin) (NZ.46.6) 23-i-1978

6a. *Chironomus* 'castaneum'. (ms name of D.J. Forsyth)

In BOLD Bin: BOLD:AAL7010

Along with C. 'paracastaneum' and C. sp. NZ9. (see under C. sp. NZ9 for further detail)

This manuscript name was allocated by D.J. Forsyth for specimens from Poutu Canal, Lake Rotoaira, South Auckland. His associated adults were not available for study.

Don described the adult abdomen as having somewhat triangular brown markings on the tergites that became progressively wider on successive segments.



DNA and cytological data indicated that there was more than one taxon initially included under this name and that North and South Island specimens differ in their mt*COI* sequences.

North Island form:

Adult:

Male: Abdomen with somewhat triangular brown markings, narrowing towards the posterior margin, on the tergites that become progressively wider on successive segments.

Pupa: Spur with 3-5 spines.

Fourth instar larva: A salinarius-type larva. Length: female about 16.23 (15.2-16.8) mm, male about 14.1 (12.8-14.8) mm. Anal tubules short and normally rounded, usually about the

same length although the ventral pair may be longer and narrower; only about 2x as long as wide – occasionally pointed and about the same width as the length.

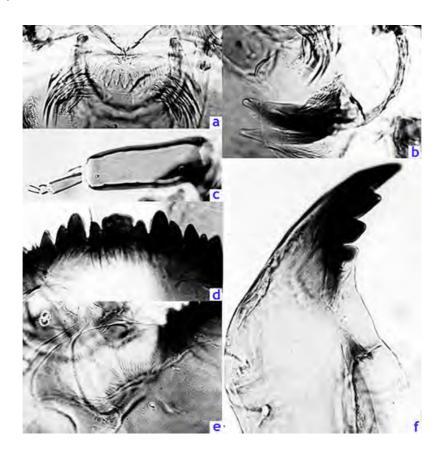
FC and posterior half to two thirds of gula, dark to very dark, sometimes with some slight darkening at posterior of head capsule. SAL relatively deep: $78 \mu m$ wide and 4.4 times wider than deep.

Mentum (Fig. d, below) width about 0.68 to 0.77 of VHL; with 4th lateral reduced to about the level of the 5th laterals (type II), c1 tooth broad (broad IIA), 6th laterals turned out. VM (Fig. e, below) about $245~\mu m$ wide and 2.95-3.23 wider than deep; about 1.03 times the mentum width and separated by about 0.4-0.5 of its width; with about 45.0 (39-55) striae, which appear to extend almost to anterior margin, particularly at the outer edges; VMR about 0.27. PE (Fig. a, below) with about 11.6 (10-14) teeth, mostly worn but narrowing towards the edges. Premandible (Fig. b, below) of type B2, with outer tooth about 3-5x the width of the outer tooth and coming to a broad point.

Distance between antennal bases (167.7 (139-245)) less than the distance between the S4 setae (183 (147-263)); S4 setae separated by about 0.94 of the FC width.

Antennae (Fig. c, below) with basal segment relatively short (0.35-0.43 of VHL) and only about 2.2-3.1 times longer than wide, AR about 2.10-2.14; RO about 0.36 (0.27-0.45) up from base of segment 1; relative length of segments (μ m) 122 : 31 : 9 : 13 : 7; A3 shorter than A4, but longer than A5.

Mandible (Fig. f, below) about 249-326 μ m long, of type IIB or occasionally IIC, and with about 18.2 (11-24) furrows on outer surface near base; 12.5 (10-15) taeniae in PecM; Mdt-Mat about 25, MTR about 0.27.



Third instar larva: A single larva was available, about 8.7 mm long; dorsal and ventral AT equal size: $160 \mu m$ long and twice as long as wide. Gula slightly darkened over posterior 1/3 to 1/2; FC pale. Mentum width about 0.4 of VHL. Ventromental plates separated by about 0.4 of the mentum width; with 28-30 striae; VMR 0.31-0.38. Premandible with inner tooth about 2.5 times wider than the outer tooth. PE with 11 teeth.

Distance between antennal bases about 0.85 of that between the S4 setae. The relationships of A1 to the VHL about the same as in the 4th instar; RO further up from base of segment (0.41-0.53); relative length of segments (μ m) 75 : 24 : 8 : 13: 8; AR about 1.42.

Mandible about 175 µm long, of type IIB; with 22 furrows and 9 taeniae in the PecM.

South Island form:

Adult:

Male:

A rather poor reared male from Lake Hawea, Otago is available:

No information on wings, head, or thorax.

Leg lengths (micron) and proportions:

	Fe	Ti	Ta1	Ta2	Ta3	Ta4	Ta5	LR	F/T
PI	1422	1295	2055	-	-	-	-	1.59	1.1
PII	1575	1435	850	520	405	265	203	0.59	1.1
PIII	1830	1854	1320	857	560	305	229	0.71	0.99

No information on abdominal markings or setae on TIX.

SVo closest to the E(h) type of Strenzke (1959); IVo reaches to end of anal point, which is narrow at the base. Gonostylus moderately swollen and narrows gently over posterior half.

Pupa: Only a few measurements from two South Island specimens (one a damaged male, the other a female prepupa) are available. No total length, but inner margin of wing case 2.18 mm. CT about 89x84 μ m, seta not seen. The female prepupa had about 94 recurved hooks of segment II. The PSA of segment IV was about 66μ m wide. Spur of segment VIII with 4-6 appressed spines; abt. 89-130 taeniae in anal fringe.

Fourth instar larva: A salinarius-type larva. Length: Female 14.7 mm., male 10.7-12.8 mm. Anal tubules short and normally rounded, dorsal pair longer (120-190 μ m) than ventral pair (110-160 μ m) which appear to be narrower (l/w 2.0 c.f. 1.75).

Posterior half to two thirds of gula sl. dark to dark, wider than the mentum and widest part way up from posterior margin. FC also sl. dark to dark, one larva with 2 stripes outside the FC. SAL about 68.5-81 µm wide and 3-3.6 times wider than deep.

Mentum width about 0.67-0.68 of VHL; with 4th lateral reduced to about the level of the 5th laterals (type II), c1 tooth broad (type IV).

VM about 207-208 µm wide and 3.42-3.62 wider than deep; about 0.99-1.17 times the mentum width and separated by about 0.42-0.46 of its width; with about 44.3 (41-47) striae;

VMR about 0.32 (0.29-0.36. PE with about 12 teeth. Premandible of type B2 orB3, with outer tooth about 3.8-4.6x the width of the outer tooth and coming to moderate points. Distance between antennal bases (159.5 (144-175)) less than the distance between the S4 setae (190 (178-206)); S4 setae separated by about 0.9 of the FC width.

Antennae with basal segment relatively short (0.32-0.34 of VHL) and only about 2.3-2.5 times longer than wide, AR about 1.64-1.93; RO about 0.31 (0.22-0.38) up from base of segment 1; relative length of segments (μ m) 94.5 : 26.5 : 7 : 12 : 7 ; A3 shorter than A4, but longer than A5.

Mandible about 240-254 μ m long, of type II-IIIB, and with about 17 (16-18) furrows on outer surface near base; 11(10-12) taeniae in PecM; Mdt-Mat about 25, MTR about 0.3 (0.28-0.31).

Cytology: 4 polytene chromosomes, with pseudothummi group arm combination (BF, CD, EA, G).

Distinguished by two nucleoli in chromosome 1 (one proximal in B and 1 proximal in F). Arm A with a sequence differing from oppA4 by a simple inversion which is also found in *C. forsythi* and other species; arm E commonly differs from E1 of Australian species by a large inversion, although that E1 is still present. Arm F appears identical to that of *C. oppositus* F3 and *C. australis*F1. Arm G often partly unpaired.

Polymorphic in all arms except arm F.

casA1: 1a-e, 2e - 3e, 1f - 2c, 10 - 11, 2d, 8 - 9, 3f-i, 12c-a, 4 - 7, 13 - 19 i.e. as forA2

casA3: 1a-e, 12a-c, 3i-f, 9 – 8, 2d, 11 -10, 2c – 1f, 3 – 2e, 4 - 7, 13 – 19 from casA1 (Poutu & also Winton as camA3)

casB1: Nucleolus just distal to 4 characteristic bands (groups 24 – 26), and puff with proximal dark bands (groups 8-7) near distal end
i.e. by small inversion from forB2

casB2: Inversion of distal third of arm taking the puff and distal dark bands (groups 7-8) close to the telomere, from casB1 (South Island).

casC1: groups 4-3 about 1/3 from distal end, with groups 6-5 distal to them. as nzlC1

casD1: as forD1

casD2: as forD2

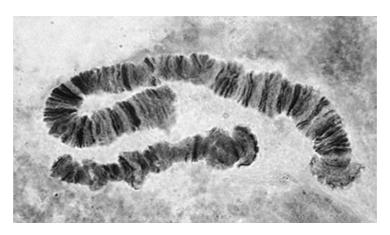
casD3: small proximal inversion from casD1, just proximal to breakpoint of D2.

casE1: 1 - 3e, 10b - 3f, 10c - 13 i.e. as oppE1, anlE1, forE1, nzlE1

casE2: 1a-e, 3f - 10b, 3e - 2a, 10c - 13 i.e. as forE2

casF1: 1 - 2a, 10 - 2c, 15c - 11a, 2b, 15d - 23 i.e. as oppF3, forF1, anlF1, nzlF1

casG1: with subterminal nucleolus. Differs from forG1 by an inversion around the median BR.



Arms A1E1 and G1 of C. sp. 'castaneum'

Localities:

North Island:

Lake Karapiro (-37.94°S, 175.56°E), South Auckland (Sofia Ibarrarán) (NZ.77.1) 27-viii-2007

Lake Okaro (-38.30°S, 176.40°E), South Auckland (Sofia Ibarrarán) (NZ.10.9) 14-ii-2007

Lake Okareka (-38.285°S, 176.603°E) Wellington District (Sofia Ibarrarán) (NZ.82.1) 29-viii-2007

Poutu Canal (-39.07°S, 175.75°E), Lake Rotoaira (D.J.Forsyth) (NZ.70.1) 8-x-1990 (Forsyth's type locality)

Lake Rotoaira (-39.056°S, 175.705°E), South Auckland (Sofia Ibarrarán) (NZ.70.2) 14-ii-2007 and 27-viii-2007

South Island

Haast junction, 3 km w Haast (-43.88°S, 169.05°E), Westland (Jon Martin) (NZ.67.1) 25-i-1978

Lake Te Anau, nr. Te Anau (-45.10°S, 167.50°E). Fiordland (Jon Martin) (NZ.46.2) 7-i-1974

Lake Hawea (-44.50°S, 169.17°E), Otago (Jon & C.J. Martin) (NZ.48.1) 7-i-1974 Winton (-46.10°S, 168.20°E), Southland (Jon Martin) (NZ.43.1) 5-i-1974

The evidence for the presence of more than one species comes from both the cytology and the mt*COI* sequence. At the type locality there is A1 and A2; B1 and B2; C1; mainly D2 with D1 and D3; E1 and E2; F1; and G1.

At Haast there is A2; B1 and B3; C2; D1; E1; F1; and G2.

MtCOI sequence: Sequence from the North Island differs at 24 bases of the BARCODE sequence from that of specimens from the South Island (see above)..

6b. Chironomus 'paracastaneum'

This species is recognized as close to C. 'castaneum' on the basis of the cytology and mtCOI sequences, and also close to C. sp.NZ9.

In BOLD Bin: BOLD:AAL7010

Along with *C*. 'castaneum' and *C*. sp. NZ9. (see under *C*. sp.NZ9 for further detail) Adult:

Two males, one a somewhat incomplete reared male, were available for study.

The adult male from Lake Hawea, Otago, South Island NZ.48.1 male 1 (7.I.1974) has been chosen as Holotype since there is also DNA barcode data for this specimen: Colouration not recorded before DNA extraction and slide mounting, but some could be inferred: legs pale, no indication tarsi darkened.

AR about 3.7; Wing length 5.33 mm, width 0.99 mm.; VR 0.95; LR 1.38; anterior tarsi without a beard, BR 1.8.

FT present 30-40 µm long and 1.7-2.5 time longer than wide.

Palpal proportions (micron): 98 : 77 : 300, 283 : 420; P5/P4 about 1.5, P5/P3 about 1.38. Clypeus 1.15 times wider than the antennal pedicel; 27 clypeal setae.

Thoracic setae: at least 10 acrostichal; 22-23 dorsocentral; 6 prealar; 1 supraalar; 25 scutellar (8 in anterior row, 17 in posterior row).

Leg lengths (micron) and proportions:

	Fe	Ti	Ta1	Ta2	Ta3	Ta4	Ta5	LR	F/T	Ta5/Ti
PI	1700	1680	2240	1320	1000	820	340	1.33	1.01	0.15
PII	1820	1810	1030	630	440	290	230	0.57	1.01	-
PIII	2120	2260	1540	940	680	420	250	0.68	0.94	-

Abdomen somewhat triangular patches or saddle spots on segments II-IV, then covering most of the tergites. TIX with 8 setae in a single pale area.



Hypopygium of the proposed type male.

Anal point narrow at base and widening a little at the distal end. SVo closest to the E(h) type of Strenzke (1959); IVo reaching about to the end of the anal point or a quarter to a third along the gonostylus, with simple setae. GS moderately swollen and narrows markedly from about the distal third to half; about 6+1, 7+1 setae at tip.

Additional data from the other male: AR about 3.7. Wings damaged LR about 1.33-1.52, anterior tarsi without a beard (BR about 1.7).

FT present, length about 69 μm ; for palp only P2 (50 μm) and P3 (241 μm) could be measured. about 22 clypeal setae.

Thoracic setae – acrostichals – at least 10; dorsocentrals - about 22; supra-alars 1; scutellar in two rows - 10 anterior, 17 posterior.

Leg lengths (micron) and proportions:

	Fe	Ti	Ta1	Ta2	Ta3	Ta4	Ta5	LR	F/T	BR
PI	1390	1315	2000	1140	810	633	304	1.52	1.06	1.67
PII	1545	1468	873	557	380	265	203	0.59	1.05	
PIII	1797	1835	1342	835	557	355	228	0.73	0.98	

Fore Ta5/Ti about 0.23. Sensilla chaetica difficult to see, perhaps 4 mTa1, 7 hTa1.

SVo closest to the E(h) type of Strenzke (1959); gonostylus narrows over posterior half.

Female: There is data for a female collected at the same time and from the same site as the proposed type male. It is included here although there is no proof that it belongs to this species (*C. forsythi* was also present at the location, but the leg ratios are more similar to those of the type male than to those of *C. forsythi* females).

Wing length: 5.37 mm; wing width: 1.14 mm; VR 1.09

AR: 0 35; flagella length (micron): 225: 130: 155: 140: 230, A5/A1 1.02.

Palpal segments (micron): -: -: 105: 275: 301; P5/P4 1.35; P5/P3 2.86. Clypeal width 1.8 times antennal pedicel; 37 setae.

Thoracic setae: About 16 acrostichal; 4-5 humeral; 25-29 dorsocentral (30-33 dl+humls); 8 prealars; scutellars in two approximate rows - anterior row about 15, posterior row about 19 (34 total) setae.

Leg lengths (micron) and proportions:

	Fe	Ti	Ta1	Ta2	Ta3	Ta4	Ta5	LR	F/T	Ta4/Ti
PI	1620	1560	2100	1080	780	680	340	1.35	1.04	0.45
PII	1620	1700	930	500	360	260	200	0.55	0.95	
PIII	1900	2140	1400	820	610	360	220	0.65	0.89	

BR about 1.46. Sensilla chaetica 14 mTa1, 16 hTa1.

Colour of abdominal tergites undetermined but apparently over most of the segment. GcIX with 3 setae; segment X appressed to cercus, crescent shaped, about 280 µm long and 5.9 times longer than its greatest width, with 16 setae. Cercus (below) with rounded outline; ventral margin longer.



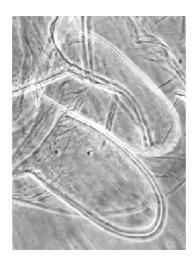
Cercus with closely applied segment X (at left)

Pupa: Length about 9.4 mm. Pedes spurii B developed on segment II, and about 55 hooks on posterior margin. Pedes spurii A well developed on segment IV. Caudolateral spur of segment VIII with about 3-6 appressed spines.

Anal lobe with a hair fringe of about 132 taeniae (on each side).

Fourth instar larva: A salinarius-type larva, length 12.7-17.2 mm (female); 12.3-14.8 mm (male).

A larva from Winton showed a small bump for the posterior VT (about 0.06mm). Anal tubules (below) short, about 200-400 μ m long and 2-3x (1.8-3.2) longer than wide.



Frontoclypeus and posterior half to two thirds of gula, dark to very dark, sometimes with some slight darkening at posterior of head capsule.

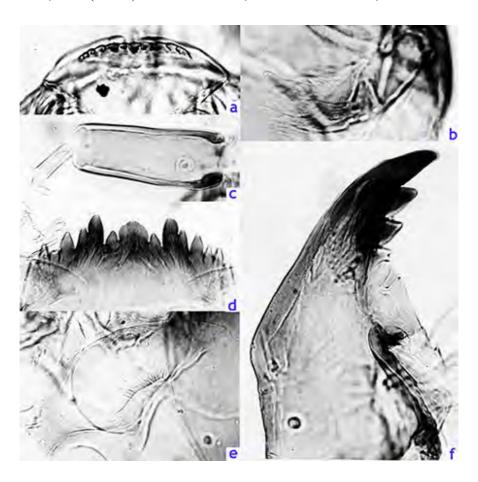
Mentum (Fig. c, below) of type II, c1 tooth of mentum broad (broad IIA), 6th laterals turned out.

Ventromentum (Fig. d, below) about 223.5 (190-248) μ m wide and 3.26-3.7 times wider than deep; with about 37.4 (35-40) striae, which reach almost to the anterior margin, particularly at the lateral edges; VMR 0.36 (0.30-0.39).

Pecten epipharyngis (Fig. a, below) with about 10-14 teeth narrowing towards the edges. Premandible (Fig. b, below) with broad teeth, inner tooth about 3-5x the width of the outer tooth and coming to a broad point.

Distance between antennal bases, $164.9 (157-184.5) \mu m$, less than the distance between the S4 setae, $181.3 (164-195) \mu m$.

Antennae (Fig. c, below) with basal segment about 40% of the VHL and 2.81 (2.61-3.21) times longer than wide, AR 1.84 (1.59 - 2.07); RO about one third (0.29-0.36) up from base of segment; proportions (μm) 125 : 35 ; 10 : 15 : 7 ; A3 shorter than A4, but longer than A5. Mandible (Fig. f, below) about 297.75 (245-328) μm long; generally type IIB, but sometimes 3rd inner tooth darker and more distinct (IIC), and with about 22.5 (18-26) furrows on outer surface near base; 12.4 (11-14) taeniae in PecM; Mdt-Mat 22.5-40.5, MTR 0.26-0.36.



Cytology: Four polytene chromosomes with the pseudothummi arm combination AE, BF, CD, G. There may be a smaller nucleolus in arm G, but otherwise basic sequences as in *C. castaneum*, with a nucleolus in both arms B and F. Polymorphic in all arms, some unique to this species. Arm G often partly unpaired.

pacA1: 1a-e, 2e - 3e, 1f - 2c, 10 - 11, 2d, 8 - 9, 3f-i, 12c-a, 4 - 7, 13 - 19 i.e. as casA1 pacA2: 1a-e, 2e - 3, 1f - 2c, 10 - 11, 2d, 8 - 9, 3f-i, 12c-a, 4 - 7, 13 - 19 i.e. as casA2 pacA3: 1a-e, 12a-c, 3i-f, 9 - 8, 2d, 11 -10, 2c - 1f, 3 - 2e, 4 - 7, 13 - 19 from camA1 (Winton, & also Poutu as casA3)

pacB1: Nucleolus just distal to 4 characteristic bands (groups 24 – 26), and puff with proximal dark bands (groups 8-7) near distal end i.e. as casB1

pacB2: Inversion of distal third of arm, as nzlB3, taking the puff and proximal dark bands (groups 7-8) close to the telomere i.e. as casB2.

pacB3: Inversion of region of the nucleolus, from camB1 (different breakpoints to the difference of camB1 to forB2).

pacC1: groups 4-3 about 1/3 from distal end, with groups 6-5 distal to it. i.e. as casC1

pacC2: simple inversion of distal third from camC1. (in NZ.67.1Em#1)

pacD1: as casD1

pacD2: as casD2

pacE1: 1 - 3e, 10b - 3f, 10c - 13 i.e. as oppE1, etc., casE1

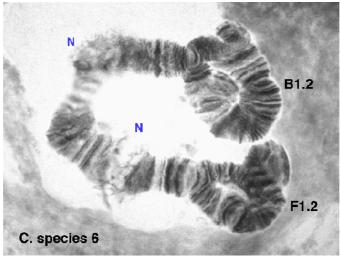
pacE2: 1a-e, 3f - 10b, 3e - 2a, 10c - 13 i.e. as casE2

pacF1: 1 - 2a, 10 - 2c, 15c - 11a, 2b, 15d - 23 i.e. as oppF3, forF1, casF1

pacF2: (approx.) 1-2a, 10-9c, 4-9b, 3-2c, 15c-11, 2b, 15d-23 as forF2

pacG1: with small subterminal nucleolus.

pacG2: from camG1 by a smaller inversion around the median BR than that differentiating pacG1 from forG1. (NZ.67.1 Em#1)



Chromosome BF of a larva from Winton, South Island, showing the two nucleoli.

Also heterozygous for an inversion in both arms.

North Island

Lake Karapiro (-37.94°S, 175.56°E), South Auckland (Sofia Ibarrarán) (NZ.77.1) 27-viii-2007

Lake Okaro (-38.30°S, 176.40°E), South Auckland (Sofia Ibarrarán) (NZ.10.9) 14-ii-2007

Lake Rotoaira (-39.056°S, 175.705°E), nr. Turangi, South Auckland (Sofia Ibarrarán) (NZ.70.2) 14-ii-2007

Poutu Canal (-39.07°S, 175.75°E), Lake Rotoaira (D.J. Forsyth) (NZ.70.1)

South Island:

abt.1 m in Lake Te Anau (-45.17°S, 167.50°E), Te Anau, Southland (Jon Martin) (NZ.46.2) 7-i-1974

Lake Hawea (-44.50°S, 169.17°E), Otago (Jon & C.J. Martin) (NZ.48.1) 7-i-1974 Owaka River (-46.42°S, 169.60°E), South Otago (T. Dodgshun & J.S. Pillai) (NZ.56.1) 14-iii-1974

Creek at Winton (-46.10°S, 168.20°E), Westland (Jon Martin) (NZ.43.1) 5-i-1974

Mt*COI*: There is sequence in BOLD.

The evidence for this species comes from the cytology and the MtCOI sequence. The species is apparently also close to *C. analis* and may hybridize with it, since at least one larva has been found with the *C.* 'paracastaneum' cytology, but the *C. analis COI* sequence.

7. Chironomus n.sp. NZ7 (C. 'tewaipounamu')

Adult: not known.

Pupa: not known.

Fourth instar larva: A bathophilus-type larva, although ventral tubules shorter than *C. novaezelandiae* (ant. abt. 0.48-0.88 mm; post abt. 0.52-1.0 mm). Medium size species, about size of *C. forsythi, C. analis*, and larger *C. novaezelandiae*, length about 14.2–19.2 mm. Head capsule relatively broad, width of mentum at least 2/3 of VHL; generally with gular region dark, a darkened FC and sometimes in surrounding regions.

Mentum with 4th laterals slightly reduced, sometimes higher than 5th laterals (i.e. between type I & II), c1 tooth relatively broad, c2 teeth usually separated, but occasionally still partially on shoulders of c1 (i.e. usually type IIA, but sometimes type III).

VM with about 34-46 striae; VMR about 0.32-0.47. Distance between VMs about 0.31-0.46 of mentum width.. PE with 11-17 teeth, some of which are reduced in size.

Antennae with basal segment at least 3.5 to 4.4 times as long as wide; AR about 1.7-2.3; A2 about 0.28 length of A1; ratio of segments (micron): 168: 40: 11: 16: 8.

Distance between antennal bases generally smaller than distance between S4 setae, but may be about equal or even greater (1 of 6 larvae).

Mandible of type I-IIB, with about 23-27 furrows on outer surface near the base; 12–14 taeniae in PMa.

Cytology: 4 polytene chromosomes with unique chromosome arm combination BG, CD, EA, F. One large nucleolus proximal on arm G. Arm E as E1 of Australian species; arm F as *oppositus* F3, *australis*, etc.

Arm A1: (approx) 1a-e, 11 - 10, 2c - 1f, 6 - 4,12a-c, 8 - 9, 2d - 3,17 - 13, 7d-a, 18 - 19

Arm B1: Puff (group 7) with proximal dark bands (group 8) near middle of arm.

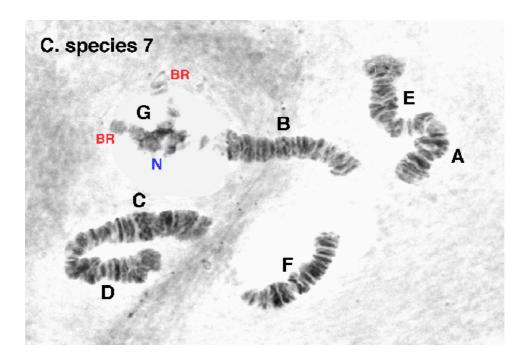
Arm C1:

Arm D1:

Arm E1: 1 - 3e, 10b - 3f, 10c - 13 i.e. as E1 oppositus, analis, forsythi, novaezelandiae, 'thermarum'

Arm F1: 1 - 2a, 10 - 2c, 15c - 11a, 2b, 15d - 23 i.e. as novaezelandiae F1, analis, forsythi, oppositus, F3

Arm G1: Nucleolus near attachment to arm B, with BR towards the distal end.



Localities:

South Island:

10m+ in Lake Pearson (-43.10°S, 171.47°E), near Cass, Canterbury (B.V. Timms) (NZ.59.1, 2, & 3) 1978/79.

Lake Lochie (-44.83°S, 168.12°E), c.87 km n.w. Te Anau, Fiordland (Jon & H.I. Martin) (NZ.45.1) 6-i-1974

The unique chromosome arm combination was noted by Wülker & Morath (1989)

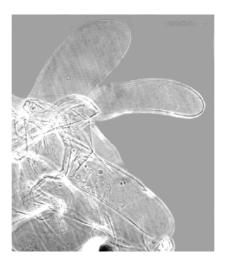
8. Chironomus n.sp. NZ8

(check Maori name for South Island)

Adult: not known.

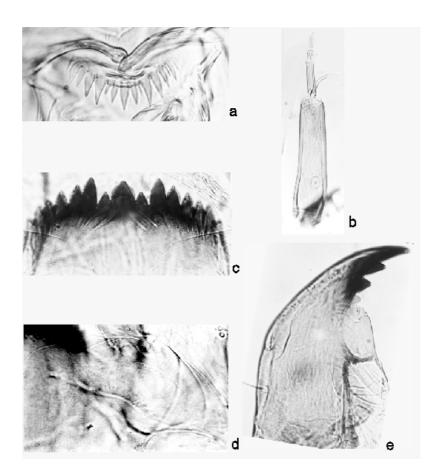
Pupa: not known.

Fourth instar larva: bathophilus-type larva. Length about 13-18.7 mm. Head capsule with pale or very slightly darkened FA, pale or some darkening in posterior region of gula. VT about equal, or anterior pair longer (Ant. 1.0-1.48 mm, post 0.96-1.42mm). Anal tubules relatively long and rounded, about 2.5x as long as wide.



Head relatively narrow, mentum width only about half the VHL.

Mentum (c, below) with 4th lateral only slightly reduced (i.e. between type I and II); centre trifid tooth with a relatively broad, squared-off c1 and distinct c2 teeth (i.e. Type IIA). VM (d, below) with about 43 striae on only specimen counted. PE (a, below) with about 14-17 rather uneven teeth. Basal segment of antenna (b, below) relatively long and narrow, about 4 times as long as wide; 4th segment relatively short, only about 20% longer than segment 3. Mandible (e, below) with 3rd inner tooth darkened and almost completely separated (i.e. type IIB); about 18-20 furrows on outer surface near the base.



Cytology: 3 polytene chromosomes, modified pseudothummi arm combination (BF, CD, GEA). Arm G is attached to arm E by a loose nucleolar connection, but the reality of this connection is confirmed by the presence of only six chromosomes in mitotic metaphases. The nucleolus in arm F is either reduced or absent. Arm A has the sequence, derived from A4 by a simple inversion, found in *C. novaezelandiae*. Arm E appears identical to *oppositus* E1, while arm F differs from F1 of *C. zealandicus* by an inversion of about the region from 11-9e. One poor specimen appears to be heterozygous in one chromosome arm, but the arm could not be identified.

Arm A1: 1a-e, 11 - 10, 2c - 1f, 3e - 2e, 7 - 4, 12a-c, 3i-f, 9 - 8, 2d, 13 - 19 i.e. as A2 of novaezelandiae

Arm B1: Puff (group 7) with proximal dark bands (group 8) near middle of arm.

Arm C1:

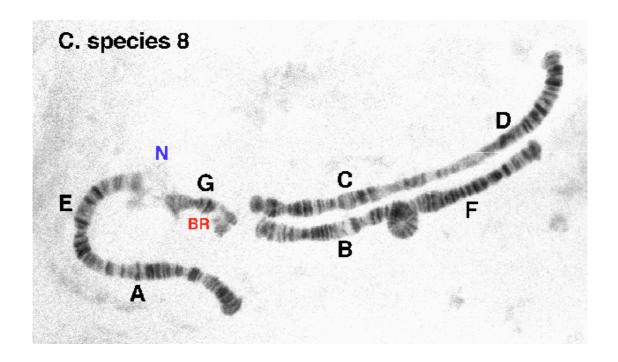
Arm D1:

Arm E1: 1 - 3e, 10b - 3f, 10c - 13 i.e. as *oppositus* E1 (appears no more than 1 band

missing)

Arm F1 1 - 2a, 10 - 9f, 11 - 9e, 12 - 15c, 2c - 7, 2b, 15d - 23

Arm G1: attached to arm E.



Localities:

South Island:

13 km north of Haast, Westland (Jon Martin) (NZ.49.1) 8-i-1974. Lake Pearson (-43.10°S, 171.78°E), nr. Cass, Canterbury South (B.V. Timms) (NZ.59.1) 12-ix-1978.

9. *Chironomus* n.sp. NZ9 (*C.* 'rotorua')

In BOLD Bin: BOLD: AAL7010

The nearest neighbor BIN was listed as BOLD: AAL7011.

The *COI* sequence suggests a relationship to the *C. castaneum*-group, which are in the same BOLD Bin, and there are some common sequences in the cytology.

Adult: Male not known.

Female: (from a specimen collected by I. Hogg, in BOLD database):



Wing length about 4.3 mm. Colour brown with darker brown postnotum and dark bands across basal part of abdominal segments at least II-VI. Legs apparently relatively pale with no darkening at knees. Mid femur abt. 1480 μm ; mid tibia about 1240 μm (F/T abt 1.19); hind femur abt. 1920 μm , tibia at least 1900 μm (F/T about 1).

Pupa: Not known

Fourth instar larva: salinarius type larva, length 15.5-15.8 mm (female), 12.5-15.7 mm (male). Head capsule with gular region darkened on posterior third, frontoclypeus very slightly to slightly darkened, but with some darkening of the posterior border of the frontoclypeus. Anal tubules about $300\text{-}360~\mu\text{m}$ long, without a constriction, and about 1.5-2.3 times longer than wide, dorsal and ventral pairs generally similar in size. SAL $83\text{-}124~\mu\text{m}$ wide and 2.9-4.9 times wider than deep.

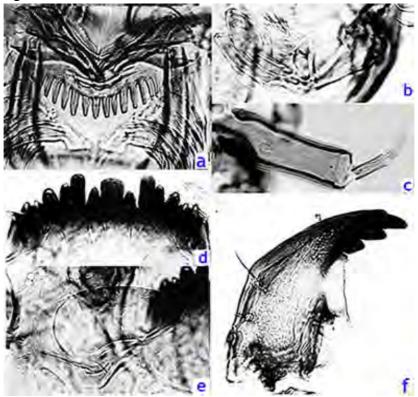
Mentum (Fig. d, below) with c1 tooth characteristically broad and flat on top, c2 teeth relatively well separated (type broad IIA); 4th lateral reduced to about the level of the 5th lateral (type II), 6th lateral arising at a slightly lower level than other teeth; width 0.71-0.77 of VHL (i.e. head relatively wide).

VM (Fig. e, below) about 3.6 times wider than deep; with about 36-40 striae; VMR about 0.35-0.38. PE (Fig. a, below) with about 14-15 relatively even teeth (Ty. B). Premandible (Fig. b, below) of type D, with robust teeth, inner tooth about 4-5 times wider than the outer tooth, which narrows to a fine point when not worn.

Antenna (Fig. c, below) with basal segment about 0.4 of the VHL; about 2.81 (2.6-3.2) x longer than wide, RO about a third (0.29-0.36) up from base; AR 1.84 (1.59-2.07); antennal proportions (micron) 125 : 35 : 10 : 15 : 7.

Distance between the antennal bases (199.75 μ m) generally similar to that between the S4 setae (204.88 μ m) but quite variable; S4 setae separated by 87-95% of the FC width, i.e. often very close to the edge of the FC. S5 setae about level, or anterior to nearby RO. Mandible (Fig. f, below) about 300 (245-328) μ m in length; 3rd inner tooth only partly separated and coloured (type IIB), with about 22.5 (18-26) furrows on outer surface near the

base; PecM of 12.4 (11-14) taeniae; Mdt-Mat 22.5-40.5 μ m, MTR about 0.26-0.36, dependant on degree of tooth wear.



Gut of most larvae almost filled with sand particles.

Cytology: 4 polytene chromosomes with thummi cytocomplex arm combination (AB, CD, EF, G). Centromeres heterochromatic. Two nucleoli, one proximal in arm F; the other about one third from the heterochromatic end of arm G. A Balbiani ring developed near distal end of arm G. Arm A with the sequence A4 found in Australian species; arm E with sequence E1 of the Australian species; and arm F as in *oppositus* F3 and *australis*. No polymorphism in small sample examined.

Arm A1: 1a-e, 11-10, 2c-1f, 3e-2d, 8-9, 3f-i, 12c-a, 4-7, 13-19 i.e. as A4 oppositus

Arm B1: Puff (group 7) and about 2 distal dark bands (8ab), close to 4 characteristic bands.

Arm C1: possibly 10A-10D6, 11B11-10D7, 11B12-11E i.e. as C2 of *forsythi*

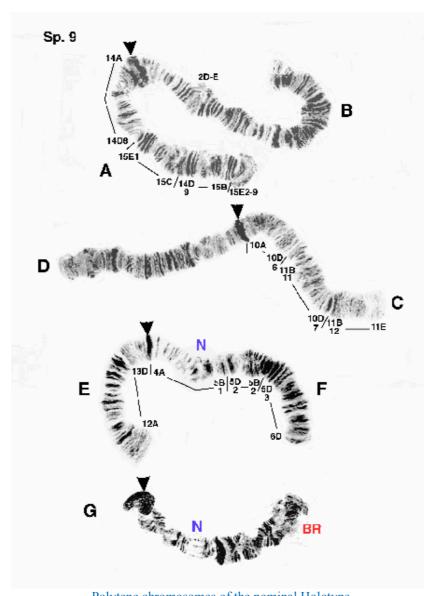
Arm D1: 1 - 2, 16c-a, 17e-a, 10c-a, 3e - 9, 3d-a, 10d - 15, 18 - 24 i.e. as for D1, nzlD2

Arm E1: 1 - 3e, 10b - 3f, 10c - 13 i.e. as oppositus E1, analis, 'castaneum'-gp

Arm F1: 1 - 2a, 10 - 2c, 15c - 11a, 2b, 15d - 23 i.e. as oppositus F3, australis,

analis, forsythi, 'castaneum'-gp., etc.

Arm G1: heterochromatic terminal centromere, small nucleolus (or BR?) in middle and BR near the distal end



Polytene chromosomes of the nominal Holotype Note the heterochromatic centromeres and the median nucleolus (N) in arm G

Note the heterochromatic centromeres and the median nucleolus (N) in arm G Mapping is according to the old Australian standard

Localities:

North Island

Lake Karapiro (-37.94°S, 175.56°E) South Auckland (S. Ibarraran) 15-ii-2007. (NZ.77.1)

Lake Okaro (-38.30°S, 176.40°E), Rotorua area (D.J. Forsyth) (NZ.10.8) 14-ix-1982 and (S. Ibarrarán) NZ.10.9 14-ii-2007)

Lake Okareka (-38.285°S, 176.603°E), Rotorua area (S. Ibarrarán) (NZ.82.1) 15-ii-2007.

nr. Te Awamutu, Waikato area (-37.802°S, 175.334°E) (Ian Hogg) 29-iv-2013

South Island:

Lake Hayes (-44.98°S, 168.80°E), c.13km n.e. Queenstown, Westland (Jon Martin) (NZ.47.1) 7-i-1974

MtCOI sequence suggests that there may be hybridization with C. 'castaneum' particularly where they occur together in the same lake, although this is unlikely due to the whole arm translocation difference. On the other hand, there is a possibility that this is a recent whole-arm translocation event from a related pseudothummi-complex species (probably C. 'castaneum', sp. 6a, on the basis of current information – see below), with no connection to species of the thummi-cytocomplex. As such, this species would be immediately reproductively isolated from its pseudothummi-cytocomplex parent. The limited mitochondrial DNA difference would then suggest that this event occurred relatively recently.

There are differences at 14 base positions between the three members of the 'castaneum-group':

Base

C. 'rotorua' (spNZ9) C/G G GC Т G G Α Т C. 'castaneum' (spNZ6a) T/C A/T/G G/A G T/C T/A G/A GC A/G A/G T/A A/G T/G Α C. 'paracastaneum' (spNZ6b) C C G Α AA G G Α Α Α Α Α G G

The similarity of many of the polymorphic bases of *C*. 'castaneum' with the equivalent base of spNZ9 suggests that the former is the ancestral species in which the translocation occurred. On the other hand, the similarity of the polymorphic bases to those of *C*. 'paracastaneum' could be the result of hybridization between these two closely related species.

10. Chironomus n. sp. NZ10

Some specimens originally considered this species are probably variants of *C*. 'thermarum' but others show differences in cytology and possibly BARCODE sequence.

Currently in Bold Bin AAJ0168with C. 'thermarum'; and C. novaezelandiae, but see comments below.

Adult:

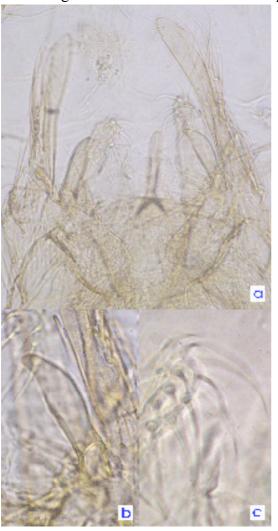
The adult of this species is amongst specimens that D.J. Forsyth called C. 'spilleri' (specimens now in the care of Ian Boothroyd?). These males were similar to those of C.

species NZ12 in having bands across the anterior two thirds of abdominal segments II to VI and on four fifths of segments VII and VIII.

A pupa with a pharate male (see right) is available, from which the following characters could be determined (Note this is same specimen as included in *C*. 'thermarum' (above):

(Note: These details may refer to Species 10)

Tergite IX with 5 setae in individual pale areas. Anal point narrow at base, wider at tip.



SVo essentially E(h)-type of Strenzke (1959); IVo reaching about to the end of the anal point with simple setae. Gonostylus GS quite swollen, reducing significantly on distal third.

SVo (b) with just a small hook at the tip (D(e)-type of Strenzke 1959); IVo (a, above) extending to about middle of gonostylus with setae simple (c). Gonostylus (a) moderately broad but narrowing over distal third, about 4+1 setae at tip.

Pupa: about 6.9-10.2 mm (2 males) - 8.3 mm (1 female) long; IMW about 1.44-2.34 mm.
Cephalic tubercle about 85-150 x 80 μm long.
Basal ring about 115-170 μm long and 57-65 μm wide, HR 1.77-2.56. Thorax and muscle scars yellow-brown, otherwise pale.
Fine shagreen on posterior half of segment II, increasing to whole of segments IV-VI, little on segments VII & VIII. Hook row with 70-92 hooks, occupying about 0.6-0.8 of segment width. PSB on segs. II and III; PSA of segment

IV large (202-218 μ m long, 101-145 μ m wide) and about 0.21-0.28 length of segment; PSA of

segment V of spines. Caudolateral spur of segment VIII with about 2 or 3 spines, at least 1 small. 68-93 taeniae on each side of anal lobe, mostly in a single row, but some double at posterior end.

Fourth instar larva: A bathophilus-type larva, with both short anterior and posterior VT (over 0.32 mm in length) to halophilus with only poster pair (up to about 0.12 mm) to salinarius-type (The latter two forms are likely to be *C*. 'thermarum'). Generally smaller due to growing at higher temperature (but larger when reared in the lab at lower temperatures), length about 10-14 mm (fem. 10.3-14.0; male 10.7-11.2 mm). Where present, Ventral tubules approximately equal in length, anterior 0.63 (0.44-0.96) mm and posterior 0.64 (0.32-1.01)

mm. Anal tubules about equal in size or ventral pair longer and 1.5 to twice as long as wide (abt. 200-265 x 100-165 μ m).

Head capsule usually with pale or slightly darkened frontoclypeus but may be dark; some darkening in posterior region of gula, much as on frontoclypeus, and can be broader than the mentum width.

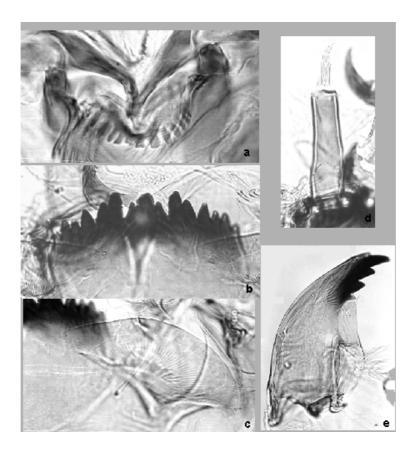
Head relatively narrow, mentum width about 0.54-0.60 of ventral head length.

Mentum (Fig. b) with 4th lateral reduced to about level of 5th laterals (type II-III); centre trifid tooth usually with distinct, small c2 teeth (i.e. Type IB or occasionally III).

Ventromentum (Fig. c) about 182-205 μ m wide and 3.04-3.86 times wider than deep, and 1.0-1.1 time wider than the mentum, with about 37.9 (33-41) striae; IPD 0.28-0.35; VMR 0.24-0.35. Pecten epipharyngis (Fig. a) with about 11-16 sometimes uneven teeth (type B). Basal segment of antenna (Fig. d) about 3-4 times longer than wide, RO about 29 (24-38)% up from base; A4 relatively short, only about 20% longer than segment 3; AR about 2.23 (2.00-2.44); proportions (micron) 117: 27: 7: 10: 7. i.e. A5 about as long as A3 (0.73-1.08 times as long).

Premandible with the usual 2 teeth, inner tooth about 2+-5 times the width of the outer tooth, both coming to a broad, or occasionally a narrow, point.

Mandible (Fig. e) about 220-252 μ m long (heel to Mdt) with 3rd inner tooth at least partly darkened and somewhat separated (i.e. type IIB, to occasionally type IIIC); at least 10-17 furrows on outer surface near the base; 11-13 taeniae in Pecten mandibularis; Mdt-Mat 22-30 μ m; MTR 0.30-0.43.



Cytology: 4 polytene chromosomes with the pseudothummi cytocomplex arm combination (AE, BF, CD, G).

Arm G without a nucleolus, but with a large subterminal BR, and another may be developed about the middle of the arm. The nucleolus is in arm F.

Arm A has the sequence identical to A4 of C. oppositus and A1 of C. 'thermarum'.

Arms B, C, D, E, F and G appear identical to B1, C1, E1, F1 and G1 of *C*. 'thermarum' while D appears similar to that of *C. novaezelandiae*. No polymorphism known.

Arm A1: 1a-e, 11 - 10, 2c - 1f, 3e - 2d, 8 - 9, 3f-i, 12c-a, 4 - 7, 13 - 19; i.e. as A4 *oppositus* and A1 of 'thermarum'.

Arm B1: Derived from nzlB1 by a long inversion of the distal region. Puff with proximal dark bands (groups 7-8) towards distal end.

i.e. as 'thermarum';

Arm C1: Appears similar to C1 of *tepperi*, 'pseudoppositus', nzlC1. i.e. as 'thermarum'

Arm D1: 1 -2, 16 - 11, 4c - 9, 3d-a, 4ba - 3e, 10a-c, 17 - 24 as D3 of novaezelandiae

Arm E1: 1 - 3e, 10b - 3f, 10c - 13 i.e. as E1 *oppositus*, 'thermarum', etc.

Arm F1: 1 - 2a, 10 - 2c, 15c - 11a, 2b, 15d - 23 i.e. as F3 *oppositus*, 'thermarum', etc. & F1 of ';thermarum';

Arm G1: subterminal BR as 'thermarum'?

Localities:

North Island:

Lake Ngahewa (38.31°S, 176.37°E), South Auckland (S. Ibarraran) (NZ.76.1) 28-viii-2007.

Lake Rotowhero (38.30°S, 176.40°E), c.24 Km s. Rotorua, South Auckland (NZ.11.1-5) (D.J. Forsyth & Jon Martin) 1970-1973; (S. Ibarraran) (NZ.11.6) abt 15-ii-2007 (these specimens are likely to be *C*. 'thermarum' as this lake is thermal) ?Queen Elizabeth Park (37.94°S,175.56°E), South Auckland (S. Ibarraran) (NZ.80.1) abt 15-ii-2007.

Initially included with *C. novaezelandiae*, but shows consistent differences in the lighter larval head colour and some unique banding patterns. It is very similar in cytology to *C*. 'thermarum', differing mainly in the better development of the ventral tubules. Phylogenetic analysis of the mitochondrial *COI* sequence places it in the *C. novaezelandiae* complex (see under *C. novaezelandiae*) along with *C.* 'thermarum'.

11. Chironomus 'spilleri' n.sp. (ms name from D.J. Forsyth)

In BOLD Bin: BOLD AAL7009

Adult:

The adult male of this species is not known for certain because no rearings associated with this larval type are available (such specimens may have been in the collection of D.J. Forsyth, subsequently with Ian Boothroyd?).

Some details of the adult female can be determined from a nice clear photograph by Grant Broomfield (below) in the BOLD Database:



Adult female of *C.* 'spilleri', from BOLD database NZINS0065+1349147079

Dark brown, halteres pale, abdomen apparently yellow brown dorsally with a dark anterior band on segments II-IV and then a dark saddle spot on segments V-VIII. Palps longer than the antennae but details not able to be determined. Legs with femur and tibia yellow-brown, tarsal segments dark brown; anterior Ta4 not longer than the preceding segments. Cercus apparently with a rounded outline.

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	Fe	Ti	Ta1	Ta2	Ta3	Ta4	Ta5	LR	F/T	Ta4/Ti
PI	58	54	70	36	26	24	10	1.30	1.07	0.44
PII	52	54	32	20	16	10	6	0.47	0.96	
PIII	84	76	36	28	22	14	8	0.65	1.10	

Fourth instar larva: A bathophilus-type larva. Length about 13-15 mm. VT from 0.6-1.5 mm, anterior pair usually longer. Anal tubules: dorsal pair about three to four times as long as wide, ventral pair often longer with a constriction and about three times as long as wide. Head capsule with pale FA, slightly dark or dark on posterior region of gula.

Mentum with 4th lateral reduced to about level of 5th laterals (type II); centre trifid tooth with distinct c2 teeth (i.e. type II or worn type III).

VM separated by about 0.29-0.37 of mentum width, with about 43-50 striae. PE with about 10-20 rather uneven teeth.

Basal segment of antenna about 2.7-4.2 times as long as wide; 4th segment relatively short, generally no more than 30% longer than segment 3, which is longer than segment 5; segment proportions (μ m) 128 : 32 : 10 : 13: 7. Premandible with inner tooth about 2.2-2.7 times as wide as outer tooth, outer tooth usually shorter.

Mandible about 292 (230-327) µm long, with 3rd inner tooth darkened but only slight separation (i.e. type IIB-C); about 18 (14-26) furrows on outer surface near the base; about 12(10-14) PecM of 12 (10-14) taeniae.

Cytology: 4 polytene chromosomes with the pseudothummi arm combination (AE, BF, CD, G). Arm G with a subterminal nucleolus and nearby BR. No nucleolus in long chromosomes. Unique sequence in arm B, with puff or BR near the centromere. Polymorphism in arm C.

spiA1: 1a-e, 11 - 10, 2c - 1f, 3e - 2d, 8 - 9, 3f-i, 12c-a, 4 - 7, 13 - 19 i.e. as A4 oppositus and A1 of novaezelandiae, analis, forsythi

spiB1: Bulb (group7) close to centromere, dark bands further to distal end.

spiC1: as nzlC1

spiC2: inversion of about the extent of nzlC3

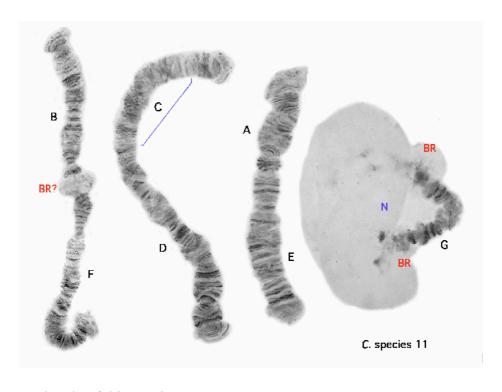
spiD1: 1 - 2, 16 - 10d, 3a-d, 9 - 3e, 10a-c, 17 - 24 i.e. as nzlD1

spiE1: 1 - 3e, 11 - 10c, 3f - 10b, 12 - 13 simple inversion from nzlE1

spiF1: 1 - 2a, 10 - 2c, 15c - 11a, 2b, 15d - 23 i.e. as oppositus F3, and F1 of

novaezelandiae, analis, forsythi, but with no nucleolus

spiG1: Virtually terminal nucleolus with nearby BR.



Possible reared male of this species:

Adult: Colour not determined as specimen very bleached.

Wing length: 5.0 mm, width 0.96, VR 1.02. Crossvein slightly darkened.

Molecular:

Mt *COI* – a number of sequences are in the BOLD database, where they are placed in the BIN AAL7009 or AAL7010.

Localities:

North Island:

Cambridge, South Auckland (-37.89°S, 175.44°E) (Sofia Ibarrarán) (NZ.74.1) 29-viii-2007.

Lake Ngahewa, South Auckland (-38.31°S, 176.37°E) (Sofia Ibarrarán) (NZ.76.1) 27 August 2007

Lake Rotoiti (-37.28°S, 174.67°E), about 16 Km north east of Rotorua, South Auckland (-37.28°S, 174.67°E) (Jon Martin & D.J. Forsyth) (NZ.19.1) 7-xii-1973. Lake Tikitapu, South Auckland (-37.94°S, 175.56°E) (Sofia Ibarrarán) (NZ.81.1) Feb. 2007.

Waikato, South Auckland (-37.809°S, 175.137°E) (Ian Hogg) 29-iv-2013, and (BOLD NZIN5065-12) (Grant Broomfield) 26-ix-2012.

Initially included with *C. novaezelandiae*, but shows consistent differences in the lighter larval dorsal head colour, some unique chromosomal banding patterns, and the lack of a nucleolus in arm F. The existence of this species was first indicated by the barcoding analyses of Sofia Ibarrarán, and the BIN analyses of the BOLD database place it in a separate BIN.

12. *Chironomus* n.sp. NZ12 (*C.* 'ibarraranae')

In BOLD Bin: BOLD ABZ5458

This Bin also contains some specimens of *C. novaezelandiae* (Ty. 2), to which this species is closely related.

Adult and Pupa of this species are not known for certain. Therefore a larva with polytene chromosomes is nominated as the potential holotype of *C*. 'ibarraranae': Lake Rotoiti NZ.19.1, Sl. 12M. The reared male with associated pupal exuvia, Lake Ngapouri NZ.9.3 reared M1 is nominated as a paratype.

Three probable adult males (NZ.9.3 rear M1 with associated pupal exuvia: & NZ.17.1 reared M2 & M3).

Wing length: 4.06-4.16 mm, width 0.88-1.01 mm; VR 0.93-1.02.

AR about 2.86-3.35.

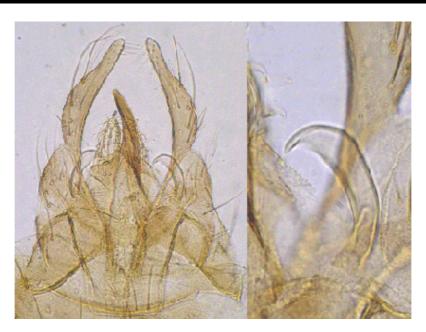
Small frontal tubercles, about 21.8 (13-30) micron and 1.4-2.75 longer than wide; about 33.7 (29-38) clypeal setae.

Relative length of palpal segments (micron): 68 : 70 : 251 : 249 : 346. P5/P4 1.39-1.4; P5/P3 1.4-1.45.

Thoracic setae: Acrostichal - 13-15; Dorsocentral - 19-21; Supra-alar - 1; Prealar - 6; Scutellar in two rows, anterior – abt 7-14, posterior – 11-16 (total 23-25).

Anterior tibiae and tarsus with sparse beard, BR about 3.8-5.15. Leg proportions (micron): Leg lengths (micron) and proportions:

	Fe	Ti	Ta1	Ta2	Ta3	Ta4	Ta5	LR	F/T	Ta5/Ti
PI	1433	1305	1823	1062	787	628	302	1.35-1.38	1.06-1.12	0.22
PII	1530	1455	850	577	370	260	190	0.56-0.61	1.03-1.08	
PIII	1820	1870	1200	770	540	340	210	0.62-0.70	0.97-0.99	



Abdomen with seg II darkened over anterior half and progressively further posterior on III-V, then whole tergite darkened. 5-10 setae in individual pale areas on TIX. SVo closest to E(h) of Strenzke 1959; IVo reaching about to tip of relatively narrow anal point, but to about basal third of gonostyle, with simple setae. Anal point is quite narrow at base; gonostylus moderately swollen (width/length 0.19-0.24) and narrows over distal third to half.

Possible pupa: Exuvial length about 8.8 mm; length of inner margin of wing case about 1.8 mm. Indications of small frontal warts. Cephalic tubercles (below) abt 86 x132 micron (l/w 0.65).

About 95 recurved hooks on segment II. L-seta on margin of III-IV at least 101 μ m. Pedes spurii A of seg. IV about 197x104 μ m and about 0.27 of segment length; those of segments V and VI not visible. Caudolateral spur (below) of segment VIII with 6 & 7 appressed spines. About 96 taeniae in incomplete double row, then a double row at posterior end of the hair fringe on each side of the swim fin. Shagreen quite heavy post 1/2 II, 3/4 III then all IV-VI, weak post VI and 2 post patches around seta on VIII; Muscle scars on tergites faint.



Fourth instar larva: bathophilus-type larva. Length about 17.2-18 mm (female) and 13.8-16.0 (male); smaller larvae from thermal habitats. Anterior VT about 0.96 (0.61-1.60) mm; posterior about 0.93 (0.25-1.46) mm. Dorsal AT possibly longer (205-367 μ m) than the ventral pair (200-321 μ m), both about 1.6-2.2 times longer than wide.

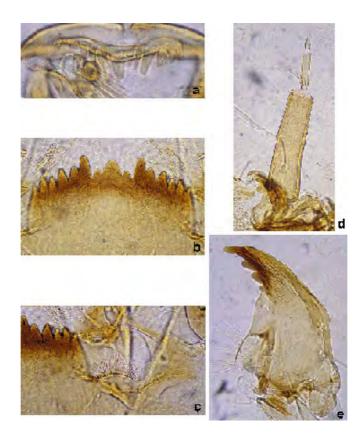
Head capsule with pale or slightly dark posterior third to half of gula; frontoclypeus usually pale but may be slightly darkened.

Mentum (Fig. b, below) with 4th laterals reduced, generally to height of 5th laterals (type I-II); central tooth with c2 teeth relatively well separated (type III, or IIA if worn).

VMs (Fig. c, below) about 191 (182-199) μ m wide and 3.5 (3.3-3.65) times wider than deep; 1.05 (0.92-1.17) times the mentum width; IPD about 83.5 (51-101) μ m (0.4 of MW), with about 41 (36-45) striae; VMR 0.31 (0.26-0.41). PE (Fig. a, below) with about 12.7 (11-15) irregular teeth. Premandible with inner tooth about 4.25-5 times the width of the outer tooth, outer tooth narrowing to a fine point (usually type D, but may be ty. C).

Antenna (Fig. d, below) with A1 about 0.38 (0.31-0.49) of VHL, and 4.0 (3.3-4.8) times longer than wide; AR about 2.44 (2.33-2.70); RO about one third to almost halfway up from base of segment 1; ratio of antennal segments (μ m): 160 : 35 : 9 : 13 : 7.5.

Mandible (Fig. e, below) about 292 (230—327) µm long, with 3rd inner tooth partly darkened and only slightly separated (type IIB); about 18 (14-26) furrows on outer surface near the base; 12 (10-14) striae in the PecM; Mdt-Mat 23-38, MTR 0.31-0.32.



Third instar larva: A single larva was available, anterior VT 1.25 mm long. Gula and FC pale. Mentum width about 0.6 of VHL.

Ventromental plates by about 0.4 of the mentum width; 132 μ m wide; 3.1 times wider than deep, and about the same width as the mentum, with 25-27 striae; VMR 0.35-0.36. Premandible with inner tooth about 2.5 times wider than the outer tooth. PE with 12 teeth. Distance between antennal bases 100 μ m, about 0.95 of that between the S4 setae. The relationships of A1 to the VHL and the distance of the RO from base about the same as in the 4th instar; AR about 1.5, relative length of segments (μ m) 83.5 : 32 : 9.5 : 12.5: 6. Mandible about 190 μ m long, of type IIB; with 13 furrows and 10-11 taeniae in the PecM; Mdt-Mat 28 μ m, MTR 0.42.

Cytology: 4 polytene chromosomes with the pseudothummi arm combination (AE, BF, CD, G).

Arm G without a nucleolus, but with an obvious subterminal BR. Nucleolus in arm F near the centromere, at about group 19. Polymorphism in arm B, with B2 apparently the more common sequence.

Arm A1: 1a-e, 11 - 10, 2c - 1f, 3e - 2e, 7 - 4, 12a-c, 3i-f, 9 - 8, 2d, 13 - 19 as nzlA2

Arm B1: Puff with reduced dark bands nearer distal end of arm as nzlB2

Arm B2: Distal inversion including the puff (group 7) that returns it to near the middle

of the arm.) as nzlB3

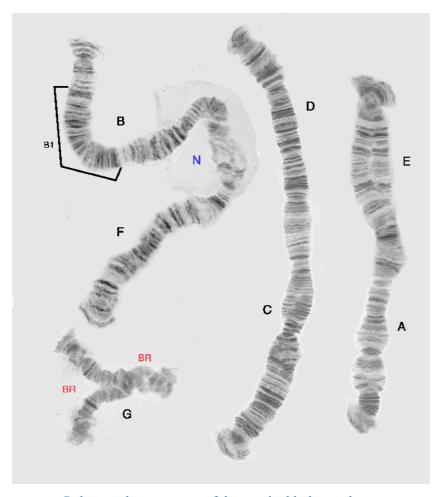
Arm C1: as nzlC3

 Arm D1:
 1 - 2, 16c-a, 17e-a, 10c-a, 3e - 9, 3d-a, 10d - 15, 18 - 24
 as nzlD2

 Arm E1:
 1a-c, 5 - 7c, 10g-c, 3f - 4, 1d - 3e, 10b - 7d, 11 - 13
 as nzlE3

 Arm F1:
 1a-e, 12 - 15c, 2c - 10, 2a, 11i-a, 2b, 15d - 23
 as nzlF2

 Arm G1:
 No nucleolus, but an obvious subterminal BR
 as nzlG2?



Polytene chromosomes of the nominal holotype larva

Molecular:

Mt *COI* – a number of sequences are in the BOLD database, where they are placed in the BIN BOLD: ABZ5458. Phylogenetic analysis of the mitochondrial *CO1* sequence places it in the *C. novaezelandiae* complex (see under *C. novaezelandiae*).

Localities:

North Island:

Lake Ngapouri (-38.00°S, 176.50°E), Waiotapu, South Auckland (D.J. Forsyth) (NZ.9.1) 15-ii-1972; (D.J. Forsyth & Jon Martin) (NZ.9.6) 5-xii-1973; and (S. Ibarrarán) (NZ.9.7) 29-viii-2007.

Lake Rotoiti (-37.28°S, 174.67°E), about 16 Km north east of Rotorua, South Auckland (Jon Martin, D.J. Forsyth) (NZ.19.1) 7-xii-1973; and (S. Ibarrarán) (NZ.19.3) 29-viii-2007.

Lake Rotowhero (-38.33°S, 176.40°E), about 24 Km south of Rotorua, South Auckland (S. Ibarrarán) (NZ.11.6) 5-ii-2007.

?Ngapuna (-38.15°S, 176.27°E), Rotorua, South Auckland (Jon Martin, D.J. Forsyth) (NZ.17.1) 7-xii-1973.

Kinloch Marina, Lake Taupo, South Auckland (D.J. Forsyth) (NZ.12.1) 18-ix-1972. Waikato area (-37.802°S, 175.334°E) (I. Hogg) 16.vii.2012 and (E. Doyle & N. Binks) 12.xi.2013.

South Island

Lake Rotoiti (-41.82°S, 172.84°E), Nelson Lakes National Park, Tasman region (B.V. Timms) (NZ.83.1) 1-x-1968.

Initially included with *C. novaezelandiae*, but shows consistent differences in the lighter larval head colour and some unique banding patterns. The existence of this species was partially supported by the initial barcoding studies of Sofia Ibarrarán, and there are some differences in the ITS-1 sequence. More detailed analysis of the *CO1* barcode sequence places it in the *C. novaezelandiae* complex (see under *C. novaezelandiae*) but differing at a number of base positions. The existence of polymorphic sites in *C. novaezelandiae* may result in some specimens being incorrectly assigned to the *C.*spNZ12 Bin.

13. Chironomus sp. nr. antipodensis

Chironomus antipodensis was described by Sublette and Wirth (1980) from the Antipodes Islands. There has been no record of it from New Zealand itself, but a single, incomplete, adult male was collected near Dunedin, which is much darker than other known New Zealand Chironomus specimens, and has a mtCO1 DNA sequence that differs from other available COI sequences. While it lacks a number of key identification characters, it seems to have relationship to C. antipodensis.

Adult:

Male:

A dark species, with dark setae that make those on the abdomen and the femurs much more obvious, i.e. it looks a quite "hairy" species.

AR, LR unknown.

Wing length 5.12 mm, width 1.09 mm; VR - 1.04. About 4 SCf on brachiolum, about 23 setae in squamal fringe.

Head: Antennae missing, FT about 25 μ m long and 13 μ m wide, i.e. about twice as long as wide. Palpal proportions (μ m) 73 : 65 : 276 : 311 ; (missing). 27 clypeal setae. Thoracic setae: Acrostichal 11; Dorsocentral - 20-22 in up to 3 rows; prealar - 6;

supraalar - 1; scutellar in three rows - 7, 7, and 12.

Leg proportions (micron):

	Fe	Ti	Ta1	Ta2	Ta3	Ta4	Ta5	LR	F/T
PI	1680	1620	-	-	-	-	-	-	1.04
PII	1800	1690	1000	640	440	280	200	0.59	1.07
PIII	2080	2120	-	-	-	-	-	-	0.98

abt 15 sensilla chaetica on mid Ta1

Abdomen with dark band covering anterior two thirds of tergites; setae very obvious because of their dark colour. About 3 setae centrally on tergite IX, SVo closest to E(i) type of Strenzke (1959). Setae of IVo appear simple, although not all are clear. GC reduces for about the distal third.

Pupa: Not known.

Fourth instar larva: Larvae collected at the same time as this adult were *C. forsythi*, so the larva of this species is not known.

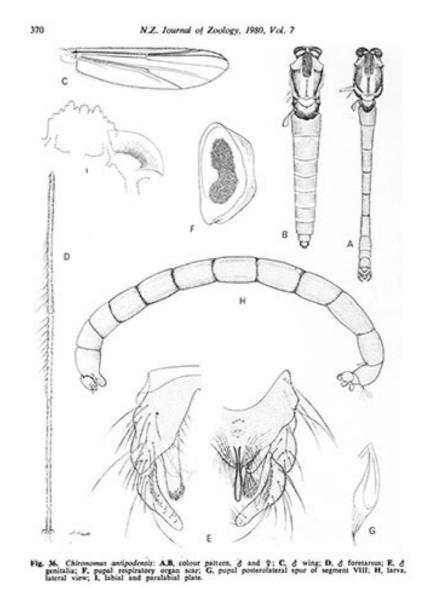
DNA sequence: Obtained although fixed in 85% ethanol until 2011 mt*COI* – does not cluster with current sequences in GenBank or BOLD but may show descent from a common ancestor of Australian species.

Localities:

South Island:

City reservoir, Dunedin, Otago (-45.80°S, 170.87°E) (J.S. Pillai) (NZ.6.2) 12-iii-1968.

The details of *C. antipodensis* Sublette & Wirth, 1980 are included in case the species does occur on the South Island:



Morphology of various parts of *C. antipodensis* from Subette & Wirth (1980)

Adult:

Male: Almost entirely blackish brown; haltere, scutellum and narrow posterolateral spot on terga VII and VIII slightly paler. Foretarsus with distinct beard, BR 4.16-6.36. AR 3.86-4.30; LR1 1.26-1.39; LR2 0.55-0.58; LR3 0.61-0.64.

Wing length 3.86-5.15 mm, VR 1.04; cross vein dark brown; 20 setae on squamal fringe.

Head: FT 25-31 μ m; clypeal width 0.86-0.98 of diameter of antennal pedicel, with 32-62 setae. Palpal proportions (segs 2-5, micron): 76 : 260 : 296 : 316 (holotype).

Thoracic setae: Acrostichal – not recorded; Dorsocentral 16-27 in partial double row; Prealar 5-10; Supra-alar 1; Scutellar in partially double staggered row 26-30.

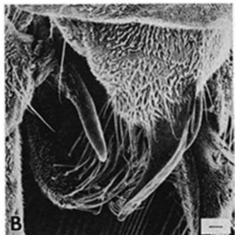
Leg proportions (Holotype)

	Fe	Ti	Ta1	Ta2	Ta3	Ta4	Ta5	LR	F/T	Ta5/Ti
PI	95	92	122	75	54	40	20	1.26-1.39	1.03	0.22
PII	105	105	59	38	28	19	13	0.55-0.58	1.00	
PIII	127	140	86	60	45	26	15	0.61-0.64	0.91	

BR 4.16-6.36

Abdomen heavily setose, each seta in a paler alveolus. About 7 setae on tergite IX, either in individual pale patches or with a few setae in some patches.





SEMs of hypopygium from Fig. 37 of Sublette & Wirth (1980)

Anal point narrow at base, SVo almost tubular – not like any of the Strenzke types, closest to a D-type. IVo about to tip of anal point and mid-point of the GC which is quite swollen, reducing over posterior third with about 5 setae at the end.

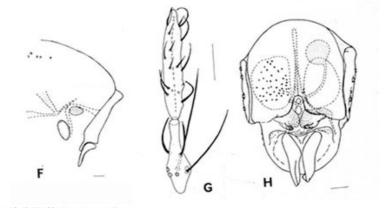


Fig. 35. F-H, Chironomus antipodentis:
F A anteoronotum. lateral view (0.1 mm); G, ♀ antenna (0.05 mm); H, ♀ genitalia.

Male pronotum, female A5 and genitalia (Sublette & Wirth 1980

Female: (Allotype):

Coloration similar to male, but femora and tibiae paler and terga II-VII with a narrow apical pale fascia.

Wing length 5.82 mm; VR 1.12; 33 setae in squamal fringe.

Antennal proportions (micron) 204: 143: 153: 153: 214; AR 0.35; A5/A1 1.05.

Palpal proportion (segs 2-5)(micron): 71: 229: 296: 347.

Thoracic setae: Acrostichal not noted; Dorsocentral 30 in partially double row; Prealar

7; Supra-alar 1: Scutellum with a staggered double row of 30 setae.

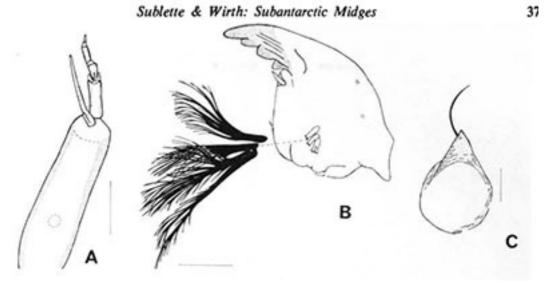
Legs similar to those of male: LR1 1.26; LR2 0.49; LR3 0.60.

Abdominal segment X sickle-shaped; cercus slightly elongated, with ventral margin longer, with a rounded posterior margin.

Pupa: Exuvial length 11.7-12.0 mm. Cephalothorax blackish brown; abdomen paler brown. Cephalic tubercles as Fig. C (below); respiratory base (Fig. F, above) moderately constricted in middle, HR about 2. Shagreen of TII coarse and covering middle third of segment; that of TIII-VI covering central half, VII with a small anterolateral patch of fine shagreen on either side of midline; TVIII largely covered by fine shagreen. Respiratory base partly constricted in middle (Fig. 36F, above).

Spur of segment VIII with about 7 appressed spines; about 80-107 taeniae on swim fin.

Fourth instar larva: a halophilus-type, i.e. some development of posterior VT. Head capsule yellowish brown, VHL 0.42-0.43 mm. Mentum (Fig. 36E, above) appears to be type II-III, with central trifid tooth of type IIA.



Antenna (Fig. A above) with A1 about 4 times longer than wide; RO about 0.34 up from base, AR about 2. PE with about 11 sharp teeth (type 2). Mandible (Fig. B, above) possibly type IIB-C.

Cytology: not known.

Sublette and Wirth (1980) suggest the species resembles *C. zealandicus*, although at that time the true identity and that of *C. novaezelandiae* was confused. However, they may well have been correct in suggesting that it derived from the *C. zealandicus*-group

14. Chironomus n.sp. NZ14

Known only from two larvae and the mt*COI* sequence.

Tentatively in Bold Bin: <u>BOLD ADF7908</u> (private data) or BOLDAAL7015?

Adult and Pupa: not known.

Fourth instar larva: (for only one larva) A salinarius type larva; head measures suggest it should be at least 15 mm long. Anal tubules $311x150~\mu m$ (dorsal) and $296x140~\mu m$ (ventral), so 2.1 times longer than wide. SAL (Fig. f) $73.5~x~23~\mu m$ (3.2 times longer than wide). Most of gula very dark, wider than mentum, highest at centre of mentum and widest just above rear margin; FC also dark and with tesselate appearance. VHL 366 μm , mentum width 0.73 of VHL and VMs separated by 0.44 of width of mentum.

Mentum (Fig. c) with 4th laterals reduced almost to level of 5th laterals (type II), central trifid tooth rather square, with c2 teeth well separated (type IIA).

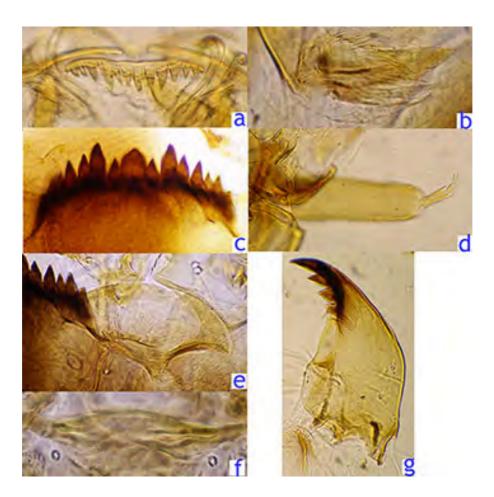
VM (Fig. e) about 275.5 μ m wide, 3.4 times wider than deep, with about 44-45 moderately spaced striae, VMR abt. 0.29.

PE (Fig. a) with 14 regular teeth (type B). Premandible (Fig. b) with inner tooth about 4.5 times wider than outer tooth and coming to a broad point (type B2).

Distance between S4 setae 1.04 times greater than that between the antennal bases. S5 setae anterior to nearby RO.

Antenna (Fig. d) with A1 about 3.1 times longer than wide and 0.44 of the VHL; A2/A1 0.23; A5/A3 0.78; AR 2.50; RO about a third up from base of segment; antennal proportions (micron): 160: 37: 9:11:7.

Mandible (Fig. g) with 3rd inner tooth well developed and darkened (type IIIB-C); about 24-26 furrows on the outer surface at the base; 10-12 taeniae in the PMan; Mdt-Mat 38, MTR about 0.33.



Localities:

North Island:

Lake Rotoaira, nr Turangi, South Auckland (-39.056°S, 175.705°E) and (S. Ibarrarán) (NZ.70.2) 14-ii-2007 & (NZ.70.3) 29-viii-2007

mtCOI - - sequence of larva in BOLD.

15. Chironomus 'pseudoppositus'

Adults of this Australian species have been collected in the Waikato area, and identified on the basis of the *COI* barcode sequence. It is not certain how close the morphology is to that of Australian material but it is reasonable to assume it will be similar as this is most likely a recent introduction since it was not identified in extensive sampling in the area in the 1960s-1970s.

Chironomus alternans b - Martin and Porter 1977; Lentzios and Stocker 1979; Lentzios et al. 1980, Martin and Lee 1981 and 1984, Martin and Cranston 1995. Chironomus 'pseudoppositus' (manuscript name) - Martin 1974. Chironomus pseudoppositus (nomen nudum) - Martin 1969b; Cranston and Martin 1989

The species is in BOLD Bin: BOLD:AAW3993





A few characters can be recorded for a male; Wing length about 3.8 mm., width about 0.8 mm, VR about 1.1. Thorax, scutellum, etc., dark brown. Legs yellowish, darkened at knees. Leg proportions (micron):

	Fe	Ti	Ta1	Ta2	Ta3	Ta4	Ta5	LR	F/T
PI	1530	-	-	-	-	-	-	-	-
PII	1530	-	-	-	-	-	-	-	-
PIII	1730	1730	-	-	-	-	ı	ı	1.0

Abdominal tergites with dark bands covering about half the segment, arising about 1/4 from anterior margin. This would be slightly lighter than the Australian specimens.

Further data from Australian specimens:

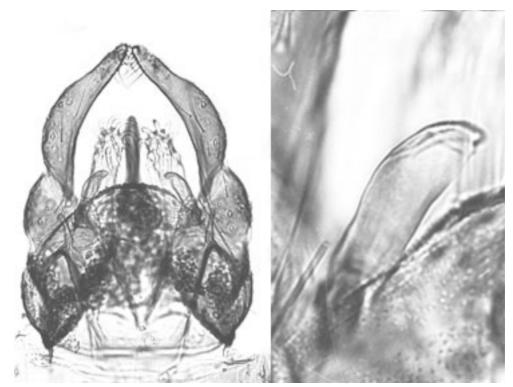
FT variable, length about 21.9 (10-29) μ m, abt 2.2 times longer than width at base. About 23 (19-25) clypeal setae; clypeal width about 0.6 of antennal pedicel diameter. Mean palpal proportions (micron): 52 : 66 : 199 : 224 : 354; P5/P4 1.5-1.71, P5/P3 1.71-1.95.

Thoracic setae: Acrostichal - abt. 10-17, Dorsocentral 15.9 (11-20), Prealar – 5.1 (4-8), Supraalar - 1.5 (1-2); Scutellar - 2 approximate rows, Ant. - 5.8 (2-8) small, Post. – 12.7 (10-14) larger; total scutellar 17.3 (14-22).

Wing length 3.12 (2.95-3.38) mm, width at crossvein 0.74 (0.68-0.80) mm, VR. 1.03-1.06; Scf on brachiolum 2-3; setae on squamal fringe 24-27; anterior veins hardly darker than posterior, crossvein slightly darkened.

Mean leg lengths (microns) and proportions as follows:

Males	Fe	Ti	Ta1	Ta2	Ta3	Ta4	Ta5	LR	F/T	BR
PI	1258	1047	1805	922	746	648	289	1.5-1.8	1.14-1.37	1.6-2.1
PII	1332	1166	743	405	297	192	143	0.58-0.63	1.04-1.14	=
PIII	1484	1436	1069	595	463	284	172	0.69-0.75	0.96-1.08	-



Male terminalia (left) and SVo (right) of type male

11.4 (7-16) setae on TIX probably in separate pale areas, 4-5+1 setae at tip of gonostylus which is as in holotype (above). SVo generally of the D-type, but occasionally E-type, varying from Strenzke's Fig. d to h.

IVo reaching almost to end of anal point, recurved setae simple. Anal point narrower at base. Gonostylus moderately swollen, narrowing over distal third.

Females:

Wing length 3.60 (2.8-4.2) mm; width at cross vein 1.03 (0.96-1.20) mm, VR 1.08-1.11; 3 or 4 SCf on brachiolum.

Head with FT of variable length: $29.3~(19-48)~\mu m$; about 35.5~(27-55) clypeal setae. Mean antennal proportions (micron): 198:128:136:132:171; AR - 0.31~(0.29-0.32); A5/A1 - 0.96~(0.90-1.00). Mean palpal proportions: 59:61:208:245:377; P5/P4 1.83-2.13; P5/P3 1.57-1.83.

Thoracic coloration similar to males. Thoracic setae: Acrostichal abt. 15.8 (11-22; Humeral not counted; Dorsocentral (incl. Humeral) 27.2 (23-35); Prealar 6.2 (5-7); Supraalar 1, Scutellum, anterior row 11 (9-14), posterior row 13 (11-20), total Scutellars 25 (20-34).

Mean leg lengths (microns) and proportions as follows:

		0 0	(,	1 1					
Female	Fe	Ti	Ta1	Ta2	Ta3	Ta4	Ta5	LR	F/T	Ta4/Ti
PI	1568	1175	2310	1140	925	860	373	1.68-1.82	1.23-1.32	0.61-0.73
PII	1546	1392	821	416	305	198	149	0.56-0.61	1.09-1.11	-
PIII	1668	1669	1199	651	511	301	190	0.67-0.72	0.98-0.99	-

BR - 1.6-1.7

Abdomen relatively dark.

Pupa: Length about 8.4 mm. 54 hooks in row on segment II, 2.8 (1-5) spines on caudolateral spur of segment VIII; 86 taeniae on each side of the swim fin.



Caudolateral spur on 8th abdominal segment of pupa

Fourth instar larva generally a medium sized bathophilus-type; length about $13.4 \ (10.2\text{-}15.3)$ mm (female), $11.45 \ (9.7\text{-}13.3)$ (male), VT well developed, posterior pair usually slightly longer, length of anterior $1.06 \ (0.42\text{-}1.67)$ mm; of posterior about $1.07 \ (0.46\text{-}2.92)$ mm. Anal tubules with ventral pair usually longer, about $382 \ (365\text{-}400)$ µm, than the dorsal pair, about $351 \ (250\text{-}420)$; and 2.6-4.2 times longer than wide.

Gula varying from pale to posterior third dark, FC often slightly or moderately darkened. SAL (below) 63-68 µm wide and 4.2 times wider than deep.



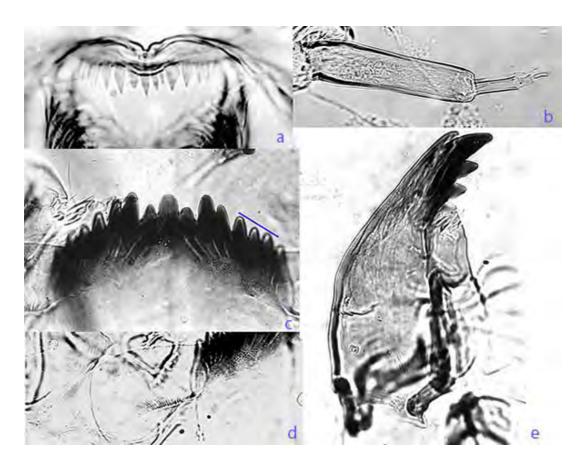
Mentum (c, below) with 4th laterals slightly reduced, sometimes to level of fifth laterals (i.e. between type I-II), and c2 teeth well separated, sometimes the c1 tooth is quite long (i.e. type III, but can appear as IIA when worn).

Ventromentum (d, below) about 3.6 times longer than deep; 1.08-1.11 times the mentum width; with about 30-40 striae; VMR 0.23-0.33. PE (a, below) with about 11-18 sharp teeth (i.e. type A of Proulx *et al.* 2013), occasionally with some reduced teeth.

Premandible with the normal two teeth, about equal in length, and coming to a relatively fine point, inner tooth two to three times the width of the outer.

Basal segment of antenna (b, below) relatively long and narrow, about 3.3-4.5 times as long as wide, RO about a third to a half way up from base; AR about 2.13 (1.89-2.31); A2/A1 about 0.22-0.27; A4/A3 about 0.9-1.3; segment proportions (micron) 124 : 31 : 9 : 12 : 7. Distance between the antennal bases, 148.25 (114-166) usually greater than distance between S4 setae, 141.6 (119-154), but may be equal or even less in about 1/3 of specimens.

Mandible (e, below) generally of type IA or B, but may be IIB, with about 14.8 (11-18) furrows on outer surface at base; about 11-17 taeniae in PMa; Mdt-Mat 20-23; MTR about 0.28-0.36.



Localities:

North Island:

Waikato area (-37.802°S, 175.334°E) (I. Hogg) 13.viii.2012.

For the description of the species in Australia, go to http://www.chironomidae.net/Martin/AustChironfiles/psoppadt.htm

There are a few other New Zealand larvae in other BOLD Bins, which require further investigation.

Chironomus bicoloris Tokunaga, 1964

Chironomus (Chironomus) bicoloris Tokunaga, n. sp. (fig. 10, f).

Large yellow species, allied to plumatisetigerus, but distinguished from it by smaller frontal tubercles (at most as long as two facets) and dark brown subtriangular or oval markings of scutal vittae. AR about 3.1; LR 1.6-1.7; frontal tubercles small, only as long as 1.5 times of diameter of facet in male and two facets in female; scutal vittae of thorax yellow and with four dark-brown spots, two of these spots elongate subtriangular on posterior half of median vittae and other two on anterior parts of lateral vittae; legs with knee parts dark brown, but knee joints very narrowly pale and usually posterior two pairs with knee parts faintly brownish, rarely tibial base without brown marking; tibial apical ends usually brownish, but in fore leg very faint and sometimes quite pale.

Male: Body 6.18-6.24 mm. long; wings 2.96-3.15 mm. by 0.8-0.82 mm. Head vellowish brown or yellowish pale brown, with mouthparts more brownish, frontal tubercles small, at most as long as 1.5 times diameter of facet, eyes separated above by one-seventh to one-eighth length of eye; palpal segments about 22.5: 17.5: 70.5: 84.5: 112.5; antenna with scape yellowish brown, flagellum and plumose hairs brown, AR 3.1 (3.08-3.11). Thorax mainly yellow, scutum pale yellow, with four yellow vittae and four subtriangular dark brown spots on vittae, scutellum pale yellow, with 15 to 17 bristles along caudal margin and 19 to 27 small setae scattered on anterior part, postscutellum dark brown on anterior half and yellow on caudal half. Legs mainly yellow or pale brownish yellow, but all knee parts dark brown and joints very narrowly pale, all tarsal segments apically brown, last one or two segments somewhat more brownish, fore tibia sometimes pale brown at tip, other tibiae usually more brownish at distal ends and sometimes basal brownish bands absent; LR about 1.69, RL-FT about 115: 99.5. Wing with veins very pale, but fR and r-m dark and covered by small dark spot, fMCu under end of r-m, RL-V 94.3: 70: 110: 94.3. Halter white or yellowish white. Abdomen pale brown, gradually fuscus brown caudad, tergites with somewhat T-shaped basal bands; hypopygium (fig. 10, f) brown, anal point slender, style slender, apical half suddenly tapered, dorsal appendage with basal pubescent part oval and setigerous, bare caudal projection not distinctly swollen or curved at tip, ventral appendage almost straight, slightly clavate, with 13 to 15 long curved apical bristles. some of these bristles finely plumose apically.

Female: Body about 6.76 mm. long; wings about 3.33 mm. by 0.98 mm. Generally similar to male, but lateral scutal vitta more brownish. Head with eyes separated above by one-sixth to one-seventh length of eye, frontal tubercles small and about as long as two facets; palpal segments about 25: 25: 85: 99: 143; antenna almost entirely pale brownish yellow, neck parts of intermediate flagellar segments as long as half of segments, six-segmented (25: 64: 45: 50: 41: 69). Scutellum with about 20 bristles along caudal margin and about 25 small setae scattered on anterior part. Leg with RL-FT about 130: 111. Wing with fMCu under origin of r-m, RL-V about 96: 87: 135: 102. Abdomen almost uniformly very pale brown or pale brownish yellow, anterior tergites 2 to 6 with very faint broad fuscus clouds, ultimate segment and cerci brown.

Tokunaga's (1964) description of C. bicoloris.

Type data: holotype USNM US66552 adult male, paratype(s) USNM 2 adult males.

Type locality: Dugor, Weloy, Yap Island.

<u>Australian</u>	specimens

Adult:

Male:

Wing length: 3.34-3.76 mm; wing width 0.82-0.86 mm; VR about 1.0. Brown spot over crossvein; usually 4, or 3, Scf on brachiolum; 22-23 setae on squamal fringe. AR about 3.10-3.16.

FT longer than in Micronesian specimens - about 50-60 micron and 2.5-2.8 times longer than wide - longer than the width of two eye facets. Clypeus width about 157-170 μ m, about 0.75 of diameter of antennal pedicel; with about 27-33 setae. Palp proportions (micron): 66: 64: 253: 249: 436; P5/P4 1.75.

Thorax green, vittae, postnotum and sternopleuron reddish brown, vittae with 2-3 darker markings; setae - about 19 acrostichal; 26-30 dorsocentral; 1 supraalar; 6-7 prealars; 28-37 scutellar – 12-17 in 1 or 2 anterior rows and 16-20 in posterior row. Legs with femur and tibia greenish, tarsi yellowish with at least slight darkening of knees and at tips of tarsi.

Lengths (microns) and proportions as follows:

	Fe	Ti	Ta1	Ta2	Ta3	Ta4	Ta5	LR	F/T	BR
PI	1460	1405	2275-	-	ı	-	-	1.62	1.04	-
PII	1540	1485	940	530	390	215	150	0.63-0.67	1.06	-
PIII	1675	1825	1355	750	600	350	155	0.73-0.75	0.93-0.94	-

Abdomen greenish proximally, anterior segments with proximal dark band which becomes larger in the more posterior segments until whole segment is darkened.



C. bicoloris: Male hypopygium (left) and superior volsella (right)

Tergite IX with 14.3 (13-16) setae in a large single pale patch. IVo reaching to about the end of the anal point; setae forked. SVo of the E-type (see above) (most like h of Strenzke 1959) but with what appears to be slight folds at the tip; gonostylus usually slender and tapers gently over posterior half, with 4 long and 1 shorter setae at the distal end.

Female: (based on single specimen from near Sarina Beach, Queensland (AQ.66.1) Colour yellowish, with brown vittae; legs as in males.

Wing length 3.42 mm., width 0.89 mm.; VR 1.09. 3-4 Scf on brachiolum; 17-18 setae on squamal fringe.

Relative length of antennal segments (micron) (percentage of neck in brackets): 164 (25): 109 (38): 124 (44): 118 (45): 182; AR 0.35, A5/A1 1.11. FT about 22 μ m long and 2.5 times longer than wide. Clypeus about 1.7 times the diameter of the antennal pedicel, with 35 setae. Palpal proportions (micron) 63: 48: 188: 250: 385 (P5/P4 1.54; P5/P3 2.05).

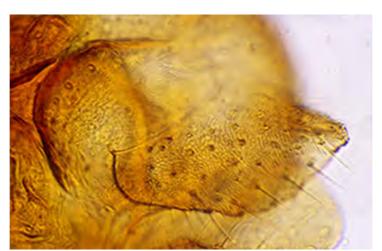
Thoracic setae: Acrostichal abt 14; Humeral 5-6 linear; Dorsocentral 22 (humeral + dorsocentral 27-28); Prealar 3; Supraalar 1; Scutellar with 2 rows – 7 in anterior row and 10 in posterior row.

Leg lengths (micron) and proportions:

	Fe	Ti	Ta1	Ta2	Ta3	Ta4	Ta5	LR	F/T	Ta4/Ti
PI	1315	935	1770	860	760	705	340	1.89	1.41	0.73
PII	1265	1140	660	350	250	190	135	0.58	1.11	-
PIII	1355	1380	950	510	410	270	160	0.69	0.98	-

BR 1.52

Segment X crescent shaped, about 3.9 times longer than its greatest width and with 9 setae.



Cercus and segment X (at left) of C. bicoloris

Cercus shorter on the dorsal margin, posterior margin slightly curved towards ventral end and merging into the longer ventral margin with a basal bulge.

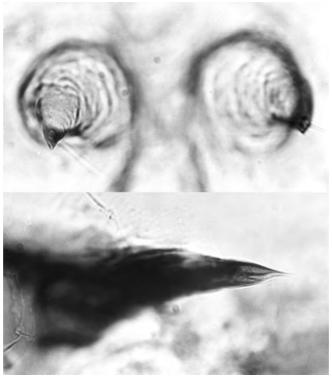
Pupa: Not previously described. One male and one female exuviae are available. Colour yellow brown, with darker yellow brown cephalothorax and muscle scars. Shagreen sparse in centre of TI, on posterior 2/3 of TII, posterior 3/4 on TIII and TIV, 5/6 on TV, more midline constrained on TVI, mainly on centre line of TVII but with gap along midline, and sparse on anterior with gap in midline of TVIII.

Length about 6.75 mm (male) and 7.07 mm (female), IMW 1.42 (f)-1.59 (m) mm. FT small, 53 x 46 μ m (male) (below); 45x50 μ m (female), with subterminal seta at least 43-53 μ m, no indication of frontal warts. Female antennal sheath about 660 μ m.

Basal scar about 122 x 68 μ m, slightly narrowed in middle, and respiratory base filling almost whole space; HR about 1.71-1.96. Irregular patch lateral/ventral of scar, about 150x75 μ m in size.

L-seta at anterior margin of intersegment of III/IV not seen, on IV/V about 56 μ m long. Hook row of segment II occupying about 76-79% of segment width, 92-94 simple recurved hooks.

PSB relatively large on segment II, small on segment III; large PSA on segment IV (abt 151-177 x 101-110 μ m) about 26-32% of the segment length. PSB on segment V still relatively large; that on segment VI small with just spines.



Frontal tubercles and spur of segment VIII of male pupa

Caudolateral spurs of segment VIII have 1-2 spines. About 72 (male) -83 (female) taeniae, initially uniserial, then partly biserial, with some places triserial, on each side of swim fin.

Fourth instar larva: a medium sized plumosus-type larva (length (female) about 15.2 mm). Anterior VT (1.06 mm) shorter than posterior pair (1.40 mm); TLt about 320 μ m. Gula pale or sl. dark over post 1/2; FC pale or very slightly darkened. Clypeal aperture about 99x15 μ m, i.e. 6 times longer than wide.

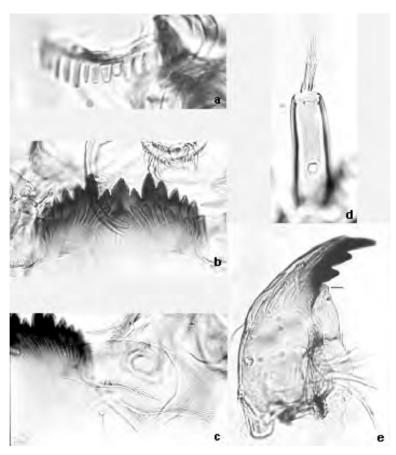
Mentum (b, below) with sculpturing along ventral surface and relatively sharp broad teeth; c2 teeth of central trifid tooth well separated from c1 tooth (type IB-IIA), 4th laterals slightly reduced (type I-II).

PE (a, below) with about 16-18 teeth (type B). Ventromentum (c, below) about 4.3 times longer than deep; with about 36-39 striae; distance between VM plates about 0.28-0.38 of mentum width; VMR about 0.32-0.33.

Distance between the antennal bases probably greater than that between the S4 setae, which are separated by about 70% of FC width at that point.

Antenna (d, below) with basal segment about 3.5 times as long as wide, RO just over a third up from the base; AR about 2.43-2.54; A2/A1 about 0.19-0.20; antennal proportions: 127 : 24 : 7 : 13 : 6.

Mandible (d, below) with third inner tooth only slightly separated and darkened (Type IA-B), and with about 13-20 furrows on outer surface at the base and about 7-10 taeniae in PMa, which appears to run down and away from the inner teeth (just visible in d, below).



Larval mouthparts of *C. bicoloris* a. Pecten epipharyngis; b. Mentum; c. Ventromental plate; d. Antenna; e. Mandible.

Cytology: 4 polytene chromosomes with the pseudothummi arm combination AE, BF, CD, G. Centromeres heterochromatic.

Arm G closely paired with a small subterminal nucleolus. Main nucleolus near middle of arm C. Polymorphism at least in arm A.

bifA2: approx 1 - 2c, 10 - 12, 3ih, <u>6 - 9, 2d - 3g</u>, 5 - 4, 13 - 19

bicB1: typical bands (groups 23-28) near centromere, puff (group 7) near middle of the arm

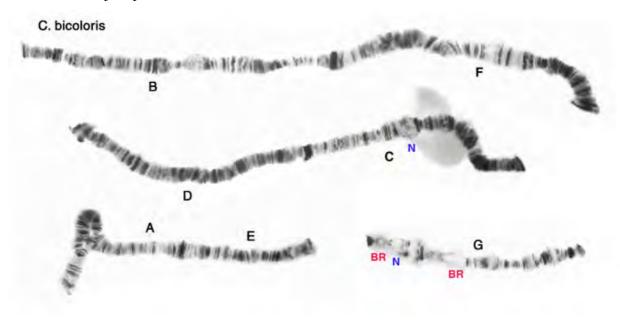
bicC1: NOR near middle of the arm with groups 3-4 immediately distal to it

bicD1:

bicE1: possibly 1 - 3a, 5 - 10b, 4h - 3f, 10c - 13 i.e. as in aprilinus, atrella, athalassicus

bicF1: Groups 9-7, 14-15 about 1/3 from end of arm.

bicG1: Small subterminal nucleolus, with a BR between the NOR and the centromere, and another just proximal of the middle of the arm.



Polytene chromosome complement of *C. bicoloris*Note arm A is heterozygous A1.2

The arrangement of the PMa on the larval mandible appears to be unique among *Chironomus* species. The other distinguishing features are the male SVo and the darker markings on the thoracic vittae.

Found: Micronesia - Caroline Islands: Yap Island (Type locality).

Queensland - Lake Boemingen and Lake Wabby (--25.27°S; 153.80°E), Fraser Island (H. Burton, light trap); 3 km w. Sarina Beach (-21.40°S; 149.25°E).

Chironomus circumdatus (Kieffer 1916)

Syn.: C. basitibialis Tokunaga 1936 (Yamamoto 2013)

C. bharati Singh & Kulshretha 1976 (doubtful synonymy – see description above)

C. costatus sensu Karunakaran 1969 (mtCOI - Wong, unpubl.; cytology - Martin unpubl.)

C. daitoefeus Sasa & Suzuki 2001 (probable synonym – Yamamoto, unpubl.)

C. plumatisetigerus Tokunaga, 1945 (Martin & Saxena 2009)

C. setonis Tokunaga 1936 (Yamamoto 2013)

A member of the broader "C. flaviplumus complex"

In BOLD Bin: BOLD:AAG5483

Adult:

Male (based partly on Tokunaga (1964):

AR about 3.24 (3.02-3.41). LR 1.54-1.64.

FT about 25-43 μ m long, 10-17 μ m wide. Palpal proportions: 14 : 15 : 68 : 70 : 77; P5/P4 1.10, P5/P3 1.13. Clypeal setae - 17-34.

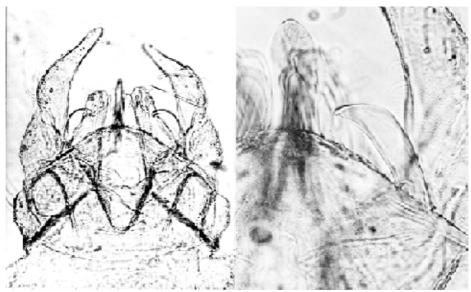
Thorax greenish, scutal stripes conspicuous with dark brown margins; scutellum pale yellow, postnotum dark brown. Thoracic setae: Scutellum with 14 in anterior row, 14 in posterior row; other thoracic setae (from other areas): acrostichals - 13-18; dorsocentrals 18-22; prealar 7; supraalar 1;

Wing length: 2.47-3.04 mm; wing width 0.65-0.74 mm. VR about 1.02-1.05 Wings without darkening of the crossvein. 25-27 setae in squamal fringe. Legs pale, with darkening at distal ends, also on distal half of Ti4 and all of Ta5.

Leg lengths (microns) and proportions (from other areas) as follows:

	Fe	Ti	Ta1	Ta2	Ta3	Ta4	Ta5	LR	F/T	BR
PI	1145	1060	1610	835	745	645	325	1.42-1.67	1.04-1.12	1.64-1.9
PII	1230	1110	705	395	295	165	135	0.62-0.66	1.05-1.13	
PIII	1350	1350	1000	560	475	250	170	0.72-0.81	0.97-1.02	

Abdominal segments pale, but with increasing central dark oval patch, so that tergites V-VIII are virtually all dark.



Male terminalia of *C. circumdatus*Anal point relatively narrow, superior volsella D-type curved at the tip.

Anal point narrow; 1-16 setae on tergite IX. SVo of the D-type, between d and e of Strenzke (1959), but tip may be more bent. IVo, with plumose setae, reaching to about 1/3 along gonostylus which is moderately swollen and narrows relatively evenly over posterior third.

Female (based mostly on Tokunaga 1964):

Coloration as male but darker markings more obvious

Wing length 2.53 (2.28-2.78) mm., width 0.85 (0.75-0.91) mm.

Antennal proportions: 54 : 39 : 40 : 39 : 72; AR 0.42; A5/A1 1.3. Necks of segments 1-4 about half the segment length.

FT short and stout, 24 µm long and 17 µm wide.

Palpal proportions: 19:16:75:84:113; P5/P4 1.35, P5/P3 1.51.

Leg lengths (microns) and proportions (from other areas) as follows:

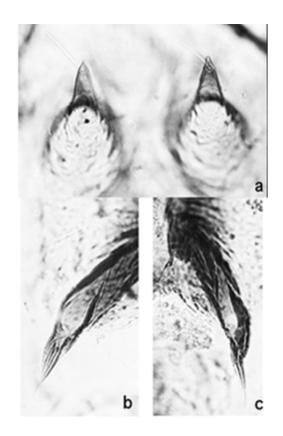
	Fe	Ti	Ta1	Ta2	Ta3	Ta4	Ta5	LR	F/T	Ta4/Ti
PI	1440	1150	2050	1000	930	880	410	1.79	1.25	0.58
PII	1630	1340	810	410	290	180	150	0.60	1.22	0.13
PIII	1490	1490	1150	560	460	270	180	0.77	1.00	0.18

Abdominal tergites brown, with narrow apical pale bands or spots on tergites I to VI.

Pupa: Brown. Exuviae pale brown.

Body about 6.6-7.7 mm (male) and 6.5-7.6 mm (female). Cephalic tubercles (a, right) about 70-100 μm, with a subapical seta (40-80 μm). Thorax rugose, with 2 pairs of precorneal setae. Abdominal tergite II with median shagreen and about 52-68 hooklets, tergites III-V entirely with shagreen, tergite VI with T-shaped shagreen, tergites VII-VIII with 2 broad patches of shagreen.

Caudolateral spur of segment VIII (b & c, right) with about 2-4 spines.



Fourth instar larva: a medium plumosus-type (length 10.6-13.7 mm; females 11.2-12.5 mm), lateral tubules well developed (about $480 \mu m$). Posterior pair of VT generally longer than anterior pair (ant. 1.84; post. 2.48) and coiled. Anal tubules may vary in size in different areas, from about twice as long as wide (Allahabad) to almost three times as long as wide (Jammu), length 290- $440 \mu m$, width $165 \mu m$.

Gular region darkened, FC variable from very slightly darkened to dark.

Mentum (c, below) with fourth laterals reduced to about the level of the 5th laterals (type II), 6th laterals pointed outwards; c2 teeth of the central tooth (type III) well separated.

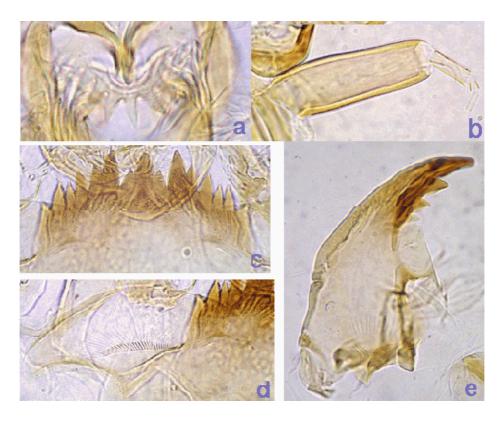
VM (d, below) with about 30-36 striae. PE (a, below) with about 12-15 teeth.

Premandible with inner tooth shorter and about twice the width of the outer.

Antenna (b, below) with basal segment less than 3.5 times as long as wide; A2/A1 about 0.24; A4/A3 about 2.3-2.6. AR about 2-2.3.

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

Mandible (e, below) with third inner tooth slightly darkened and only partly separated (type IIB).

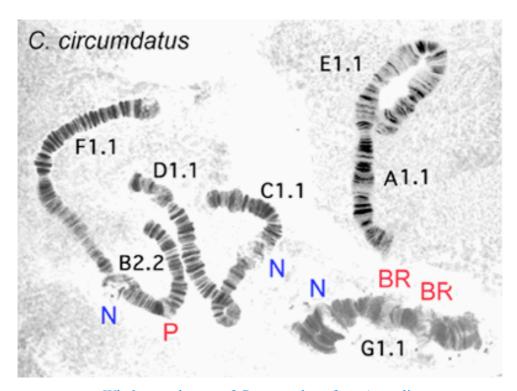


Cytology: Four polytene chromosomes with the pseudothummi-cytocomplex combination BF, CD, AE, G. Nucleoli in arm B and C, with a small one also present subterminal in arm G (which is not always present/active). Arm G closely paired with generally 3 obvious BRs from near the nucleolus to the opposite end, depending upon the sequence.

The cytology of Pacific region specimens is not known – so only widespread sequences are listed below.

Polymorphism in arms A, B, C, D and G, although Pramual *et al.* report pericentric inversions involving the AE and BF chromosomes. Most polymorphism in arm B.

```
cirA1: 1 - 3, 12 - 4, 13 - 19
                                                      as pseudothummi
                                                                          (widespread)
cirA2: 1 - 2c, <u>10 - 12</u>, <u>3 - 2d</u>, 9 - 4, 13 - 19 as holomelas, incertipenis
                                                                          (widespread)
cirB1: Puff just beyond middle of arm with dark bands distal (gps. 8 - 7)
                                                                            (widespread)
cirB2: Puff near nucleolus, with dark bands on proximal side (gps. 7 - 8)
                                                                            (widespread)
cirC1: Median nucleolus
                                                                            (widespread)
cirC2: Inversion of about a third of the arm distal of the nucleolus
                                                                            (widespread)
cirD1: differs from oppD1 by at least one inversion
                                                                            (widespread)
cirD2: Inversion of approximately the middle third of the arm
                                                                            (widespread)
cirE1: 1 - 2, 4 - 10ab, 3e-a, 3f, 10c - 13
                                                                   from aprilinus by Inv4-3a
cirF1: 1 - 2a, 10d - 2c, 15c - 11a, 2b, 15d - 23
                                                                          as oppositus F3
cirG1: Nucleolus near one end, three BRs towards the other end (most distal not always
       visible)
                                                                              (widespread)
cirG2: Inversion of over two thirds of the arm, from proximal of the nucleolus to between the
       two larger BRs
                                                                              (widespread)
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Whole complement of *C. circumdatus* from Australia

Found: Type locality – Tainan (abt 23.0°N, 120.0°E), Yentempo, (formerly Takao Prefecture), FORMOSA (TAIWAN).

New Guinea - Lake Wisdom (-5.33°S, 147.10°E), Long Island, Madang District. Micronesia – Guam, S. Mariana Is., Palau, Yap, Caroline Islands (Tokunaga 1964, as *C. plumatisetigerus*)

Also found in Australia, India, Malaysia, Singapore, and Thailand.

The morphology was redescribed by Tokunaga (1964), Sasa (1978) and Chaudhuri *et al.* (1992). Chaudhuri *et al.* claim the larval VT are not coiled.

Chromosomes described by Kumar & Gupta (1990) and Pramual *et al.* (2008) as *C. circumdatus*, by Kuvangkadilok (1985) from Thailand, and for arms A, E and F (with some errors) by Saxena (1995) as *C. plumatisetigerus*.

There have been numerous studies of mitochondrial *COI* sequence (indicated below). The species can be bred in the laboratory (Kuvangkadilok 1994).

DNA Sequence:

mt*CO1*: sequence is in GenBank for India (acc. no. KX271850), Pakistan (acc. no. KJ768129), Malaysia acc. no.), Thailand (acc. nos.GU944724, JQ287743-51, KT212956 - 977), Singapore (acc. no. KJ530964-69, KP462069-74, KP462468-69, KP462389-94,68-70, KP462650, 53-56, 59, 62-70, 84), Australia (acc. no. AF19225), China (acc. no. KP902724-29), Japan (acc. no. LC050935).

Chironomus claggi Tokunaga, 1965

Syn: *Chironomus simantobeceus* Sasa, Sukuki and Sakai 1998 (Yamamoto & Yamamoto, 2018)

In BOLD Bin: BOLD:ACQ6925

Adult:

(based on Tokunaga (1964)

Large yellow or dark brown or brown species.

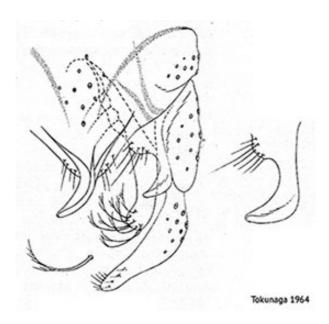
Male: Body length 5.33 (5.01-5.66) mm, wings 2.67-2.69 mm long and 0.68-0.72 mm. wide, crossvein not darkened, VR about 1.

FT cylindrical about the length of 2 eye facets. Palps brown, segment lengths: 15.5 : 16.5 : 70.5 : 70.5 : 84; P5/P4 & P5/P3 1.19. AR 2.7 (2.5-2.85).

Thorax widely yellow, vittae entirely yellow, scutum white. 9-11 small setae in anterior row and 10-11 larger setae in posterior row (total 19-22) on scutellum.

Legs with coxae and trochanters usually yellow, all femurs mainly yellow but apically brownish, fore tibia mainly dark but other tibiae dark only on basal parts; fore tarsi entirely black or dark brown, other tarsal segments pale or yellowish brown on basal 1 or 2 segments but apical part black or more brownish as on following segments; LR 1.7 (1.66-1.77); Fe1/Ti1 1.22.

Abdomen mainly very pale brown, but TII-VI with basal bands brown and broad, other segments uniformly brown. TIX with about 8 setae in a pale area.



SVo a boot shape closest to S-type of Strenzke (1959), similar to that of *C. indiaensis*. Inferior volsella long, to half way along gonostylus, setae bi- or trifurcate apically; gonostylus moderately swollen and reduces gradually over posterior third. Anal point narrowed at base.

Female:

Wing length 3.04 mm, width 0.89 mm. LR about 1.81. Frontal tubercles about 1.5 times as long as the diameter; palp proportions about 20 : 18 : 70.5 : 82.5 : 107; P5/P4 1.30, P5/P3 1.52.

Scutal vittae entirely brown; scutellum with 12 setae along caudal margin and 7-10 smaller setae anteriorly (total 19-22).

Abdomen mainly yellow with dark tergal bands on all but last two segments which are dark brown.

Pupa: It is not clear whether this known pupa is definitely associated with this species. It was collected along with a female, but there is no detail as to how they were associated - the caudo-lateral spur is rather unusual for a *Chironomus* species, more like that of a *Stictochironomus*, as is a "rounded" anal swim fin. With that proviso, Tokunaga's description gives the following information:

Length about 7 mm. Cephalic tubercles small on a basal blunt swelling and with a small lateral seta. Abdominal surface very weakly spinulous, sternite IV with lateral groups of spinules hardly visible; tergite II with about 71 yellow simple hooklets; caudolateral spur black with 5-6 lateral and 3-5 apical spines.



Pupal cephalic tubercles and spur of *C. claggi* from Tokunaga 1964

Larva and Cytology: not known.

Allied to *crassicaudus* in coloration and to *carolinense* in structure of the male hypopygium., but separable from *carolinense* by the different coloration. Separable from *crassicaudus* by the longer FT, larger LR and different structure of the hypopygium.

DNA Sequence:

The mtCO1 sequence for Japan in GenBank is AB740233-34.

Found: Type localities - Futami-ko; Camp Beach, Omura; Gen's beach, Minato-ko, Yatsue Region; all Chichi Jima, Micronesia.

Japan- Tokyo Metropolitan; Chicijima Island, and Hahajima Is., Bonin Isles, Tokyo; Shimanto River, Nakamura, Kochi Pref. (type locality of *C. simantobeceus*) (all Yamamoto & Yamamoto, 2018); and Ogasawara, Kanto (GenBank & BOLD).

According to Yamamoto & Yamamoto (2018), *C. claggi* superficially resembles *C. flaviplumus* and *C. yoshimatsui* but is separable by the more slender superior volsella. In scutal coloration and SVo shape it resembles *C. circumdatus*.

Chironomus magnivalva Kieffer, 1917

Placed in the subgenus *Camptochironomus* by Cranston & Martin 1989, but this is an artificial grouping of species that mate on the substrate and have enlarged male terminalia.

Returned to *Chironomus* by Bugledich et al. (1999).

Synonyms: *Chironomus crassiforceps* - incorrect synonymy by Guryev *et al.* (2001) and Peck *et al.* (2002).

Tokunaga (1964) misidentified C. magnivalva from Micronesia as C. crassiforceps.

In BOLD Bin: BOLD: AAJ4269

The nearest neighbour is <u>BOLD:ACC5271</u> which contains *C. crassiforceps*.

Adult

A greenish brown species with an enlarged hypopygium, typical of species that mate on substrate.

CHIRONOMUS (CHIRONOMUS) MAGNIVALVA Kieffer

Chironomus magnivalva Kieffer, 1917, p. 219.

Antennae and legs rather short, thorax greenish, stripes brown, abdomen somewhat flattened, male hypopygium not unlike *tepperi* at first sight, but appendage 2 of a more normal, elongate form. This species seems allied to *tepperi* on account of the shape of the styles; it is readily recognized from Kieffer's figure and appears only to be known from Townsville.

Wing length.—2.4-2.5 mm.

Male.—Head and mouthparts greenish, antennae browner, A.R. about 2.5, antennae rather short. Thorax greenish, stripes either brown or partially brown, dorsocentral bristles not arising from very distinct pits, thorax slightly shining.

Legs darkened at the knees, somewhat shorter than in most species but not as short as in tepperi; L.R. 17, posterior L.R. about 0.5, whole posterior tarsus less than one and a half times length of tibia. Abdomen rather flattened, brownish green. Hypopygium similar at first sight to tepperi, but examination shows resemblance confined to anal point, appendage 1, and styles; appendage 2 not swollen, but of normal elongate form, styles not rounded as they are in tepperi.

Female.—Resembles male in colour and general appearance.

Type.—Holotype & from Townsville, Qld., was in the Hungarian National Museum.

Specimens seen.—Townsville, Qld., A. K. O'Gower, 22.i.1959–12.ii.1959, 31 ♦ ♦, 16 ♀ ♀.

From Freeman (1961

Male:

Antennal ratio (AR): Kieffer gave the AR as about 2, while Freeman pushed this up to about 2.5. However in one specimen that he measured, it is only about 1.5. Tokunaga (1964) gives the AR of Micronesian specimens as 1.55 (1.48-1.65). Wing Length: 2.54 (2.32-2.83) mm; wing width 0.64 (0.48 - 0.68), VR 1.08 (0.94-1.15); 2.3 (2-3) Scf on brachiolum; 11.1 (10-13) setae on squamal fringe.

Head: FT about 23-36 μ m in length, generally columnar, and about twice as long as wide; 28.6 (19-44) clypeal setae. Palpal proportions (micron): 48 : 46 : 155 : 147 : 213; P5/P4 1.42 (1.20-1.75); P5/P3 1.37 (1.15-1.57).

Thoracic setae: Acrostichals 12.1 (10-13); Dorsocentrals 17 (15-21); prealars 5 (4-6); scutellars in two rough rows, 4-9 in anterior row, 8-11 in posterior row, total 15.3 (13-17).

LR about 1.59 (1.6-1.72).

Mean leg measurements ((microns)	and pro	portions	as follows:
Trican ica incasarcincing	(IIII OI OIIO)	, and pro	POLUTIO	as iono ii.

Male	Fe	Ti	Ta1	Ta2	Ta3	Ta4	Ta5	LR	F/T	BR
PI	1143	1021	1615	800	699	695	341	1.46-1.74	1.04-1.29	1.27-1.67
PII	1172	1149	569	329	266	192	161	0.47-0.52	0.94-1.08	-
PIII	1323	1338	791	446	396	234	187	0.55-0.69	0.97-1.00	-

Ant Ta4/Ti - 0.68; FeI/FeII - 0.92-1.03

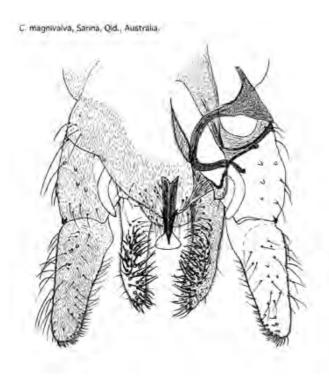


Illustration of the hypopygium of C. magnivalva

No setae on TIX; anal point downturned with a flattened triangular tip. SVo normal, closest to E(i) type of Strenzke (1959); IVo also relatively normal, with simple setae on inner margin only, and extending to mid-point or tip of gonostylus. Gonostyle normally broadest at base, but sometimes quite swollen, narrowing slightly and evenly along its length; with numerous spines rather than setae at the tip.

Female:

Coloration essentially as in male; brownish green darkening over the abdominal tergites.

Wing length 2.97 (2.64-3.44) mm, width 0.95 (0.85-1.01) mm; VR 1.05 (1.03-1.13); 2.8 (2-3) Scf on brachiolum; 16.3 (13-20) setae on squamal fringe.

Head: FT about 25-40 μ m long and 11-18 μ m wide, about twice as long as wide; antennal proportions (micron, with percentage of neck in brackets) 129 (26): 91 (39): 98 (39): 97 (45): 170. AR about 0.40 (0.37-0.45); A5/A1 1.33 (1.15-1.60). Palpal proportions (micron): 48: 43: 146: 168: 231.

Thoracic setae: about 13 (10-17) acrostichals; 3.85 (3-6) linear humerals; 20.7 (14-27) Dorsocentrals; 5.1 (4-6) prealars; 1 supraalar; scutellars in two rows -6.2 (3-8) in anterior row and 10.6 (9-13) in posterior row.

Mean leg measurements (microns) and proportions as follows:

Female	Fe	Ti	Ta1	Ta2	Ta3	Ta4	Ta5	LR	F/T	Ta4/Ti
PI	1138	954	1513	715	631	645	323	1.50-1.69	1.13-1.24	0.57-0.78
PII	1160	1150	600	325	250	185	155	0.52-0.53	1.00-1.02	-
PIII	1300	1370	860	470	405	230	190	0.62-0.65	0.94-0.97	-

BR 1.38-1.62. FeI/FeII 0.96-0.99

There are stiff setae ('pseudospurs') on the mid and hind tarsomeres (Yamamoto & Yamomoto 2018)

GcIX with abut 4 setae; segment X larger than usual and oval in shape with about 19.5 (14-24) setae. Cercus (below) essentially oval/rectangular with at best a very small basal extension on dorsal side.

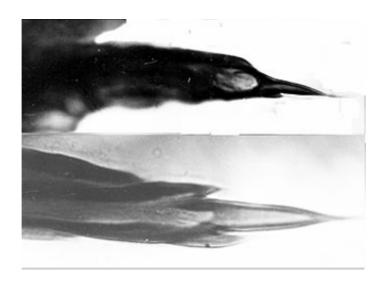


Cercus and segment X of C. magnivalva

Pupa: Colour yellowish brown, muscle scars slightly darker; shagreen complete on tergites II-VII, anterior quarter on TVIII, none on TIX.

Length about 6.55 (6.3-6.8) mm (female) and 6.33 (5.04-6.7) mm (male); posterior margin of wingcase about 1.44 (1.27-1.52) μ m (female), about 1.34 (1.04-1.49) μ m (male); female antennal sheath about 450 (340-550) μ m. T about 57.3 (51-71) μ m (female), 71.0 (56-83) μ m (male) long and usually wider than long in females but longer than wide in males; some slight indication of frontal warts, also a small tubercle about 21.4 (15-28) μ m long and 1.0-2.2 times longer than wide, just anterior to the respiratory scar. Respiratory base 109-159 μ m long and 46-66 μ m wide, respiratory fibres narrowing in middle, HR 2.45 (2.1-2.7). About 87 (55-105) recurved spines on second segment, occupying just over half of the segment width (subject to mounting distortions). Pedes spurii B difficult to see on available specimens and only determined for segment II; pedes spurii A on segment IV about 116-182 μ m long and about 0.22 (0.13-0.27) of segment length. L-setae at III/IV margin at least 50-106 μ m long and at IV/V margin at least 71-96 μ m long.

Spurs dark yellow brown, with about 1-3 spines plus 1-3 small spines in many cases where there is only 1 large spine.



Variation of pupal spur (Sarina, Old above; Nadi, Fiji, below)

Taeniae on anal fin in up to 3 rows towards the posterior end; about 53-115 taeniae but more in females (mean 96.7) than in males (mean 79.3).

Fourth instar larva: a medium sized plumosus-type larva. Length (females) about 19.7 (11.3-14.5) mm.; males about 10.27 (9.8-11.2) mm. Anterior VT (1.48 (0.92-2.36) mm.) slightly shorter than the posterior pair (1.54 (1.08-2.48) mm.); TLt 272.5 (200-360) μ m long. Anal tubules about 380 (220-480) μ m long and about twice as long as wide.

Gular region at least slightly darkened on posterior half, FC also darkened to some extent, sometimes just along the edges. SAL (Fig. c, below) 76.5 (61-88) μ m wide and 4.53 (3.50-5.33) times wider than deep.

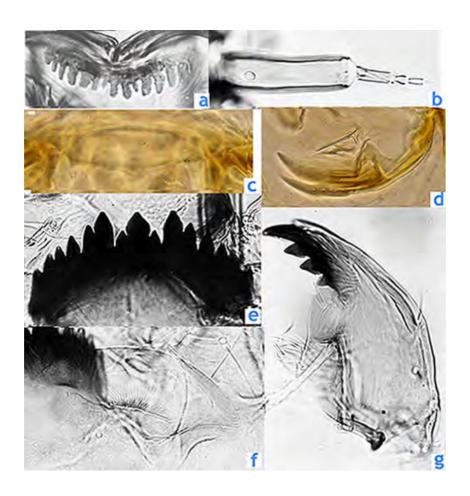
Mentum (Fig. e, below) with 4th laterals only slightly reduced (essentially type I), and c2 teeth only partly separated from c1 (type IA), 6th laterals arising at a slightly lower level. Ventromentum (Fig. f, below) about 168.13 (161-180) µm wide and 3.38 (3.18-3.65) times wider than deep; about 1.09 (1.03-1.18) times wider than the mentum and separated by about 31-39% of MW; with about 34.25 (28-38) striae; VMR 0.26 (0.21-0.32).

Premandible (Fig. d, below) of type B2, with inner tooth about 4.5 times wider than the outer tooth, with both coming to a sharp point when not worn. PE (Fig. a, below) with about 16 (14-17), usually type B teeth.

Antenna (Fig. b, below) with basal segment only about a third of the VHL, but relatively long compared to the other antennal segments, about 3.52 (3.17-3.83) times longer than wide; AR about 1.93 (1.77-2.19); proportions (micron) 110; 27; 9.5; 12; 6.5.

Distance between the antennal bases (124-144 μ m) greater than that between the S4 setae (121-126.5 μ m), which are separated by about 74-76% of the FC width at that point; S5 setae slightly posterior to the nearby RO.

Mandible (Fig. g, below) about 220 (212-230) μ m long; with 3rd inner teeth showing some colour and usually only partly separated (type IA-IIIB); about 16.6 (15-18) furrows near the base and about 11.8 (11-13) taeniae in the PecM.



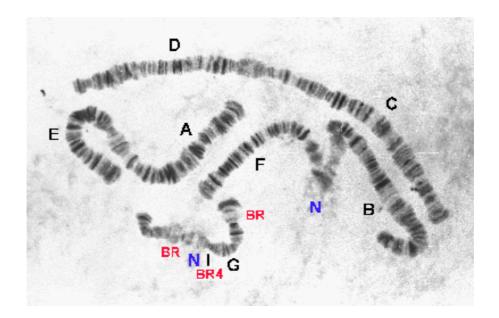
Cytology: 4 polytene chromosomes with the pseudothummi arm combination AE, BF, CD, G. Arm G closely paired with a small nucleolus near the middle of the arm, and three Balbiani rings (BRs), two just distal to the NOR and the other near the somewhat heterochromatic centromere. The most distal BR is not always developed. Nucleolus in arm F with NOR at about group 19. No polymorphism known in Australian samples. Irradiation experiments suggest the MD may be on either arm B or on chromosome CD.

magA1: 1 - 2c, 3 - 2d, 10 - 12, 14 - 13, 4 - 9, 15 - 19 as crassiforceps

magB1: A puff with some dark bands on distal side, may be developed near the distal end of the arm

magC1: not mapped. as crassiforceps
magD1: not mapped. as crassiforceps
magE1: 1 - 3e, 5 - 10b, 4 - 3f, 10c - 13
i.e. as cingulatus, etc.
magF1: 1 - 2a, 10d-a, 2b - 9, 11 - 18 NOR 19 - 23
as crassiforceps

magG1: Nucleolus and at least one BRs near centre of the arm. Site of BR4 just proximal to NOR, and further BR subterminal.



Guryev *et al.* (2001) and Peck *et al.* (2002) referred Australian specimens from the Alligator Rivers region of the Northern Territory to *C. crassiforceps*. However this is incorrect. Specimens identified *as C. magnivalva* in Australia, Fiji and Tahiti, differ from those identified as *C. crassiforceps* from Japan by a fixed inversion in arm E and more complex changes in arm G. They also differ in mt*COI* sequence.

A possible point of difference in the morphology of the males is that, while the gonostyle of *C. magnivalva* narrows evenly to the distal end, that of *C. crassiforceps* appears to remain the same width for most of its length and then rounds off.

Usually found in shallow waters, pools or the littoral of lagoons.

Molecular Data:

mt*COI* AF192212 (as *C. crassiforceps* but now corrected)

mt*cytB* AF192181 (as *C. crassiforceps* but now corrected)

Found:

Australia - Townsville (Type locality).

Also: Motu Kö. Pukapuka, Cook Islands (-10.88°S, 165.51°W) (E.H. Bryan Jr., Bishop Museum).

Micronesia – numerous specimens from S. Mariana Is., Caroline Is., Caroline Atolls, Marshall Is., Gilbert Is. (misidentified as *C. crassiforceps* by Tokunaga (1964).

Cytologically identical material has been collected from Lautoka (-17.67°S, 177.50°W) and Laucola Bay (-18.25°S, 178.33°W), Viti Levu, Fiji and Punaauia, Tahiti (-17.53°S, 149.57°W).

Chironomus pallidinubeculosus Tokunaga 1964

Synonyms:

Chironomus calipterus - misidentification in Bugledich et al. 1999., and other authors. *Chironomus kiiensis* Tokunaga 1936- incorrect synonymy by Hashimoto *et al.* 1981.

In BOLD Bin: BOLD: AEB0699

Adult

Characterized by the patterned wings. This pattern is very similar to those of other patterned wing *Chironomus* species (see below)

Initially considered to be *C. calipterus*, because of the similar wing pattern. However, cytological examination of specimens from Israel, indicated that the two species were distinct, with the Israeli, and presumably African, chromosome arm combination belonging to the thummi-cytocomplex, unlike this species, which belongs to the pseudothummi-cytocomplex.

The wing pattern is also similar to that of *C. striatipennis*, but the adults of that species are darker, and they differ in mt*COI* sequences.

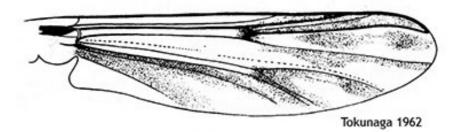


Tokunaga's original description (1964):

40. Chironomus (Chironomus) pallidinubeculosus Tokunaga, n. sp.

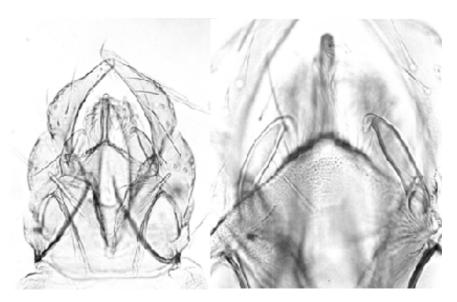
Rather large yellow species, wings with well-developed gray clouds and seams closely resembling African calipterus Kieffer; leg marking, value of LR, and structure of male hypopygium also similar to those of calipterus. Frontal tubercles large; AR 2.82-2.94, female antenna with last segment much shorter than preceding two together; scutum with yellow vittae on white ground color, lateral scutal vittae each with slender fuscus stripes on both sides, median scutal vittae each with similar fuscus stripe only on outer side in male and uniformly brownish in female. Legs with femora each with narrow preapical pale brown ring, LR 1.78-1.94. Wing with anal lobe well developed, gray clouds and seams rather distinct.

Male: Body 4.2 mm. long; wings 2.07-2.35 mm. by 0.53-0.64 mm. Head pale brownish yellow, with mouthparts pale brown, eyes separated above by about one-fourth length of eye, frontal tubercles large, subcylindrical and as long as about two or two and one-half facets together; palp pale brown and five-segmented (13.3: 13.7: 41.7: 41.3:64); AR 2.89 (2.82-2.94), scape yellowish brown, other segments and plumose hairs brown, last segments pointed at apex. Thorax mainly white, scutum with four yellow vittae, fuscus slender stripes on both sides of lateral vitta and only outside of median vitta, anterior part of scutum, just behind head, pale fuscus, postscutellum pale brownish yellow, pleural sclerites beneath wing base pale brownish, sternum yellow, scutellum with six bristles along caudal margin. Legs mainly yellow or yellowish white, coxa somewhat brownish, femur with narrow pale-brown ring on preapical part, tarsal segments with apical end brown, last one or two tarsal segments unitormly brown; pulvilli large, LR 1.85 (1.78-1.94), RL-FT 82.7: 64.3. Wing (fig. 10, a) with anal lobe well-developed, pale gray clouds and seams rather distinct but ill-defined, main veins white, but r-m and fR dark, fMCu under r-m, RL-V 70.7: 43.7: 79.3: 76.5. Halter white. Abdomen mainly pale brown or yellowish pale brown, tergites 1 to 6 slightly fuscus; hypopygium (fig. 10, b) with anal point slightly beyond tip of ventral appendage, oblong at apex and curved downward, style normal, dorsal appendage with basal area subtriangular, setigerous and pubescent, apical bare projection almost straight, slightly curved and not painted at tip, ventral appendage almost straight, with 10 to 11 curved apical bristles, some unequally bifid at tip.



Male:

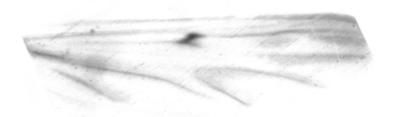
In details below, values for Micronesian specimens are followed by the range for Australia, given in brackets. Note that the Australian specimens were raised from larvae in the laboratory at a temperature of 20°C and so some measurements and ratios may be different from those of Tokunaga's wild-caught adults.



Male hypopygium of Australian specimen of *C. pallidinubeculosus* (left) and superior appendage (right)

Wings with dark spot over the crossvein and with obvious dark clouds and seams, particularly in cell R5. 19.2 (17-22) setae in squamal fringe, 2 SCf on brachiolum. Haltere pale.

Wing length 2.07-2.35 (2.44-2.82) mm; width 0.53-0.64 (0.60-0.68) mm; VR 1.04-1.10 (1.02-1.07).



Wing of an Australian female specimen

Face yellowish brown, antennae and palps brown. AR about 2.82-2.94 (3.00-3.13). FT quite large, about 30-60 (15-56) μ m long and 10-15 μ m wide, 1-4 times longer than wide. Abt. 16-33 (11-19) clypeal setae. Palpal proportions (μ m): 40 (45) : 40 (48) : 136 (143) : 136 (161) : 200 (234); P5/P4 1.24 (1.37-1.48); P5/P3 1.24 (1.58-1.79).

Thorax yellowish green with brown stripes, lateral stripes darker along the medial edge, and ending in a darker spot; postnotum and sternopleuron brown.

Setae (only Australian data available): acrostichals - 9–11; dorsocentrals - 9-11; prealar - 4-5; scutellar in two rows – 2-3 in anterior row, 6-9 in posterior row (total 8-12).

Legs yellow, femur with a dark band just before the knee; tarsal joints darkened. LR 1.78-1.94 (1.59-1.94).

Leg lengths (micron) (Australia, n = 3) and proportions as follows:

	Fe	Ti	Ta1	Ta2	Ta3	Ta4	Ta5	LR	F/T	BR
PI	1000	915	1470	800	580	480	234	1.59-1.63	0.97-1.20	2.1-3.0
PII	1045	1050	600	353	265	190	138	0.52-0.63	0.98-1.00	-

DIII	1150	1205	005	(00	157	272	172	0.75.0.00	0.01.0.02	
PIII	1150	1285	985	600	45/	2/3	1/3	0.75-0.80	0.91-0.93	-

Ant Ta5 about 0.26-0.30 (0.32) length of Ti. About 7 sensilla chaetica on mid Ta1, and perhaps 5 on hind Ta1.

Abdomen brown, no obvious bands on the segments. 14 (5-6) setae near centre of TIX probably in a single pale area. SVo essentially an E-type, perhaps closest to fig. h of Strenzke 1959, but end more sharply curved. IVo reaching about to end of the anal point. Gonostylus abruptly narrowing at distal third.

Female (Micronesian):

40. Chironomus (Chironomus) pallidinubeculosus Tokunaga, n. sp.

Female: Body about 3.12 mm. long; wings about 2.13 mm. by 0.65 mm. Coloration somewhat more brownish than in male, but structures mainly as in male with usual sexual differences. Head brown, with mouthparts dark brown, eyes separated above by one-fifth length of eye; palp five-segmented (10: 10: 32: 40: 55); antenna fuscus pale brown, last segment dark, neck parts of intermediate flagellar segments little shorter than one-half of segments, six-segmented (17: 40: 29: 31: 29: 41). Thorax mainly pale yellow, median scutal vittae uniformly brown or pale brown, postscutellum brown, sternum pale brown, scutellum with six bristles along caudal margin and two small accessory setae on anterior part, RL-FT about 70: 55. Wings with anterior veins pale brown, RL-V about 63: 50: 85: 70. Abdomen, including cerci, uniformly pale

Original description of C. pallidinubeculosus female from Tokunaga 1964

Additional data from Paratype female:

Wing length 2.60 mm, width 0.73 mm, VR 1.11. LR not available.

Antennal proportions (μm): 142 : 99 : 114 : 91 : 165 . Cephalic tubercles about 390 μm.

Palpal proportions (segs. 2 - 5) (μm): 40 : 136 : 136 : 170; P5/P4 1.25-1.38; P5/P3 1.38-

1.75. 14 clypeal setae.

thoracic setae: acrostichals - 14; dorsocentrals - abt 22; prealar - at least 2; scutellar in two rows - 5 in anterior row, 8 in posterior row.

2 SCf on brachiolum of wing.

Leg lengths (µm) and proportions as follows:

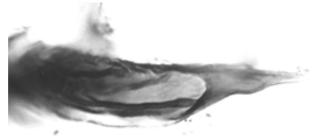
	Fe	Ti	Ta1	Ta2	Ta3	Ta4	Ta5	LR	F/T	BR
PI	1040	860	1	-	ı	-	-	ı	1.21	1
PII	1060	1060	600	320	250	170	110	0.57	1.00	-
PIII	1220	1320	-	-	-	-	-	0.92	-	-

Pupa: (based on Australian specimens) Length about 6.7-7.0 mm, posterior margin of wing case about 1.35-1.60 mm.

Head: Cephalic tubercles about $63-79~\mu m$ long and about $40-67~\mu m$ wide at the base, with a subterminal seta about $40-57~\mu m$ long. Small frontal warts present, about $9-15~\mu m$ high and $18-38~\mu m$ wide.

Thorax: Basal scar of respiratory horn with edge thicker at the anterior end, and pinched at the centre, about $113-116 \mu m$ long and $49-57 \mu m$ wide; HR 2.1-2.4. There are a number of small pits (2–4) immediately anterior to the basal scar, and a large, possibly muscle scar just posterior to it.

Abdomen: About 61--75 recurved hooks on posterior margin of segment II, the hook row covering about 60--85% of the width of the segment. Pedes spurii B on segment II, and larger on segment III; pedes spurii A on segment IV abt $107 \times 93 \, \mu m$ and occupying about 0.32 of segment length; that of segment V abt $76 \times 38 \, \mu m$, and that of segment VI is very small with spinules. Only 3 L-setae seen on segment III, while the last one on segment IV is at the junction with segment V. Segment VIII with two pairs of larval ventral tubules; caudolateral spur with one main spine and sometimes with 1 or 2 smaller ones (ave. 1+1). Anal lobe with about $60.3 \, (59\text{-}62)$ taeniae on each side, mostly in a single row but multiple rows at posterior end.



Fourth instar larva: (based on 3 Australian specimens) A medium sized (8.0 (male)-10.5 (fem.) mm) plumosus-type larva. Gula pale or darkened on posterior third; FC slightly dark or dark. Ventral tubules moderately long (ant. 0.84-1.36 mm; post. 0.76-1.48 mm) shorter in the male, relative length of anterior and posterior pair variable.

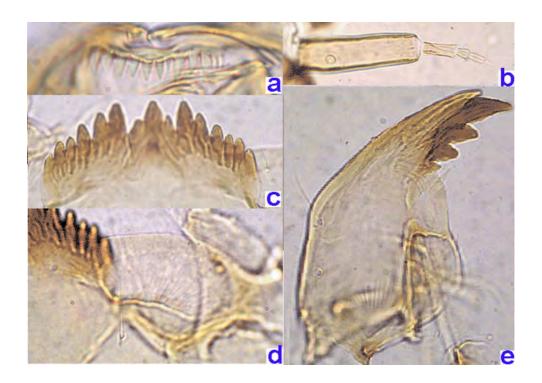
Anal tubules of the male larva about 220 µm long and 2.4 times longer than wide.

Mentum (c, below) with c2 teeth of central trifid tooth well separated from c1 tooth (type III), 4th laterals slightly reduced (type I).

PE (a, below) with about 16-21 variable but sharp teeth. Ventromental plates (d, below) abt. 168 µm wide, separated by about 0.32-0.38 of width of mentum; with about 31 (29-32) striae; VMR 0.25. Prm with the two teeth about equal in length, and coming to relatively fine points, inner tooth about 1.3-2.5 wider than the outer tooth (Ty. B1).

Antenna (b, below) with a moderately long basal segment, which is about 4.04-4.05 times as long as wide; RO about 0.3-0.4 up from base; AR about 2.02-2.05. Antennal proportions: 115:27:10.5:13:6.

Mandible (e, below) with third inner tooth only slightly darkened (Type I-IIB), and with about 13 (12-14) furrows on the outer surface at the base; also reported to have a double bulge on the inner contour; MTR about 0.33.



Cytology: 4 polytene chromosomes with the pseudothummi arm combination AE, BF, CD, G. Nucleolus virtually terminal in arm G, with very large BR adjacent, separated by only 4-5 bands; normally closely paired, but NOR and BR region may be unpaired. No nucleolus in long chromosomes. Polymorphism in arm F seen in Australian specimen examined.

pnbA1: 1 - 2c, 11 - 7, 4 - 6, 2d - 3, 12 - 19 as *striatipennis*

pnbB1: Puff near the middle of the arm with the dark bands proximal.

pnbC1: Constriction (groups 3 and 4) about one quarter from distal end.

pnbD1:

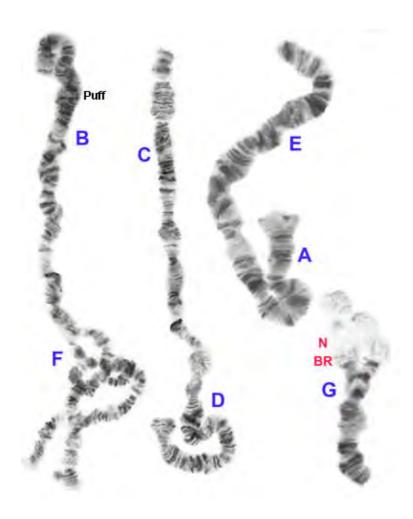
pnbD2: a simple inversion of about 1/3 of the arm towards the distal end.

pnbE1: 1 - 13 as piger, striatipennis.

pnbF1: 1 - 2a, 10 - 6, 15 - 11, 2b - 5, 16 - 23 i.e. from *oppositus* F1 by Inv15-5

pnbF2: A simple inversion of about the distal half of the arm

pnbG1: Virtually terminal nucleolus with adjacent BR.



Found: Micronesia - Ulimang, Babelthaup Island, Palau Islands (Type locality).

Australia - Queensland - Noosaville.

Molecular

mtCOI: There is mtCOI sequence in BOLD.

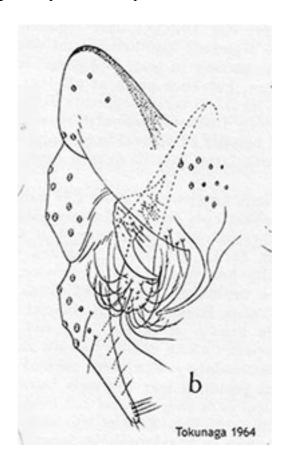
Chironomus preapicalis Tokunaga 1964

Male:

Body about 6.96 mm long, wings abt. 3.19 mm long and 0.9 mm wide. Head entirely brown, FT cylindrical, as long as the width of two facets. Palps five segmented, proportions 15: 15: 65: 83: 90; P5/P4 1.08, P5/P3 1.38.; antenna with scape yellowish brown, flagellum and plumose hairs brown, AR about 3.15. Thorax mainly pale brownish yellow, mesonotum white, vittae yellow and fuscous just behind the head, postnotum brownish on anterior part and yellow on caudal part. Scutellum with 24 small anterior setae and 13 larger posterior setae (total 37). Legs with coxae and trochanters pale brownish yellow, other segments mainly yellow.; all femora with small preapical dark rings, fore tibia with sub-basal broad dark band. LR about 1.58.

Wing with veins pale brown, r-m dark and covered by a small dark spot, VR about 1; 3 Scf on brachiolum, about 23-24 setae on squamal fringe. Haltere white.

Abdomen very pale brown with T-shaped fuscous basal bands on segments II-VI; TIX illustrated as having about 11 setae anterior to anal point. Hypopygium pale brown with anal point slender and curved ventrad, IVo reaching about to end of anal point, setae finely plumous at ends; SVo closest to D(f) of Strenzke (1959). Gonostyle moderately swollen and reduces gradually over about posterior half.



Female:

Body length about 6.16 mm; wings 3.16-3.9 mm, width 0.96-1.26 mm. Coloration essentially as in male but vittae pale brownish yellow, abdomen with tergal spots triangular.

Palp segments 20.3 : 23.3 : 77 : 86.7 : 132; P5/P4 1.52, P5/P3 1.71. Antenna with scape yellowish brown, flagellum yellow but last segment and neck parts of penultimate two segments fuscous, six segmented (26.7 (pedicel) : 66 : 46.3 : 54.7 : 44 : 81.7), neck parts of intermediate flagellar segments about one half of segments: (AR 0.39; A5/A1 1.24).

Scutellum with more setae than in male. RL-FT 148.7; 122.3. Wing with RL-V 98: 84.5: 135: 103.5.

Possible specimens have been collected in Fiji: Males:

Colouration: Face yellow-brown, antennae brown, palps light brown. Thorax yellow brown (although may be faded from green) Thoracic vittae dark along edges but pale anteriorly on central, and in middle of all three vittae. Postnotum and sternopleuron brown but darker at posterior postnotum. Legs pale with joints somewhat darkened. Abdomen greenish with saddle spots on segments III-VII. Terminal segments somewhat brownish. Wings pale, anterior veins somewhat darker distally; crossvein slightly darker. Haltere yellowish green.

Head: AR abt 3.21-3.23. FT present, about 15-37 μ m long and 6-12.5 μ m wide at base (2.4-2.8 times longer than wide). Postocular setae in a double row, reaching to the dorsal apex of the eyes. Palpal proportions: 60 : 47 : 231 : 232 : 380; P5/P4 1.51-1.78, P5/P3 1.59-170. 18-23 clypeal setae.

Wing length 2.84-2.96 mm, width 0.67-0.68 mm; VR 1-102; 2-3 Scf on brachiolum, 19-24 setae on squamal fringe.

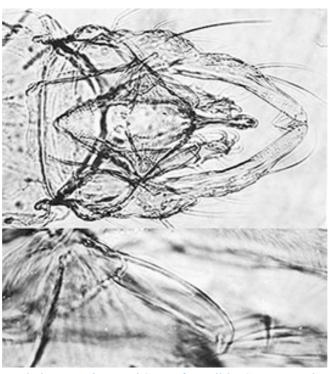
Thoracic setae: at least 13 acrostichal; 18-19 dorsocentral in 2 rows; 3-4 prealar; 1 supraalar; Scutellum anteriorly with 5+4-15 setae in two indefinite rows, 12-13 setae in posterior row (total 22-27 setae).

Legs: Anterior tarsi without a beard.

Leg lengths (micron) and proportions:

	Fe	Ti	Ta1	Ta2	Ta3	Ta4	Ta5	LR	F/T	BR
PI	1175	1035	1650	875	820	695	275	1.57-1.62	1.11-1.17	1.53-1.54
PII	1230	1115	700	395	285	160	120	0.63	1.08-1.12	-
PIII	1350	1355	990	510	400	235	150	0.73-0.74	0.97-1.03	-

Setae on TIX: 11-12 in individual spots.



Male hypopygium and SVo of possible C. preapicalis

Anal point narrow at base and widening at tip; IVo almost to end of anal point; SVo closest to D(f) type of Strenzke 1959. Gonostyle only moderately swollen and narrows gently over posterior half, with 1+3-6 setae at tip.

Localities:

Holotype male (US66555), Ngarsung, Airai, Babelthaup I., Palau. Allotype female, Ulimang, Babelthaup I, Palau.

Found: Kolonia, Yap I; Agric. Expt. Sta., Colonia, Ponape; all Micronesia. Fiji: Nadi (-16.33°S, 179.50°E), Viti Levu.

Chironomus samoensis Edwards 1928

Tokunaga's (1964) description of *C. samoensis* seems to be the most reliable description of the adult with much more information than in the original description.

Chironomus (Chironomus) samoensis Edwards (fig. 12, a). Chironomus samoensis Edwards, 1928, Insects of Samoa 6 (2):67. Chironomus dorsalis, Tokunaga, 1940, Philippine Jour. Sci. 71:220. Chironomus eximius Johannsen, 1946, B. P. Bishop Mus., Bull. 189:193.

Large yellow species, scutal vittae yellow; legs yellow, but all tarsal segments apically black or brown; frontal tubercles cylindrical or oblong; AR 2.7-3.09; L,R 1.75-1.92, in female fore tarsus with segment 4 far longer than 3 and slightly longer than 2; wing with fR and r-m usually more brownish or fuscus than other veins; abdomen pale brownish yellow or yellow, tergites of basal segments 2 to 6 of male and 2 to 4 of female with round or rhombic pale fuscus spots; male hypopygium of dorsalis type.

Male: Body about 4.5 mm. long; wings 2.2-2.3 mm. by 0.59-0.61 mm. Almost entirely yellow. Head with mouthparts pale brownish yellow, eyes separated above by one-fourth length of eye, frontal tubercles subcylindrical and slightly shorter than width of two facets; palp five-segmented (about 15.7: 14: 62.7: 69.3: 100); antenna with scape yellowish brown, other segments brown, plumose hairs very pale brown, AR 2.86 (27-3.09). Thorax mainly yellow, scutum white, with vittae yellow, scutellum white, with 9 to 10 bristles along caudal margin and seven to nine small setae on anterior part, postscutellum faintly fuscus on middle part. Legs yellow, only dark or brown at distal ends of all tarsal segments; LR 1.81 (1.75-1.84), RL-FT 85: 70. Halter yellowish white. Wing with fR and r-m usually somewhat fuscus, fMCu under origin of r-m, RL-V 72: 49.5: 81.5: 75.2. Abdomen pale brownish yellow, basal segments 2 to 6 with oval or rhombic faint spots on tergites; hypopygium (fig. 12, a) of dorsalis type, anal point rather large, style normal, dorsal appendage setigerous (with eight to nine setae) on basal part and bare caudal projection stout and subtriangular, ventral appendage stout, with 12 to 18 strong apical bristles, some of these bristles bifid or trifid apically.

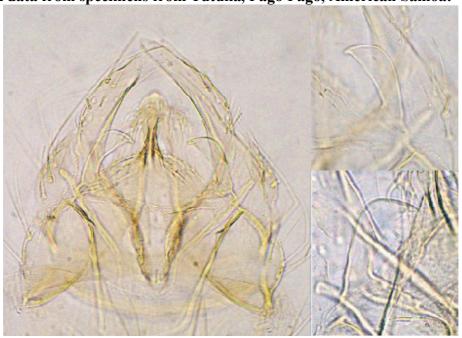
Female: Body 4.92 (4.68-5.07) mm. long; wings 2.88 (2.52-3.12) mm. by 0.84 (0.78-0.91) mm. Similar to male in color and structure with usual sexual differences. Head with eyes separated above by one-sixth length of eye, frontal tubercles oblong; palp five-segmented (13.5:12.5:57.5:70:87); antenna with scape and basal two-thirds of segment 2 yellow, other parts fuscus, neck parts rather long, six-segmented (22:49.5:38:39.5:37.3:55.3). Scutellum with 13 to 14 bristles and 8 to 11 small setae. LR 1.86-1.92, RL-FT 110:86.5, RL-T 163.5:84.5:81.5:88.5:38.5. Wing with fMCu under or just beyond origin of r-m, RL-V 85.3:67.3:110:92.7. Abdomen yellow, with tergal oval faint fuscus spots on segments 2 to 4.

Specimens from Micronesia are probably *C. samoensis*, and the illustration is presumably intended to depict the somewhat beaked SVo seen in some specimens. The misinterpretation of this illustration may be partly responsible for the identification of *C. samoensis* in other locations, which have an S-type SVo. However, they also differ in other characters and are mis-identifications.

While the females are largely dismissed as "like the male apart from the usual sexual differences", the relative lengths of the fore leg segments appear to be useful in separating the species of this group.

Tokunaga makes the important point that the fore tarsus has Ta4 far longer than Ta3, and slightly longer than Ta2, although examination of a pharate female from Tutuila, American Samoa, suggests that Ta2 and Ta4 can be about equal in length.





Male hypopygium of *Chironomus samoensis* (left), superior volsella (right) Note the appearance of a 'beak' in the SVo of left hand figure.

Male

Head: AR - 2.94 (2.51-3.23, 4); FT 33 μm (29-38, 4) long and 15μm (14-17, 3) wide; palpal proportions (micron) 46 : 46 : 193 : 234 : 354; P5/P4 1.51, P5/P3 1.83. Clypeal setae 17-23.

Thoracic setae: Acrostichal - at least 14 or 15; Dorsocentral - 17-21; Prealar - 4-5; Scutellar in two rough rows, ant. 5-12, post. 12-15 (total 17-25).

Wing length 2.58 mm (2.40-2.68, 4), width 0.63 mm (0.60-0.66, 4), VR 1.03 (1.02-1.04, 4).

Legs, pale, tarsi slightly darker.

Relative length of leg segments (micron) (4):

	Fe	Ti	Ta1	Ta2	Ta3	Ta4	Ta5	LR	F/T	BR
PI	1107	1000	1507	810	750	670	330	1.50-1.52	1.08-1.12	1.54-1.75
PII	1290	1155	720	380	270	175	120	0.62	1.12	-

Abdomen pale, with darkening as described by Edward. Hypopygium (above) similar to that of *C. dorsalis*, with the SVo of the D type, similar to fig. e of Strenzke (1959), but sometimes with the development of a beak. The IV has mainly simple, curved setae, but a small number appear to have a small simple fork near the tip. About 4-6 setae on the 9th tergite near the base of the anal point.

Female:

No females are available amongst the material, but some characters could be obtained from a pupa with a pharate female. An important character is the relative proportions of the fore leg, particularly the tarsi, as Tokunaga (1964) notes that the Ta4 of specimens he assigned to *C. samoensis* was unusually long. The approximate lengths of these segments were measured (in micron) as:

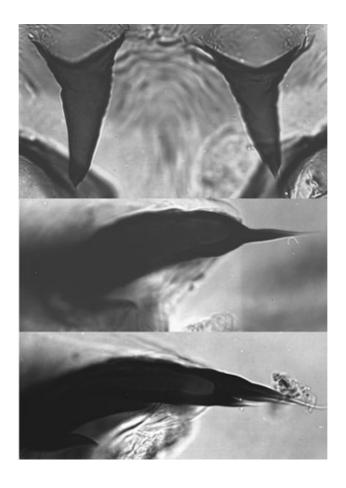
Fe 900; Ti 750; Ta1 1020; Ta2 620: Ta3 470: Ta4 610: Ta5 340; Ta4 about same length as Ta2, about one third longer than Ta3, and 0.81 of the Ti length. Other characters:

Head - FT - length $23\mu m$, width $13~\mu m$, 1.8 times longer than wide. Antennal segments (μm) 144:109:116:106:215. About 24 clypeal setae.

Thoracic setae: Acrostichal 14, Dorsocentral 31, Prealar 5, Scutellar in two rows, ant. 14, post. 14 (total 28).

Pupa:

Exuviae length (male) 6.8 (6.5-7.0, 3) mm., IMW about 1.34 (1.27-1.42, 3) mm (male). Pale, with darkened caudolateral spurs. Cephalic tubules 93 (76-115, 4) μ m long and 78 (56-94, 4) μ m across the base, subterminal bristle about 68-80 μ m in length. Basal ring about 142 (129-164,5) by 68 (54-85,5) μ m. About 67-77 hooks in row on segment II. Slight development of PSB on segment II, PSA largest in segment IV, reducing in segments V and VI. Caudolateral spur of segment VIII about 180 µm and 1-3 spines. About 78-88 taeniae on each side of the anal lobe of male.



Fourth instar larva:

A medium sized plumosus-type; length about 12.5-12.7 mm (female) and 10.8-11.8 mm (male); TLt about 280-360 μ m; VT relatively long (anterior 1.76-2.16 mm; posterior 1.80-2.68 mm), posterior pair longer and coiled; AT moderately long (about 1.6-2.6 times longer than wide), dorsal pair (240-410 μ m) slightly longer than ventral pair (215-370 μ m). Head capsule pale with darkening of the posterior half of the gula, FC sometimes pale but mostly with slight darkening, ventral head length 261-318 μ m. Distance between antennal bases greater than the distance between the S4 setae.

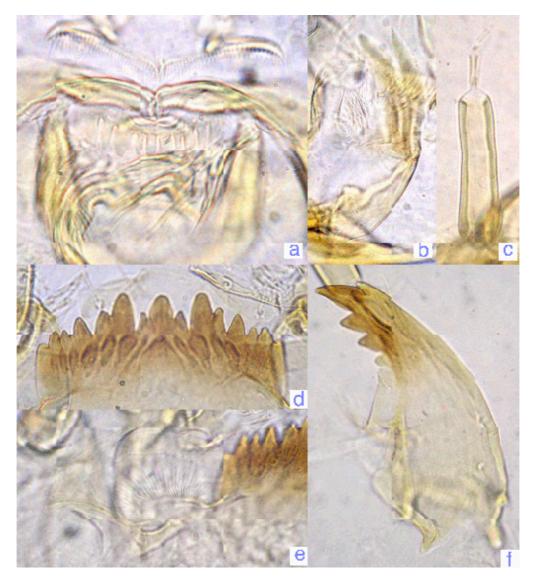
Mentum (Fig. d, below) wider than usual, about 0.6 of ventral head length; centre trifid teeth with c2 teeth well developed (essentially type IV); 4th laterals reduced to about the level of 5th laterals (type II), 6th lateral variable, sometimes arising at same level as other laterals but generally appearing to be at a slightly lower level, apparently due to wear.

Ventromental plates (Fig. f, below) separated by about 35-41% of the width of the mentum; each with about 32-35 striae; VMR about 0.36. PE (Fig. a, below) with about 13 (10-16, 8) sharp pointed teeth.

Premandible (Fig. b, below) with sharp teeth, outer tooth shorter than inner tooth, which is about twice as wide as the outer tooth.

Antenna (Fig. c, below) with moderately long A1, almost 4 times longer than wide, RO between 0.4 and 0.5 up from the base of the segment; relative length of antennal segments (micron) 110: 24: 6: 11: 7; AR 2.03-2.30.

Mandible (Fig. f, below) about 208-228 mm long, with 3rd inner tooth relatively pale and only partly separated (type IIA), about 13 (12-14,8) furrows on outer surface at base, PMa sparse, with about 8 (7-10,5) setae.



Larval head capsule characters of C. samoensis

Cytology: 4 polytene chromosomes with the pseudothummi arm combination AE, BF, CD, G.

Nucleolus medial in arm G; two Balbiani rings distal to the nucleolus.

A further nucleolus at about group 20 of arm F and there is a large puff in arm C that might also be a nucleolus.

All chromosomes closely paired. No polymorphism in the available specimens.

samA1: 1 - 2c, 10 - 12, 3 - 2d, 9 - 4, 13 - 19 as holomelas

samB1: Puff of group 7 near distal end of the arm with dark bands proximal to it.

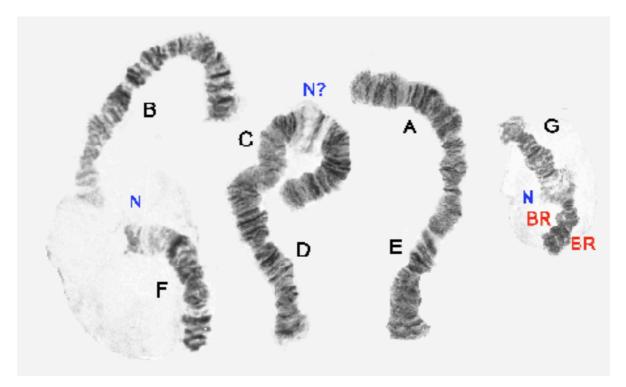
samC1: Characteristic groups 3-4 just proximal to the large puff.

samD1:

samE1: Groups 11-13 near centromere.

samF1: Groups 20-23 near centromere, with nucleolus about group 20.

The polytene chromosomes of *C. samoensis* also differ from those described for the others species in the group. The arm combination is pseudothummi-cytocomplex, as in the other species, but the most obvious difference is that the nucleolus in arm G is near the middle of the arm, rather than almost terminal. There is a second nucleolus near the diagnostic bands of arm F, and generally a large puff, which may be a nucleolus, near the middle of arm C.



Polytene chromosome complement of *C. samoensis*

Diagnosis

Based on these descriptions, diagnostic features of the species are: FT relatively long; LR about 1.50-1.52, fore Ta5 about one third of the length of the fore tibia, SVo of the D-type, or "beaked"; in female fore Ta4 longer than Ta3 and about the same length as Ta2. In larva, antennal segment 3 relatively short, usually shorter than A5. In the polytene chromosomes, the nucleolus in arm G is median, and there is a further nucleolus about region 20 of arm F and usually a large puff in arm C.

Found: Type localities - Apia, Western Samoa; Faratogo, Tutuila (now American Samoa); Tonga.

American Samoa - Mapusaga, Tutuila.

Micronesia – South Mariana Is.; Palau, Yap, Truk, Ponanpe, Kusaie, , Caroline Atolls, Marshall Is.(Tokunaga 1964)

Chironomus vitellinus Freeman 1961

Synonyms:

Incorrectly placed as a synonym of *C. javanus* by Chaudhuri *et al.* 1992 and Tokunaga 1964

Yamamoto (2002) has suggested that this species (as *C. javanus*) should be in a separate subgenus *Austrochironomus*.

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as C. javanus, but most specimens are actually C. vitellinus.

Adult:

This is rather variable species across its wide distribution. This is likely due to different selection pressures in different habitats but also. in some cases, to genetic drift if island populations are re-established by a small number of founders.

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

There are a male and female paratype from Mafulu, PNG:

Paratype male from Mafulu, Papua New Guinea (BM 1933-427):

AR: 3.18

Cephalic tubercles about 30 μ m; clypeus about 0.5 of antennal pedicel diameter, with 14 setae. Postocular setae in multiple rows, reaching almost to the midline of the head

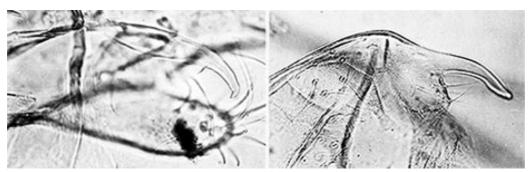
Palpal proportions (segs 2-5 (μm)) 60 : 170 : 200 : 140 (shrivelled).

Thoracic setae: 6-7 dorsocentral; prealar 4; supraalar 1; others not evident.

Leg lengths (micron) and proportions:

Male	Fe	Ti	Ta1	Ta2	Ta3	Ta4	Ta5	LR	F/T
PI	1220	1040	-	1	-	ı	-	-	1.17
PII	1300	1140	800	420	280	200	140	0.70	1.14
PIII	1420	1440	1140	620	460	280	160	0.79	0.99

10 setae on TIX. Setae on IVo simple.



SVo and IVo (left) and lateral view of anal point of paratype male of C. vitellinus

Further descriptions:

Male

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 the AR as about 4.5, but in other populations the AR is lower: 3.34 (2.89-4.5).

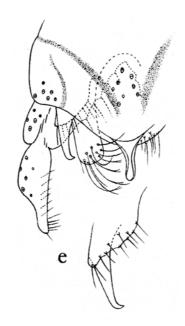
Head: FT 43.7 (30-55) μ m long and 2.4-2.6 times longer than wide. Palpal proportions (micron): 49: 46: 157.5: 191: 272: P5/P4 1.45 (1.25-1.74); P5/P3 1.79 (1.40-2.17). Clypeus about 0.60 (0.47-0.75) of antennal pedicel, with about 18.2 (11-23) setae.

Thoracic setae: acrostichal at least 11-14; 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	1245	970	1480	895	715	740	340	1.56-1.93	1.11-1.27	0.46-1.60
PII	1215	1085	715	370	270-	180	135	0.64-0.70	1.10-1.17	-
PIII	1325	1345	1075	575	425	270	165	0.77-0.83	0.95-1.00	-

Tergite IX with 6.3 (5-9) setae in individual pale patches.

Hypopygium with narrow anal point expanded at distal end, strongly turned down and narrow in lateral aspect (see above); SVo well developed and curved, perhaps closest to E(g) of Strenzke (1959); IVo 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 but narrows conspicuously over posterior third to half, with 5+1 setae at the tip.



Male terminalia from Tokunaga 1964

Female

Freeman's (1961) description of *C. vitellinus* – 'Resembles male; sensory hairs on apical antennal segment longer than usual.'

There is a paratype female from Mafulu, Papua (BM 1933-427):

Wing length 3.16 mm, width 0.90 mm, VR 1.11; 3 SCf on brachiolum.

Head: Antennal segments (micron): 180 : 140 : 140 : 130 : 220; AR 0.37, A5/A1 1.22.

Palpal segments (2-5)(micron): 60 : 160 : 220 : 140 shrivelled). Clypeus about 1.2

times the diameter of the antennal pedicel, with abt 16 setae.

Thoracic setae: abt 14 Acrostichals; Humerals + Dorsocentrals 12-13; Prealar 6; Supraalar 1; Scutellars in 2 rows, abt 6 in anterior row, 8 larger in posterior row (total 14).

Leg lengths (microns) and proportions as follows:

BM1933-427)	Fe	Ti	Ta1	Ta2	Ta3	Ta4	Ta5	LR	F/T	Ta4/Ti
PI	1345	950	1715	975	825	875	390	1.80	1.16	0.91
PII	1320	1240	780	400	260	160	120	0.63	1.06	
PIII	1560	1580	1160	600	480	280	180	0.73	0.99	

Anterior Ta4 longer than Ta3.

Abdomen brown.

Other specimens (including those of Tokunaga 1964):

Wing length 2.70 (2.07-2.93) mm, width 0.81 (0.66-0.96) mm; VR 1.09 (1.07-1.11); 2–3 Scf on brachiolum; 15 (14-16) setae in squamal fringe.

Coloration essentially as in male.

Head: FT present but may be small (6 μ m long and equally wide). Antennal segments (micron) with percentage neck in brackets: 265 (27): 126 (46): 130 (49): 129 (49): 240; AR 0.37 (0.34-0.43); A5/A1 1.26 (1.07-1.45).

Palpal segments (micron): 55 : 54 : 206 : 220 : 366; P5/P4 1.6-1.7; P5/P3 1.8.

Clypeus heart-shaped, about 1.32-1.45 wider than antennal pedicel; abt 25 (20-31) setae.

Thoracic setae: Acrostichals – abt 10-12; Humerals – 3.6 (3-5), mostly linear but may be grouped (e.g. as a triangle); Dorsocentrals – 17.8 (11-25); 21.5 (16-28) including the Humerals; Prealars – 5.5 (4-7); Scutellars in two rows – 2.3 (0-6) in anterior row and 10 (6-12) in posterior row (total abt 6-18).

Leg lengths (microns) and proportions as follows:

Female	Fe	Ti	Ta1	Ta2	Ta3	Ta4	Ta5	LR	F/T	Ta4/Ti
PI	1475	925	1635	1025	875	885	355	1.69-1.84	1.55-1.63	0.94-0.97
PII	1270	1155	660	320	240	180	127.5	0.55-0.58	1.08	
PIII	1350	1470	925	455	400	265	160	0.58-0.63	0.92-1.08	

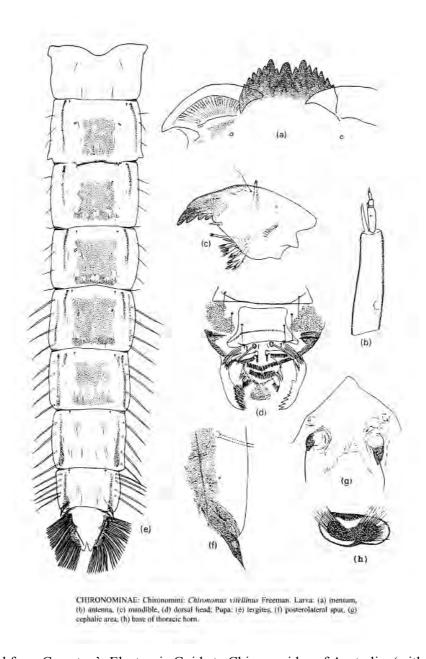
Anterior Ta4 longer than Ta3.

GcIX with abt 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 6.38 (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 FT. 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 70.29 (61-78) taeniae in two rough rows (particularly distally).



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Fourth instar larva: a medium sized (11.6 (9.2-13.7) mm, female; 10.8 (9.0-13.0) mm, male), essentially plumosus-type larva, although TLt (437 (320-800) μ m long are more ventrally placed than in other species. VT long, anterior pair 1.49 (0.8-2.48) μ m generally longer than posterior pair 1.28 (0.7-2.16) μ m. AT with median constriction, dorsal about 550 (406-600) μ m long and 3.4 times longer than wide; ventral 330 (220-440) μ m long and 3.67 times longer than wide. SAL (2 specimens) 92.25 (86-98.5) x 18.75 (15-22) μ m, 3.14 (2.6-3.7) times wider than deep.

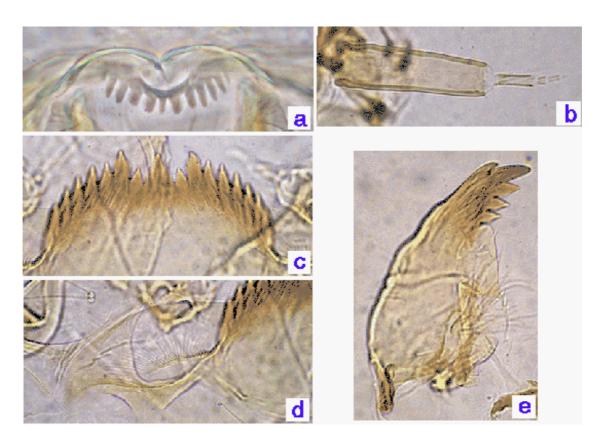
Gula pale or more commonly slightly darkened on posterior third, slightly wider than mentum width and widest at the posterior margin; FC pale. SAL about 78 (55-98) x 18 (15-20) μ m, and about 4.3 (3.1-5.3) times wider than deep.

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). One specimen from Sogeri had a mentum with only 13 teeth. PE (a, below) with about 13.9 (12-17) often irregular teeth (Type D). Ventromental plates (d, below) about 172 (159-183) μ m wide, 3.6-4.0 times wider than deep and1.1-1.2 times wider than the mentum; separated by about 0.37 (0.33-0.40) of the mentum width, with about 32.8 (29-36) striae.

Antenna (b, below) with the basal segment about 3.5 times as long as wide, about a third to a quarter of the ventral head length; AR about 2.56 (2.3-3.3); ratio of segments 111 : 25 : 4.5 : 7.5 : 5. RO about a fifth to a third up from base of segment.

Distance between S4 setae (143 μ m) larger than with between antennal bases (126 μ m); setae separated by about 0.7-0.9 of the frontoclypeal width at that point. S5 setae mostly posterior to the nearby RO, but occasionally about level.

Mandible (e, below) about 207 (202-216) µm long, with third inner tooth variably darkened and completely separated (type IIIA-C), with three spines on inner margin, and about 12-17 furrows on the outer surface at the base; Mdt/Mat about 24 (19-26.5), MTR 0.38 (0.26-0.44).



The larva is most readily recognised by the unusual premandible, which has 7 teeth (as opposed to *C. javanus* which has only 6) rather than the usual two.



Premandible of C. vitellinus larva with 7 teeth.

Some larval characters have also been illustrated by P.S. Cranston in his Electronic Guide to Chironomidae of Australia, as C. vitellinus. These are reproduced with the pupa above (with permission).

Cytology: 4 polytene chromosomes, possibly with the thummi arm combination AB, CD, EF, G, but Keyl arms very difficult to recognize. Nucleolus virtually terminal in arm G, with large BR near middle of the arm; closely paired. No nucleolus in long chromosomes.

vitA1:

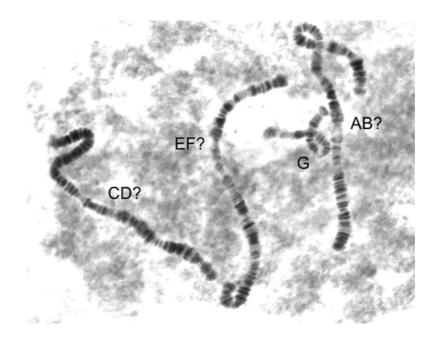
vitB1: Puff (gp. 7) about one third from distal end of the arm with dark bands distal.

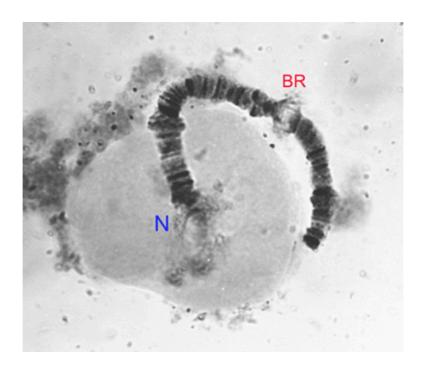
vitC1: vitD1:

vitE1:

vitF1:

vitG1: Nucleolus subterminal, BR about one third from other end; closely paired.





Molecular: The mitochondrial *COI* barcode sequence is available in GenBank (Accession number DQ648203) for a specimen from Japan.

It seems likely that the majority of specimens identified as *C. javanus* in the BOLD database are actually *C. vitellinus*.

The males are easily recognised by the unusual genitalia and the larvae by the multitoothed premandible.

The pupa is a fairly typical for *Chironomus* but the possibility of frontal warts is unusual. The female may be distinguished by the generally wider segment X (often semi-oval) and the fore Ta4 being longer than Ta3.

Found: Type locality - Darwin, NORTHERN TERRITORY.

Fiji: Nadi (-16.33°S, 179.50°E), Viti Levu ; Labasa (-16.33°S, 179.50°E), Macuata Province, Vanua Levu.

Papua New Guinea: Mafulu (1200 m) Lae-Goroka Road (-8.50°S, 146.00°E) (Paratype)

Micronesia: Caroline Islands and Marshall Islands (as *C. javanus*) (Tokunaga 1964) Broadly distributed through Indonesia, Japan and Pacific Islands. Also recorded at Blantyre, Malawi.

Chironomus species Fiji1 (Chironomus "harrisi")

Larvae and reared adult male from a channel with thick red gravel bottom, going under a road

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Adult:

Male; Wing length 3.0 mm, VR about 1; 3 Scf on brachiolum; squama fringed. Crossvein and posterior fork both darkened.

Head: AR 2.43; FT 50.5 μ m and 3.3 times longer than wide. Clypeus broader (1.06) than diameter of antennal pedicel; 34 clypeal setae. Palp proportions: 50 : 70 : 230 : 270 : 360 : P5/P4 1.33.

Thorax appears pale. Setae: Acrostichal at least 9; Dorsocentral 9 in about 2 rows; prealar - 6; supraalar - 1; scutellar 22, with about 9 in anterior row, 13 in posterior row. Leg lengths (micron) and proportions:

Pupa: Exuvia quite dark. Cephalic tubercles about as wide as long, subterminal setae about as long as tubercle. Spur of segment VIII with about 7 closely applied spines.

Fourth instar larva: Small bathophilus-type larva, 12.3-14.7 mm (female 14.2-14.7 mm; male 12.3.mm); anterior VT longer 0.64 (0.48-0.72) mm. than posterior pair 0.58 (0.48-0.62 μ m); dorsal anal tubules longer (260-361 μ m long and 110-150 μ m wide; 2.17-2.72 times longer than wide), than ventral pair (280-365 μ m long and 113-180 μ m wide; 2-2.7 times longer than wide). SAL about 86-88 wide and 20 μ m deep (4.25-4.4 times wider than deep). Head with pale gula and frontoclypeus. Mentum with central trifid tooth of type IIA or III, VM (Fig. d, below) width 190-209 μ m, about 3.2-3.6 times wider than deep; 1.2-1.27 times wider than mentum and separated by about 0.29-0.31 of the mentum width; about 34-43 striae; VMR about 0.22-0.30.

PE (Fig. a, below) with about 11-14 teeth, probably of type B when not worn. Premandible (Fig. a, below) probably of type B, of the normal *Chironomus*-type, clearly distinguishing it from *C. vitellinus*; outer tooth about 3-3.5 times wider than the outer tooth.

Antenna ((Fig. b, below) with A1 relatively short (0.38 of VHL); 3.2 times longer than wide, with RO about 1/3 up from base of segment; AR 1.71-1.74; A2 about 0.29 of A1; segment proportions (micron) 105 : 30 : 9.5 : 13 : 7.5.

Mandible (Fig. e, below) of type IA-B; length 200-216 μm; 17-19 furrows on outer surface near base; 9-11 taeniae in PMan; Mdt-Mat 30 (MTR 0.43-0.44).



Cytology: Four polytene chromosomes of the pseudothummi-cytocomplex – AE, BF, CD, G. No polymorphism in available specimens. Main nucleolus appears to be distal on arm F. Arm G closely paired with a nucleolus or large BR just anterior to middle of the chromosome. Other BRs may be developed near each end.



Tokunaga (1964) described 6 species from Micronesia that have dark spots over the crossveins, but none of these fit the characteristics of this species and so it is considered to be a new species.

Holotype: Adult male with pupal and larval exuvia from Wiaqele, Vanua Levu, Fiji, FVA.3.1 reared male 1 (8 September 1967), JM; Paratypes: larvae, same data as holotype. Other specimens: Skylodge Hotel, Nadi, Viti Levu, FVI.2.2 larvae reared from an egg mass (27 February 1971), JM.

The proposed species name is an acknowledgement to Howard and Merran Harris who provided accommodation and assisted with my field work on Vanua Levu.

Found:

Fiji: 3Km w. Waiqele (-16.55°S, 179.50°E), Macuata Province, Vanua Levu. Skylodge Hotel, Nadi (-18.13°S, 178.45°E), Viti Levu.

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