

## Appendix to paper by C. P. Madden, A. D. Austin, &amp; P. J. Suter - Pollution monitoring using chironomid larvae. What is a deformity? pp.89-94

## APPENDIX

Description of *Chironomus maddeni* sp. novJon Martin<sup>1</sup> and Peter S. Cranston<sup>2</sup><sup>1</sup>Genetics Department, University of Melbourne,  
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## Introduction

In the preceding study (Madden *et al.*, 1995), the investigated species of *Chironomus* evidently is undescribed. This judgement is based upon comparison with the cytology of all known species of Australian *Chironomus* by the senior author of this appendix contribution. Here we provide details of the cytology and morphology of all stages and provide a formal description of *Chironomus maddeni* as a species new to science.

*Chironomus maddeni* n.sp.

*Type-locality*: South Australia, Glen Osmond, pool in grounds of Waite Institute, University of Adelaide.

*Description*. Conforms in all stages with the generic diagnoses of Cranston *et al.* (1989; adult male), Pinder and Reiss (1986; pupa) and Pinder and Reiss, (1983; larva). All measurements in  $\mu\text{m}$  unless stated (as mm).

*Adult Male* (n=2) (Fig. 4a,b)

Body length 5.3-5.6 mm, wing length 2.4-2.5 mm, colour yellow to pale brown with mid-brown scutal vittae, scutellum, and pre-episternum. Abdomen pale, with brown, broadly diamond-shaped transverse area in basal half of tergites I-IV. Legs pale, unbanded.

Head. Without frontal tubercles, with 25-27 temporal setae in a continuous multiserial row from frontals to postoculars, 19-21 clypeals. Antenna with apical flagellomere 850-920 $\mu\text{m}$  long, basal 10 flagellomeres 320-355 long, Antennal Ratio 2.55-2.60. Palp segment 2-5 lengths: 50-65, 140-150, 180-205, 240-315.

Thorax with quite prominent scutal tubercle. Thoracic setation: acrostichals about 8, biserial, dorsocentrals 10-12 anteriorly uniserial, posteriorly biserial, 5 prealars, 12-14 scutellars. Wing with Venarum Ratio 1.03. Vein setation: R with 31, R<sub>2+3</sub> 27-29, R<sub>4+5</sub> 31-34; squama with 14-16.

Leg lengths and proportions as follows:

	Fe	Ti	Ta1	Ta2	Ta3	Ta4
PI	1045-1080	865-900	1510-1620	765-790	570-580	500-505
PII	1115-1225	1010-1045	610-615	325-360	250-320	175-180
PIII	1260-1295	1225-1295	900-935	505-540	360-395	215-220
	Ta5	LR	BV	SV	BR	
PI	215-250	1.75-1.80	1.69-1.70	1.22-1.26	2.0-2.5	
PII	105-110	0.58-0.60	2.92-3.20	3.47-3.70	3.0-4.0	
PIII	140-145	0.72-0.73	2.72-2.76	2.75-2.76	3.0-4.0	

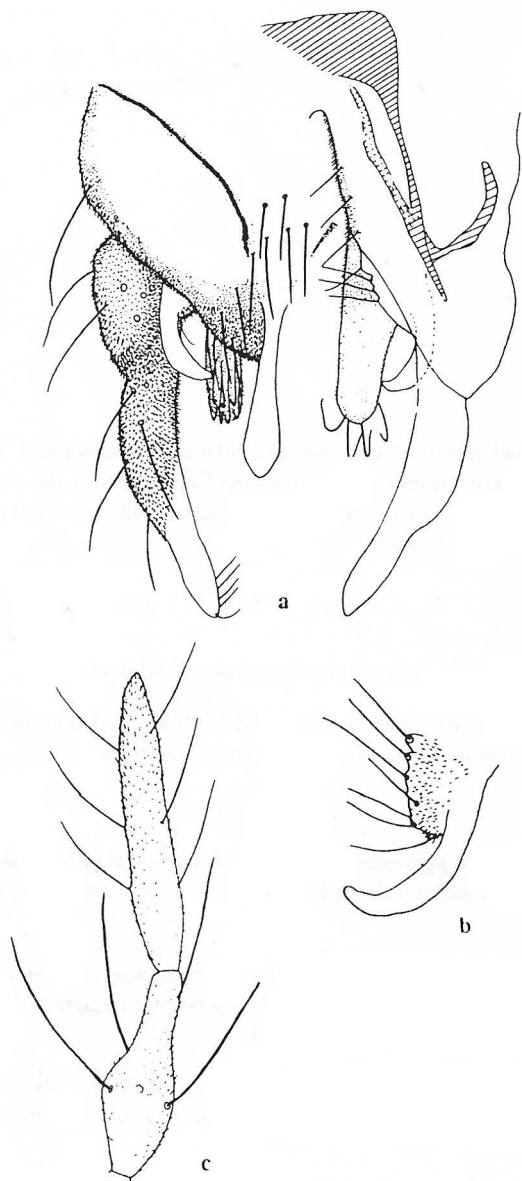


Figure 4. *Chironomus maddenii* sp. nov., adult: (a) male hypopygium; (b) superior volsella, (c) female antenna apex.

Sensilla chaetica: PII with 5-6 on subapex of  $T_{11}$ , PIII with 7-8 on subapex of  $T_{11}$ .

Hypopygium: see Figure 4a, b.

*Adult female* (n=2)

Body length 5.3-5.4mm, wing length 2.8-2.9mm, colour as male; abdomen less well marked than in male. Head. Without frontal tubercles, with 22+ temporal setae in a continuous multiserial row from inner verticals, 26 clypeals. Antenna (Fig. 4c) with apical flagellomere 400-420 long, basal 5 flagellomeres 156-158 long, Antennal Ratio 0.35-0.37. Palp segment 2-5 lengths: 52-60, 140-155, 190-210, 315-335.

Thoracic setation: anteprenotals absent, acrostichals c. 17, biserial, dorsocentrals 27-28 biserial, 6 prealars, 14-21 scutellars. Wing with Venarum Ratio 1.02-1.09; setation: R with 35-40, R<sub>1</sub> 32-35, R<sub>4+5</sub> 78-80, squama with 13-16.

Leg lengths and proportions as follows:

	Fe	Ti	Ta1	Ta2	Ta3	Ta4
PI	1190-1260	972-1010	-	-	-	-
PII	1290-1295	1190-1260	650-685	325-360	250-255	180
PIII	1405-1440	1440-1475	1010-1045	540-576	430-435	250
	Ta5	LR	BV	SV	BR	
PI			-		-	
PII	145	1.74-1.83	3.38-3.48	3.63-3.94	4.0-5.5	
PIII	145	1.40-1.46	2.79-2.84	2.75-2.85	3.5-4.0	

Sensilla chaetica: PII with 50-60 uniserial, absent only on basal 10% of Ti<sub>1</sub>, PIII with 60-70 uniserial, absent only on basal 10% of Ti<sub>1</sub>.

Genitalia: typical for *Chironomus* s.str.

*Pupa* (n=10) (Fig. 5d-g).

Pale brown, with basal ring and tracheal bases, abdominal apophyses, posterolateral spur and bases of anal lobe setae all darker brown. Cephalic tubercles (Fig. 5d) mid-brown, 122-157 long subapically bearing 28-25 long frontal seta. Thorax with dense brown rugulosity to midpoint (Fig. 5e). Basal ring oval, 120-132 long, 58-98 wide, with elliptical fused double tracheal bases.

Abdomen (Fig. 5f) with very restricted shagreen, present only along apophyses on TV and TVI. Pedes spurii B quite strong on II. Pedes spurii A on IV, much weaker on V, absent on VI. Tergal spinule pattern as in Figure 5f. Hook row with 60-75 hooks, extending about 55-60% of the width of tergite II. Conjunctives all bare. Paratergite IV with narrow spines, remainder bare. Setation ('O', D, V and L) typical for genus. Posterolateral spur (Fig. 5g) simple, narrow, tapering to fine point, sometimes with one or two small fine spines at base. Anal lobe with about 150 multiple ranked setae in fringe (not drawn).

*Fourth-instar larva* (n=10) (Fig. 5a-c).

Medium sized, length about 15-18mm (female), 13-14mm (male). Head capsule with mentum mid-brown, mandibular teeth brown, remainder of mandible yellow, gular region slightly darkened towards posterior margin, very slight darkening of frontoclypeus and dark brown to black occipital margin. Ventral tubules relatively short (c. 0.65-1.3mm), not coiled, anterior pair usually slightly shorter than the posterior pair; no lateral projections on eighth abdominal segment. Similar to larvae of *C. oppositus*-group.

Measurements. Head capsule length 580-660. Antenna (Fig. 5c) segments 100-112, 26-31, 8-9, 9-11, 6-8; Antennal Ratio 1.9-2.2; Ring organ 33-42 from base of 1st segment; style 39-44 bifid; Lauterborn organs and peg 5-6, about half length of third antennal segment. Mandible (Fig. 5b) 202-225, with yellow-brown outer tooth, dark apical and two inner teeth, mola yellow. Mentum (Fig. 5a) 160-170 wide, with typical triple-toothed median part and six pairs of lateral teeth of which the innermost pair are rather more closely appressed than are the remainder; ventromental plate 108-117 with 29-35 striae across median part.

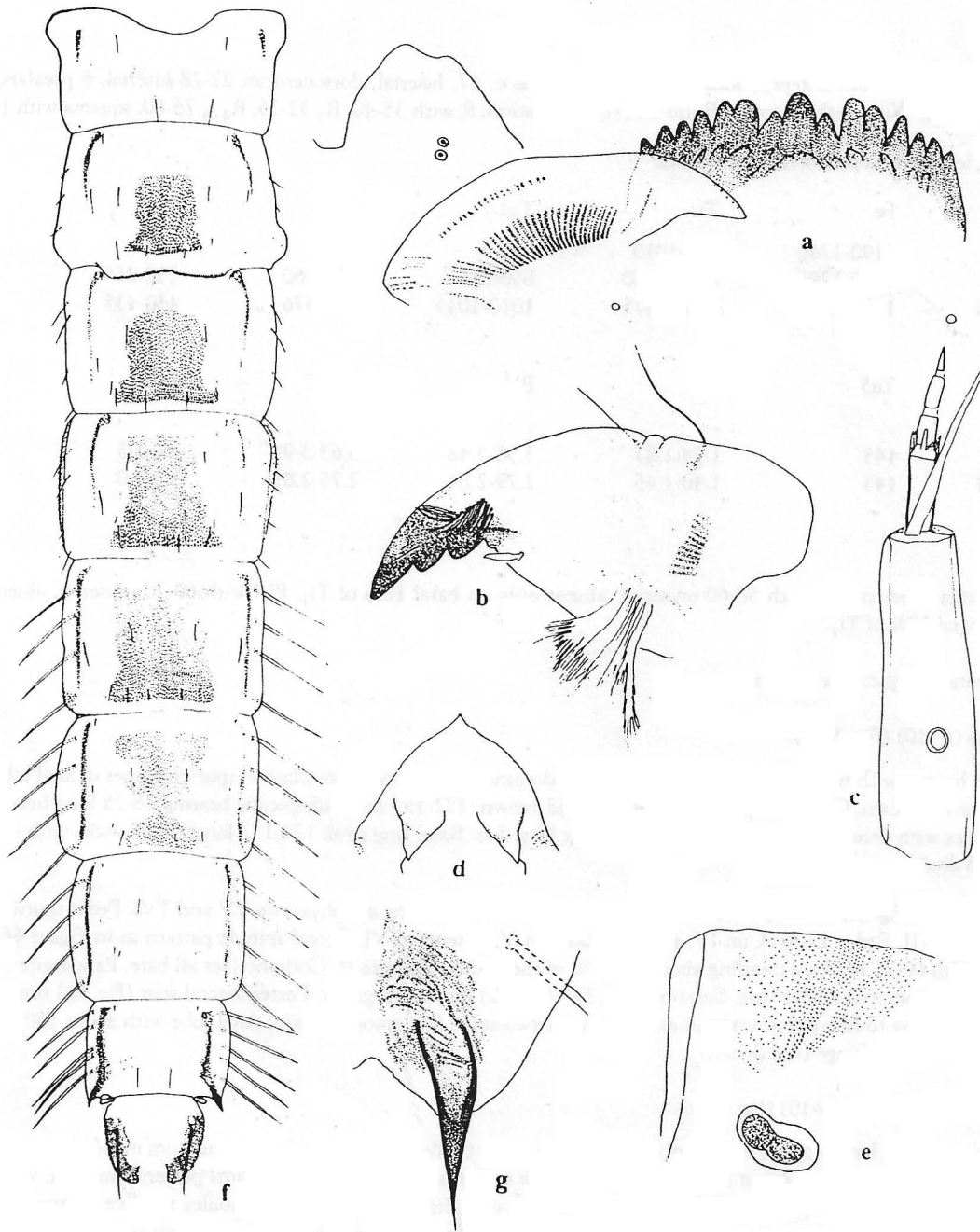


Figure 5. *Chironomus maddeni* sp. nov. larva and pupa. (a)-(c) Larva: (a) mentum, (b) mandible, (c) antenna; (d)-(g) Pupa: (d) cephalic tubercles and frontal setae, (e) anterior thorax, (f) tergites (anal lobe fringe not figured), (g) posterolateral comb.

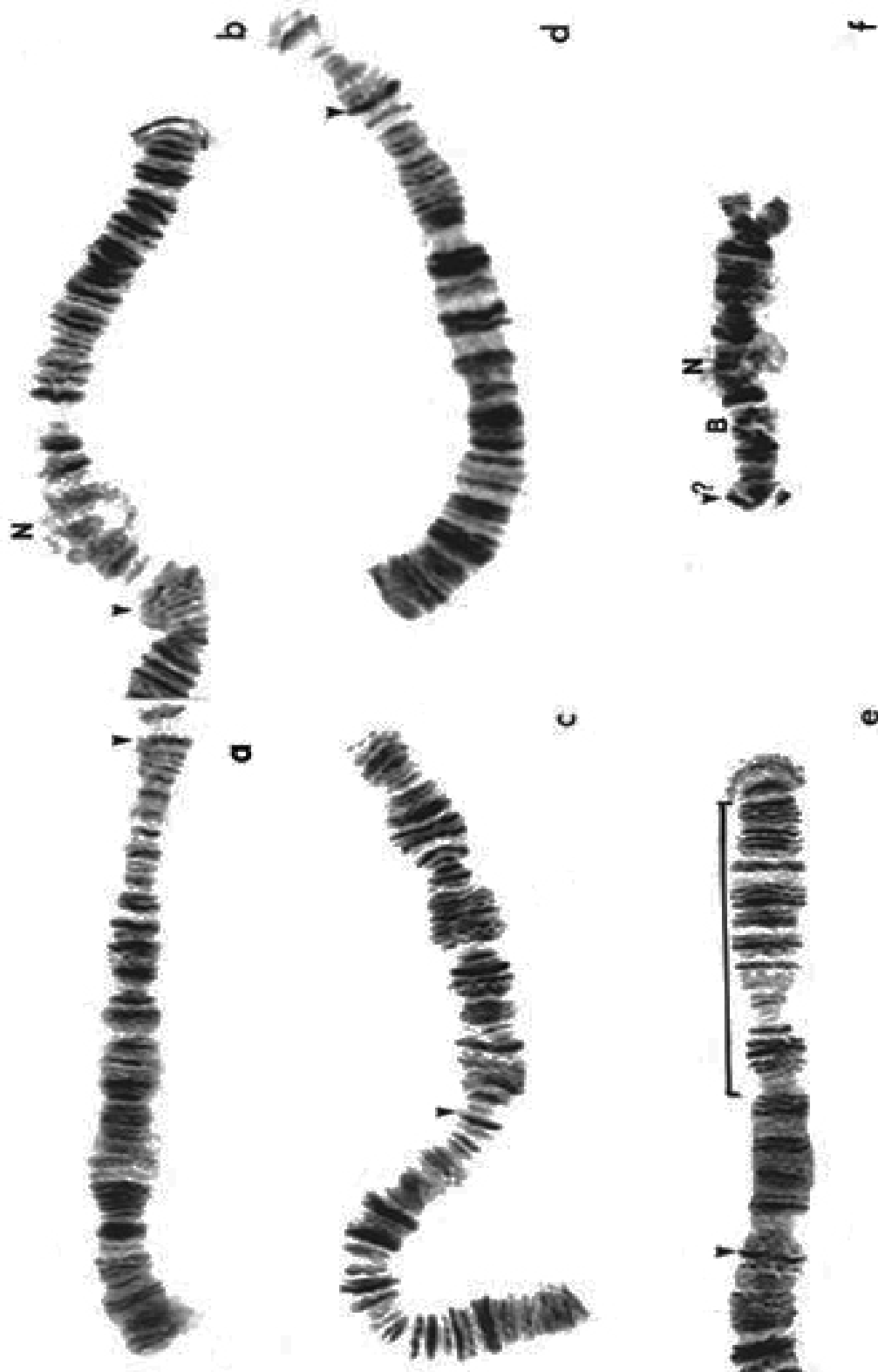


Figure 6. *Chronomus maddenti* sp. nov., polytene chromosomes: (a) Arm B, (b) Arm F, (c) Arms D (left) and C (right), (d) Arm A, (the bracket above the arm indicates the approximate extent of the heterozygous inversion observed in one individual), (e) Arm E, (the bracket above the arm indicates the approximate extent of the heterozygous inversion observed in one individual), (f) Arm G. The postulated centromere is at the left end. Centromeres are indicated by arrow heads; N - nucleolus, B - Balbiani ring.

*Larval cytology* (Fig. 6a-f)

Four polytene chromosomes (Fig. 6) with the arm combination of the pseudothummi-group, i.e. BF, CD, AE, G. Large nucleolus developed near the centromere of arm F, with a smaller one developed medially in arm G. Only one inversion polymorphism, in arm A, noted in the material available for study.

Arm B (Fig. 6a): Sequence as B2 of *C. oppositus* (Martin 1969; Martin *et al.*, 1978), i.e. large puff of 2D is near the distal end with the dark bands of 2E towards the telomere.

Arm F (Fig. 6b): Sequence as F1 of *C. oppositus* (Martin 1969; Martin *et al.*, 1978), including the large nucleolus at 4C.

Arms D and C (Fig. 6c): Banding pattern does not appear to correspond to any previously described sequence.

Arm E (Fig. 6d): Sequence as E1 of *C. oppositus* (Martin 1969; Martin *et al.*, 1978).

Arm A (Fig. 6e): Differs from the previously described sequences by an inversion of approximately the region 14D1-15C8 of sequence A4. This results in the ball of 15E having a similar position and orientation to that of A5. However it can be distinguished from that sequence because the distal region remains similar to A4. One specimen was heterozygous for an inversion of the region from about 15D4-15E.

Arm G (Fig. 6f): Differs from arm G of *C. oppositus* by the nucleolus being near the middle of the arm, with an obvious Balbiani ring between the nucleolus and the postulated centromere.

*Material examined*

Holotype male slide-mounted in Euparal, ex-South Australia, Glen Osmond, pool in grounds of Waite Institute, University of Adelaide (Madden), reared in culture in Melbourne University Genetic Department by Jon Martin, Australian National Insect Collection (ANIC).

Paratypes, 1 male, 2 females, 10 pupal exuviae, 10 larvae, all mounted in Euparal, same data as holotype.

*Comments*

Cytologically this species is similar to those of the broader *C. oppositus*-group (i.e. including the undescribed species *C. alternans* b and *C. alternans* c (Martin and Lee, 1984)). It can be distinguished from *C. oppositus* by the lack of a subterminal nucleolus in arm G, and from *C. alternans* b and *C. alternans* c by the orientation of group 15E of arm A, since both the latter species have sequence A4.

Although this species is distinguishable on the basis of cytology, it has not been possible to find unambiguous character states to differentiate from related species in the *C. oppositus*-group. However, characters that may prove of value in future differentiation include the following:

Adult: "normal" male genitalia, unpatterned wing and legs, short bristle ratio (BR) on the fore leg, longer on mid and hind legs; presence of acrostichal setae and absence of frontal tubercles.

Pupa: this stages provides less diagnostic information than in many other genera. The tergal spinule pattern, presence of spines on paratergite IV, thoracic rugosity and pigment distribution may prove significant in species differentiation. Larva: the short, uncoiled ventral tubules with the anterior pair usually slightly shorter than the posterior pair and lack of lateral projections on eighth abdominal segment typify the *oppositus*-group. Within this group the number of striae in the basal part of the ventromentum (29-35), and the distribution of pigment on the head capsule may allow separation from other members of the group.