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## *Chironomus melanotus* Keyl and its taxonomical and ecological relations to *C. riihimakiensis* Wülker

(Diptera, Chironomidae)

By Mauri Hirvenoja and Paraskeva Michailova

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The karyological, taxonomical and ecological relationships between sympatric populations of *Chironomus melanotus* Keyl and *C. riihimakiensis* Wülker from Riihimäki in southern Finland are described.

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### Introduction

*Chironomus riihimakiensis* Wülker (1973) was originally found in a small, old clay pond on the Hirvenoja farm, about 5 km north of the town of Riihimäki, in southern Finland. This find first led to a confusion of species when specimens of *C. melanotus* Keyl (1961) were reared from the main ditch, which is flowing through the same farm very near the type locality of *C. riihimakiensis*. *C. melanotus* was identified karyologically by the second author. Possibilities of morphological differences between the species have been sought.

### Karyological studies

Larvae of *Chironomus melanotus* Keyl from Riihimäki, October 1986, November 1987 and June 1988, family Hirvenoja leg., were used. The well known acetorceinic method was applied. The distribution and localization of the constitutive heterochromatin were determined by the “C.” banding technique (Michailova 1987). Paratype chromosome preparation of *C. riihimakiensis* from the Zoological Museum, University of Helsinki was used for comparison.

Karyotype ( $N = 11$ ) of *C. melanotus* (Fig. 1)

$2n = 8$ . This species was karyologically described by Keyl (1961) and later by Wülker (1973). *C. melanotus* belongs to the *thummi* complex. The I<sub>st</sub> and II<sub>nd</sub> chromosomes are metacentric, III<sub>rd</sub> submetacentric and IV<sub>th</sub> acrocentric. Unlike the German population, the Finnish population has large dark heterochromatin blocks not only in the centromere regions of chromosomes AB, EF and G but also in the CD chromosome. The band sequences of arms A, E and F have been described and compared with other *Chironomus* species by Keyl (1962) and Wülker (1973). Arms A, E, F have been divided according to Keyl (1962).

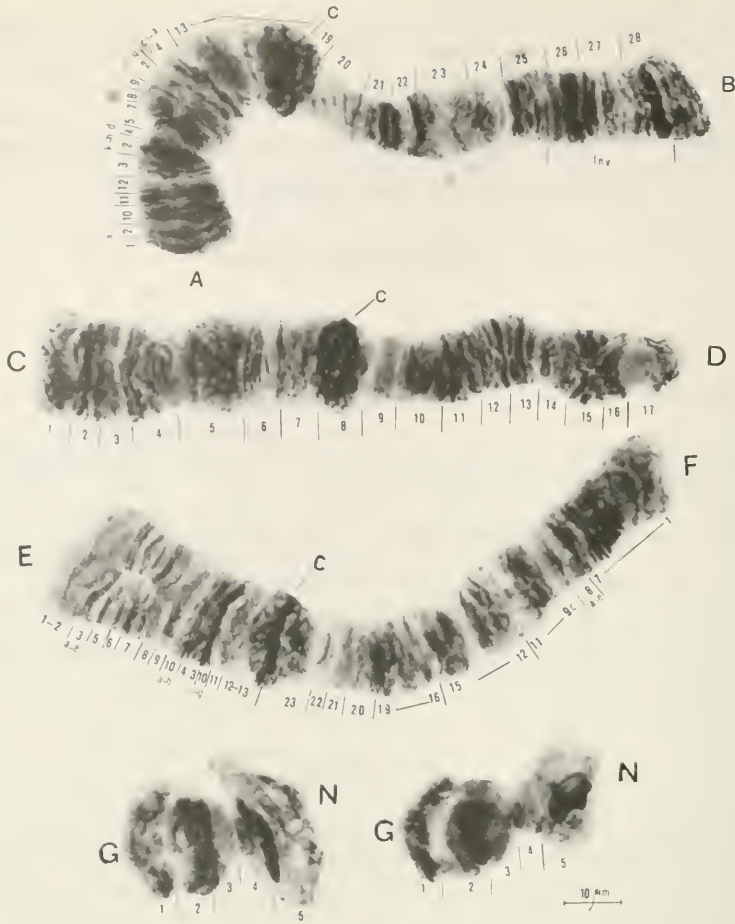


Fig. 1. Chromosomes of *C. melanotus* Keyl; Ist chromosome (AB), IIrd chromosome (CD), IIIrd chromosome (E), IVth chromosome (G), nucleolus (N), centromere region (C).

Arm B. Similar to *C. riibimakiensis*: near the centromere region is a light zone followed by two dark bands. Not far from these is a homozygous inversion (in section 26–28) if compared with *C. riibimakiensis*.

Arm C. In the centre of the arm is a constriction, a typical feature of the *C. plumosus* group (Ryser & al. 1983). Near to this is an active zone, section 5. *C. riibimakiensis* has in the middle of this arm a nucleolus, a region often unpaired (Wülker 1973).

Arm D. This is quite different from that of *C. riibimakiensis* but similar to that of *C. plumosus*. At the telomere region of this arm is a group of dark bands.

Arm G. The homologues are unpaired with heterochromatin in a heterozygous state.

“C” banding (Fig. 2). Heterochromatin bands only in the centromere regions.

This species has no inversion polymorphism in other populations (Keyl 1962, Wülker 1973). The Finnish population has a heterozygous inversion in arm D (20%) (Fig. 3).

The larvae of the present two species can be identified by their karyotypes: sequences of bands in arms A, D, F and G; large heterochromatin blocks present in the centromere regions of *C. melanotus*

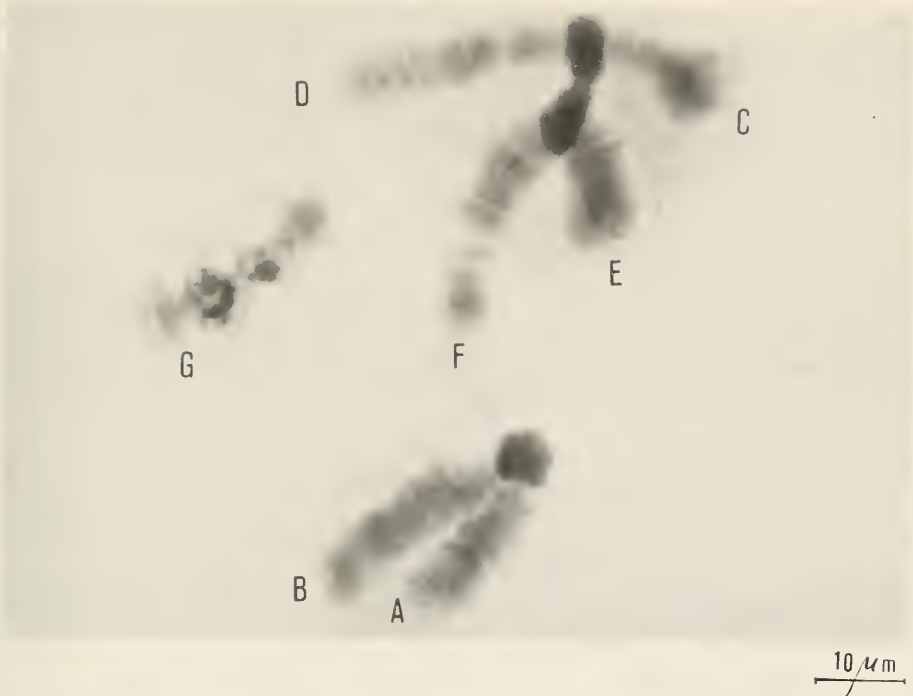


Fig. 2. AB, CD, EF and G chromosomes of *C. melanotus* Keyl stained by "C" banding.



Fig. 3. A heterozygous inversion in IInd chromosome of *C. melanotus* Keyl.

only. Up to now the latter appeared to be rare in genus *Chironomus*: in *C. crassimanus* (Keyl 1962), *C. cucini* (Martin 1979) and some Swiss populations of *C. plumosus* (Michailova, Fischer 1986) and USSR populations of the same species (Petrova, Kiknadze, Michailova 1985). Perhaps the duplication has played an important role in the formation of the centromere regions. The amount of "C" heterochromatin seems to have increased during evolution. These cytogenetic features allow us to consider *C. melanotus* a derivative species.

Summarizing differences, the species can be distinguished as follows:

- 1 (2) All chromosomes without centromere heterochromatin. Arm B with a standard sequence, IVth chromosome paired. . . . . *C. riihimakiensis*
- 2 (1) All polytene chromosomes with large centromere heterochromatin. Arm B with homozygous inversion in comparison with *C. riihimakiensis*. IVth chromosome unpaired. . . . . *C. melanotus*

### Morphological studies

#### *Chironomus melanotus* Keyl, 1961

Field material from Riihimäki, Hirvenoja, Finland, 1981–1988.

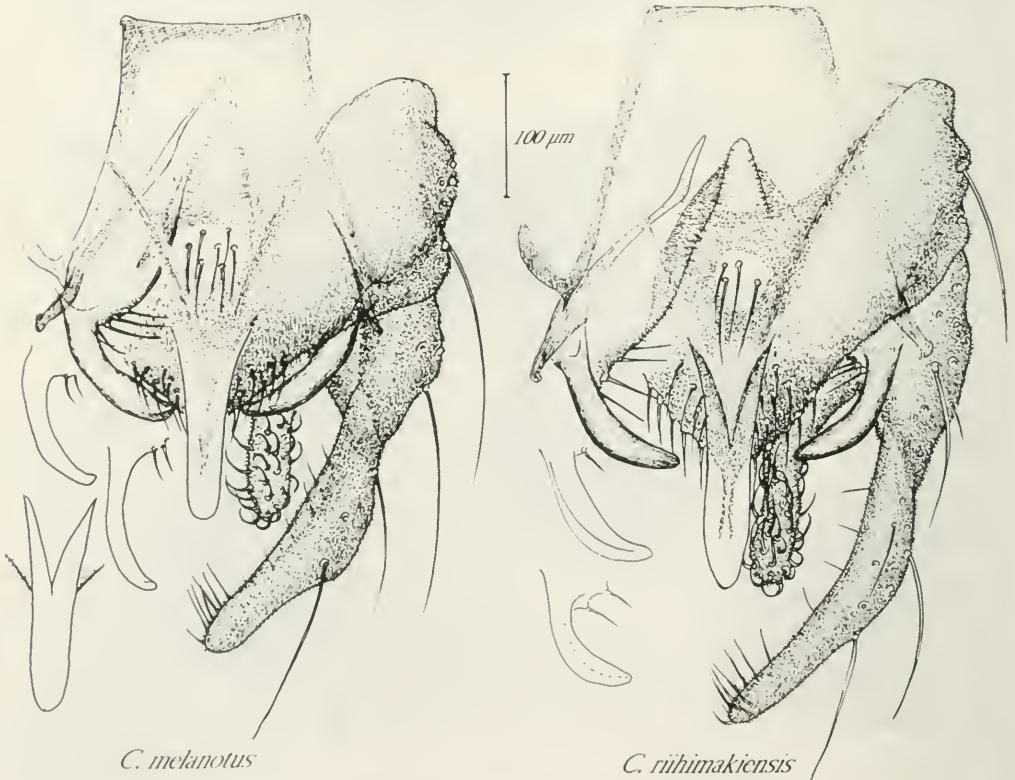


Fig. 4. Male genitalia of *C. melanotus* Keyl and *C. riihimakiensis* Wülker.

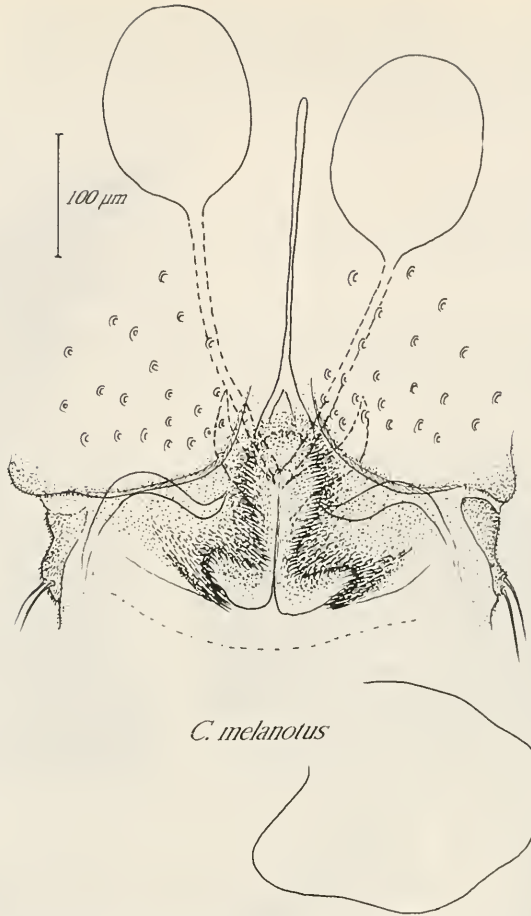


Fig. 5. Female genitalia and shape of cercus of *C. melanotus* Keyl.

Male (N = 11)

A blackish species, with a more or less greenish tint. Abdomen unicolorous; thorax dorsally more or less dark. Legs somewhat pale, but femora apically and tibiae proximally clearly darkened and tarsi darkening towards the distal joints.

AR 3.78–4.40 (according to Wülker 1973 the lowest value 3.43). Segments of the palps (2–5) 70–90, 240–285, 250–270 and 315–375 μm. Clypeus with about 30 setae like vertex and postocular region. Frontal tubercles about 40–50 μm. Distance between eyes less than twice breadth of dorsal extension.

Chaetotaxy of thorax and abdomen as usually in this genus; dorsocentral setae in 1–3 rows, 20–35 in number; about 30–45 setae of the scutellum are standing irregularly, but the most caudal setae are nearly in a row. Tracheal scar of pupal respiratory organ (“thoracic sensory pit”, Edwards 1929) about one half of the length of postpronotum. Wings 4.3–5.0 mm. Costa not extended over *R*<sub>4</sub>+*5*; setae on *R*<sub>1</sub> and on the distal part of *R*<sub>4</sub>+*5*; squama with about 30 setae.

Sensilla chaetica (about 15 μ) near distal end of the basitarsus; in the middle leg 7–14 and hind leg 4–16 in number observed. *P1:LR* 1.43–1.54 (according to Wülker 1973 1.29–1.56), *BR* 2.0–4.4. *P2:LR* 0.59–0.64, *BR* 2.2–4.0. *P3:LR* 0.69–0.74, *BR* 3.9–6.8. Legs (Fe–Ta<sub>5</sub>) in μm:

P1 1205-1640 1150-1565 1750-2280 955-1260 685-900 575-825 260-360

P2 1305-1760 1250-1740 785- 980 480- 640 335-445 230-305 150-215

P3 1520-2000 1550-2085 1130-1520 675- 955 480-685 280-425 175-250

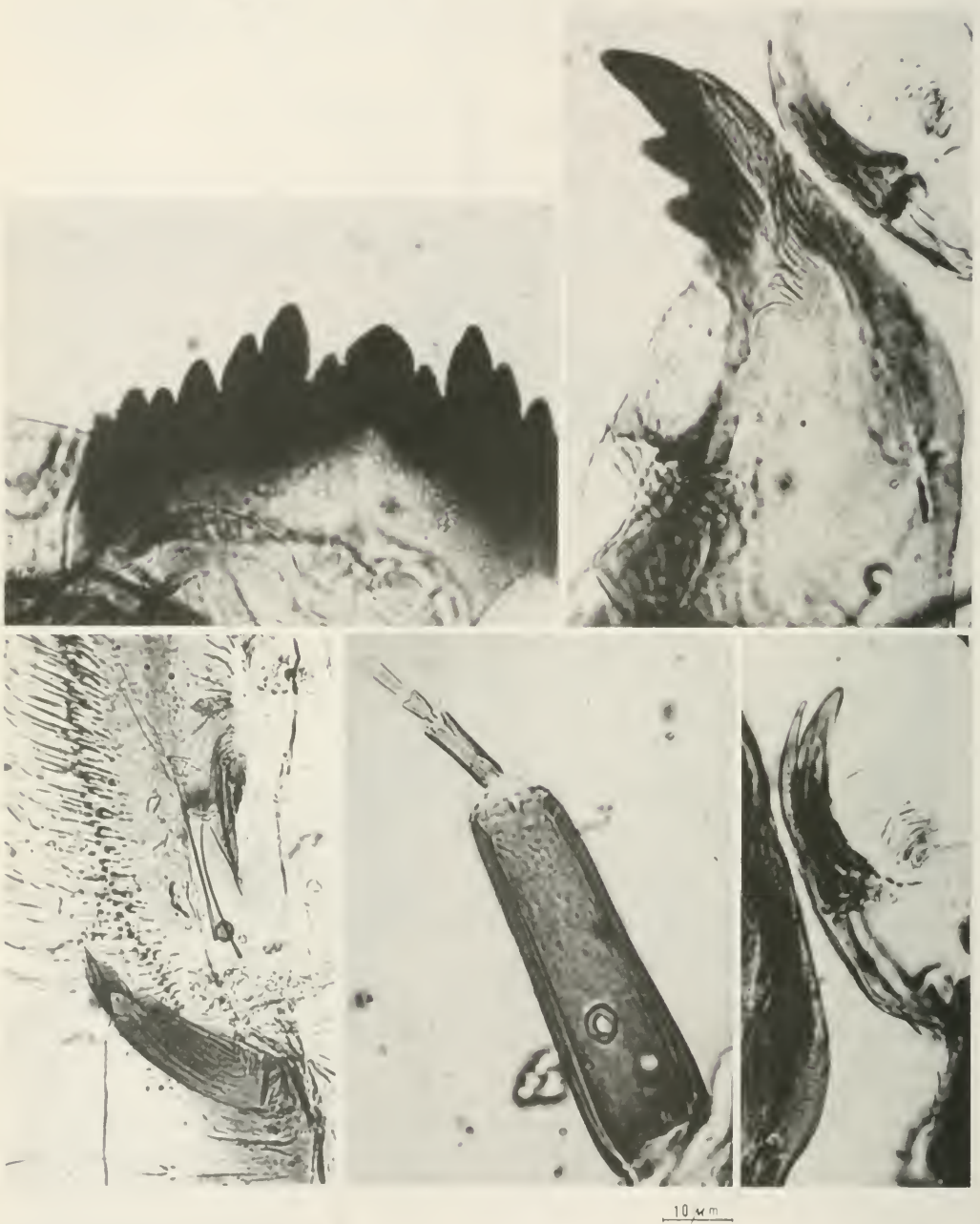


Fig. 6. *C. melanotus* Keyl; region of chin (hypochilum), mandible, premandible and antenna of the larva, anal comb of the pupa.

Anal tergite of the hypopygium with 7–10 bristles. Characteristic features (Fig. 4) are the moderate broadened (proximally about 65  $\mu\text{m}$ ) anal point (length/width < 3; cf. Lindeberg & Wiederholm 1979, Fig. 1 a) and long appendages 1 (Fig. 1 in Reiss & Fittkau 1971), which are at least 4–5 times longer than broad, usually with a knob-like projection or a fold apically; the styli are more suddenly tapering than in *C. riibimakiensis*.

Female (N = 9)

The coloration as in male or thorax sometimes light with a greenish touch; abdomen always dark. Chaetotaxy about as in the male, up to 55 dorsocentrals. Antennae with 5 flagellomeres. Segments of the palps (2–5) 70–80, 250–260, 240–265 and 340–365  $\mu\text{m}$ . Wings 4.5–5.0 mm; setae as in the male, but some also proximad to *R4+5*, and up to 44 squamal setae.

Sensilla chaetica nearly all the distance along basitarsus, in mid leg 59–79 and hind leg 58–82 in number. *P1:LR* 1.40–1.57, *BR* 1.6–2.3. *P2:LR* 0.53–0.58, *BR* 1.6–2.6. *P3:LR* 0.53–0.58, *BR* 2.4–3.6. Legs in  $\mu\text{m}$ :

*P1* 1350–1630 1260–1520 1890–2175 945–1110 715–825 675–740 260–350

*P2* 1435–1715 1415–1740 760– 945 415– 520 260–380 195–250 175–215

*P3* 1565–1935 1740–2085 1175–1455 655– 825 510–640 270–325 175–230

Genitalia as usually in the genus; spermathecae oval, 140–190  $\mu\text{m}$  long (Fig. 5).

Pupa (N = 5)

Length of the somewhat pigmented exuvie 8–9 mm. Frontal apotome with about 100–150  $\mu\text{m}$  long cephalic tubercles. Thorax dorsally broadly granulated. Shagreen on tergites 2–8 lacking laterally and clearly seen on tergite 7 at the oral half and on tergite 8 at the anal half only as in many other species of this genus. *PB* and about 60–80 intersegmental hooklets on segment 2. *PA* clearly present on the hind corners of segments 4–6 and nearly invisible on the hind corner of segment 7. Dorsal setae on all tergites present. Lateral setae on the abdominal segments 1–8: 0, 3, 3, 3, and filamentous setae 4, 4, 4, (4)5. Anal comb (Fig. 6) of the segment 8 quite large. Anal lobe fringe of filaments multiserial, number of setae 119–141.

Larva (N = 9)

Length up to 14–15 mm. Claws of anterior parapods apically nearly invisibly serrated. Lateral tubules of segment 10 very short, about 100  $\mu\text{m}$ . Two pairs of ventral tubules present, about 1 mm long (Fig. 7). Head, 650–700  $\mu\text{m}$  broad (according to Geiger & al. up to 730  $\mu\text{m}$ ), IC 81–88%. Borders of foramen occipitale dark and head capsule ventrally on the postgenal bridge usually as dark as border of foramen occipitale. Extent of darkened area varies laterally; one pigmented area behind mandibles and pigmented dots on the lateral sklerites of the labrum and antennal sockets. Postgenal bridge light on proximal part of postmentum (Fig. 6), but on the chin or hypochilum (Gouin 1968) where postmentum and postgenal bridge of the head capsule have fused forming the teeth, is dark. Paralabials striated less than a half of their breadth. Distance between the dorsal eyes seen from above 3 times the length of first antennal joint.

Sensory field of labrum with the usual structure of chironomids. Seta *S<sub>I</sub>* (Fig. 7) about 70  $\mu\text{m}$  long, quite narrow, apically strongly serrate and the plumose structure on one side only. Setae *S<sub>II</sub>* simple but the 3 long *Ch* setae have weakly plumose sides, clearly to be seen in *Ch* nearest to *S<sub>I</sub>*. Pecten epipharyngis with 12–15 teeth, only one *Chb* seta found, *Chl* setae apically strongly serrate.

Premandibles darkened with 2 apical teeth, inner (dorsal) tooth quite narrow, at least 4 times as long as broad, the outer (ventral) tooth about 2.5 times broader (if seen from apex). Teeth of mandible dark or sometimes the fourth inner tooth more or less light; proximal part of inner side of mandible proximally with few small denticles.

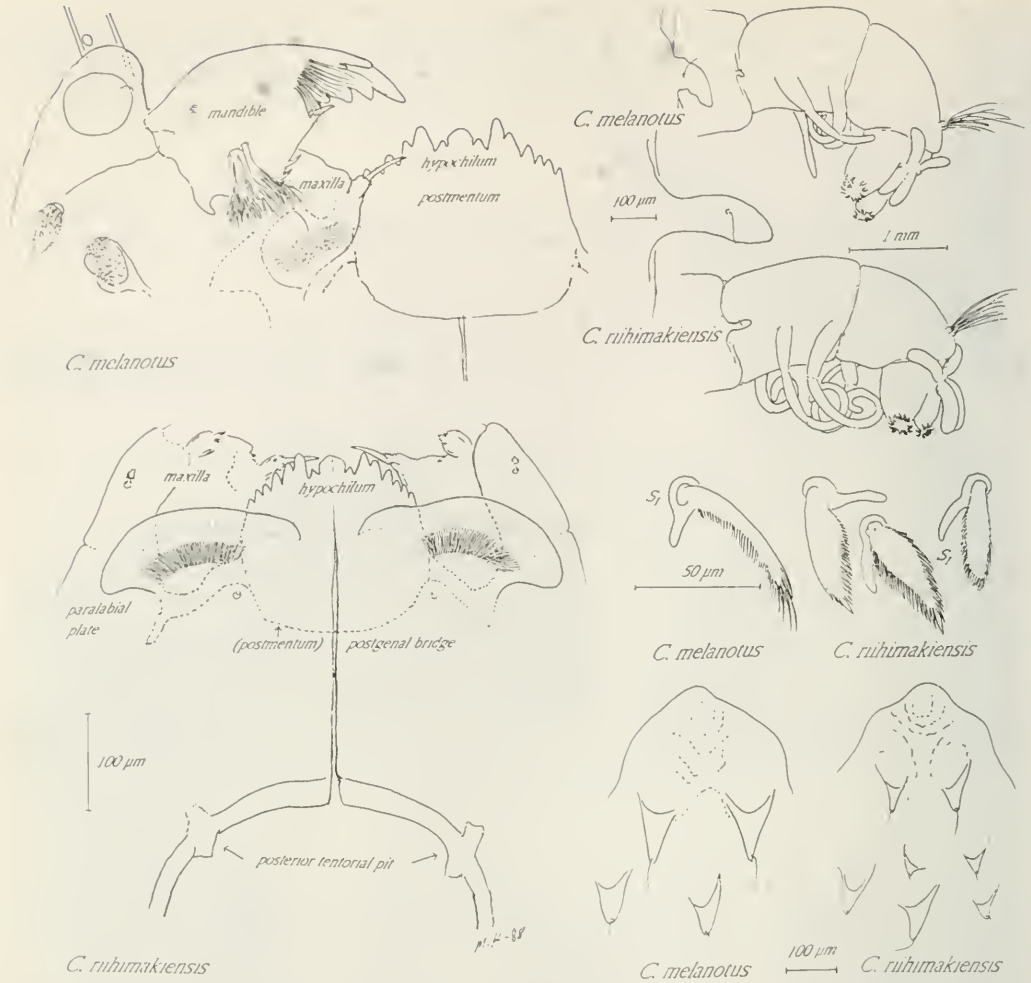


Fig. 7. *C. melanotus* Keyl and *C. rühimakiensis* Wülker; top right: last segments and lateral tubules of abdominal segment 7 of the larvae, setae  $S_1$  and below variation of cephalic tubercles of the pupae; left: parts of the head capsules of the larvae dorsal view (*melanotus*) and ventral view (*rühimakiensis*).

Median tooth of hypochilum sometimes slightly constricted proximally (as in Fig. 2 in Wülker 1973), but often this cannot be observed; the fifth (in the Russian literature fourth) lateral tooth a little smaller and shorter than the sixth.

Antennae <math>< 230 \mu\text{m}</math>; *LO* very small, difficult to see; *RO* from base of basal segment in distance about  $\frac{1}{4}$ – $\frac{1}{3}$  the length of this segment; proportions of the segments in the fourth instar:

100 : 29 : 10 : 12 : 8  
 100 : 28 : 9 : 11 : 7  
 100 : 27 : 9 : 10 : 7  
 100 : 25 : 9 : 10 : 6  
 100 : 25 : 8 : 10 : 6  
 100 : 22 : 9 : 10 : 6



*Chironomus riibimakiensis* Wülker, 1973

Material. One paratype male, deposited in the Zoological Museum, University of Helsinki. Other specimens, the measured males and females, pupae and larvae from laboratory stock, originally from Riihimäki, Hirvenoja, Finland, reared by M. Hirvenoja in Helsinki, 1970. From this stock eggs were in 1970 sent to professor Wülker (cf. Wülker 1973: 371).

Male (n = 6)

AR 3.25–3.38 (according to Wülker 1973 up to 3.68). Palp segments (2–5) 70–90, 270–290, 270–290 and 350–390  $\mu\text{m}$ . Clypeus with about 40–50, vertex and postocular about 20–30 setae. Length of frontal tubercles about 40–50  $\mu\text{m}$ .

Thorax and abdomen darkened. Tracheal scar of pupal respiratory organ about one half the length of postpronotum. Chaetotaxy as usually in the genus *Chironomus* with more than 30 dorsocentrals in 1–3 rows. Scutellum with about 30–40 setae from which the most anal are nearly in a row. Wings 4.0–4.4 mm with bristles on *R1* and on the apical half of *R4+5*; costa not lengthened over *R4+5*. Squama with 18–32 setae.

Number of sensilla chaetica (about 10  $\mu\text{m}$  long) 8–15 on mid leg and 5–13 on hind leg, situated near distal end of basitarsus as usual in this genus. *P1:LR* 1.44–1.50 (according to Wülker up to 1.58), *BR* < 2.0. *P2:LR* 0.60–0.66, *LR* 2.0–3.6. *P3:LR* 0.70–0.74, *BR* 2.7–4.7. Legs in  $\mu\text{m}$ :

*P1* 1565–1715 1480–1665 2215–2480 1175–1280 870–900 715–760 335–345

*P2* 1675–1880 1530–1805 980–1065 600– 675 415–445 280–315 195–200

*P3* 1870–2045 1935–2175 1435–1555 740– 915 565–650 350–400 215–240

Anal tergite of hypopygium with 6–10 bristles. Characteristic structures (cf. fig. 3 in Wülker 1973) are the moderately broadened anal point (about 70  $\mu\text{m}$ , length/width about 3) and the long, quite evenly apically narrowing, and curved first appendages without a clear fold or knob-like projection; first appendage about 3–4 times as long as broad.

Female (n = 8)

Colouration as in male. Antennae with 5 flagellomeres. Segments of palps (2–5) 55–90, 225–270, 240–285 and 315–355  $\mu\text{m}$ . Chaetotaxy as in male but number of setae may be higher; dorsocentrals usually 40–60 in number, in 1–4 rows. Wings 4.0–4.4 mm with veins and setae as in the male but, *R4+5* with setae also in proximal part.

Observed number of sensilla chaetica 88–118 almost all the length of basitarsus of mid leg and 98–129 in hind leg. *P1:LR* 1.44–1.58, *BR* 1.3–2.0. *P2:LR* 0.57–0.64, *BR* 1.4–2.2. *P3:LR* 0.69–0.72, *BR* 2.3–2.7. Legs in  $\mu\text{m}$ :

*P1* 1560–1850 1415–1685 2230–2545 1085–1260 760–900 670–780 320–370

*P2* 1695–1915 1480–1780 835–1065 425– 610 305–435 175–285 150–215

*P3* 1825–2065 1760–2175 1250–1520 725– 870 475–650 280–380 195–230

Genitalia about as in *C. melanotus*; spermathecae oval, 130–190  $\mu\text{m}$  long.

Pupa (N = 8)

Length of the quite pale exuvie 8–9 mm. Frontal apotome with about < 100  $\mu\text{m}$  long cephalic tubercles. Thorax broadly but not very densely or strongly granulated. Shagreen of abdominal tergites 2–8 present, about as in *C. melanotus*; *PB* and about 80 intersegmental hooklets on second segment. *PA* as in *C. melanotus*. Dorsal setae on all segments present. Lateral setae of segments 1–8: 0, 3, 3, 3 and filamentous setae 4, 4, 4, (4) 5. Anal combs of eighth segment as in *C. melanotus*. Observed number of uni- or biserial laterally arranged filaments of the anal segment 59–77.

Larva (n = 9)

Length up to about 14 mm. Claws of anterior parapods apically nearly invisible serrated. Lateral papillae on tenth segment 150–250  $\mu\text{m}$  long; two pairs present of weakly strangulated ventral tubules about 2 mm long (Fig. 7). Head 650–850  $\mu\text{m}$  broad, IC 80–85%. Borders of foramen occipitale dark, postgenal bridge, labral sklerites and antennal sockets only weakly darkened.

Sensory field of labrum as in *C. melanotus*, but seta  $S_1$  shorter, about 50  $\mu\text{m}$ , plumose all round (Fig. 7);  $S_1$  with hairs seemingly only on one side found, but this depends on mounting position in the slide. All *Cb* setae plumose. Pecten epipharyngis with 13–16 teeth.

Mouthparts typical of the genus. Premandibles with two apical teeth; inner (dorsal) tooth about 3 times as long as broad, outer (ventral) tooth about 2 times broader than the inner (seen from the apex). Mandible with pale proximal (fourth) tooth; inner edge proximally weakly serrated. In hypochilum the unpaired median tooth narrowed proximally as in *C. melanotus*, the fifth and sixth lateral teeth about equal in size; this depends perhaps on orientation on the slide, the fifth tooth may be as short as the sixth.

Antenna <200  $\mu\text{m}$ ; RO from the base of basal segment in distance about  $1/4$ – $1/3$  of the length of this segment; proportions of segments in fourth instar:

100 : 30 : 11 : 11 : 5

100 : 28 : 11 : 10 : 5

100 : 28 : 10 : 10 : 6

100 : 27 : 13 : 11 : 4

100 : 26 : 11 : 10 : 6

100 : 24 : 11 : 10 : 6

### Ecological comparison

As mentioned, *C. riihimakiensis* was originally found in an old, permanent clay pond (2×3 m, depth <0.5 m), some meters from a similar but somewhat smaller, ephemeral pond (R2 in Fig. 3 in Hirvenoja 1962: 98). The bottom consisted of clay ooze covered with alder leaves (*Alnus incana* [L.] Willd.) and *Callitriche* vegetation. Conductivity > 8 mS/m, pH about 6 and colour > 80 mg Pt/l depending on the amount of water.

*C. riihimakiensis*, identified as adult females and males, was collected also by Mr. Petri Nummi in June 1987 in a beaver pond in the small Saukonoja river, (Evo, Lammi), south Finland. The mean depth of the biotope was 0.49 m, velocity of the water 3–40 cm/sec, the conductivity 4.4–4.5 mS/m and pH 6.1–6.2 (Nummi, 1989). It thus can live also in a running-water habitat, but is probably less waste-water-tolerant than is *C. melanotus*.

According to Wülker (1973: 362), *C. melanotus* has been found in humic or eutrophicated ponds. The biotope of *C. melanotus* in Riihimäki is loaded with waste waters from the direction of the town of Riihimäki and with the humic waters of a peat bog. Some features of the monitoring station on 10.V.–26.IX.1981, when *C. clarus* Hirv. was the dominating species of *Chironomus* in the habitat, were as follows:

width m	depth m	velocity cm/s	temp. °C	conduct. mS/m ( $\gamma$ 25)	pH	color Pt mg/l
1.5–2.5	0.2–1.0	<25	<+22	18.0–45.3	6.6–7.3	140–300

A single water sample on 5.7.1981 gave the following results in mg/l:

$P_{\text{tot}}$	$N_{\text{tot}}$	K	Ca	Mg	Cl	Fe	S	$\text{KMnO}_4$ cons.
0.249	1.71	3.75	10.1	4.1	26.5	0.44	9.9	101.8

### Taxonomical remarks

The male of *C. melanotus* is very similar to *C. riihimakiensis* on the basis of the structure of the hypopygium or of the morphometrical data.

The key of Lindeberg and Wiederholm (1979), on males, gives the group of *C. aberratus* Keyl, *C. sororius* Wülk., *C. striatus* Str., *C. melanotus* Keyl, *C. lacunarius* Wülk. and *C. riihimakiensis* Wülk. According to the authors, these species are not readily identifiable. The identification of the males of the two present species from reared or trapped samples from the field is possible from the differences in the females and pupae as follows:

- 1 (2) About 90–130 sensilla chaetica on the Ta1 of P2 and P3 of the female. Pupae with about 60–70 lateral filaments on anal segment; cephalic tubercles shorter than 100  $\mu\text{m}$  ..... *C. riihimakiensis*
- 2 (1) About 50–90 sensilla chaetica on Ta1 of P2 and P3 of the female. Multiserial set of 120–140 filaments on each side of the anal segment; cephalic tubercles longer than 100  $\mu\text{m}$  ..... *C. melanotus*

According to the literature, the size of the abdominal tubules is influenced by the water chemistry of the habitat. We can, however, try to summarize the differences between the species in local populations of the larvae from Riihimäki as follows:

- 1 (2) Lateral tubules of seventh abdominal segment about 100  $\mu\text{m}$ .  $S_1$  longer than 70  $\mu\text{m}$ , one side plumose, apically coarsely serrated. Borders of foramen occipitale and head ventrally very dark. Ventral tubules of eighth abdominal segment about 1 mm. Fourth antennal segment longer than the third. Inner (dorsal) tooth of premandibles at least four times as long as proximally broad. Median tooth of hypochilum proximally weakly narrowed and the fifth lateral teeth a little shorter (or lower from the line of the apices of the teeth) than the sixth ..... *C. melanotus*
- 2 (1) Lateral tubules of seventh abdominal segment 150–250  $\mu\text{m}$ . Setae  $S_1$  about 50  $\mu\text{m}$ , all round plumose. Borders of foramen occipitale dark, head ventrally weakly darkened. Fourth antennal segment not longer than third. Inner (dorsal) tooth of premandibles about three times as long as proximally broad. Median tooth of hypochilum proximally weakly narrowed and fifth lateral weakly shorter. Ventral tubules of the eighth abdominal segment 2 mm ..... *C. riihimakiensis*

### Acknowledgements

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