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Cytotaxonomic characteristics of *Chironomus clarus* (Diptera, Chironomidae) with completion of the description of morphology of adults and immature stages

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The karyotype and external morphology of the larva, pupa and female of *Chironomus clarus* are described here for the first time. Cytologically *C. clarus* belongs to the *thummi* complex (chromosome arm combination: AB CD EF G). On the basis of external morphology and karyotype this species should be combined with *C. obtusidens* Goetghebuer and *C. acutiventris* Wülker, Ryser & Scholl in the *obtusidens* group. *C. clarus* and *C. obtusidens* are homosequental sister species (if not even synonyms) with one and the same band sequences. Completions of the male morphology are also given and the species is compared with members of the *obtusidens* group.

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1. Introduction

The type locality (near Station 6 in Fig. 10 and Fig. 13 in Hirvenoja 1962b, p. 101) of *Chironomus clarus* was a small channel between a pond and a small, quite acid (pH 5.4–6.5) littoral lake (sensu Brundin 1956:196) Posolampi in Sodankylä, north Finland. The current of flow in this channel was very slow because neither the pond nor the lake had any outlet; the water soaked into the peat of Posoaapa, which was one of the largest aapa bogs (a subarctic type of sedge-bog) in Finland. The type locality no longer exists as a channel, but was inundated, as well as the other adjacent water habitats, to become part of the large Lokka dam basin (about 40 km in diameter).

Originally (Hirvenoja 1962a) only one individual of *C. clarus* was described. Two additional male individuals were later found in the original material (1961). It is quite impossible to complete the material from the precise type locality, or (attempts have been made!) from the same area, which is today ecologically totally different. Therefore, new material found quite abundantly in Riihimäki, south Finland, has been used here for the present re-description of *C. clarus*. This material or description lacks, of course, the same nomenclatural significance as the holotype (in the Zoological Museum, Helsinki).

The morphometrical variation of the new

southern material includes the key characters of the specimens from north Finland. The adults reared in June–July 1988 in south Finland at an exceptionally high room temperature represent the smallest measured individuals, and the free-living individuals from north Finland are the largest. The most important difference between the illustration of the hypopygium in Hirvenoja (1962a: Fig 1) and in the present paper (Fig. 3) is the peculiar shape of appendage 1 in the previous figure, which is a function of the mounting position. Appendage 1 in the old microscope-slide of the holotype in question has, however, moved later to the more "normal"position, seen in the present paper´s illustration.

2. Karyological studies

Material: 12 larvae; 19.9.1986, 22.6.1988 and 7.6.1990 (M.H. leg.) from Hirvenoja, Riihimäki, south Finland.

Methods: Larvae fixed in alcohol:acetic acid were used. Chromosome preparations were made from salivary glands according to the aceto-orcein method adopted. The chromosome arms have been letter-indexed from A to G, using the sections homologous to *C. piger* Strenzke according to Keyl (1962). A, E, F arms were divided along the arms according to the range of designations proposed by Keyl (1962). B, C, D and G have been divided conditionally by us. Permanent mounts were made using liquid nitrogen. After freezing in liquid nitrogen the preparations were dehydrated in alcohol, rinsed in xylol and mounted in Canadabalsam.

The specimen from which the salivary glands were squashed was treated in 10% KOH and mounted on the slide beside the squashed preparation.

Karyotype 2n = 8. This species belongs to the thummi complex (Keyl 1962) having the arm combination: AB CD EF G. The polytene chromosomes have no clearly marked centromere regions. 1st and 2nd chromosomes metacentric; 3rd submetacentric and 4th acrocentric (Fig. 1–2). Sex chromosomes were not discovered. It has a nucleolus and a Balbiani ring in 4th (G) chromosome. Inversion polymorphism has not been observed.

Arm A (Fig. 1ab). It is divided into 19 sections. It is almost identical with *C. obtusidens* (Keyl 1960,1962; Petrova & Michailova 1986) with band sequences: 1ae 7–4 1fk 2ac 10–12 3 if 8 9 2dk 3 15–13 16 17 19. The bands are much thinner. Sections 8 9 17–18–19 are functionally active. The bands in sections 2–3–15–13–16 were characteristic for this arm. The narrowing in sections 7–4 (Fig. 1ab) is a marker of this arm, which is a part of the basic pattern in the genus *Chironomus* (sensu Wülker 1980).

Arm B (Fig. 1ab). It is divided conditionally into 12 sections. The band sequences almost coincide with those of *C. obtusidens* (Keyl 1960; Wülker et al. 1983; Petrova & Michailova 1986). The dark band in section 1 is a marker for this arm. Section 3–4–7 is functionally active. The band pattern in sections 9–10–11 is distinctive. The band pattern in sections 3–4 is similar to bands in sections 17–18 of *C. plumosus* (Linnaeus) (Maximova 1976, Kiknadze 1987, Michailova 1989). The bands of this arm are thinner than those of *C. obtusidens*.

Arm C (Fig. 1cd). It is divided into 11 sections. Arm C begins with light bands, section 1. Typical is the constriction in section 2 (Fig. 1cd). This feature is a typical sign for the species of the *thummi* complex. The bands in section 3–4–5–6 are dark. Sections 9–10 were functionally active. The bands in sections 3–4–5–6 are markers for this arm. Band sequences: 1–5 are similar to those of *C. acutiventris* 11 (Wülker et al. 1983; Petrova & Michailova 1986). All bands are thinner than those of *C. obtusidens*.

Arm D. (Fig. 1cd). It is divided conditionally into 12 sections. The arm is characterized by bands in sections 7–8–9–10–11–12. The bands in sections 20–21 are similar to those in sections 6–7 of species from the *plumosus* group. In section 4 there is a "bulb"-like formation. The band sequence is almost identical to those of *C. obtusidens* (Keyl 1960, Wülker et al. 1983, Petrova & Michailova 1986).

Arm E (Fig. 2 abc). It is divided into 13 sections. This arm has band sequences: 1 2 3a 11ba 10cg ´3–4 10ba 9 8 7 6 5 3eb 11ed 12 13. The band sequence is identical to those of *C. obtusidens* (Keyl 1962). It is distinguished from *C. aberratus* Keyl by homozygous inversion (Keyl 1962).

Arm F (Fig. 2abc). It is similar to *C. obtusidens* (Keyl 1960, 1962; Wülker et al. 1983; Petrova & Michailova 1986). The bands are: 1 9–2 17–14 13–11 18 19ac 10 19d 20.

Arm G (Fig. 2d). It is divided into 5 sections. It has a nucleolus and a Balbiani ring, and always is paired. At the region of the nucleolus is a "heterochromatic dark knob".

3. Morphological studies

Material and methods: For the re-description, material (M.H. leg.) is available as mentioned below, but used (mounted in Euparal) to a limited extent in the measurements as mentioned (N) in the text.

1) Holotype, male (Zoological Museum, Helsinki) and 2 other males June, 1961, Posolampi, Korvanen, Sodankylä, north Finland.

2) Numerous adults, pupal skins and larvae 1981–1988 from the brook Hirvenoja, a few specimens from the river Punkanjoki and one male with pupal exuviae 1962 from a clay pond; all these in Riihimäki, south Finland.

3.1. Male

Wings 4.1 (2.9-4.6) mm. AR (antennal ratio, N = 8) 3.83(3.50–4.10). Palps (2–5, N = 8) in µm: 70–80, 280–300, 280–300, 315–390. LR (leg ratio): P1(N = 15) 1.43 (1.32–1.56), P_2(N = 8) 0.58(0.57–0.59), P_3(N = 8) 0.73(0.68–0.77). Number (N = 8) of sensilla chaetica: (distally in) P_2/Ta_1 12.3(7–17), P_3/Ta_1 11. (8–18). BR (tarsal beard ratio, N = 8): P_1 2.2(1.9–2.4), P_2 2.5(1.7–3.6), P_3 4.1(2.9–5.9). Legs (P_1–P_3, N = 8) in µm:

	Fore leg	Mid leg	Hind leg
Femur	1150–1750	1250–1830	1450–2060
Tibia	1050–1650	1140–1740	1400–2130
Tarsus1	1640–2390	670–1000	1080–1550
Tarsus2	850–1280	400–640	610–930
Tarsus3	620–910	300–450	420–660
Tarsus4	550–760	170–300	250–390
Tarsus5	250–360	140–200	120–230

Hypopygium as in Fig. 3. Processus analis apically always rounded, width (N = 10) proximally < 88.3(60–100) µm; width of anal point / width of appendage 1 > 3.0 (cf. Lindeberg & Wiederholm 1979).

3.2. Female

Coloration similar to the male, which is relatively pale (Hirvenoja 1962, Fig. 1). Wing (N = 6) 4.0(3.0–4.9) mm. Palps (2–4 , N = 6) in µm: 60–90, 190–300, 200–340, 310–420. LR : P_1 (N = 13) 1.56 (1.45–1.67), P_2(N = 6) 0.53(0.47–0.56), P_3(N = 6) 0.69(0.67–0.71). Number (N = 6) of sensilla chaetica: P_2/Ta_1 94.0 (87–102), P_3/Ta_1 107.3(100–112). BR (N = 6): P_1 1.7(1.4–1.9), P_2 1.9(1.2–2.3), P_3 2.5(2.0–3.7). Legs (P_1–P_3, N=8) in µm:

	Fore leg	Mid leg	Hind leg
Femur	1200–2100	1300–2100	1300–2260
Tibia	1000–1650	1240–2050	1410–2400
Tarsus1	1610–2660	650–1060	970–1600
Tarsus2	800–1480	390–650	600–980
Tarsus3	620–1200	290–450	490–710
Tarsus4	550–1100	190–300	290–420
Tarsus5	260–450	150–190	150–220

Spermathecae oval, 157.7(120–170) µm long.

3.3. Pupa

Exuviae 9.5–10.5 mm long, abdomen quite pale. Frontal apotome with cephalic tubercles about 150 µm long and up to 110 µm broad, each with an apical seta. Thorax dorsally darkened and granulated to the base of the wing sheaths. Basal ring usually about 180 × 80 µm, its tracheal patch with <11–17 tracheoles across (N = 7), about 3 µm in diameter.

Shagreen on tergites 2–8 typical of the genus, on tergites (2)3–6 somewhat in the X form, extending laterally not over the most lateral anterior adhesion marks (Langton 1994; "Fensterflecken"Thienemann 1944, "Muskelmale"Fittkau 1962) at this level; on segments 5 and 6 spinules on paratergites (lateral to the row of the lateral adhesion marks). On segment 2 quite large pedes spurii and 94.3(87–101) hooklets (N = 8), the latter mostly with some minute posterior or dorsal denticles: other intersegmental short rows of orally directed spinules present close behind the armament of tergites 4 and 5; because the number of the intersegmental spinules is relatively small, the first impression may be that all are lacking (in comparison for instance with *C. riparius* Meigen).

Some 10–20 µm long points difficult to find on the pleural region, ventral to the lateral setae

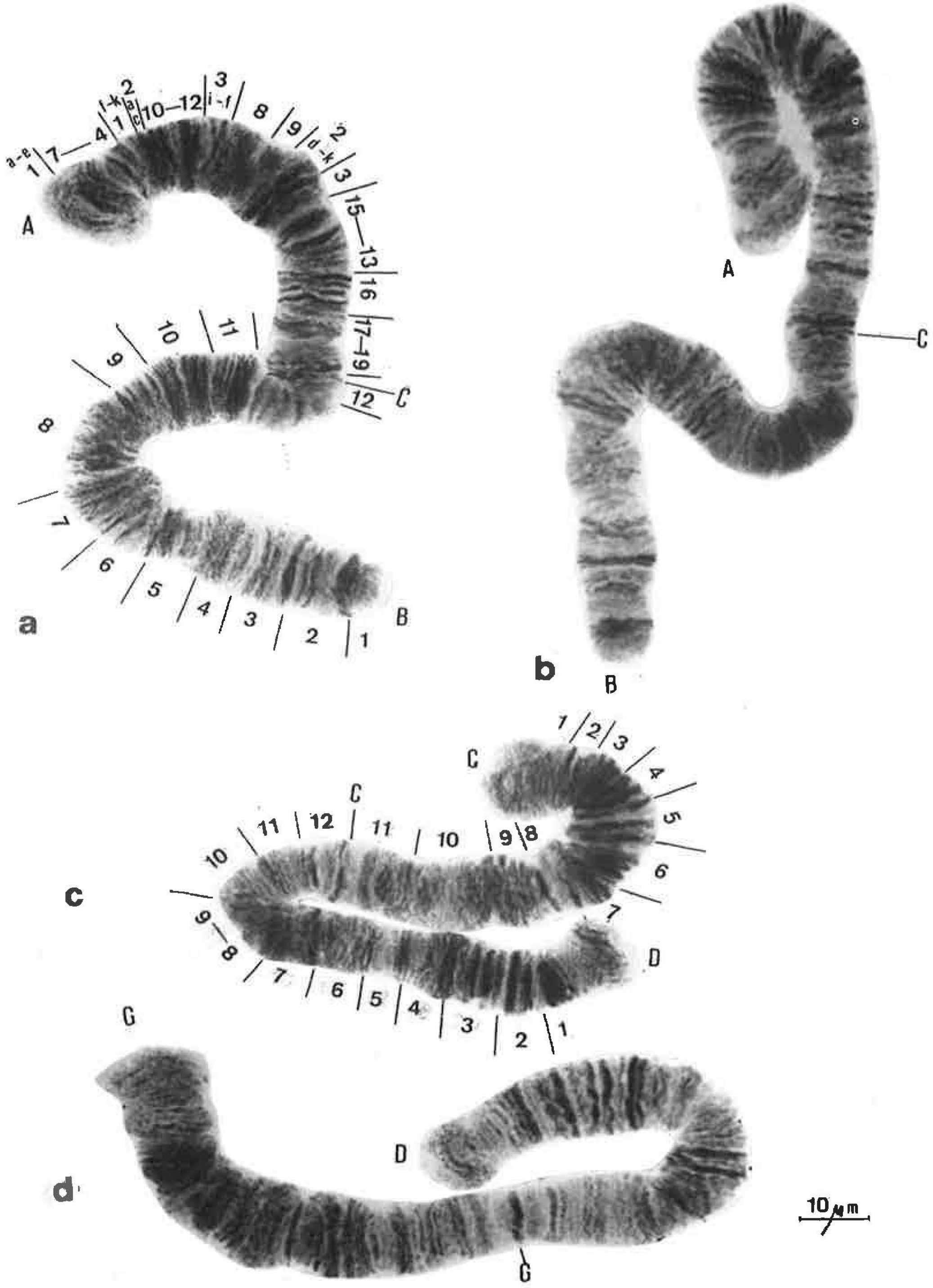

Fig. 1. Chironomus clarus Hirvenoja. a & b: Chromosome AB; c & d: Chromosome CD.

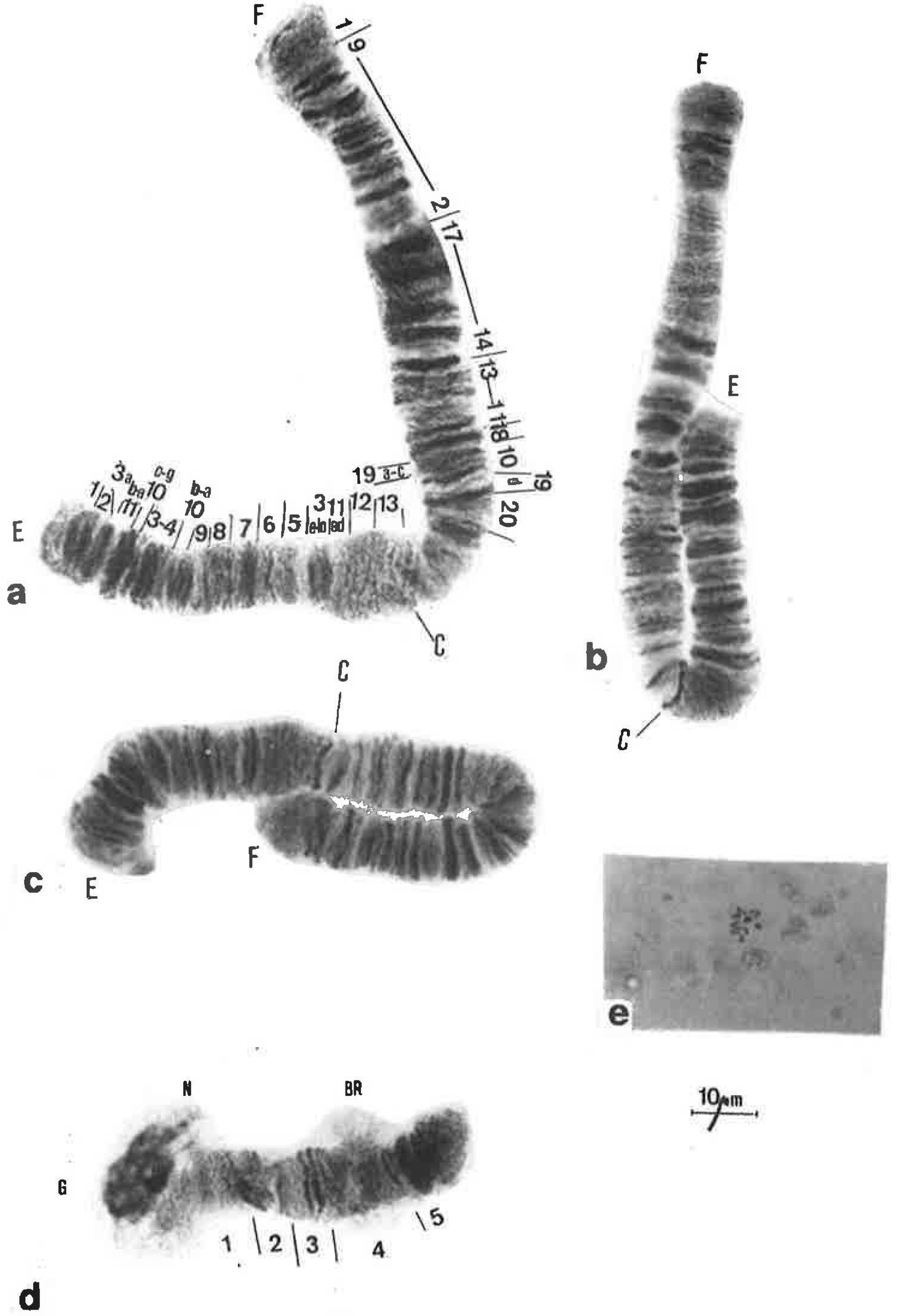

Fig. 2. Chir*onomus clarus* Hirvenoja. a, b & c: Chromosome EF; d: Chromosome G; e: Metaphase plate. — The scale does not apply to e.

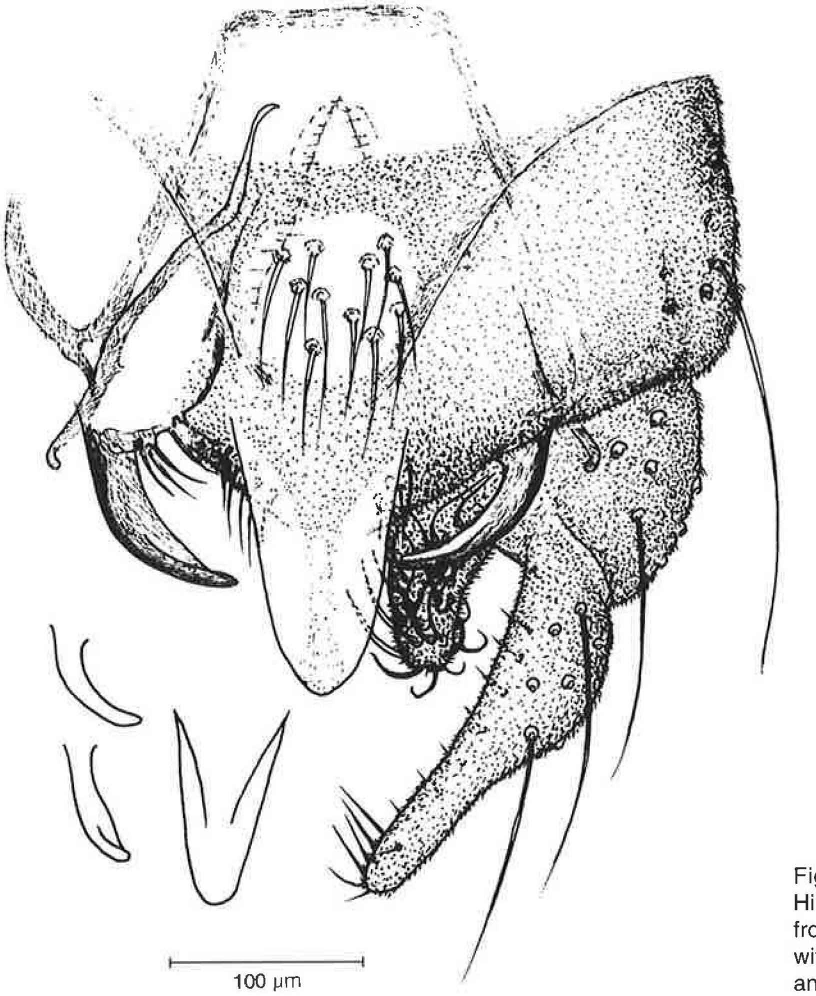

Fig. 3. *Chironomus clarus* Hirvenoja. Male hypopygium from the Riihimäki population with variation in appendage 1 and processus analis.

on segment 4. Anal-lateral to sternite1 are a few spinules in some exuviae. Lateral and medial longitudinal rows of spinules on sternite 2 clearly separated from each other, parasternite (?) also chagreened; longitudinal lateral rows present on sternite 3; a short or weak row may occasionally also be present on sternite 4; on sternite 3 is an oral-median patch of spinules (Fig. 4). Partly transverse rows, which are broader near the front corners of the sternites and narrow or lacking medially on sternites 6 and 7, and further small patches of spinules on the oral corners of sternite 5 and near seta V_1 on segment 8.

Lateral setae on segments 1–8: 0, 3, 3, 3, (and filamentous) 4, 4, 4, 4–5. Spurs on segment 8 with up to 5 fine points apically. Anal lobe fringe of filaments multiserial; number (N = 13) of setae 115.9(105–129).

3.4. Larva

Length up to 14–15 mm. Ventral tubuli (Fig. 5a) of abdominal segment 8 quite straight, about 0.9–1.3 mm long; the first pair longer than the second. No lateral tubules on abdominal segment 7. Claws of the anterior parapods smooth or very weakly serrated. Head capsule about 750 µm long, quite pale, ventrally somewhat darkened. In the sensory field of the labrum S_1 (Fig. 5b) 50–55 µm long, with hairs on one side except apically on both sides; the most apical flattened "hair" is characteristically the strongest; *Ch* setae fine plumate. Pecten epipharyngis with 11–13 teeth. Mandibles (Fig. 6a) pale except the denticles, of which also the smallest (third) proximal may be more or less darkened or pale. Paralabials with 38–45 striae, the striation about half of their breadth; the front border smooth. Hypochilum as in Fig 6c. In the premandibles the inner (dorsal) tooth apically paler and clearly thinner than the outer (ventral).

Antennae <190 μm; RO from the base of basal segment set at a distance about 1/3–1/2 the length of this segment; proportions of the segments (1–5; Fig. 6b):

100: 25: 14: 15: 6
100: 30: 9: 9: 7
100: 27: 10: 11: 7
100: 21: 7: 10: 6
100: 29: 1: 12: 6
100: 28: 9: 12: 6
100: 28: 8: 11: 6

Some relations based on the measurements (compare and see abbreviations in Wülker et al. 1983: 376–377) of the antennae and hypochilum in μm (N = 9):

Lat	L_1	L_2
64.3	117.6	31.2
(60–69)	(110–125)	(28–35)
W_1	R/L_1	$L1/W_1$
36.5	0.39	3,21
(33–40)	(0.32–0.52)	(2.75–3.48)
L_1/L_2	L_2/W_1	L_1/Lat
3.80	0.88	1.87
(3.29-4.81)	(0.70-1.00)	(1.72-2.08)
L_2/Lat	W_1/Lat	
0.48	0.54	
(0.43–0.55)	(0.49–0.56)	

4. Discussion

The larva of C. clarus belongs to the "fluviatilis larval type"of the older literature corresponding to the *obtusidens* group sensu Wülker & al. (1983). Larvae of this group have long, distally tapering, quite straight ventral tubuli on abdominal segment 8, but the lateral tubules of segment 7 are not developed. Other known European species of this group are according to Wülker & al. (1983) *C. obtusidens* and *C. acutiventris*, including two subspecies, *C. a. acutiventris* and *C. a. bavaricus* Wülker, Ryser & Scholl. According to Wülker & al. (1983) the adults of *C. acutiventris* resemble *C. cingulatus* Meigen or *C. venustus* Staeger.

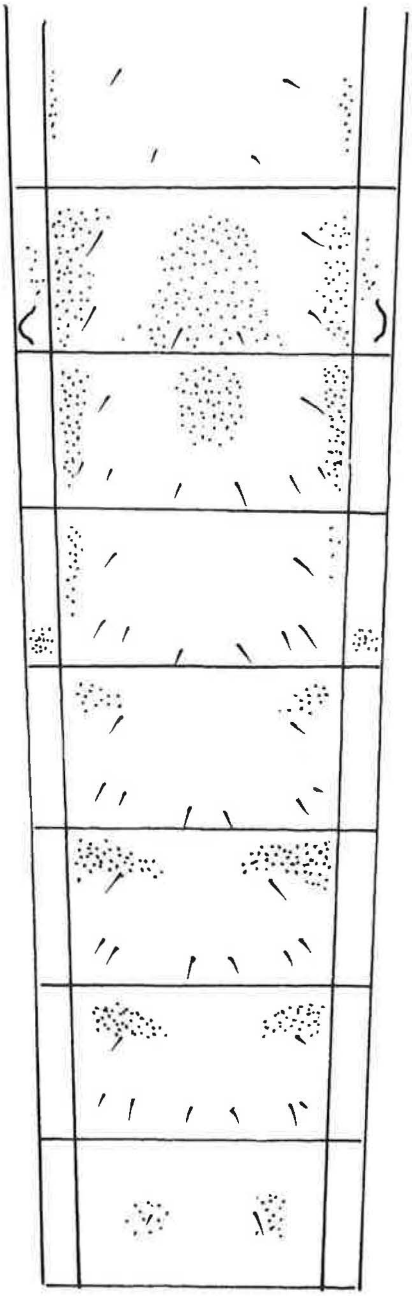

Fig. 4. *Chironomus clarus* Hirvenoja. Pupa: A scheme of the armament of sternites 1–8.

Morphometrically *C. clarus* and *C. obtusidens* are similar (cf. Strenzke 1959: 16–17). In their key to European species of *Chironomus*, Lindeberg & Wiederholm (1979 were able to differentiate be-

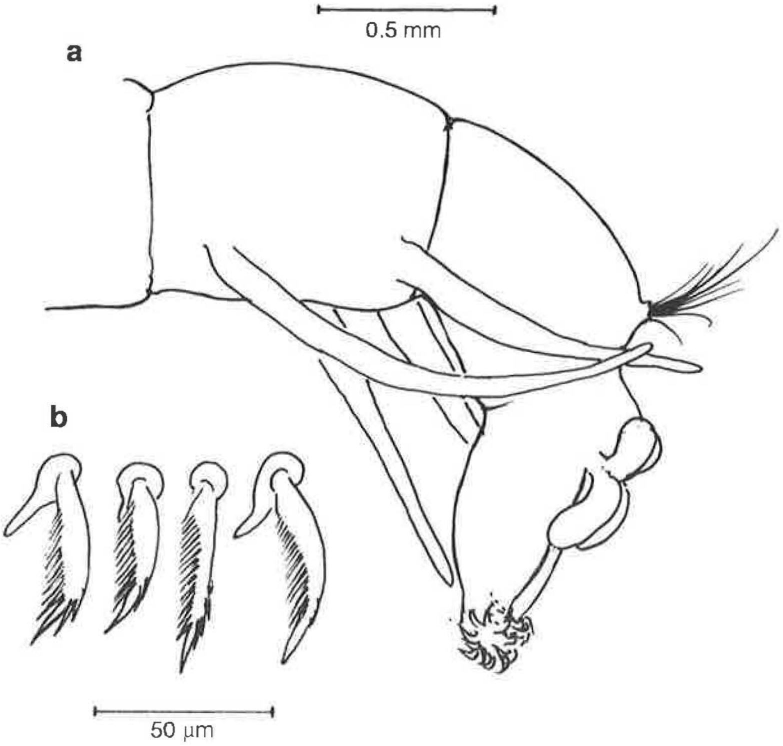

Fig. 5. *Chironomus clarus* Hirvenoja. a: Last segments of the larva, b: Aspects of seta S_1 in the sensory field of the labrum

tween the males of *C. clarus* and *C. obtusidens* because the processus analis of the hypopygium in the latter species is pointed (Fig. 7c in Strenzke 1959). No individuals with a pointed processus analis were found in our material, and the dorsal membrane seems to be narrower in *C. clarus*. Pinder (1978: Fig. 142C; cf. Goetghebuer 1921 p.151 Fig. 195, 1937–1954 Fig. 89 and Shilova 1958 Fig. 43!) has illustrated the hypopygium of *C. obtusidens,* in which the anal point is not clearly pointed and another one (sub *Chironomus* sp. a, Fig.142D) with a very broadly rounded anal point.

C. clarus is separable, according to Lindeberg & Wiederholm (1979, for instance from *C. cingulatus* because the anal point of *C. cingulatus* is narrower than in *C. clarus,* which partly makes a difference in the relation "width of anal point / width of appendage 1"as well as the length / width relation of the anal point proper.

The males of *C. acutiventris* and *C. clarus* may also be difficult to distinguish, because the anal point of the hypopygium seems, according to Fig. 11 in the original description of *C. acutiventris,* also to be quite broad, and other morphometric features are also quite similar. (Both species are distinguished by polytene chromosomes.) For instance the leg-ratio (LR) values of *C. clarus* are intermediate between the subspecies of *C. acutiventris*. Wülker et al. (1983: 738) mention, however, that in the hypopygium of *C. acutiventris* appendage 1 (or better 1a?) is not pointed.

It is probably correct to understand phylogenetically the often long, dominant apical portion of appendage 1 in Chironomini as an organ homologous to 1a of several Tanytarsini, mediatory structures being seen for instance in some species of *Einfeldia* and in some others. The reasons for some other terminological selections are the same as mentioned in Hirvenoja & Michailova (1991b).

Morphometrically, except for *C. obtusidens,* the females of *C. clarus* resemble, for instance, females of *C. annularius* auct. (see Lindeberg & Wiederholm 1979), but in local populations of Riihimäki the clearly different means of LR values between the females of *C. clarus* and *C. riparius* were useful in the determination (see also Strenzke 1959).

The pupal exuviae of *C. clarus* belong to the structural type, in which the paratergites 5 and 6

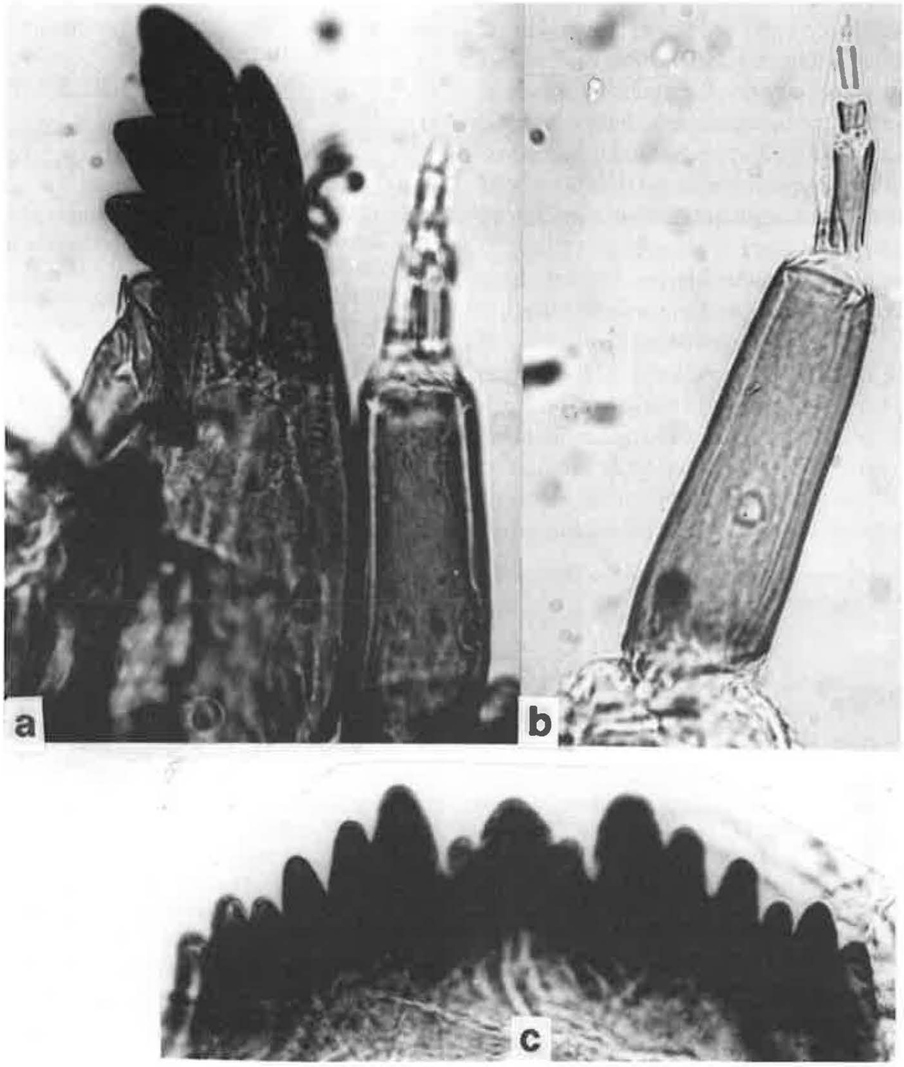

Fig. 6. *Chironomus clarus* Hirvenoja, Larva. a: Mandible; b: Antenna; c: Hypochilum

are spinulated, and also the pleura of segment 4 has few points (see Langton 1991: 246–251). It somewhat resembles also for instance *C. annularius* (cf. Langton 1991: 251). The tracheoles in the basal ring are, in *C. clarus*, however, somewhat larger, and differences exist in the shagreen of the sternites: the armament on sternite 2 is more reduced and median spinules on sternite 3 in *C. clarus* form a short patch, but in our specimens of *C. annularius* a broad longitudinal row; *C. annularius* has also longitudinal lateral rows of spinulae on sternite 4, whereas the corresponding rows are, if present, more or less reduced or may obviously also be lacking in *C. clarus*.

As in *C. obtusidens* or in *C. acutiventris* (see Wülker et al. 1983), the dentation of the hypochilum of the *C. clarus* larva is also of a type quite similar to that known as the "*anthracinus* larval type" with its small fifth lateral tooth. This type occurs in several species. Most similar larvae seem to be on the basis of the given morphometric features *C. clarus* and *C. acutiventris bavaricus*.

The data reported on the karyotype indicate that *C. clarus* and *C. obtusidens* are homosequental sister species (if not even synonyms). They have one and the same band sequences. However, *C. obtusidens* has thinner bands than those of *C. clarus*. This situation is known in the genus *Chironomus* and has been found for example in the species pairs *C. riparius C. piger* (Keyl & Strenzke 1956). According to karyotype, this species should be combined with *C. obtusidens* and *C. acutiventris* in the *obtusidens* group sensu Wülker et al. (1983). *C. clarus* is a monomorphous species. The sex-determining inversion which has been observed in arm F of *C. obtusidens* has not been found in our material.

Because of the great similarity of the species discussed here, further careful studies are desirable to confirm the constancy of the characters. Ecologically, *C. clarus* (cf. also Hirvenoja & Michailova 1991a: 318) prefers lotic biotopes or lacustrine littoral as do the other species of the *obtusidens* group.

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