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Description of *Chironomus quinnitukut*, n. sp., closely related to the *C. decorus* group in North America, with characterization of an additional larval form from halobiontic habitats

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Abstract

Chironomus quinnitukqut **n. sp.**, from halobiontic habitats in Connecticut and Massachusetts, is described on the basis of the adult and larval morphology, and the banding pattern of the salivary gland chromosomes. In previous studies, the Connecticut population has been referred to as *Chironomus atrella* Townes, but a re-examination has indicated that it can be readily differentiated from *C. atrella* in all life stages. Rather, the banding pattern of the polytene chromosomes indicates the species, is best placed as a member of the *Chironomus decorus* group. Larvae of a second halobiontic species, *C.* species Cape Cod, are morphologically very similar to *C. quinnitukqut* and this species also appears to be a member of the *C. decorus* group.

Key words: Chironomidae, *Chironomus*, new species, karyosystematics

Introduction

In 1968, Anderson and Hitchcock described the biology and tested for the control of an estuarine species of *Chironomus* that had been identified as *C. atrella* by S.S. Roback and H.K. Townes Jr. Subsequently, larvae were obtained from the same location for cytological examination, which clearly showed that this species was not in fact *C. atrella* (Martin *et al*. 2006). Consequently it is necessary to allocate a different name for the species from estuarine habitats. One possibility would be *C. halophilus* Packard (1874), but this name is rejected on three grounds: 1, As with previous attempts (e.g. Townes 1945), it has not been possible to locate Packard's material, so it is not possible to check the details of the larval morphology; 2, an additional and cytologically different larval form occurs in our Massachusetts specimens; and 3, Packard notes that the larvae possessed ventral tubules, probably a bathophilus-type, while the name *halophilus*-type has been used to describe a larval type with very reduced ventral tubules (e.g. Harnisch 1942), which would cause confusion if the name were to be resurrected for a species that does not have a halophilus-type larva. Packard's species is therefore best considered a *nomen dubium*, and the present material is described as a new species, based on the adults, larvae (including a few pupal characters from a prepupa) and cytology. The larvae of the second species at Massachusetts have the preliminary name of 'species Cape Cod' (Martin 2010), and some characters to enable separation of the two species are given.

Material and methods

Morphological terminology follows Sæther (1980), Webb & Scholl (1985) and Vallenduuk & Moller Pillot (1997). Measurements include range, median (meristics), or mean (mensurable), and, in brackets, the number

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of specimens examined. In the case of the adult males, the measurement of the holotype is given first, and the specimens examined are paratypes. Chromosome preparation techniques, the identification of chromosome arms and the standardization of the banding patterns of arms A–F are as previously described (e.g. Martin *et al*. 2006). Most larvae had been slide mounted before they could be measured, so measurements of total length and ventral tubule length were only possible on a few specimens. Consequently ventral head length (VHL) is used as a measure of comparative size.

Abbreviations. Collectors, JM—Jon Martin; SWH—Stephen W. Hitchcock, JP—John Portnoy. Institutions, UMN—University of Minnesota Insect Collection, St. Paul, MN; ZSM—Zoologische Staatssammlung Muenchen. Other: BR—Balbiani ring, I—imago, sgcl—salivary gland chromosomes with larval body, ppt—parts per thousand, resp.—respectively. Other morphological abbreviations as in Sæther (1980).

Chironomus quinnitukqut n. sp.

Chironomus halophilus Packard 1874: 245 & 415: *nomen dubium*. The description is inadequate for recognition other than generic placement as a *Chironomus* and is listed here to avoid future possible confusion.
Chironomus atrella (Townes) Anderson and Hitchcock 1968: 1597: (misidentification).
Chironomus atrella (Townes) Hitchcock and Anderson 1968: 16: (misidentification).
Chironomus species 2n Martin 2010: karyotype and associated larva.

Type material. Holotype, male: U.S.A., Old Saybrook, CT, light trap, 10-VI-1966, SWH, in UMN. Allotype female, Paratypes 59 male, 22 female, Old Saybrook, CT, light trap, 10-VI-1966, 33 sgcl, South Cove, 17-V-1966, SWH.; 14 sgcl., Old Saybrook, CT, (approx 21 ppt salinity), 28-VIII-1970, JM and SWH, UCT 2-1, in UMN and ZSM; 3 sgcl., Truro, Barnstable Co., MA, Pilgram Lake, East Harbor, Cape Cod National Seashore, (19 ppt salinity), 29-VII-2005, JP.

Etymology. A common transliteration of the Mohegan (Algonquin) word meaning “place of long tidal river”, referring to the Connecticut River, the longest in New England and the source of the state name, Connecticut. The marsh, which is the type locality, at South Cove, Old Saybrook, near the Connecticut River mouth, has been designated as “Wetland of international importance”. To be used as a noun in apposition.

Diagnostic characters. This species will key in Townes (1945, P.117) to couplet 18 containing *C. atrella*; however, it does not fit the description of *C. atrella* as this species has a lower foreleg ratio and distinctive differences in the structure of its genital apparatus. The remaining three species in the key (couplets 19 and 20) all have broad anal points or other differences. The most distinctive features, a very narrow base to the anal point and an elongate, slightly curved superior volsella with an upturned tip, clearly separate *C. quinnitukqut* n.sp. from all other members of the genus in North America. The few other species with a narrow anal point have differently shaped anal points and superior volsellae. One specimen before us may represent a new species; perhaps *C.* sp. Cape Cod; it differs slightly in genitalia, coloration, some meristics, and size, being larger. Unfortunately, its foretarsi are missing and its association with sp. Cape Cod is not certain; thus it is not described further or included in this species account. A second specimen may also belong to this variant and is also excluded due to lacking foretarsi. The only known pupal spur, with three spines, has more than that of *C. atrella*, which has only one or two.

Larvae can be differentiated from *C. atrella* by the larval type (Shobanov *et al*. 1996), which is bathophilus in *C. quinnitukqut* and plumosus in *C. atrella*. At Cape Cod it was found with *C.* species 'Cape Cod’ (sp. 4k of Martin 2010), which is also a bathophilus-type larva with a similar VHL, but is sometimes distinguishable by the lower number of teeth in the pecten epipharyngis and the greater reduction of the fourth lateral teeth of the mentum.

Cytologically the fixed pericentric inversion in the CD chromosome is diagnostic; the median position of band groups 24–28 in arm B will distinguish it from all species other than sp. Cape Cod, where the sequence appears similar, and *C. decorus* Johannsen in which these bands are in the reverse orientation. The relatively longer arm G will distinguish it from *C. atrella*, and the lack of a distinct heterochromatic band adjacent to the nucleolus, along with pairing usually only at the distal end of arm G will distinguish it from sp. Cape Cod.

Description. Male imago. Holotype male.

Coloration: Thoracic vittae, postnotum, a spot on the pleural sclerites, pre-episternum, a spot posterior to the lateral vittae , legs and abdomen largely blackish; ground color of head, thorax, including most of antepronotum and scutellum and narrow apices of the abdominal tergites paler yellowish.

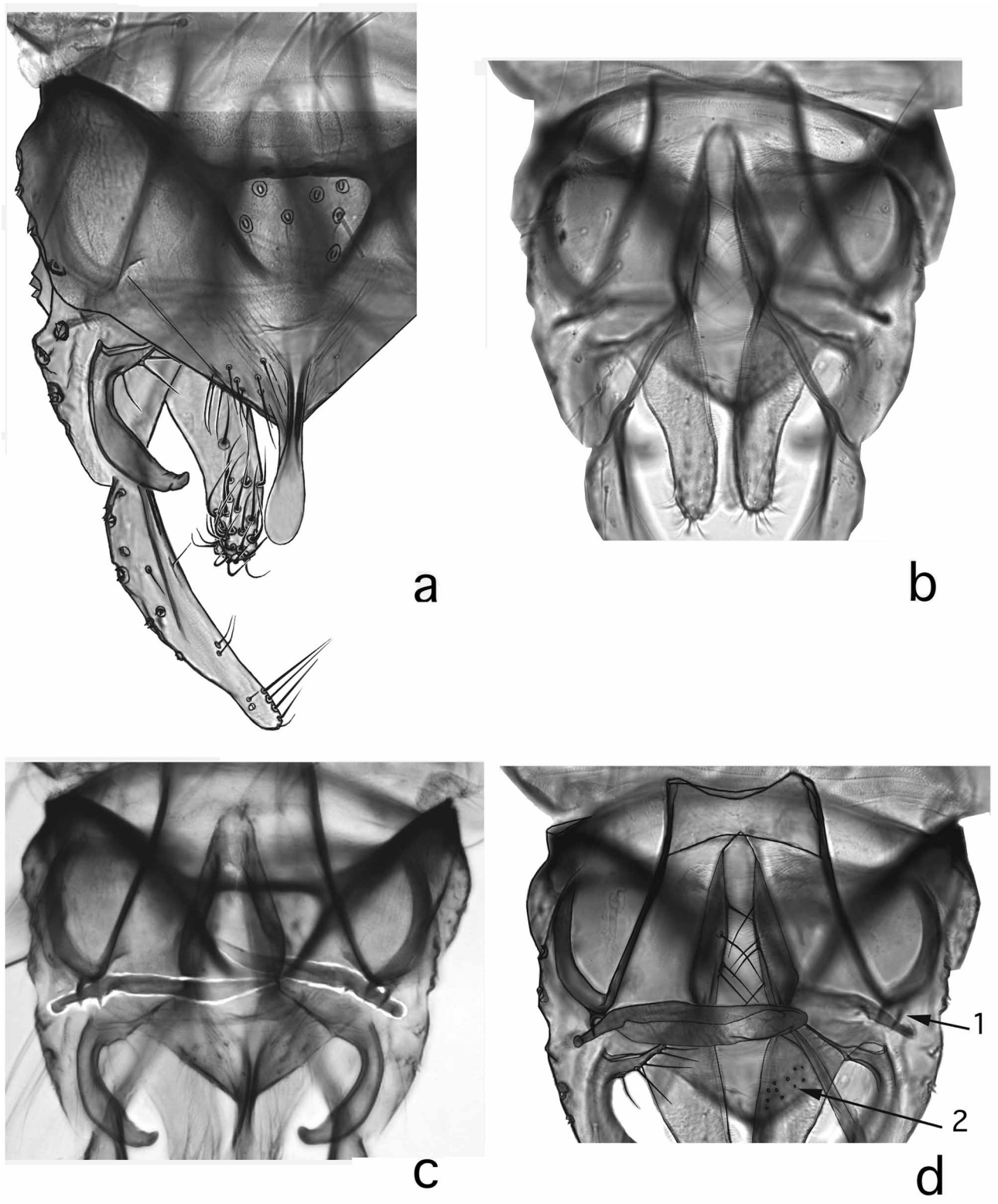


FIGURE 1. Male genitalia of *C. quinnitukqut*: a. dorsal view, b. inferior volsellae, c. apodemes, d. phallapodeme and pars centralis - 1. Note the double "fulcrum" on the transverse sternapodeme, and 2. Ventral portal setae.

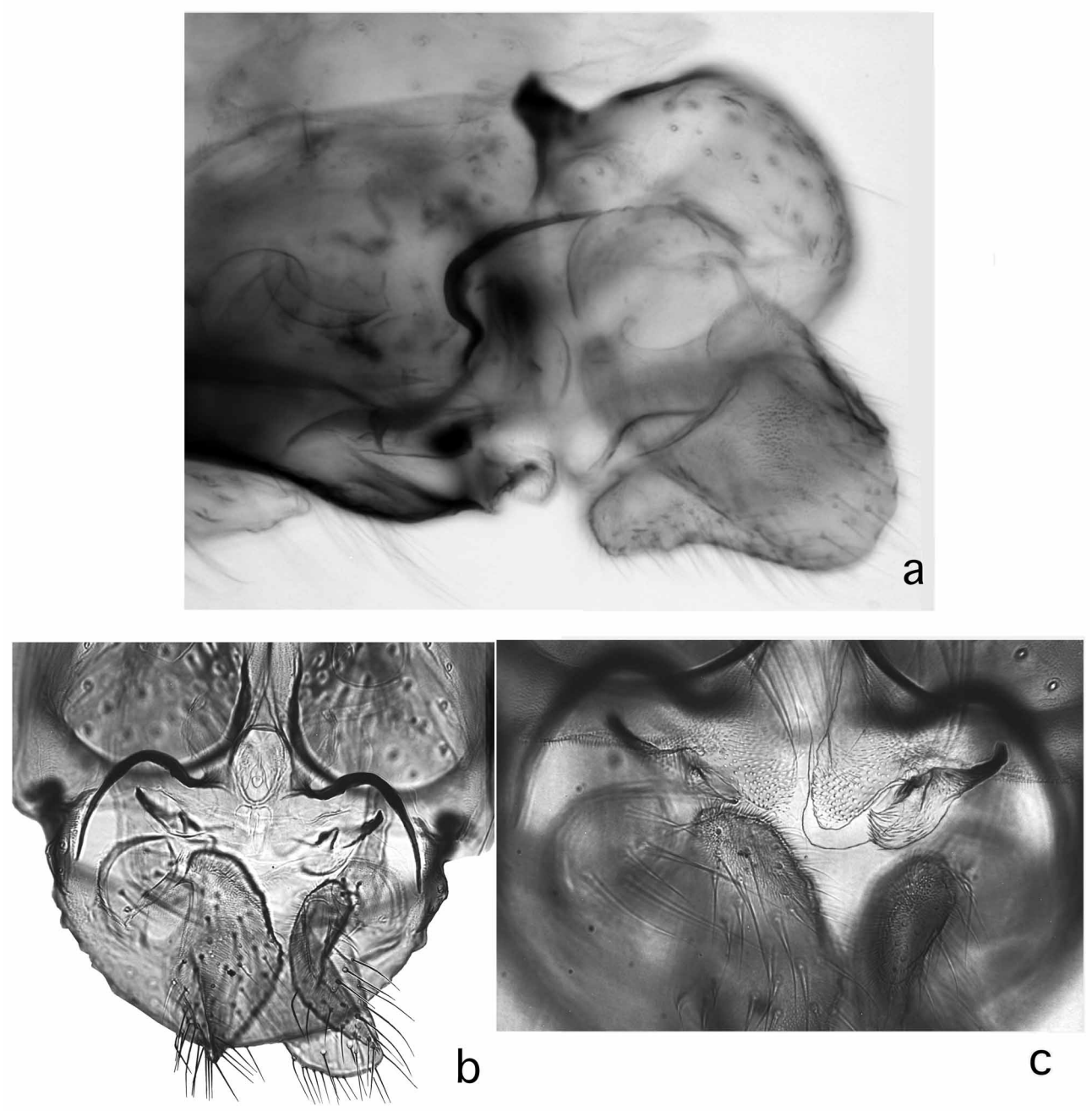


FIGURE 2. Female genitalia of *C. quinnitukqut*: a. lateral view, b. ventral view, c. enlargement to show vagina and gonocoxite IX.

Head: Antennal ratio 3.57 [3.41–4.70, 4.16 (8)] ; temporal setae 35 [22–35; 30 (10)]; clypeal setae 38 [24–56; 36 (10); ocular ratio 0.20 [0.17–0.19; 19 (4)]; frontal tubercle length 30 [25–42; 33 µm (9), almost as wide as high]; clypeal width/pedicel width 1.0 [0.7–0.86; 0.78 (7)]; palpal proportions (apical four flagellomeres): 70:203:189:224µm.

Thorax: Antepronotum rather narrow, slightly projecting at the apex. Dorsocentral setae 29 [25–42, 31(10)], in a single to mostly triple row; acrostichal setae present but obscured in lateral mount; prealar setae 7 [6–7, 7 (9)]; scutellar setae 26 [22–42, 29(10)], mostly in a single slightly staggered row laterally, becoming doubled at medial apex.

Wing: Anterior veins darkened with r-m slightly darker still; R_{2+3} ends at 0.33 of the distance between R_1 and R_{4+5}; wing length 3.70 mm [2.96–4.31, 3.57 (10)]; setae: R 24 [24–45; 27 (5)]; R_1 8 [6–19; 16 (5); R_{4+5} 7 [3–7; 6 (5)]; squamal 36 [21–43; 30 (9)].

Legs: Ratios p_1 1.16 [1.14–1.22; 1.17 (6)]; p_2 0.58 [0.55– 0.59; 0.57 (6); p_3 0.71 [0.70– 0.75; 0.72 (5)]. Beard ratio, p_1 4.15 [5.71–7.16; 6.33 (6)]. Sch, p_2 9 [12–21; 15 (7); p_3 13 [9–19; 14 (7)].

Genitalia: (Fig. 1); gonostylus width/length ratio 0.22 [0.19–0.21; 0.20 (5)]; gonostylus/gonocoxite ratio 0.73 [1.11–1.37; 1.18 (5)]; superior volsella of the E-type (Strenzke 1959), length 150 [112–165; 145 µm(9)]; inferior volsella length 220 [185–234; 209 µm (9)]; Inferior volsella setae 37 [31–46; 38 (9)]; TIX setae 2 [2–7; 5 (9)].

Female imago. Allotype female.

Coloration: Similar to male but with the paler abdominal incisures slightly more pronounced.

Head: Antennal proportions 150:85:85:105:270 µm; palpal proportions 75:210:190:240 µm; temporal setae 19; frontal tubercles about as wide as high; clypeal setae 38; ocular ratio 0.18.

Thorax: Coloration as holotype male. Antepronotum similar to holotype male; setae: dorsocentral 41 extending anteriorly to near antepronotum; acrostichal present but not counted; prealar 7; scutellar 38.

Wing: Veins coloured as male but more intensely; R_{2+3} somewhat folded under, ending at 0.17 of distance between R_1 and R_{4+5}; wing length 3.61mm; setae: R 30: R_1

28; R_{4+5} 39; squamal 38; VR 1.12.

Legs: p_1 LR 1.24; p_2 LR 0.55; p_3 LR 0.71; Sch p_2 47; p_3 54.

Genitalia: Fig. 2.

Pupa. No pupae were available, but the following pupal characters were determined from a late prepupa: Basal Ring about 140 µm long, but folded over obscuring width, setal fringe of anal lobe multi-layered, about 115 setae on each side, spur of segment VIII (Fig. 3g) about 250 µm long, with 3 spines progressively along the outer edge.

Larva (Fig. 3). A medium sized bathophilus–type, length about 11.2–13.2 (6) mm, VHL about 300µm [278–311 µm (11)], lateral tubules absent. Ventral tubules relatively short, but length may be influenced by ecological factors since those of larvae from Old Saybrook are much shorter than those from Truro [anterior about 0.38–0.46 mm (4) and 0.75–1.04 mm (3) resp., posterior about 0.28–0.46 mm (4) and 0.96 mm (2) resp]. Gular region dark to very dark over most of its surface, frontoclypeus pale, but sometimes with slightly dark lines alongside it and some darkening around the base of the antenna. Setae of labrum typical for the genus. Mentum (Fig. 3d) with somewhat rounded teeth; c1 tooth broad and relatively tall, c2 teeth little more than notches (type I–II); fourth laterals slightly reduced compared to the third and fifth laterals, sixth laterals often arising lower than the line of origin of the other laterals. Ventromental plates (Fig. 3e) with about 38 – 46 (11) striae, and separated from each other by about a third [0.29–0.37 (11)] of the total width of the mentum. Premandible (Fig. 3b) with inner tooth 2–3 times wider than outer tooth. Pecten epipharyngis (Fig. 3a) with about 13–20, 15 (11) irregular teeth. Mandible (Fig. 3f) with third inner tooth relatively well developed and almost completely separated (type II–III), with about 12–16 striae on the inner surface near the base. Antenna (Fig. 3c) relatively short, basal segment only about 0.4 times the VHL [0.39–0.44 (11)] and about 2.3–3.2, 2.9 (11) times longer than wide, ring organ a third to half way up the segment; antennal ratio 1.99–2.33, 2.13 (11); antennal segments (µm): 123:24:7:13:7. Anal tubules short and rounded, about 230–555 (12) µm long, generally less than twice as long as wide [1.2–2.4x, 1.5x (12)].

Karyotype (Fig. 4). Four polytene chromosomes in salivary gland cells, with the chromosome arm combination AB, CD, EF, G (thummi-cytocomplex). Keyl pattern difficult to recognise, particularly for chromosome AB where the characteristic bands (groups 24 to 27) of arm B are away from the centromere and the "olive" (groups 6 and 7) of arm A is not obvious. In the CD chromosome, arm D is relatively shorter than arm C, as the result of a fixed asymmetrical pericentric inversion which moves band groups D24 to 21 into arm C, while only group C22 is added to arm D. The main nucleolus is virtually terminal in arm G, but a second nucleolus may be developed immediately distal to the four characteristic bands of arm B. Arm G may be fully paired, but is usually unpaired towards the nucleolus. Only one Balbiani Ring (BR) is normally visible in arm G, about one third of the distance distal from the nucleolus, but another may be developed near the distal end. Inversion polymorphism has been observed in all arms except for arm B.

Arm A. The banding pattern is difficult to identify, but there are some points of similarity to sequences in two other members of the *decorus*-group, *C. decorus* itself (sp. 3a of Martin *et al.* 1979) and *C. decorus*-group

species 2 (Butler *et al*. 1995). The distal bands of qutA2 appear similar to those in the equivalent position of decA1 (Fig. 5a, b)., while the proximal bands labelled 'a' in Fig. 4, appear similar to the proximal bands of *C. decorus*-gp sp. 2. Sequence A2 is derived from A1 by a simple inversion of about half of the arm (Fig. 5b,c)

Arm B. As noted above, the characteristic bands, about 23a to 28e, are located away from the centromere by about one third of the length of the arm. These bands are reversed (28e - 23a) compared to the normal pattern (23a - 28e) in other species (Fig. 4), sometimes with a nucleolus developed at their distal limit (about group 28) (Fig. 5b). This nucleolus was observed in a single specimen from Connecticut (Fig 5c). It appears that group 7 is towards the distal end of the arm (Fig. 5b), although the puff often developed in this group in other species, is rarely developed.

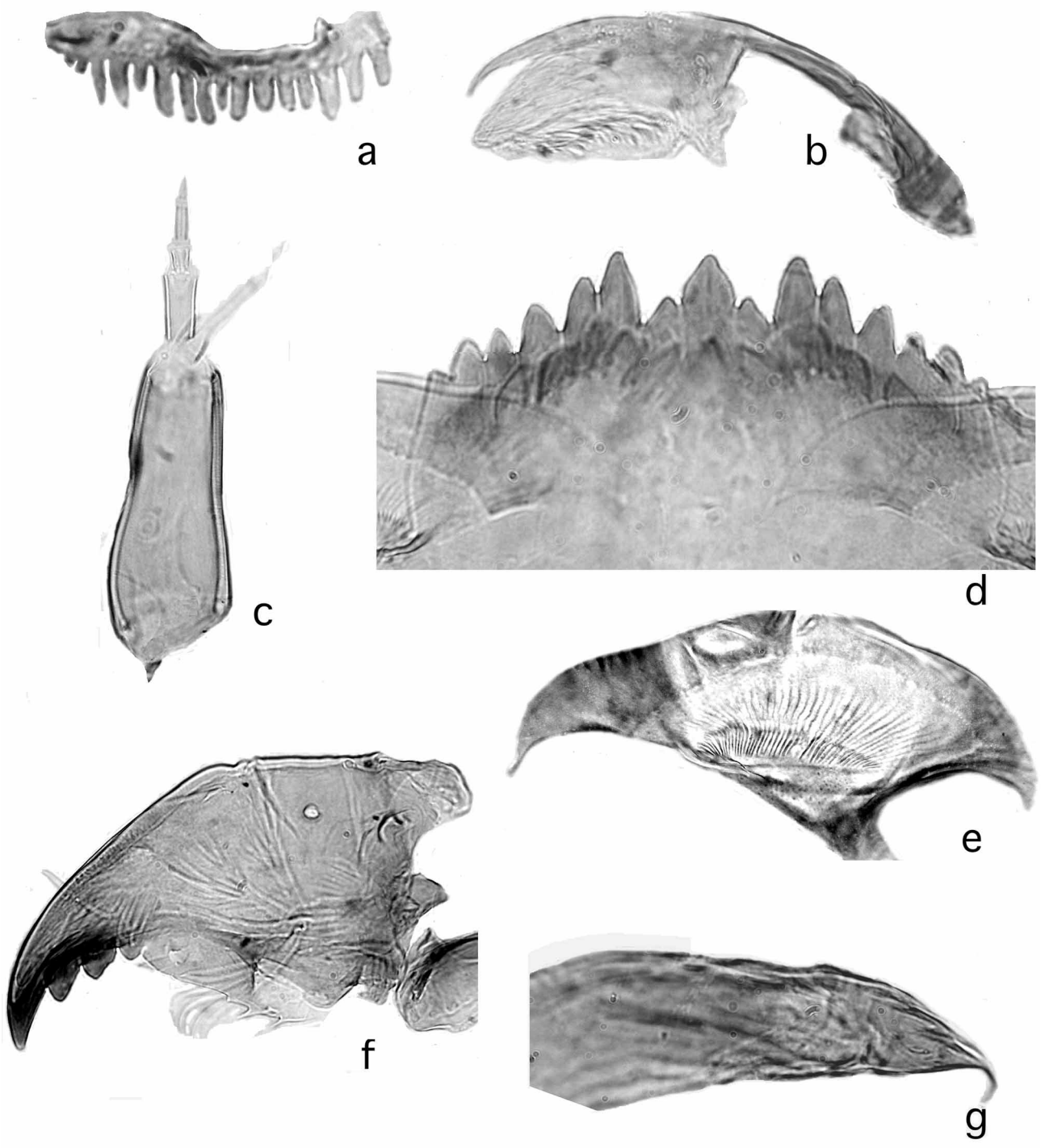


FIGURE 3. Larval characters of *C. quinnitukqut*: a. pecten epipharyngis, b. premandible, c. antenna, d. mentum, e. ventromentum, f. mandible, g. developing pupal spur from a prepupal larva.

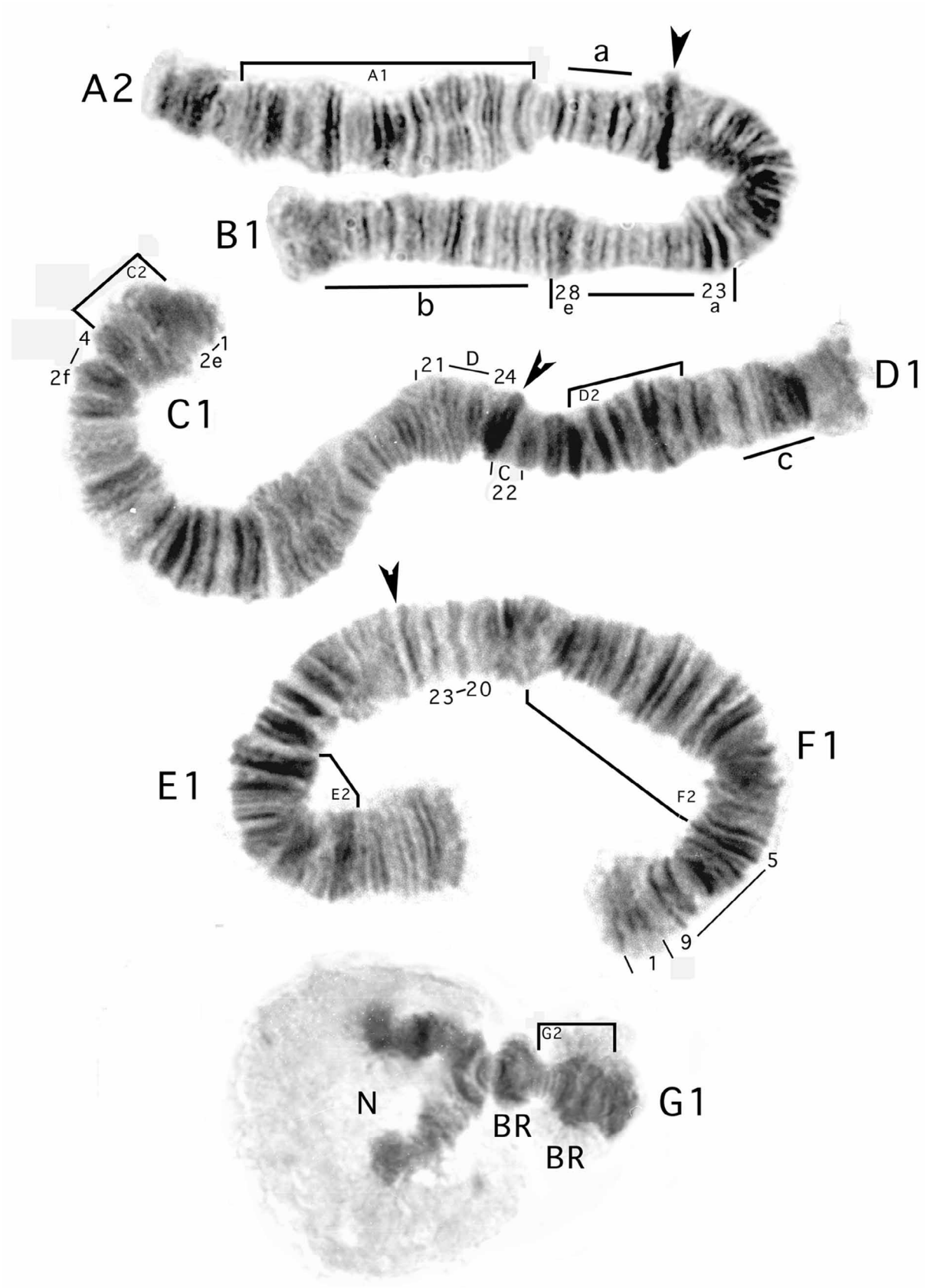


FIGURE 4. Salivary gland chromosome complement of *C. quinnitukqut*: Centromeres marked by arrowheads. Limits of intraspecific inversions marked by brackets above the region involved, lines underneath indicate regions of homology to other species (see text for details). N—nucleolus, BR—Balbiani ring.

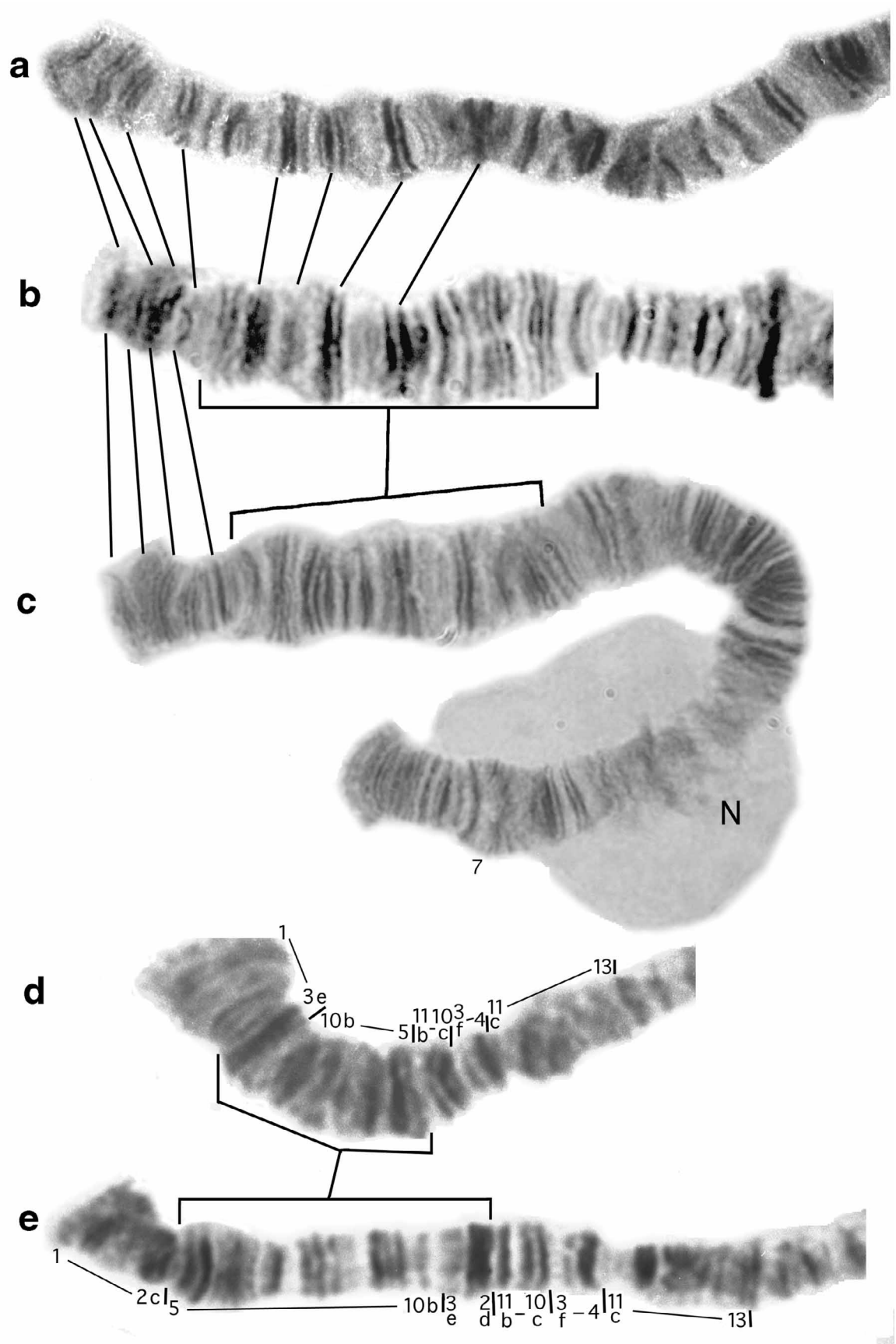


FIGURE 5. Polymorphisms and relationships of arms A, B, and E of *C. quinnitukqut*: a. decA1.1 of *C. decorus* showing region of possible homology with b. qutA2.2; c. qutA1.1 and B with nucleolus developed; d. qutE1.1; e. qutE2.2. Symbols as in Fig. 4.

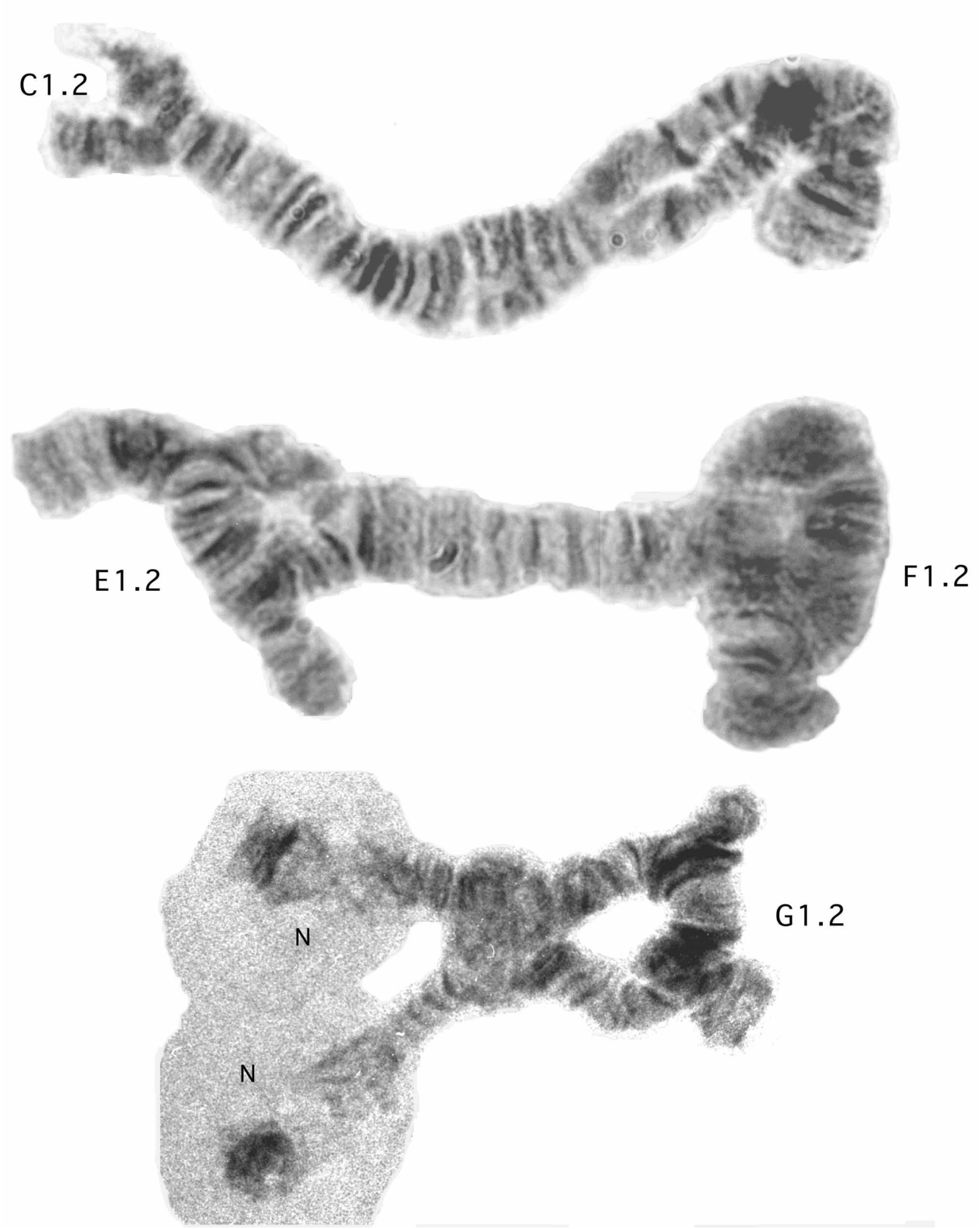


FIGURE 6. Heterozygotes for inversions in arms C, D, E, F and G of *C. quinnitukqut*.

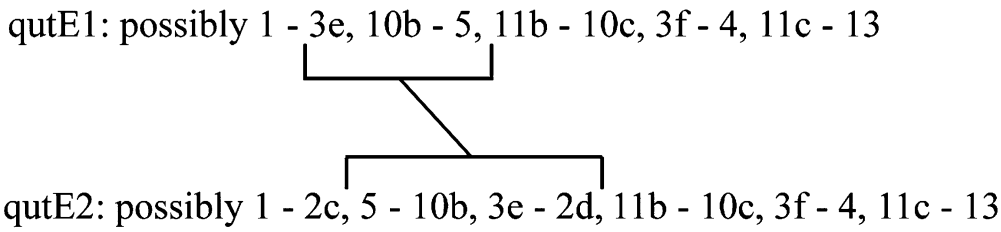
Arm C. As noted above band group C21 is followed by groups D21–24. The banding pattern from 1–12c may correspond to that of bla2 of *C. blaylocki* (Wuelker *et al*. 2009), with the typical constriction of groups 4-3 within this region (Fig. 4), although the sequence between 2e and 4 is not clear. The polymorphism in C is a

small inversion within this region (Fig. 6a), perhaps 2d-5, found only as occasional heterozygotes. The rest of the banding pattern up to the inserted regions of arm D is uncertain.

qutC1: 1 - 2e, 12b?, 6b? - 2f, 12c, ? - ?, 21, (D)21 - 24

Arm D. There are two sequences of this arm. The inversion covers about one third of the arm, starting over one third from the distal end of the arm, and appearing to extend almost to the centromere due to lack of pairing (Fig. 6), although the inversion is actually shorter (Fig. 4). The banding sequence is uncertain, but it is likely that the groups 19 and 20 are immediately distal to the inverted group 22 from arm C.

Arm E. Sequence E1 appears to be derived from the basic sequence of *C. aprilinus*, etc. (Wuelker 1980), by the small inversion In4-11b. The alternative sequence qutE2 is then derived from qutE1 by the simple inversion In2d-5 (Fig. 5d,e).



Heterozygotes qutE1.2 (Fig. 6) have been found in both populations, and a homozygote E2.2 was found in Connecticut.

Arm F. There are two sequences of arm F. It has not been possible to clarify the whole banding sequence, but band groups 9-7 are distal, following group 1 (Fig. 4), suggesting the sequence is based on the inversion 9-2, typical of the *C. decorus* cytological group. However it is not clear how much of that inversion remains beyond about group 5. The alternative sequence, qutF2 is known only from heterozygotes (Fig. 6) in both populations, indicating a simple inversion of about two thirds of the arm, with approximate limits as shown in Fig. 4.

Arm G. The general features of this arm - extent of pairing and position of the nucleolus and BRs have been given above. The virtually terminal nucleolus and the lack of pairing at this end of the chromosome is in common with a number of other members of the *C. decorus*-group, such as *C. decorus* (sp. 3a) (Martin *et al.* 1979); *C. decorus*-group species 2 (Butler *et al.* 1995), *C. decorus* (sensu Rothfels and Fairley 1957) and *C. bifurcatus* (Wuelker *et al.* 2009). Two sequences for this arm are known, with qutG2 differing by a small simple inversion at the distal end of the chromosome (Figs. 4 and 6). This sequence was found in two specimens at the Old Saybrook locality.

Discussion

Although Townes and Roback both considered males of this species belonged to *Chironomus atrella*, that similarity is superficial, with differences in leg ratio and genitalia characters. These characters clearly indicate that it represents a previously undescribed species, which we have described here as *Chironomus quinnitukqut*. The overall morphology and cytology do not suggest a relationship to the European halobiontic species *Chironomus salinarius* Kieffer, but is most consistent with it being a member of, or closely related to, the *C. decorus*-group.

Possibly the closest species, in terms of the banding patterns of the polytene chromosomes, is the true *C. decorus* (species 3a of Martin *et al.* 1979, Martin 2010). Thus, although the detailed banding pattern of arm A has not been determined for either species, the distal end of qutA is similar to that of decA1, while a more medial region of qutA2 appears to have the same band order as the medial region of decA1. As well, the typical bands (region 22–28) are removed to an interstitial place in arm B of *C. decorus*. In decB1 this sequence is reversed to that of qutB1, but it is possible that the inversion decB4 creates the same banding

pattern as that of *C. quinnitukqut*. It also seems likely that the distal region of qutB1, labelled 'b' in Fig. 4, is similar to the distal region of decB1, although the puff in group 7 is only slightly developed in most specimens of *C. quinnitukqut*. Again in arm D, the region marked 'c' in Fig. 4 is as the same part of decD4. However, the arm E sequence does not relate directly to any member of the cytological *decorus*-group, but does have a relationship to *C. whitseli* Sublette and Sublette, a member of the broader morphological *decorus* grouping (Sublette and Sublette 1974). QutE1 is most closely related to the basic sequence found in *C. aprilinus* and *C. whitseli*, while the studied members of the *decorus*-group, including *C. decorus* itself, have an arm E derived from the basic sequence seen in *C. aberratus* (Wuelker 1980, 2007; Martin *et al*. 1979; Wuelker *et al*. 2009). This may indicate that the ancestor of the cytological *decorus*-group was polymorphic for these two basic patterns in arm E.

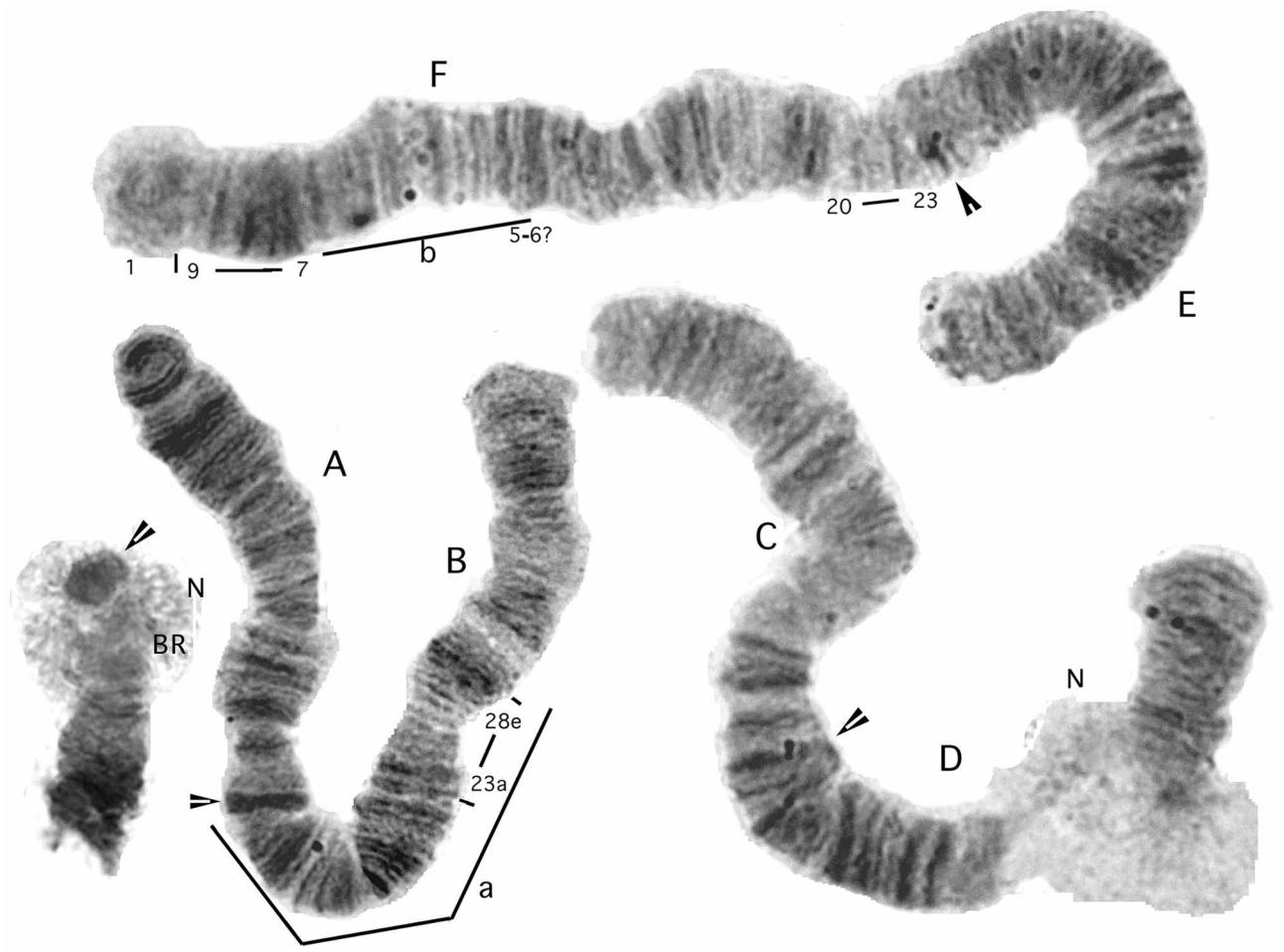


FIGURE 7. Salivary gland chromosome complement of *C.* species Cape Cod. a. basal region of arm B showing similarity to, and b. region of arm F inverted compared to those of *C. quinnitukqut*. Symbols as in Fig. 4.

Larvae of a second halobiontic species, *C.* species Cape Cod, were equally common in the sample from Truro. This species is not being formally named since the adult is not known for certain and thus these could be larvae of a previously describe adult form. The larvae are similar in gross morphology, with only slight differences to those of *C. quinnitukqut*, e.g. *C. quinnitukqut* generally has a lower number of PE teeth (13–20, 11) but the ranges overlap (19–24, 21 (5) in sp. Cape Cod), and a larva with only slightly reduced 4th laterals on the mentum is more likely to be sp. Cape Cod. While, the two species are readily separated by the cytology, there are also points of similarity that indicate that sp. Cape Cod is also closely related to the *decorus*-group, relatively close to *C. quinnitukqut*. The obvious differences are that arm G of sp. Cape Cod is normally unpaired, or paired at the small heterochromatic cap (the centromere?) proximal to the sub-terminal nucleolus (the paired chromosomes shown in Fig. 7 are unusual), with a BR immediately distal of the nucleolus. As

well, there is a second nucleolus about one third from the distal end of arm D (Fig. 7), and the fixed inversion of the CD centromere present in *C. quinnitukqut* does not appear to be present in sp. Cape Cod. Cytological relationship is indicated by the similarity of the basal part of arm B, including the shift of bands 23–28 to a more median position (a in Fig. 7), and arm F may differ by only a small median inversion (b in Fig. 7). The similarity of the distal end of arm F, including the sequence 1, 9–7 (Fig. 7), supports the suggestion that sp. Cape Cod is related to the *decorus*-group. Arm E, on the other hand, is not similar to that of *C. quinnitukqut* or to any other member of the *decorus*-group. Available specimens were not clear enough to determine the exact nature of the differences, but they appear to involve rearrangements near both ends of the arm.

There are also some homologies of *C. quinnitukqut* with *C. decorus*-group species 2 (Wuelker *et al*. 2009, Martin 2010), e.g. the proximal region of qutA2 labelled 'a' in Fig. 4, is similar to the proximal region of arm A of *C. decorus*-group species 2. The similarity of the gross morphology and pairing characteristics of arm G to other *decorus*-group species has been noted previously.

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