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Cytotaxonomic description of *Simulium kaffaense*, a new member of the *S. damnosum* complex (Diptera: Simuliidae) from south–western Ethiopia

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Although Ethiopia is one of the countries worst affected by human onchocerciasis, the exact taxonomic identity of the blackflies acting as the main vectors in the endemic areas has never been determined. A cytotaxonomic analysis of *Simulium damnosum* s.l. collected from three endemic sites in south–western Ethiopia has now revealed the existence of the 'Kisiwani' form (a non-anthropophilic cytoform that is common in East Africa) and a newly recognized species, *Simulium kaffaense*. *Simulium kaffaense* sp. nov. is differentiated from other members of the *S. damnosum* complex by six fixed inversions and dozens of 'new' floating inversions. The rearing of egg batches from some of the biting adult females, to larvae or adults, indicated that the human-biting blackflies were all *S. kaffaense*. As *S. kaffaense* is not only highly anthropophilic but also, apparently, the only anthropophilic member of the *S. damnosum* complex present, it is likely to be the main (if not the only) vector of *Onchocerca volvulus* in the study area. The presence of inversion 1S-1 and a complex inversion possibly involving 1L-3 indicates that *S. kaffaense* either belongs or is close to the 'Nile' phylogenetic group of *S. damnosum* s.l. The karyotype frequencies of the inversions in the collections from the three study sites indicate that at least two forms of *S. kaffaense*, here designated 'Bebeka' and 'Jimma', were caught. The taxonomy and medical importance of *S. kaffaense* are discussed.

Of the many African countries with endemic human onchocerciasis, Ethiopia is one of those worst affected, with >900,000 people thought to be infected with the causative parasite, *Onchocerca volvulus*, and approximately 10 million at risk of infection (Zein, 1993; Burnham, 1998). The disease is endemic in both the south–west and north–west of the country, over an area of 200,000 km^2. The south–western focus is perhaps the more noteworthy since — because the focus covers some highly fertile land with intense agricultural activity, including many coffee and tea plantations — the disease there tends to have the greater socio–economic impact.

Although about 30 *Simulium* species have been recorded in Ethiopia (Mebrahtu *et al.*, 1980), only *S. damnosum* s.l. and *S. ethiopiense* have been incriminated as vectors, with the former considered the more important (Tanaka *et al.*, 1973; Raybould and White, 1979; Gebre-Michael and Gemetchu, 1996). *Simulium damnosum* s.l. has a wide and patchy distribution over much of the country, including areas where no onchocerciasis has been reported, whereas *S. ethiopiense* is confined to the south–western highlands (Raybould and White, 1979; Mebrahtu *et al.*, 1980).

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Simulium damnosum s.l. is a complex of sibling species that differ in their vectorial competence, some acting as the main vectors of *O. volvulus* in Africa and on the Arabian Peninsula (Dunbar, 1966, 1969; Vajime and Dunbar, 1975). The design, implementation and evaluation of vector-control strategies, in any endemic area, require detailed knowledge of the behaviour of the *Simulium* species involved in the transmission of *O. volvulus*. This, in turn, depends on the correct identification of the vector(s) (Post and Boakye, 1992; Beebe and Cooper, 2000). Although there is much information available on the taxonomy of the *S. damnosum* complex, the members of the complex that occur in western Africa, especially in the area formerly covered by the World Health Organization's Onchocerciasis Control Programme (OCP), are better described than those to be found in East and Central Africa (Vajime and Dunbar, 1975; Post, 1986; Boakye, 1993; Boakye *et al.*, 1993). In East Africa, studies of the complex have concentrated mostly on the species within Tanzania (Maegga and Cupp, 1993, 1994; Procunier and Muro, 1993) and Uganda (Krüger *et al.*, 1998; Krüger and Garms, 1999).

The species of the *S. damnosum* complex that occur in Ethiopia are not fully known. The only information available is contained in the article by Dunbar (1976), who reported the existence of the 'Jimma' form in areas where onchocerciasis was endemic and the 'Kulfo' and 'Kisiwani' forms in other, onchocerciasis-free areas. Unfortunately, Dunbar (1976) failed to provide any chromosomal maps or morphological descriptions.

The African Programme for Onchocerciasis Control (APOC) has recently initiated the control of onchocerciasis in Ethiopia. Although APOC's main strategy is the use of ivermectin-based mass drug administrations (MDA) in endemic communities, vector-control activities are also being considered in areas where there is a well-defined focal distribution of the disease and vectors (such as the island of Bioko in Equatorial Guinea and some regions of Tanzania). Even where there is no vector control, the evaluation and monitoring of the MDA-based activities includes the assessment of several vector-transmission indices. Thus, whether or not APOC's managers decide that vector-control activities are appropriate in Ethiopia, the *Simulium* species that are known or thought most likely to be the vector(s) need to be carefully identified and their distributions need to be explored. The aim of the present study was to identify the species within the *S. damnosum* complex that occur in the endemic foci in south–western Ethiopia. During this investigation, a newly recognized species within the *S. damnosum* complex — designated *S. kaffaense* and identified as the probable main, vector of *O. volvulus* in the study area — was discovered.

MATERIALS AND METHODS

Study Sites

The three study sites, all of which are in south–western Ethiopia and in areas endemic for onchocerciasis (Oomen, 1969; Taticheff *et al.*, 1987), were the small Aboare stream at Bebeka, the River Gojeb at Gojeb, and the River Ghilgel Ghibe at Ghilgel Ghibe [from which White (1977) collected the 'Jimma' form (Dunbar, 1976)]. These sites (see Table 1) were known as 'Bebeka', 'Gojeb' and 'Ghilgel Ghibe', respectively.

Collection of Samples

Simulium damnosum s.l. larvae were picked off the aquatic vegetation and transferred into Carnoy's solution (three parts absolute ethanol to one part of glacial acetic acid, prepared freshly before each collection) in 'universal' sample bottles. The specimens were transported, in iceboxes under cool conditions, to the laboratory, where they were kept at 4°C until their chromosomes could be analysed and their morphologies investigated (see below).

TABLE 1. *Descriptions of the three study sites in south–western Ethiopia*

Locality	Vegetation and landscape	Altitude (m above sea level)	Co-ordinates	Date of collection
Aboare stream, Bebeka	Mix of forest and coffee plantation, in hilly area	1000	6°57′N, 35°22′E	2 October 2002
River Gojeb, Gojeb	Riverine vegetation next to savannah woodland, in mountainous area	1350	7°25′N, 36°22′E	16 October 2002
River Ghilgel Ghibe, Ghilgel Ghibe	Riverine vegetation next to cultivated fields, in flat area	1750	7°46′N, 37°12′E	19 October 2002

Pupae were collected, at the same time as the larvae, and biting flies were also caught, using human landing catches during the day, close to each sampling site; the fly collectors were from the endemic focus and gave their informed consent. Pupae and adult flies were kept in 80% alcohol, for subsequent morphological examination. A single egg batch produced by each of seven of the biting flies caught at Gojeb was reared through to larvae or adults, to allow the identity of the biting flies to be confirmed.

Preparation and Analysis of Chromosomes

The larval chromosomes were prepared as described by Dunbar (1972), with minor modifications. Briefly, the abdomen of each larva was opened while the larva was still in Carnoy's solution, in a small Petri dish. The larva was then washed four times with tap water, stained in lacto-acetic orcein for 3 h, and washed again in tap water (to remove excess stain) before its salivary glands were dissected out into 60% glacial acetic acid. The gelatin-like material was teased off the epithelial cells of the glands before these cells were placed in a drop of 60% acetic acid on a fresh microscope slide and squashed gently, under a coverslip, to spread the chromosomes. The chromosomes were then re-stained, examined under a light microscope and photographed at $\times 400$ magnification. The photographs of the chromosomes were compared with similar photographs of other *Simulium* chromosomes — firstly those of the standard 'Nyamagasani' form (Dunbar, 1969; Dunbar and Vajime, 1981), to determine the inversion breakpoints, and then with those of other relevant species or cytoforms (Vajime and Dunbar, 1975; Post, 1986; Boakye, 1993; Maegga and Cupp, 1993; Procunier and Muro, 1993), to see if any of the inversions observed had been described previously.

Morphology

The larval and adult stages were examined under a dissection microscope with overhead illumination, so that those of *S. damnosum* s.l. could be identified (Crosskey, 1973). The colours of the fore coxa, the antennae, the wing tuft, the arculus, the ninth abdominal tergite setae and the scutellar setae of each adult were scored (Wilson *et al.*, 1993).

Statistical Analysis

χ^2 tests were used to check whether the three different populations were in Hardy–Weinberg equilibrium. Hierarchical cluster analysis was carried out on the data-set of floating-inversion frequencies, for the full chromosome complement, to explore taxonomic relatedness. All of the data analyses were carried out using version 10 of the SPSS software package (SPSS Inc., Chicago, IL).

RESULTS

Cytotaxonomy

The results of the morphological examination of the 226 larvae that were collected and prepared for cytotaxonomic identification indicated that all were members of the *S. damnosum* complex. Only for 102 of the field-collected larvae of *S. damnosum* s.l. were the chromosome preparations of sufficient quality to allow complete karyotyping.

KISWANI FORM

Four specimens (two from Bebeka and one each from Gojeb and Ghilgel Ghibe) were identified as the 'Kisiwani' form, based on the presence of the diagnostic inversions 2L-5 and 3L-21 and a standard chromosome 1 (see Figures 1, 2 and 3; Maegga and Cupp, 1993).

NON-KISWANI FORM

Each of the other 98 specimens that were fully karyotyped possessed several 'new'

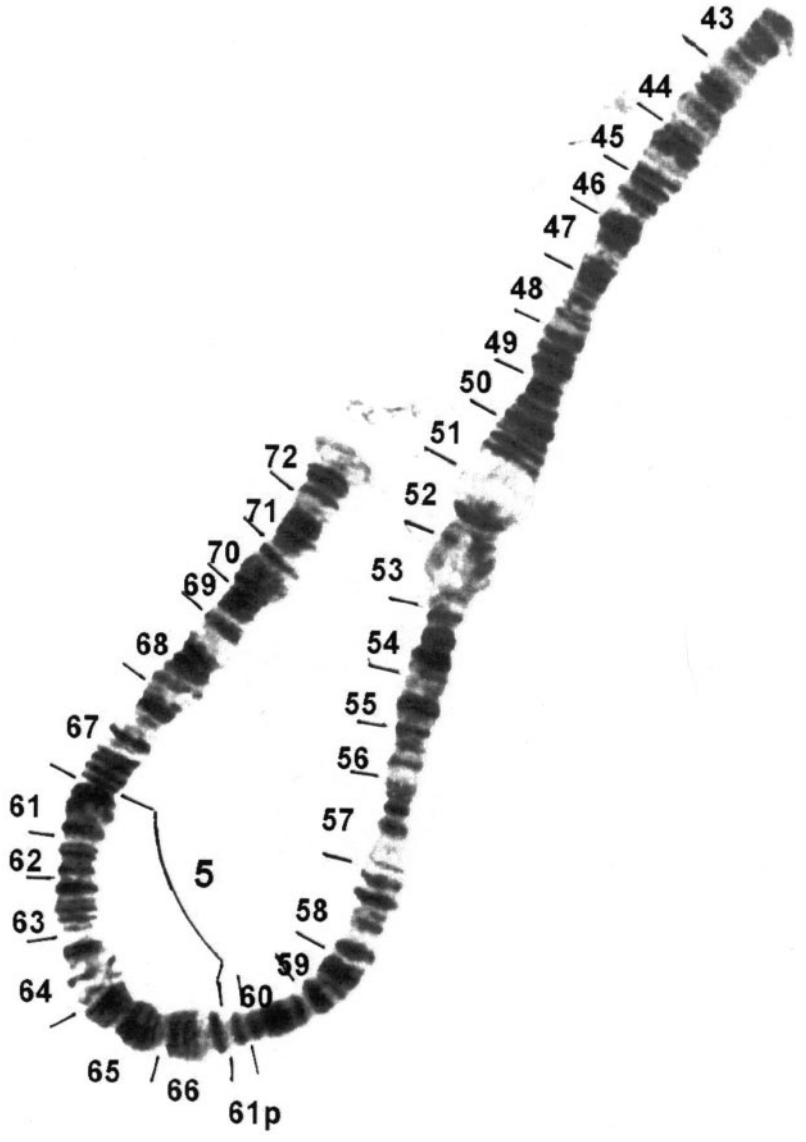


FIG. 1. Chromosome 2 of the 'Kisiwani' form (a female from Aboare stream at Bebeka), showing the diagnostic fixed inversion 2L-5.

inversions (both fixed and floating) on all three chromosomes. The frequencies of these inversions among the 'non-Kiswani' *Simulium* (nKS) caught at each of the three study sites are given in Table 2. Chromosome 1 possessed one new fixed inversion on the long arm (1L); this inversion, designated 1L-NE, is a four-step complex inversion involving segments 34p–29 (Figs 4 and 5) and was found in all 98 nKS. All the segments involved in 1L-3 (Vajime and Dunbar, 1975) are included in 1L-NE. Within 1L-NE is an inversion, 1L-P, which always appeared as heterozygote (Fig. 6). Another inversion, 1L-CE (running from part of segment 41 up to part of segment 39), was found only in the homozygous state (Fig. 4), indicating a possible heterozygous disadvantage. 1L-CE occurred at a much lower frequency in the

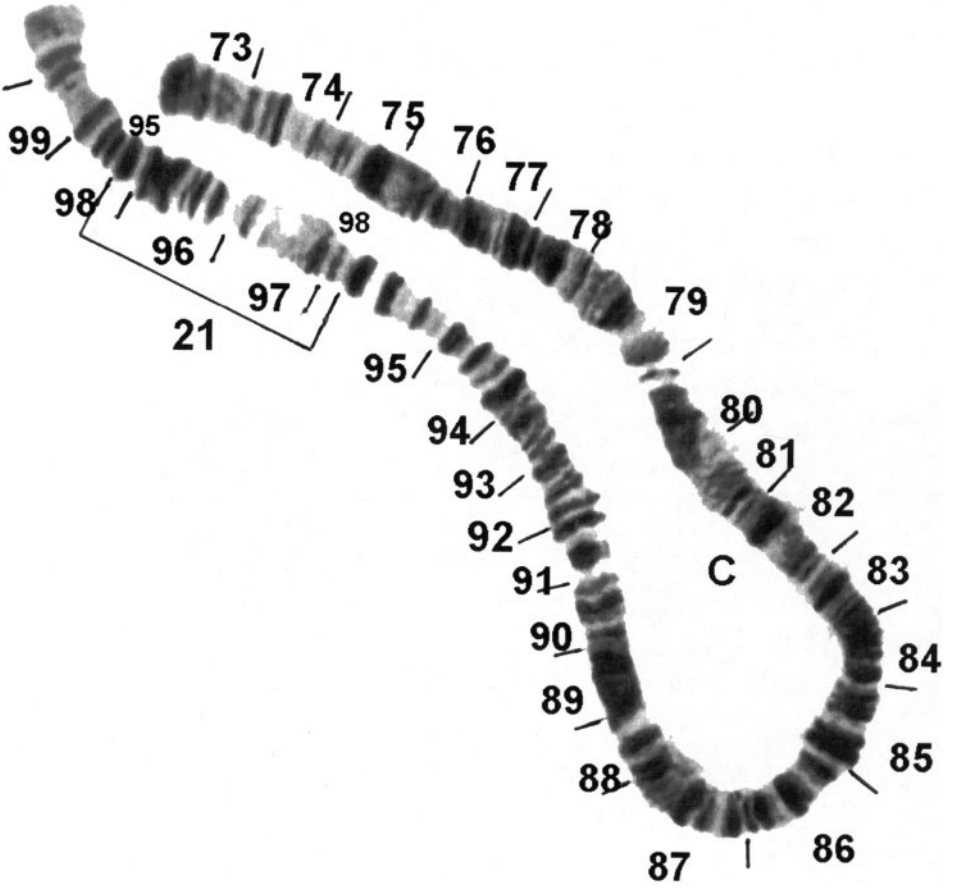


FIG. 2. Chromosome 3 of the 'Kisiwani' form (a female from Aboare stream at Bebeka), showing the diagnostic fixed inversion 3L-21.

Bebeka samples than in those from Gojeb or Ghilgel Ghibe (Table 2). In contrast, inversion 1L-1E, a two-step, floating inversion that ran from part of segment 38 to part of segment 41 (Figs 5 and 6), was much more common at Bebeka than at Gojeb or Ghilgel Ghibe (Table 2). 1S-BE (Figs 4 and 5), another floating inversion found at relatively high frequency, stretched from part of segment 14 up to segment 11; it was detected in 62% of the nKS from Ghilgel Ghibe and in all of the nKS from Bebeka and Gojeb. Inversion 1S-DE, which ran from part of segment 10 up to segment 8 (Fig. 7), was more common among the nKS from Ghilgel Ghibe (68.8%), than those from Bebeka (29.8%) or Gojeb (24.0%).

Inversion 1S-1 occurred at a similar frequency at each study site, either in the homozygous state (79.2% of the nKS) or the heterozygous (20.8%); this polymorphism of 1S-1 does not appear to have been reported before.

The rarer heterozygote inversions seen on chromosome 1 of the nKS included 1L-EX (running from segment 37 to part of 29 within 1L-NE), 1L-OE (from segment 35 up to part of 36), 1L-TA (from part of 35 up to segment 30), 1L-YE (from part of 25 to part of 27), 1L-3A (segment 36), and 1S-HA (from segment 3 up to part of segment 6).

Two new fixed inversions, designated 2L-E1 and 2L-E3, were found on chromosome

FIG. 3. Chromosome 1 of the 'Kisiwani' form (a female from Aboare stream at Bebeka), showing the standard arrangement.

2L. 2L-E1 involves bands within segments 63 and 66, whereas E3 involves segment 67 (Fig. 8). These inversions usually occurred together with three floating inversions (2L-E2, 2L-E4 and 2L-E5), leading to three different homozygous arrangements: 2L-E1.E2.E3/E1.E2.E3, 2L-E1.E2.E3.E4/E1.E2.E3.E4 and 2L-E1.E3.E5/E1.E3.E5 (Figs 8–10). These homozygous arrangements occurred at different frequencies

among the nKS from the three study sites (Table 2). The homozygous arrangements 2L-E1.E2.E3 and 2L-E1.E2.E3.E4 were found only in Bebeka, where the other homozygous arrangement, 2L-E1.E3.E5, occurred at a very low frequency. In Gojeb and Ghilgel Ghibe — except for two specimens with 2L-E1.E3/E1.E3.E5 — 2L-E1.E3.E5 was the only arrangement found. Figures 11, 13 and 14 show the heterozygous arrangements of 2L-E1.E2.E3/E1.E2.E3.E4, 2L-E1.E3.E5/E1.E3.E2 and 2L-E1.E2.E3.E4/ E1.E3.E5.

Another common floating inversion found on 2L was 2L-13, a two-step inversion presumed to be the same as that previously

TABLE 2. *The karyotype frequencies among the 'non-Kiswani'* Simulium *(i.e.* Simulium kaffaense *sp. nov.) larvae that were collected from the three study sites and fully karyotyped*

Karyotype	No. and (%) of the 'non-Kiswani' Simulium from:		
	Bebeka	Gojeb	Gilgel Ghibe
CHROMOSOME 1			
1L-NE	57(100)	25(100)	16(100)
1L-NE/NE.P	14(24.6)	1(4.0)	3(18.7)
1L-CE	14(24.6)	22(88.0)	13(81.3)
1L-1E/1E	22(38.5)	1(4.0)	2(12.5)
1L/1E	20(35.1)	2(11.8)	1(6.3)
1S-1/1	45(78.9)	18(72.0)	12(75.0)
1S/1	10(17.5)	7(28.0)	3(18.8)
1S-DE	0(0)	0(0)	3(18.8)
1S/DE	17(29.8)	6(24.0)	4(25.0)
1S-BE/BE	55(96.5)	20(80.0)	7(43.8)
1S/BE	2(3.5)	5(20.0)	3(18.7)
CHROMOSOME 2			
2L-E1.E2.E3	10(17.5)	0(0)	0(0)
2L-E1.E2.E3.E4	13(22.8)	0(0)	0(0)
2L-E1.E3.E5	2(3.5)	25(100)	14(87.5)
2L-E2/E2.E4★	17(29.8)	0(0)	0(0)
2L/E5★	0(0)	0(0)	2(12.5)
2LE2/E5★	7(12.3)	0(0)	0(0)
2L-E2.E4/.E5	5(8.8)	0(0)	0(0)
2L-13	2(3.5)	24(96.0)	16(100)
2L/13	12(21.1)	1(4.0)	0(0)
2S-DA.B.G	7(10.5)	6(24.0)	9(56.3)
2S/DA.B	22(38.6)	7(28.0)	2(12.5)
2S-DA.B/DA.B.G	0(0)	12(48.0)	5(31.3)
2S-DA	28(49.1)	0(0)	0(0)
2S/C	20(35.1)	0(0)	0(0)
CHROMOSOME 3			
3S-A	56(98.2)	25(100)	16(100)
3S/A	1(1.8)	0(0)	0(0)
3L-8	4(7.0)	20(80.0)	15(93.7)
3L/8	18(31.6)	4(16.0)	1(6.3)
3L-F†	46(100)	22(95.6)	15(100)
3L/F	0(0)	1(4.3)	0(0)
3L/D	5(29.4)	2(8.0)	0(0)
3L/E	5(29.4)	0(0)	1(6.3)

★Fixed inversions E1 and E3 are not shown.

†The percentages shown were calculated only for the chromosomes where segment 94 was not overlapped with other heterozygote inversions.

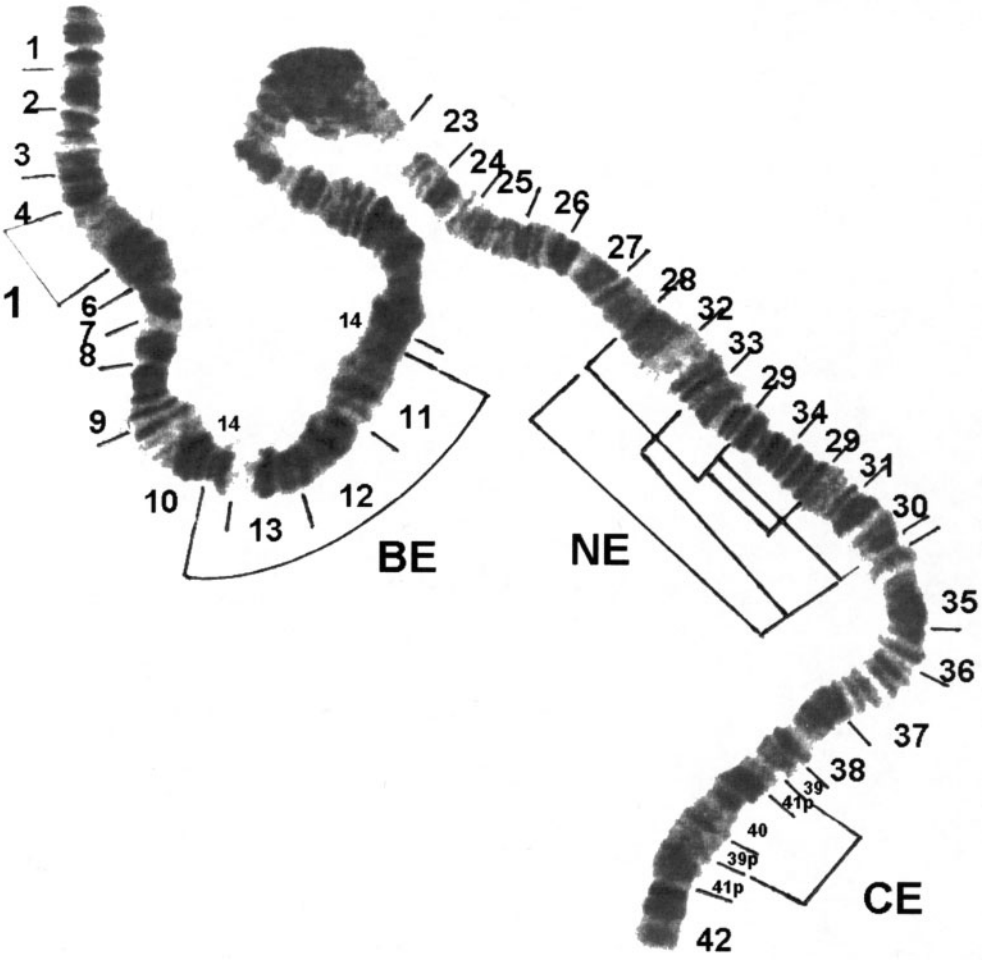


FIG. 4. Chromosome 1 of *Simulium kaffaense* (a female from Aboare stream at Bebeka), showing the fixed inversion 1L-NE, and floating inversions 1L-CE, 1S-BE and 1S-1.

mentioned (Dunbar, 1969) in the 'Kulfo' form (Fig. 10). 2L-13 was fixed in Gojeb and Ghilgel Ghibe (with 97.6% of the nKS homozygous) but only 24.6% of the nKS larvae from Bebeka had 2L-13, with 85.7% of these occurring as heterozygotes.

Chromosome 2S had one fixed inversion (2S-DA) and three common inversions (2S-B, 2S-G and 2S-CE). 2S-DA runs from segment 47 up to part of segment 51 (Fig. 11). The 2S-B inversion overlaps part of 2S-DA and includes the double bubble and ring of Balbiani (Fig. 10). Inversion 2S-G occurs within the 2S-B inversion and was found only in the heterozygote state (Fig. 12) and only in Gojeb and Ghilgel Ghibe. Among the nKS from Gojeb and Ghilgel Ghibe, 2S-B was fixed. 2S-CE was found only in the nKS from Bebeka and then only as a heterozygous inversion (Fig. 9). Two rare inversions, 2S-E (running from part of segment 50 to part of segment 52) and 2L-R (from band 63 to band 72), were also recorded.

Two new fixed inversions, 3L-F and 3S-A, were found on chromosome 3 (Fig. 15). The 3L-F inversion involves part of segment 93 to 95a. Only one 3L-F heterozygote specimen was found, and that was among the 25 fully karyotyped nKS from Gojeb.

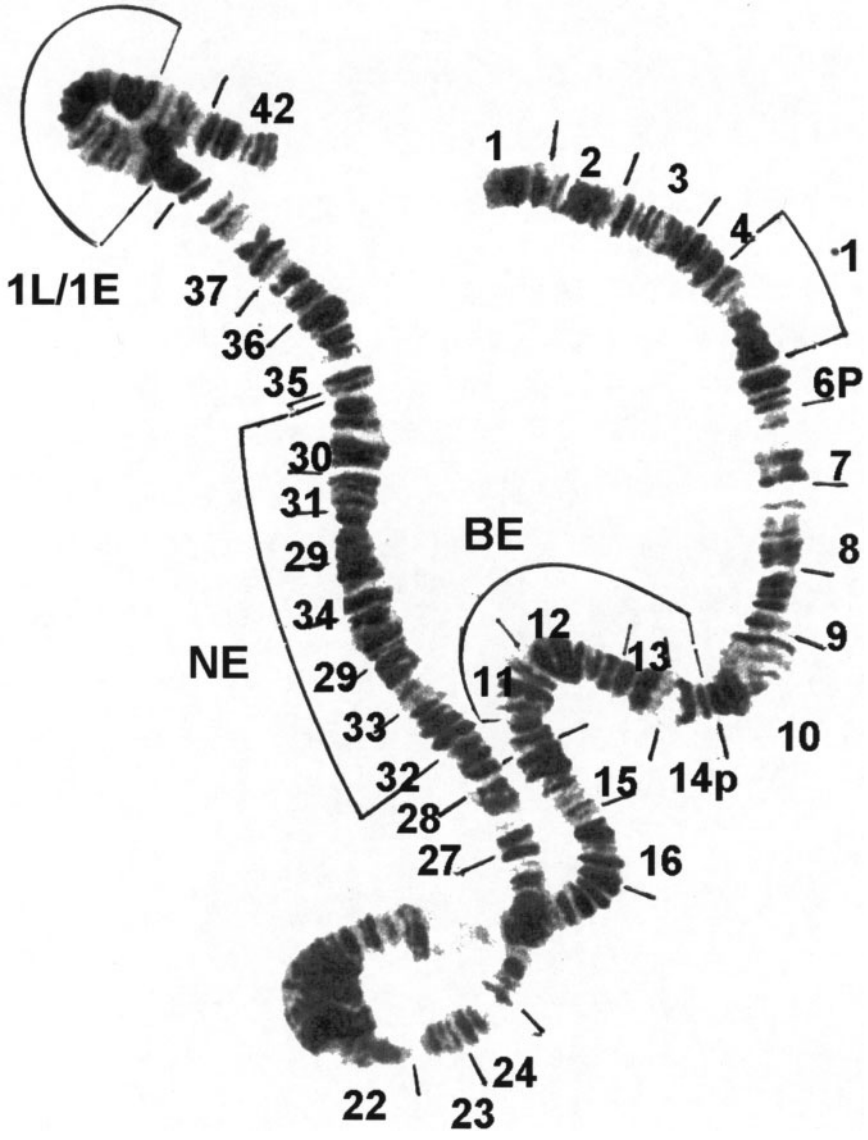


FIG. 5. Chromosome 1 of *Simulium kaffaense* (a female from River Gojeb), showing the floating inversions 1L/1E, 1S-BE, 1S-1 and the fixed inversion 1L-NE.

Thirteen of the 98 fully karyotyped nKS could not be scored for the 3L-F inversion because of another (heterozygous) inversion that also involved segment 94. Inversion 3L-8 (Dunbar, 1969) occurred as a floating inversion (Fig. 16) and was much rarer among the nKS from Bebeka (7.0%) than among those from Gojeb (80.0%) or Ghilgel Ghibe (93.7%). Rare inversions recorded on the long arm of chromosome 3 included 3L-DA (running from segment 91 to 94), 3L-EA (from segment 88 to part of 94), 3L-AA (part of segment 87 to part of 90), 3L-GA (segment 93 to 94) and 3L-JA (part of segment 83 to part of segment 86).

The fixed inversion 3S-A starts in segment 74 and ends in part of segment 76, thus overlapping the blister (Fig. 15). Its breakpoints are similar but not identical to those of 3S-1K in the 'Kibwezi' form from

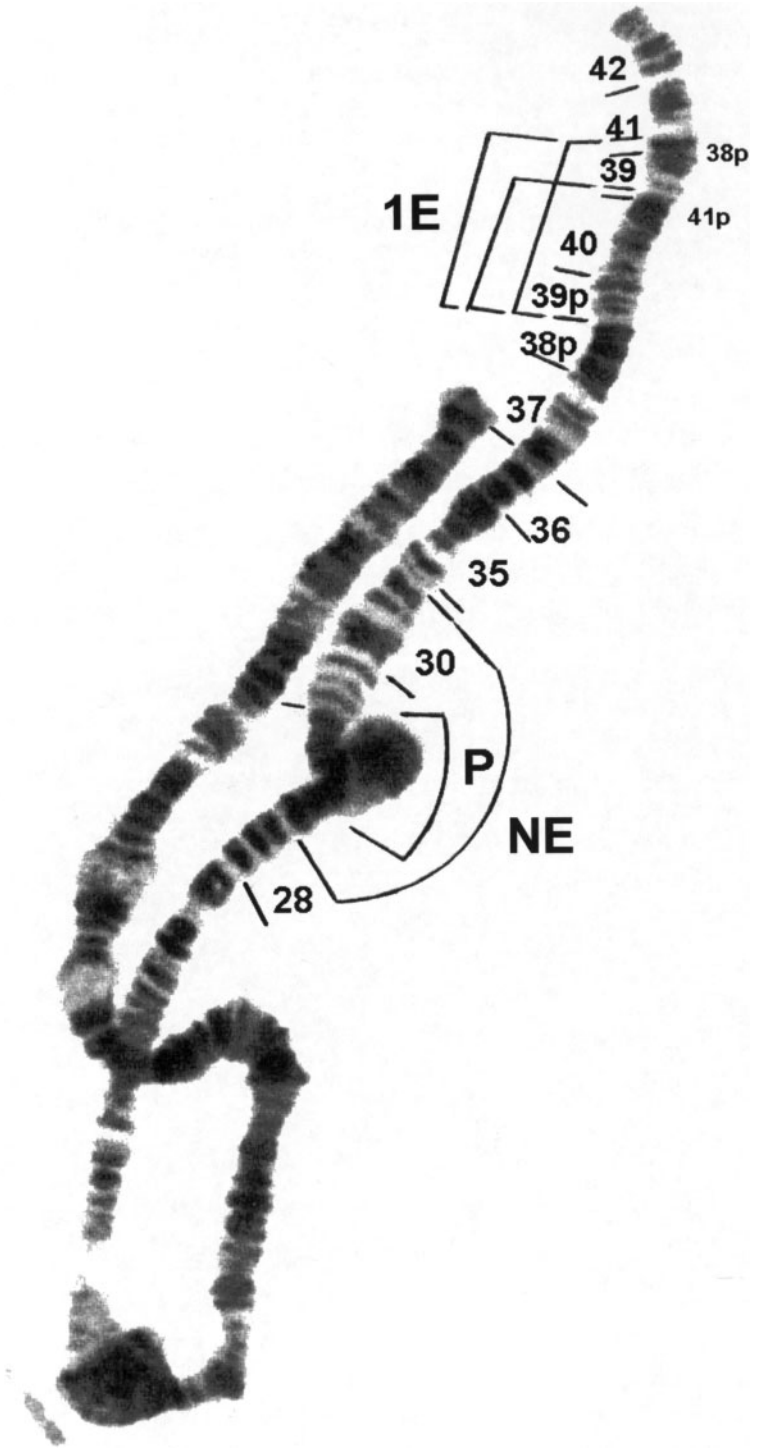


FIG. 6. Chromosome 1 of *Simulium kaffaense* (a male from Aboare stream at Bebeka), showing the floating inversions 1L-P and 1L-1E, and the fixed inversion 1L-NE.

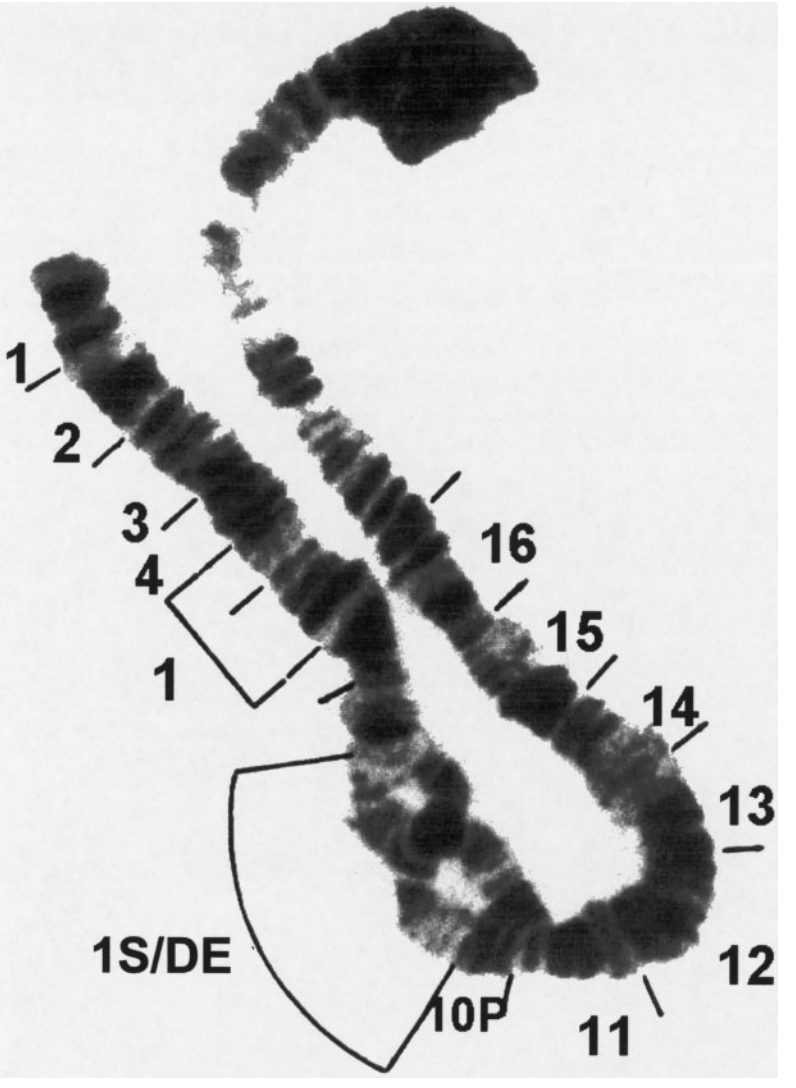


FIG. 7. Short arm of chromosome 1 of *Simulium kaffaense* (a male from the River Ghilgel Ghibe), showing 1S-DE and 1S-1.

Tanzania (Maegga and Cupp, 1993), the latter inversion running from part of segment 75 up to part of segment 77.

LARVAE REARED FROM THE EGG BATCHES OF HUMAN-BITING FLIES

Overall 106 larvae were successfully reared from the seven single egg batches. Although chromosomes from 22 of the larvae reared (from four of the egg batches) were prepared, none of the preparations was good enough to allow any of the larvae to be fully karyotyped. The preparations were therefore examined only for inversion 3S-A, which was found to be present in all 22 specimens.

DESCRIPTION OF A NEW SPECIES

The results of the cytology indicated that all 98 nKS larvae collected in the field, and the 22 larvae that were reared in the laboratory and subjected to chromosomal analysis, belonged to a newly recognized species within the *S. damnosum* complex. This newly recognized species,

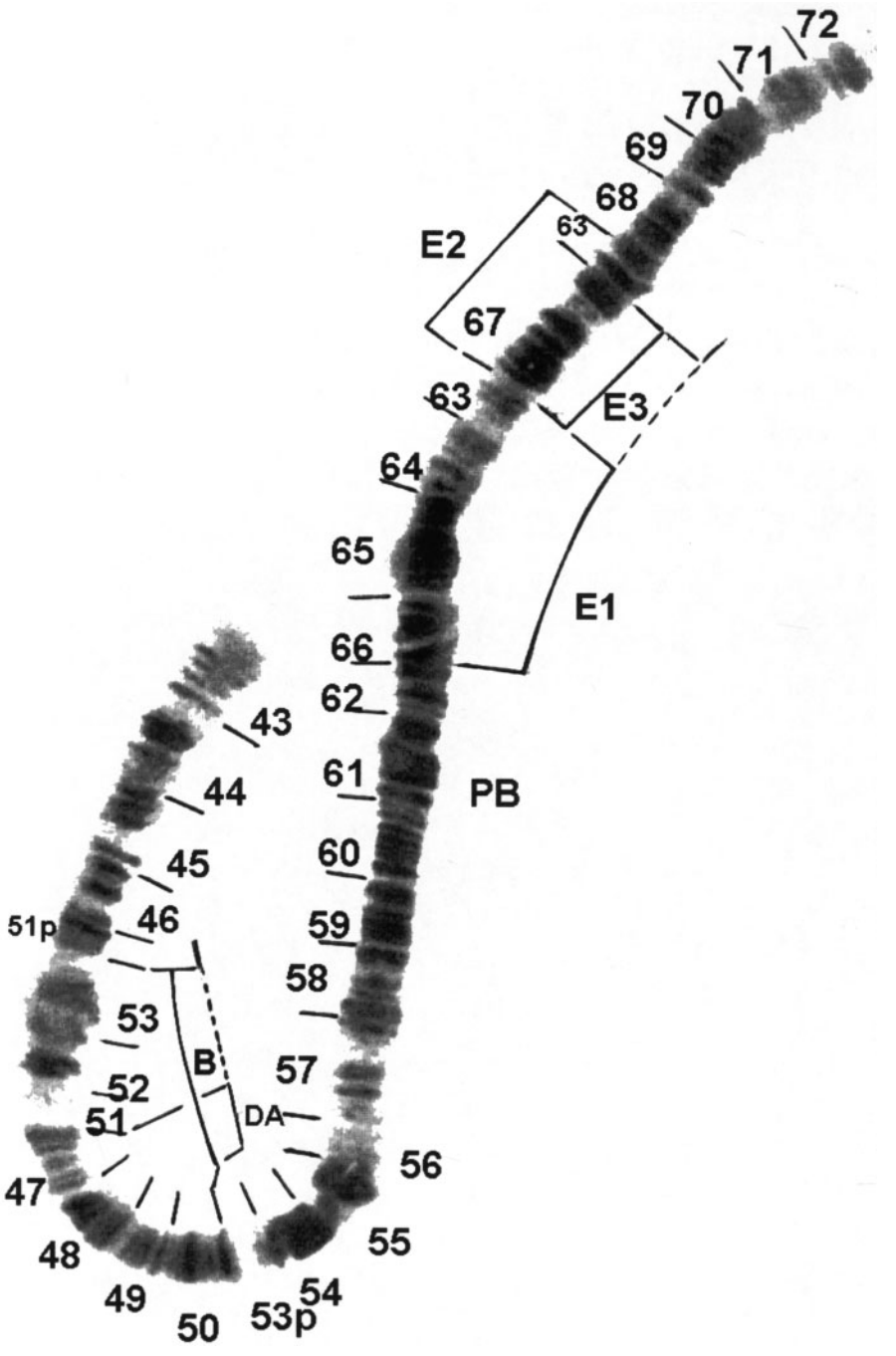


FIG. 8. Chromosome 2 of *Simulium kaffaense* (a female from Aboare stream at Bebeka), showing fixed inversions 2L-E1 and -E3 and 2S-DA, and floating inversions 2S-B and 2L-E2.

here named *Simulium kaffaense* Hadis, Boakye, Wilson and Cobblah, is described below.

Simulium kaffaense SP. NOV. *Simulium kaffaense* sp. nov. has six fixed inversions (IL-NE, 2L-E1, 2L-E3, 2S-DA, 3S-A and

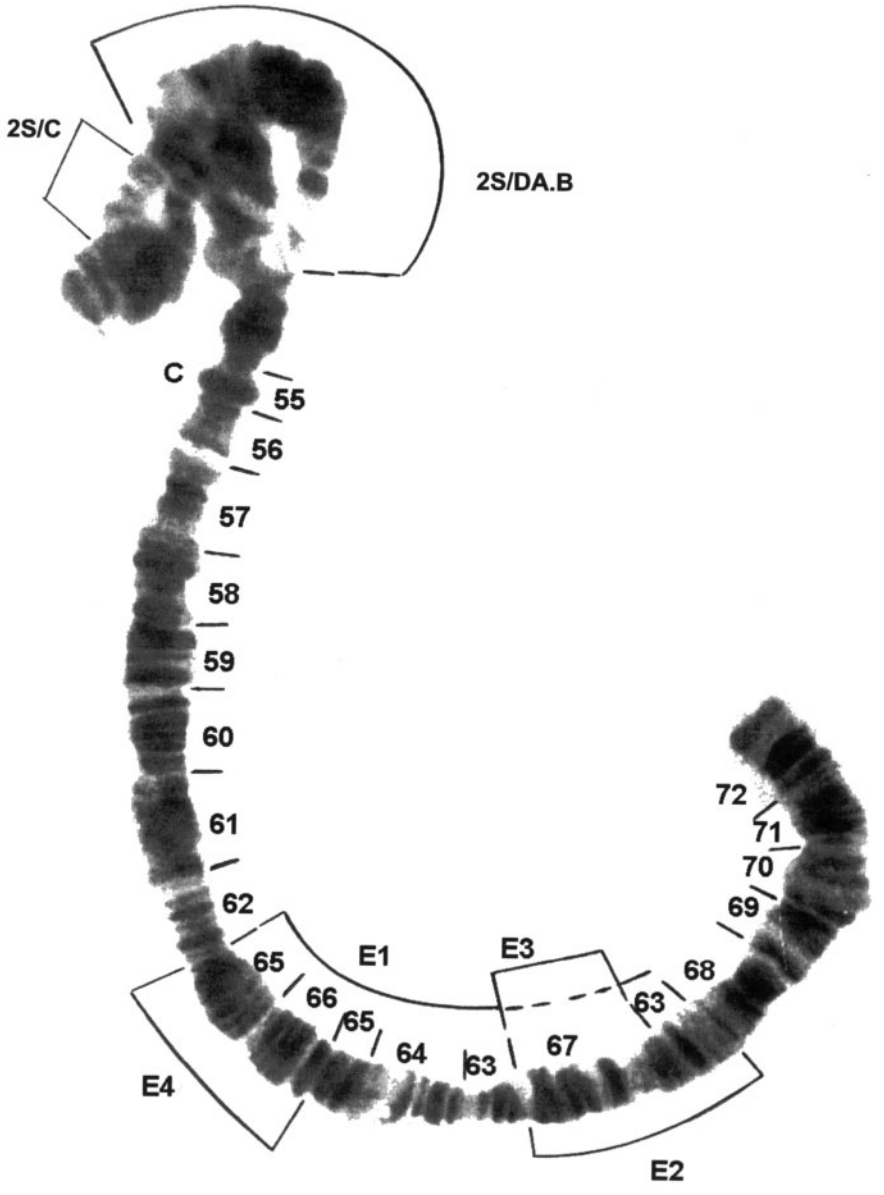


FIG. 9. Chromosome 2 of *Simulium kaffaense* (from a larva, of undetermined sex, from Aboare stream at Bebeka), showing fixed inversions 2L-E3 and -E1, and floating inversions 2L-E2 and -E4, 2S-DA.B and 2S-C.

3L-F) and 29 new floating inversions that separate it from all the other described members of the *S. damnosum* complex. The floating inversions observed within *S. kaffaense* are 1L-CE, 1L-1E, 1L-EX, 1L-OE, 1L-TA, 1L-YA, 1L-3A, 1S-1, 1S-BE, 1S-DE and 1S-HA on chromosome 1, 2L-E2, 2L-E4, 2L-E5, 2L-13, 2L-R, 2S-G, 2S-B, 2S-C and 2S-E on chromosome 2, and 3L-8, 3L-DA, 3L-EA, 3L-AA, 3L-GA and 3L-JA on chromosome 3.

In the present study, with the exception of the fixed inversions, there was a clear variation in the frequencies of the floating inversions between the nKS from Bebeka and those collected at the other two sites;

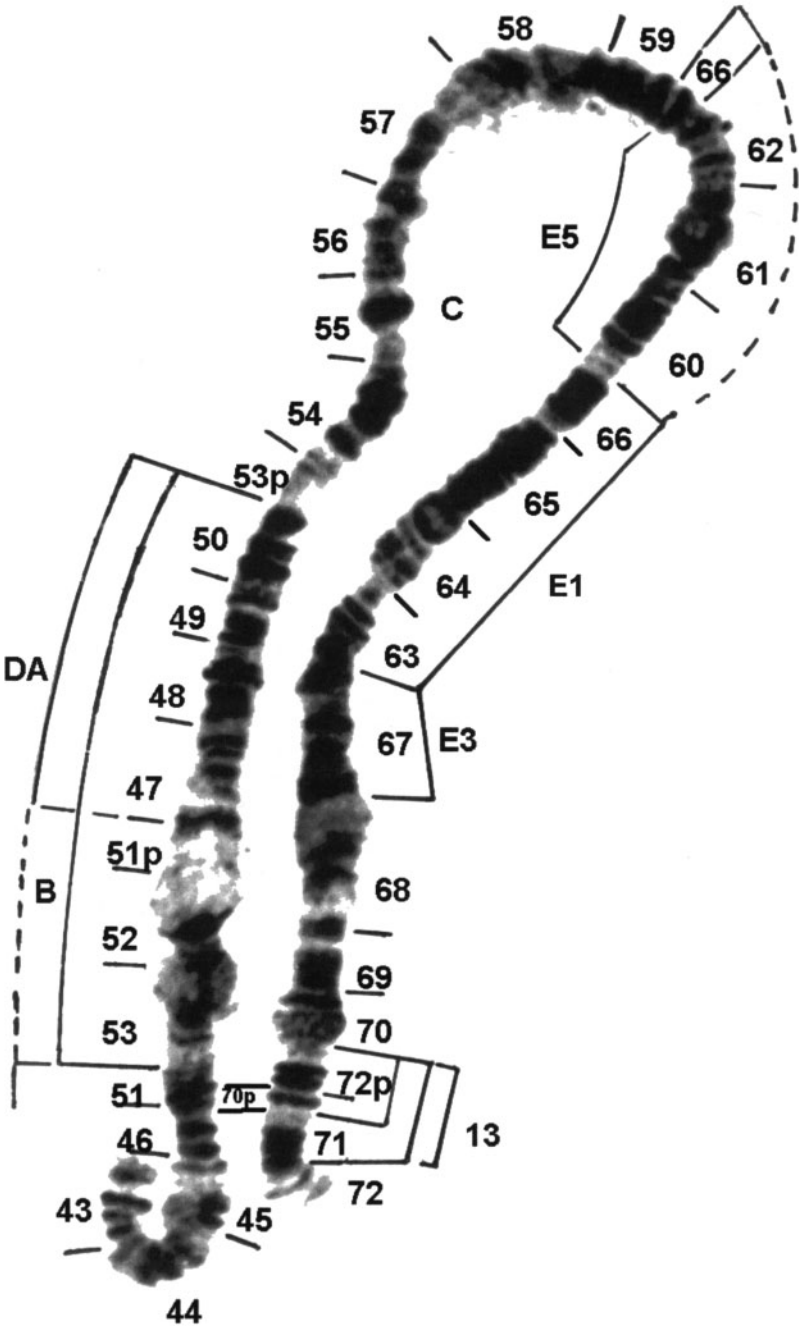


FIG. 10. Chromosome 2 of *Simulium kaffaense* (a male from the River Gojeb), showing the floating inversions 2L-E5, 2S-B and 2L-13, and the fixed inversions 2L-E3 and -E1 and 2S-DA.

the greatest differences were seen in the frequencies of inversions 1L-CE, 2L-13, 2L-E2, -E4 and -E5, and 3L-8 (Table 2). A hierarchical cluster analysis of all the floating inversions indicated the presence of two clusters within *S. kaffaense*; all of the fully karotyped nKS from Ghilgel Ghibe and Gojeb fell into one cluster whereas all of

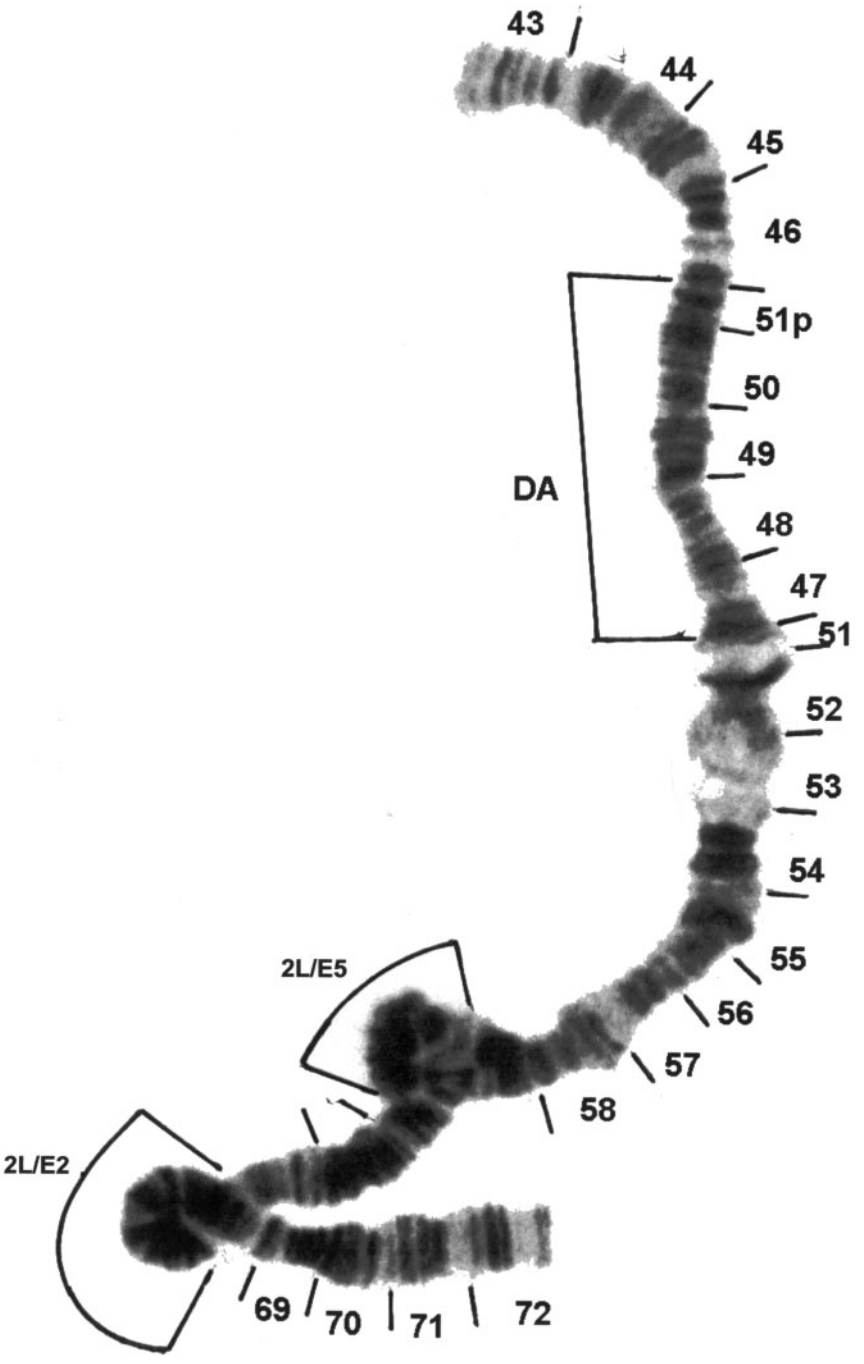


FIG. 11. Chromosome 2 of *Simulium kaffaense*, showing the fixed inversion 2S-DA and the heterozygote arrangement 2L-E1.E2.E3/ E1.E3.E5 (the loops represent the inversions 2L/E2 and 2L/E5).

the fully karotyped nKS from Bebeka fell into the other (Fig. 17). The cytotype from Ghilgel Ghibe and Gojeb is designated the 'Jimma' form (Dunbar, 1976), since this form was collected from the River Ghilgel Ghibe (White, 1977; Dunbar and Vajime,

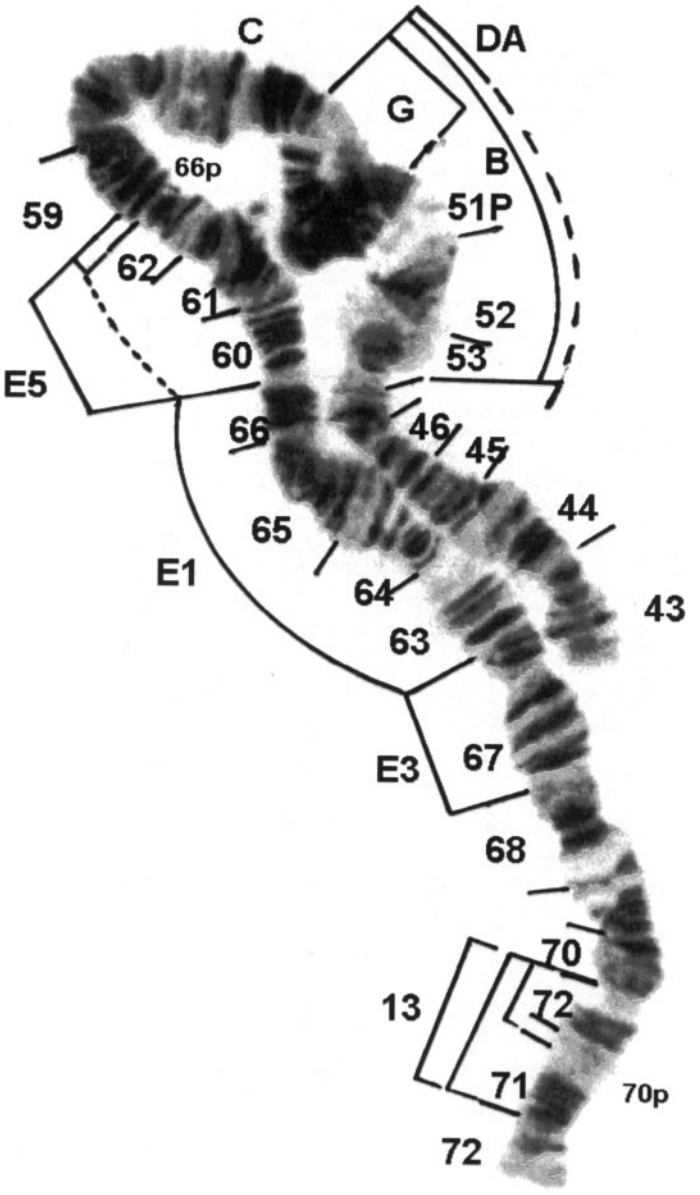


FIG. 12. Chromosome 2 of *Simulium kaffaense* (a male from the River Gojeb), showing the heterozygote arrangement 2S-DA.B/DA.B.G, and the homozygote arrangements 2L-E1.E3.E5 and 2L-13.

1981). The other cytotype, so far recorded only in Bebeka, is designated the 'Bebeka' form. The results of the appropriate χ^2 tests confirmed the results of the hierarchical cluster analysis. The results of other χ^2 tests, on the frequencies of inversion 3L-8, indicated that the entire *S. kaffaense* population was not in a Hardy–Weinberg equilibrium (if all the *S. kaffense* formed one, randomly mating population; $P<0.0001$) whereas the 'Jimma' and 'Bebeka' cytoforms were each in Hardy–Weinberg equilibrium ($P>0.05$).

Adult Morphology

Twenty adult female *S. kaffaense*, from Bebeka and Gojeb, were examined. Although each arculus, wing tuft and

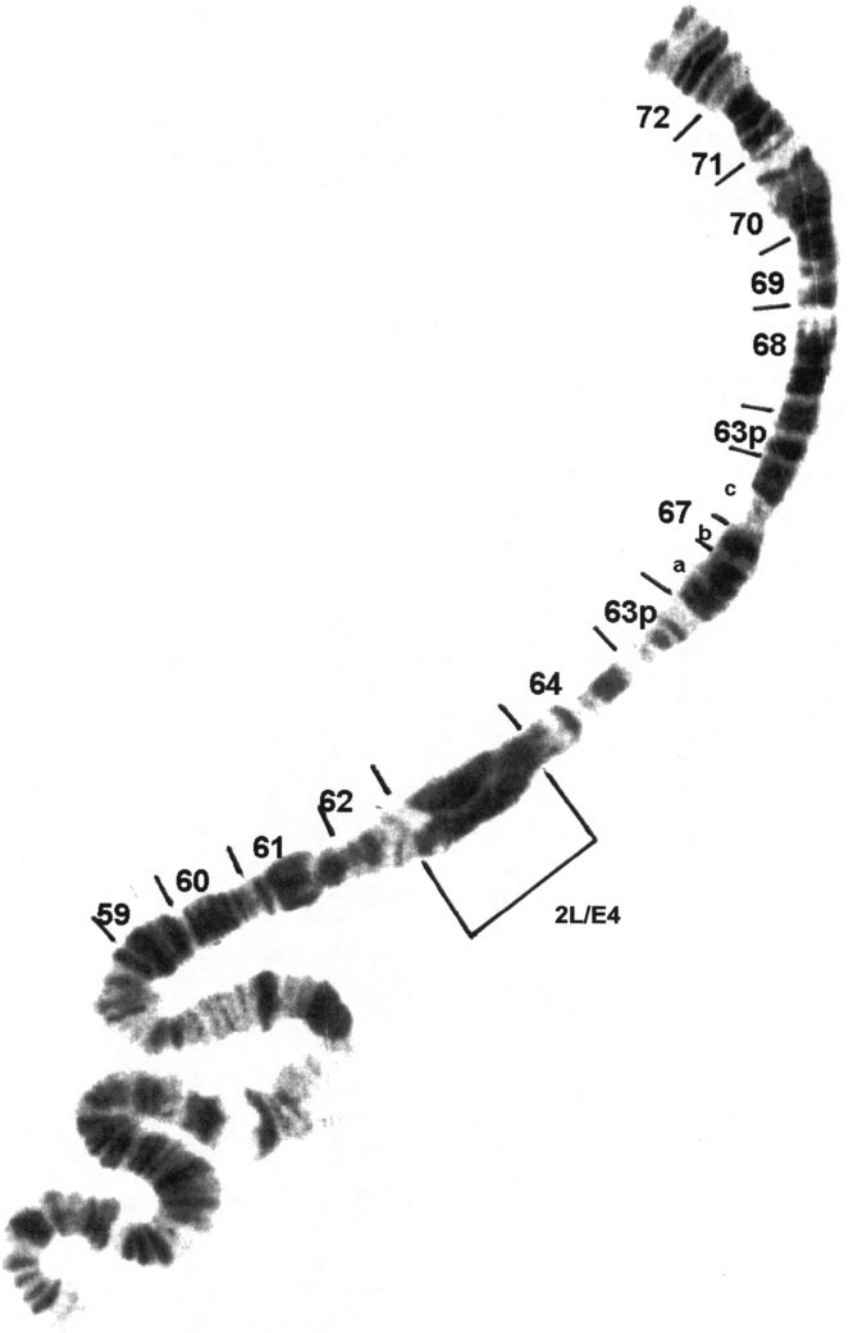


FIG. 13. Chromosome 2 of *Simulium kaffaense* (a female from Aboare stream at Bebeka), showing the heterozygote arrangement 2L-E3.E2.E1/ E3.E2.E1.E4 (the loop indicates the inversion 2L/E4).

scutelar and abdominal seta of *S. kaffaense* was pale, each antenna and fore coxa was dark.

Etymology

The newly recognized species is named kaffaense after the old name for the Ethiopian province in

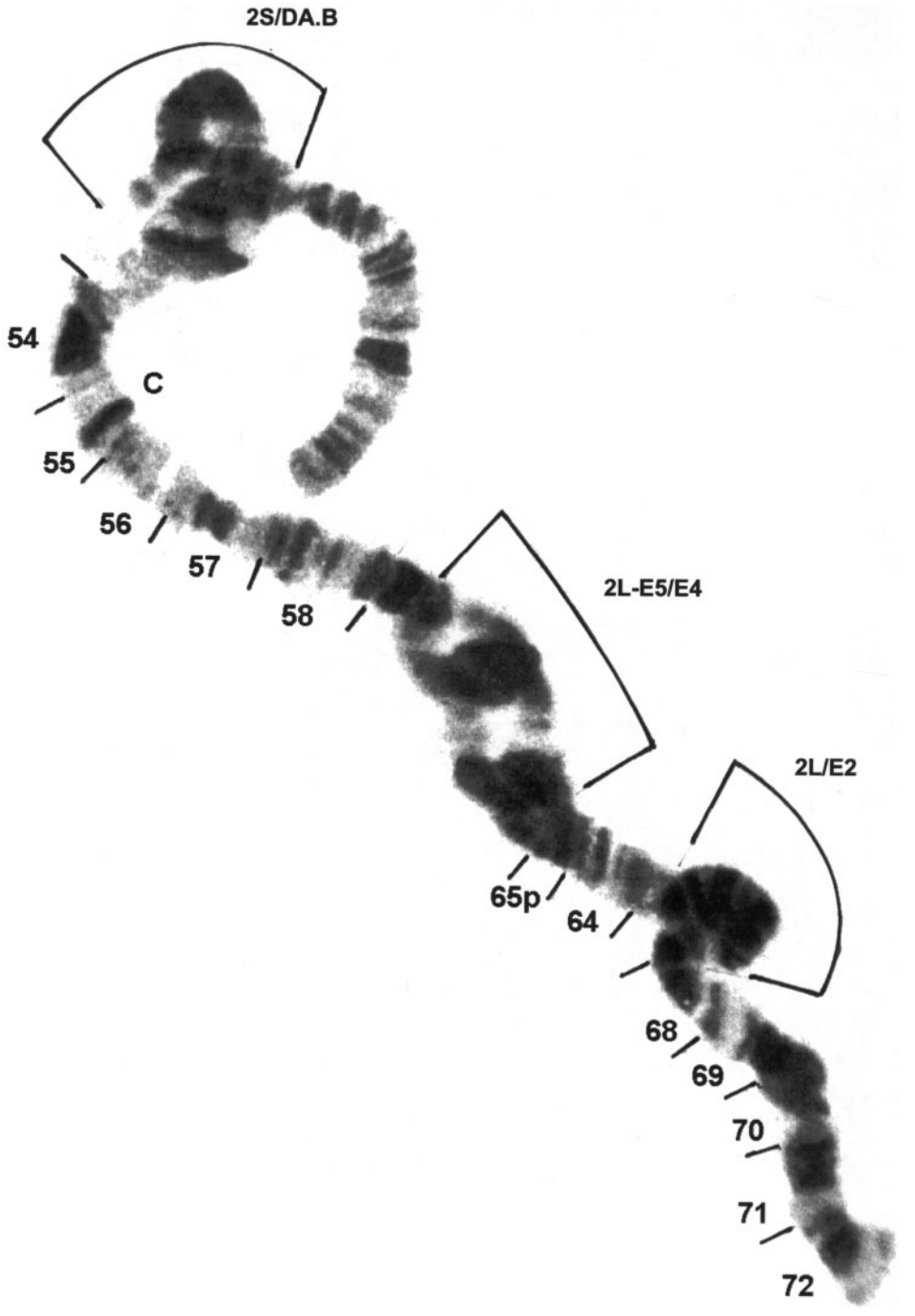


FIG. 14. Chromosome 2 of *Simulium kaffaense* (a female from Aboare stream at Bebeka), showing the heterozygote arrangements 2L-E1.E2.E3.E4/E5.E3.E1 and 2S/DA.B.

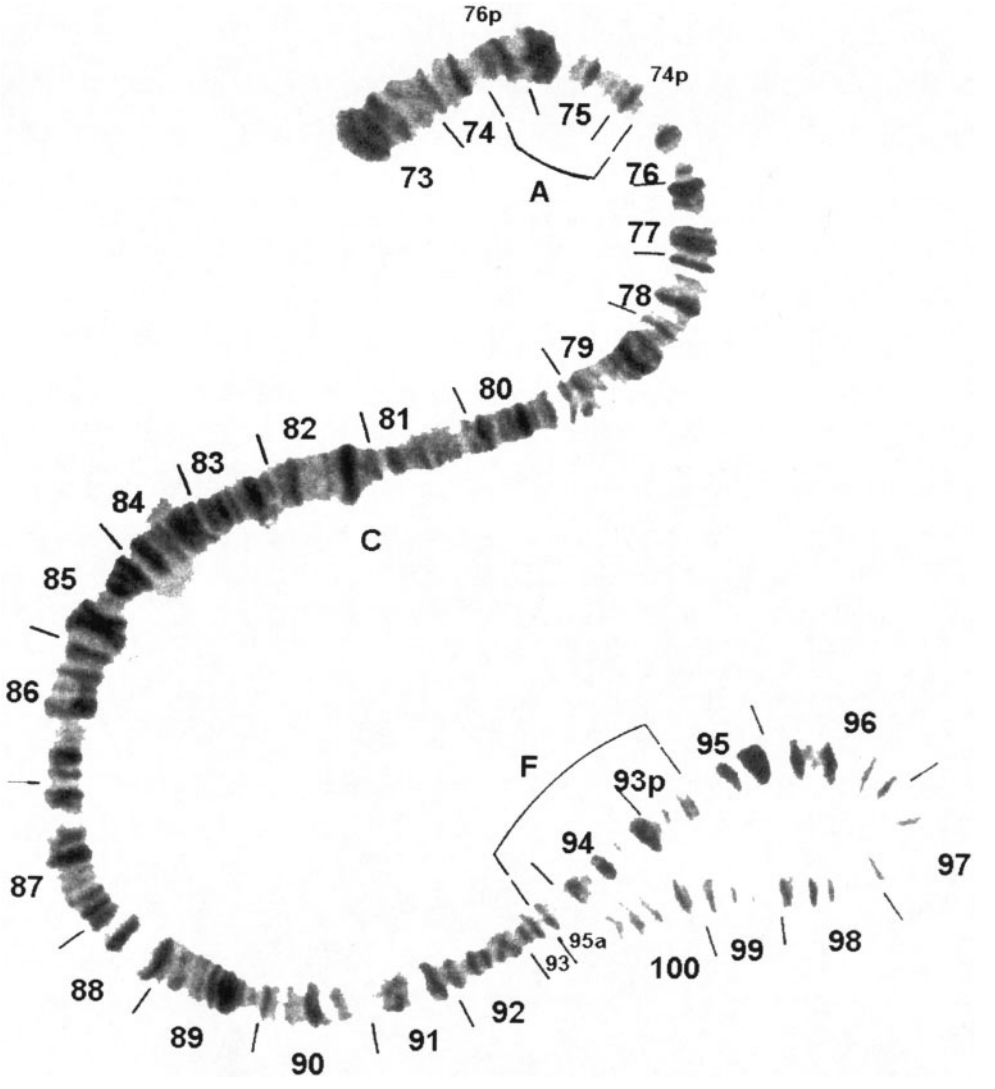


FIG. 15. Chromosome 3 of *Simulium kaffaense* (a female from Aboare stream at Bebeka), showing the fixed inversions 3S-A and 3L-F.

which all the present specimens and the 'Jimma' form (Dunbar, 1976) were collected (and in which most of studies on human onchocerciasis in Ethiopia have been conducted): Kaffa.

DISCUSSION

In the earliest reports of *S. damnosum* s.l. in Ethiopia, Dunbar (1969, 1976) mentioned the existence of three cytoforms: the 'Jimma' form (which was presumed to be the vector of *O. volvulus* in south–western Ethiopia) and the 'Kisiwani' and 'Kulfo' forms (which had then only been found in onchocerciasis-free areas). In the present study, the 'Kisiwani' form was found, in sympatry with the new cytologically distinct population here designated as *S. kaffaense* sp. nov., in areas where onchocerciasis is endemic. The same observation has recently been made by Krüger *et al.* (2005).

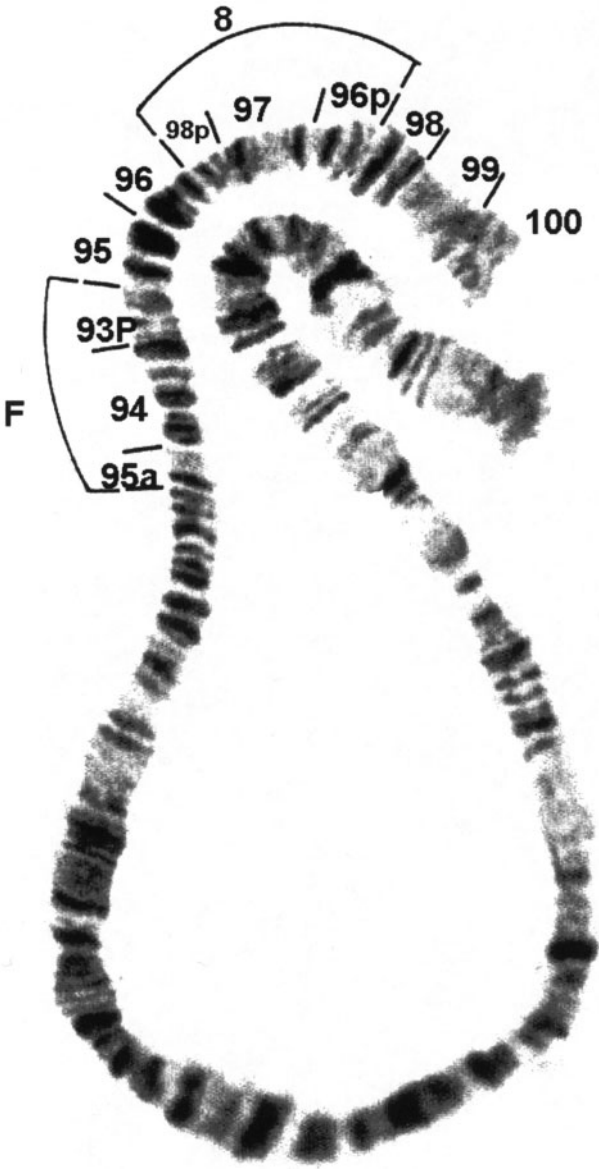


FIG. 16. Chromosome 3 of *Simulium kaffaense* (a male from the River Gojeb), showing the floating inversion 3L-8.

Although no chromosomal maps were published by Dunbar (1969, 1976), the inversions of the 'Kulfo' form were presented, in an idiogram by Dunbar (1969). The inversions observed, towards the terminal ends of chromosomes 2L and 3L, in *S. kaffaense* (present study) could well be the same as the 2L-13 and 3L-8 inversions of the 'Kulfo' form, respectively, and were therefore also named 2L-13 and 3L-8. On chromosome 2, although the 2S-2 of 'Kulfo' (Dunbar, 1969) looks very similar to the 2S-B of *S. kaffaense* (present study), the involvement of the fixed inversion 2S-DA, which overlaps 2S-B, in *S. kaffaense* makes it difficult to conclude whether the two inversions are the same or not.

In the present study, 1S-1 was found in every nKS that was fully karyotyped. Although this inversion exists as a fixed

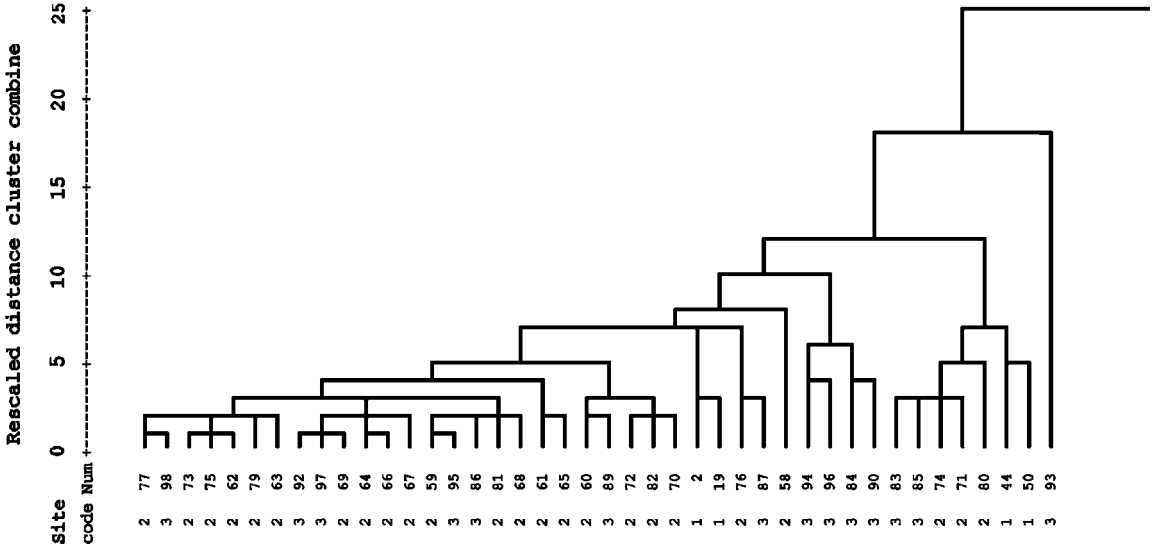
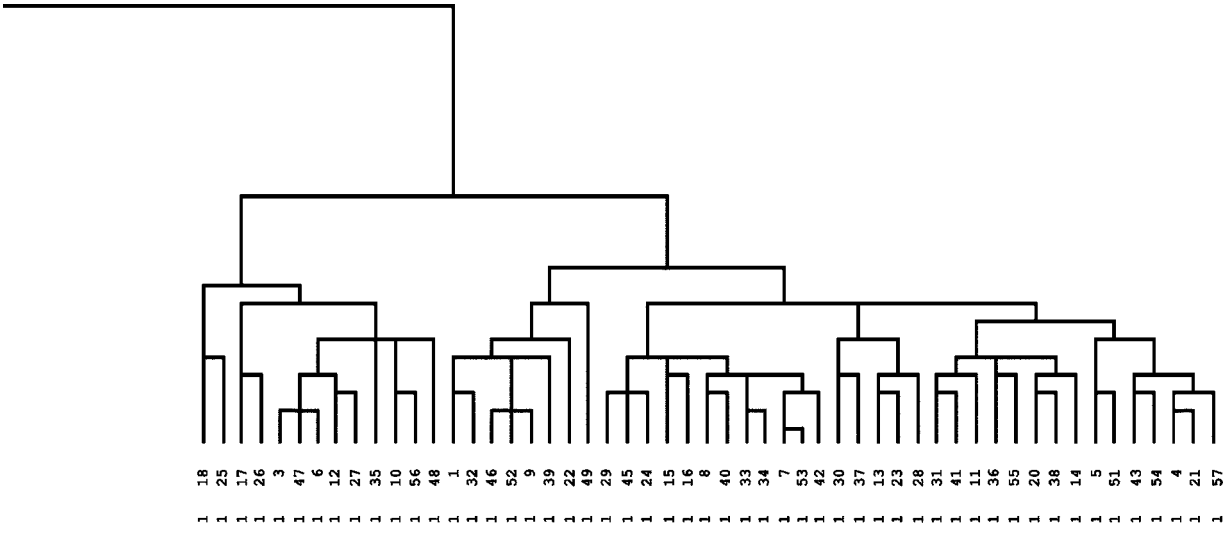


FIG. 17. The results of a hierarchical cluster analysis of the data on the frequency of floating inversions (site code=1) and the other by the larvae collected from the River Gojeb at Gojeb (site code=2) and the



among the 'non-Kiswani' larvae, indicating the two main clusters — one formed by the larvae collected at Bebeka River Ghilgel Ghibe at Ghibe (site code=3). Each specimen was given its own identification number (Num).

'Kibwezi' phylogenetic groups (Dunbar and Vajime, 1981), it was polymorphic in the *S. kaffaense*. This polymorphism in 1S-1 currently seems to be unique to *S. kaffaense*.

The presence of 1S-1 together with the complex inversion 1L-NE, possibly involving 1L-3, indicates that *S. kaffaense* is at least close to the 'Nile' group. Its dark antennae indicate that *S. kaffaense* is not a member of the savannah group found in West Africa and Sudan, since all the recognized members of this group have pale antennae (unpubl. obs.).

The results of several earlier investigations indicate that *Simulium damnosum* s.l. is the major vector of *O. volvulus* in south–western Ethiopia, with *S. ethiopiense* playing a secondary role (Tanaka *et al.*, 1973; White, 1977; Gebre-Michael and Gemetchu, 1996). As the present results and those of Krüger *et al.* (2005) indicate that *S. kaffaense* is the only anthropophilic member of the *Simulium damnosum* complex to be found in south–western Ethiopia, it is probably *S. kaffaense* that is the major vector in the area.

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