CompCytogen 5(1): 23–46 (2011)
doi: 10.3897/compcytogen.v5i1.975
www.pensoft.net/journals/compcytogen

RESEARCH ARTICLE

COMPARATIVE Cytogenetics
A peer-reviewed open-access journal
International Journal of Plant & Animal Cytogenetic, Karyosystematics, and Molecular Systematics

Karyotypes of *Chironomus* Meigen (Diptera: Chironomidae) species from Africa

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Academic editor: *Ilya Gavrilov* | Received 28 January 2011 | Accepted 15 March 2011 | Published 5 May 2011

Citation: Wülker WF, Kiknadze II, Istomina AG (2011) Karyotypes of *Chironomus* Meigen (Diptera, Chironomidae) species from Africa. Comparative Cytogenetics 5(1): 23–46. doi: 10.3897/compcytogen.v5i1.975

Abstract

The karyotypes of six African *Chironomus* species (*Ch. alluaudi* Kieffer, 1913, *Ch. transvaalensis* Kieffer, 1923, *Ch*. sp. Nakuru, *Ch. formosipennis* Kieffer, 1908, *Ch.* prope *pulcher* Wiedemann, 1830, *Ch.* sp. Kisumu) were investigated; four of these karyotypes were described for the first time (*Ch.* sp. Nakuru, *Ch. formosipennis*, *Ch*. prope *pulcher*, *Ch*. sp. Kisumu). Of the six *Chironomus* karyotypes, three had "pseudothummi" cytocomplex chromosome arms combinations AE CD BF G (*Ch. alluaudi*, *Ch. transvaalensis*, *Ch.* sp. Nakuru), two had "thummi" cytocomplex arms combinations AB CD EF G (*Ch. formosipennis*, *Ch.* prope *pulcher*), and one had "parathummi" arm combinations AC BF DE G (*Ch.* sp. Kisumu). Thus, three of the ten main cytocomplexes known were detected in Africa. Detailed photomaps of all chromosome arms, with the exception of arms B and G, were prepared for the karyotypes of *Ch. alluaudi, Ch. transvaalensis, Ch*. sp. Nakuru*, Ch.* prope *pulcher;* the karyotypes of *Ch. formosipennis, Ch.* sp. Kisumu could only be fragmentarily mapped.

Endemic African banding sequences were characteristic for most of the chromosomal arms in all species studied. However, basic sequences, which can be present in different *Chironomus* species on different continents (Wülker, 1980; Kiknadze et al., 2008), were also detected also in several African species *(Ch. alluaudi, Ch*. sp. Nakuru*,* and *Ch. formosipennis*). The banding sequences of African species studied allow discussion of the derivation of modern banding patterns from hypothetical species, living before separation of cytocomplexes and continents.

Keywords

Chironomus, karyotype, banding sequences, chromosomal polymorphism, chromosomal evolution

Introduction

As shown by cytogenetic analysis of chromosomal evolution, the divergence of animal karyotypes during speciation was mainly mediated by para- and pericentric inversions, altering the gene orders in linkage groups (Dobzhansky 1970, White 1977, King 1993, Zdobnov et al. 2002). The other types of chromosomal rearrangements (translocations, fusions, duplications) play an additional role in rearrangements of the linear structure of genome. Alteration of the gene orders in chromosomes during evolution can be visualized in Diptera, which possess polytene chromosomes with distinct banding sequences. The bands of polytene chromosomes, which form species-specific banding sequences, are considered as genetic markers to analyze divergence patterns of the linear genome structure during evolution. The use of the number of chromosomal breakpoints as a divergence measure provided establishment of phylogenetic relationships between species (Kiknadze et al. 2008). Species of the genus *Chironomus* have four giant chromosomes with seven chromosome arms (A-G). Based on the different combination of the arms, caused by whole-arm translocations, the *Chironomus* species are grouped into several cytocomplexes (Keyl 1962, Wülker 1980). Cytocomplex is not a taxonomic term. It includes the species with definite chromosome arms combinations, but not similar morphologically. Comparison of banding sequences between species from different cytocomplexes have shown that karyotypes can include species-specific sequences and so called basic sequences, common to more than one cytocomplex and in more than one continent. Such basic sequences were probably present before the separation of species and cytocomplexes (Keyl 1962, Wülker 1980).

By global analysis of banding sequences in Eurasia, North and South America, Australia, we have traced banding sequence changes during *Chironomus* species divergence and continent dispersal (Martin et al. 1974, Wülker 1980, Wülker et al, 1989, Kiknadze at al. 2003, 2008). It was shown that in Eurasia, North America, and Australia, banding sequence pools of many species were represented mainly by endemic continent-specific sequences. However, basic sequences, common for different continents were also found in karyotypes of some species in addition to the endemic sequences. Such basic sequences were noted also in two African *Chironomus* species (*Ch. alluaudi,* and *Ch*. sp. Nakuru) (Martin 1979, Wülker 1980). It was of interest to study how often such basic sequences can be found among African species. However, the data on *Chironomus* karyotypes in Africa are very scanty despite there being much information on the morphology of African chironomids. Wülker (1980) has presented photographs of seven chromosome arms of *Ch. alluaudi*; Martin (1979) has quoted the arm F banding sequence of *Ch. transvaalensis*; Wülker et al. (1989) included the banding sequences of *Ch. transvaalensis* arms A, and F in their list of *Chironomus* sequences, and have shown the position of *Ch. alluaudi* and *Ch. transvaalensis* on the phylogenetic tree.

This paper contains full descriptions of the karyotypes of six African *Chironomus* species (*Ch. alluaudi, Ch. transvaalensis, Ch*. sp. Nakuru*, Ch. formosipennis, Ch.* prope *pulcher*, *Ch.* sp. Kisumu). Among them four karyotypes are described for the first time

(*Ch.* sp. Nakuru*, Ch. formosipennis, Ch.* prope *pulcher, Ch.* sp. Kisumu). Detailed photomaps of arms A, C, D, E, and F are presented for *Ch. alluaudi, Ch. transvaalensis,* and *Ch.* sp. Nakuru. The chromosome arms could be mapped only partly for *Ch. formosipennis, Ch*. prope *pulcher* and *Ch.* sp. Kisumu.

The presence of further basic banding sequences in the karyotypes of African *Chironomus* species was discovered, along with endemic continent-specific (Ethiopian region) sequences.

Evolutionary divergence of "thummi" and "pseudothummi" cytocomplexes is discussed.

Material and methods

Forth instar larvae of African *Chironomus* species were used for karyotype study. 35 years ago, one of us (W.W.) had the opportunity to visit Kenya (22.12.1975–16.01.1976). From a base at the house of relatives in Nairobi, he went with family (wife and 3 sons) to collect chironomids to the west to Lake Nakuru and Lake Victoria, to the north to Abrader Mount Ca. 3000 m above N.N., and to the southeast to Tsavo National park, Mombasa and vicinity. Other material was contributed by colleagues: Mount Elgon and Lake Naivasha (Peter N. Cox), Mount Kenya, near 4350 m (scientific excursion of University Erlangen, Germany, under Prof. Dr. Rüppell), Zigi River, Tanzania (Dr. J. Grunewald). The list of collection sites of *Chironomus* larvae is presented in Table 1. We have not identified species *Ch.* sp. Nakuru and *Ch*. sp Kisumu, but the study of the banding sequences of their karyotypes was very important for purpose of our paper.

Larvae were fixed in ethanol-glacial acetic acid (3:1). The technique of chromosome preparation was as usual (Keyl, 1962). The identification of chromosome banding sequences follows by Keyl (1962) for arms A, E, and F, and by Dévai et. al., (1989) for arms C and D.

To trace the relationship of African *Chironomus* banding sequences with sequences from other continents, we compared them with known basic sequences; if basic sequences for some of species were unknown, we compared them with *Chironomus piger* standard (ST).

We have pointed to previous literature on morphological characteristics of species studied at the beginning of each species description. Most part of the material (larvae, pupa, adults and karyotype slides) is now deposited in Zoologische Staatssammlungen in Münich (Germany).

Equipment of the Center of Microscopy Analysis of Biological Objects of SB RAS in the Institute of Cytology and Genetics (Novosibirsk) was used in accomplishment of this work: microscope "Axiokop" 2 Plus, CCD camera AxioCam HRc, software package AxioVision 4 (Zeiss, Germany).

Table 1. Collection sites and number of specimens of African *Chironomus* species.

Species	Collection sites	Collection date	Collector	Number of specimens
Chironomus alluaudi	Kenya: drinking troughs brooks and pools at Endebess/Mt. Elgon, mountain lakes W of Nakuru, Aberdare mountains up to 3300 m, ponds at MtKenya 4350 m, near Limuru (north of Nairobi), Athi-river south of Nairobi, Amboseli-park	29.12.75 03.01.76 12.01.76 13.01.76 10.01.76	P. N. Cox, W.Wülker. G. Rüppell W. d'Oleire-Oltmanns,H. Koehler W. Wülker	120
Chironomus transvaalensis	Kenya: pool east Lake Victoria, north and south Athi-river near Nairobi, west of Mombasa, Tansania: Kikuwi-river	27.12.75 13.01.76	W. Wülker J. Grunewald	115
Chironomus sp. Nakuru	Kenya: brook south east Lake Nakuru	26.12.75	W. Wülker	9
Chironomus formosipennis	Kenya: Lake Naivasha, Tansania: Zigi-river, running waters		P. N. Cox, J. Grunewald	15
Chironomus prope *pulcher*	Kenya: two pools in short distance, River Athi south of Nairobi	13.01.76	W. Wülker	6
Chironomus sp. Kisumu	Kenya: flat pools 10 and 22 km east Lake Victoria, together with *Ch. transvaalensis,* brook in Amboseli-park (Kilaguni)	27.12.75 10.01.76	W. Wülker	7

Results

***Chironomus alluaudi* Kieffer, 1913**
http://species-id.net/wiki/Chironomus_alluaudi

Previous reports: Kieffer 1913, imago.
Freeman 1957, imago.
Wülker 1980, photo of arms A-G
Wülker et al. 1989, phylogenetic position
Kiknadze et al. 2004, list of banding sequences of arms A, C, D, E, and F

Karyotype (Fig. 1a). Haploid number n=4, arm combination AE CD BF G ("pseudothummi" cytocomplex), centromere bands not heterochromatinized, nucleolus in arm G (terminal), at least 3 Balbiani Rings (BRs) on arm G, inversion polymorphism in arms C and G.

Banding sequences (Fig. 1b-g)

Arm A (Fig. 1b) has the sequence all A1 identical with the main sequence of arm A found in many *Chironomus* species (*Ch. holomelas* Keyl, 1961, *Ch. melanescens* Keyl, 1961, etc.) and it is considered a cosmopolitan basic sequence (holA1).

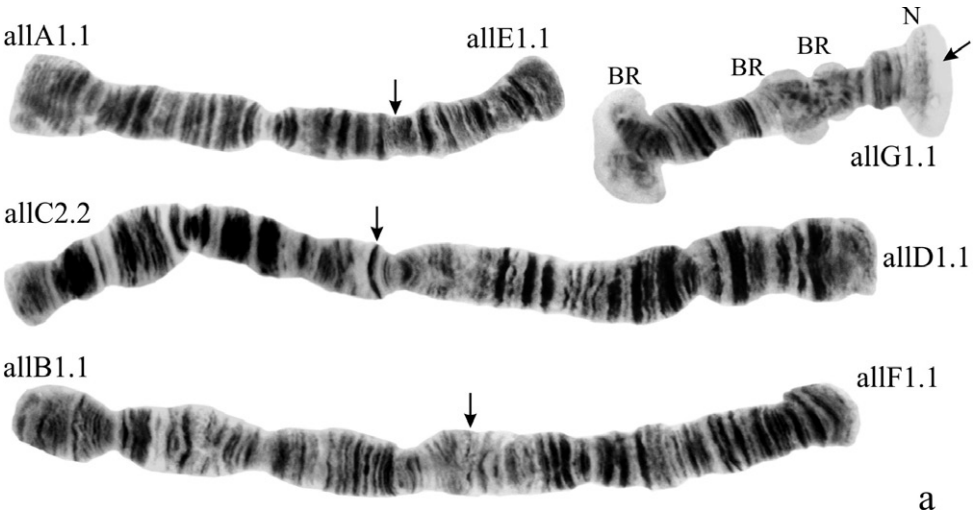

Figure 1a. Karyotype of *Chironomus alluaudi*. In this and all other Figures: **allA1.1**, **allE1.1** etc. – symbols of arm and homozygous genotypic combinations **N** – nucleolus **BR** – Balbiani ring, arrows show centromeric bands, brackets near chromosome arms show inversions.

allA1 1a-2c 10a-12c 3i-2d 9e-4a 13a-19f

Arm E (Fig. 1, c, 7, b) has the sequence allE1 identical with *Chironomus piger* ST (cosmopolitan basic sequence).

allE1 1a - 13g

Arm C (Fig. 1, d) has two sequences, allC1 and allC2, differing by a simple inversion. The sequence allC1 differs greatly from the basic sequence in arm C; therefore we have compared it with *Chironomus piger* ST: differing by seven inversion steps from pigST:

allC1 1a-2g 11c-10a 16a-17a 6h-2h 11d-15e 9f-7a 17b-22g
allC2 1a-2g 11c-10a 16a-17a 15e-11d 2h-6h 9f-7a 17b-22g
hyp 5 1a-2g 11d-15e 17a-16a 10a-11c 7a-9f 6h-2h 17b-22g
hyp 2+3+4 1a-2g 11d-15e 16a-17a 10a-11c 7a-9f 6h-2h 17b-22g
hyp 1 1a-2g 17a-16a 15e-11d 11c-10a 9f-7a 6h-2h 17b-22g
pigST 1a-2g 2h-6h 7a-9f 10a-11c 11d-15e 16a-17a 17b-22g

Arm D (Fig. 1, e) has single sequence allD1 differing by one inversion step from pigST:

allD1 1a-9e 19h-10a 20a-24g
pigST 1a-9e 10a-19h 20a-24g

Arm B (Fig. 1, a) not mapped, monomorphic. The common BR is not developed.

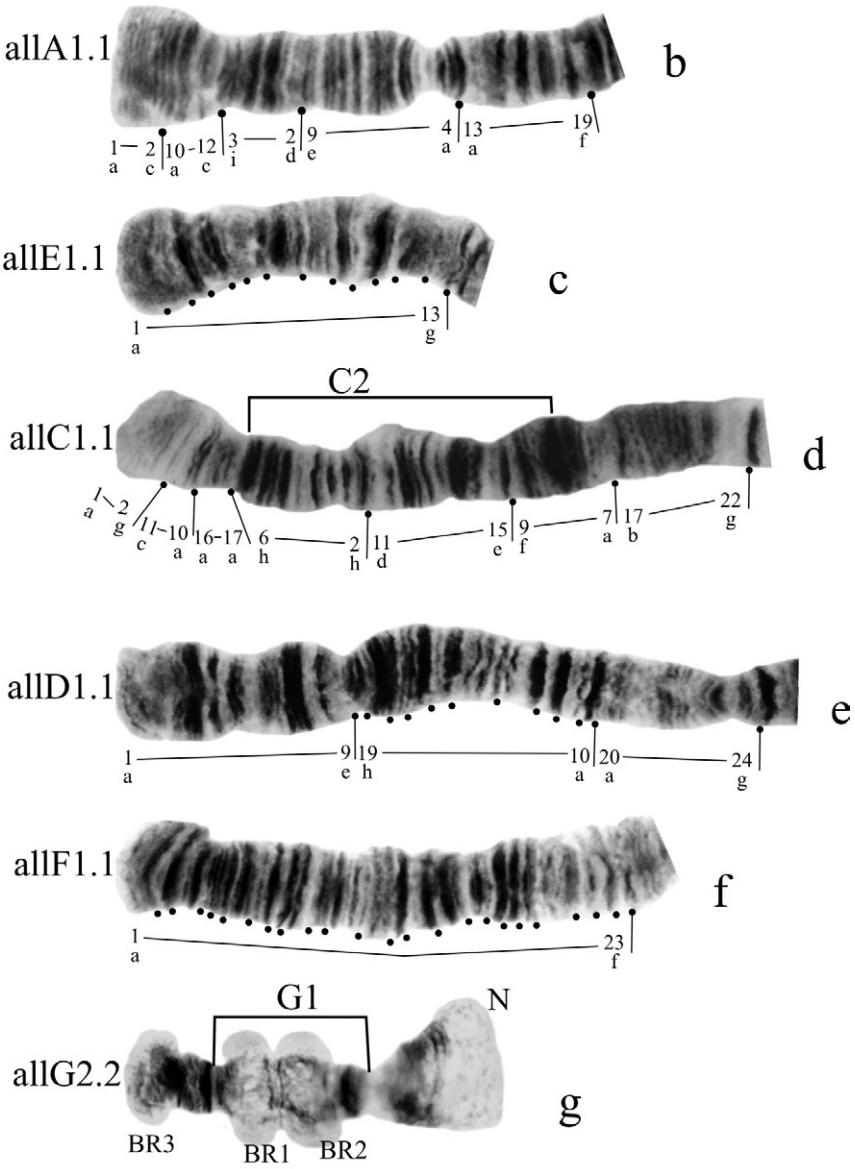

Figure 1b-g. Homozygous banding sequences of *Chironomus alluaudi* in arms A, E, C, D, F and G. The designations are the same as in Fig. 1.

Arm F (Fig. 1, f) has the sequence allF1, identical with pigST (cosmopolitan basic sequence).

allF1 1a–23f

Arm G (Figs 1, a, g) not mapped, has two sequences allG1 and allG2 differing by one simple inversion in the central part of arm G, including two of the Balbiani rings.

In total, the banding sequence pool of *Ch. alluaudi* contains 9 sequences. Six of them endemic for Africa (Ethiopian sequences), three of them (allA1, allE1, allF1) belong to the category of cosmopolitan basic sequences. *Ch. alluaudi* can be considered as a *Chironomus* species with a primitive karyotype (Wülker, 1980, 2010).

Larva: "thummi-type" (no tubuli laterales) on abdominal segment VII). Mentum with high lateral tooth, median tooth as in other *Chironomus* species, pectin epipharyngis about 11 teeth, antenna black with 4 segments, paralabial plates about 40 striae.

Distribution: different places in Africa (Freeman, 1957), Kenya (leg. Wülker, Jan. 1976). Dunking troughs brooks and pools at Endebess/Mt Elgon (N. Cox leg.); mountain lakes W of Nakuru, Aberdare mountains up to 3300m, ponds Mt. Kenya 4350m (Oltmanns leg.) near Limuru (north of Nairobi), Athi river south of Nairobi, Amboseli-park (Wülker leg.)

***Chironomus transvaalensis* Kieffer, 1923**

http://species-id.net/wiki/Chironomus_transvaalensis

Previous reports: Kieffer 1923, imago.
Mc Lachlan 1969, 1971: larva and pupa.
Freeman 1957, imago.
Martin 1979, banding sequence of chromosome arm F.
Wülker, Dévai and Dévai 1989, banding sequences of arms A, E, and F, phylogenetic position of species.

Karyotype (Fig. 2, a). Haploid number n=4, arm combination AE CD BF G ("pseudothummi" cytocomplex), centromeric bands not heterochromatinized, nucleolus in arm C, inversion polymorphism in arms C and G.

Banding sequences (Fig. 2, b-f).

Arm A (Fig. 2, b) has the sequence trvA1, differing by only one inversion step from the basic sequence holA1.

trvA1 1a-2c 10a-12c 3i-c 5a-9e 2d-3b 4d-a 13a-19f
holA1 1a-2c 10a-12c 3i-c 3b-2d 9e-5a 4d-a 13a-19f

Arm E (Fig. 2, c) has the banding sequence trvE1, differing only by one step from basic sequence aciE1 (*Ch. acidophilus* Keyl, 1960 etc.)

trvE1 1a-2b 5a-10b 3e-2c 4h-3f 10c-13g
aciE1 1a-3e 10b-5a 4h-3f 10c-13g

Arm C (Fig. 2, d, j) has two banding sequences, trvC1 and trvC2, differing by one simple inversion (Fig. 2, j). The sequence trvC1 is formed by four inversion steps from a basic sequence, (lonC1), found in several *Chironomus* species (*Ch. longistylus* Goetghebuer, 1921, *Ch. anthracinus* Zetterstedt, 1860 etc.).

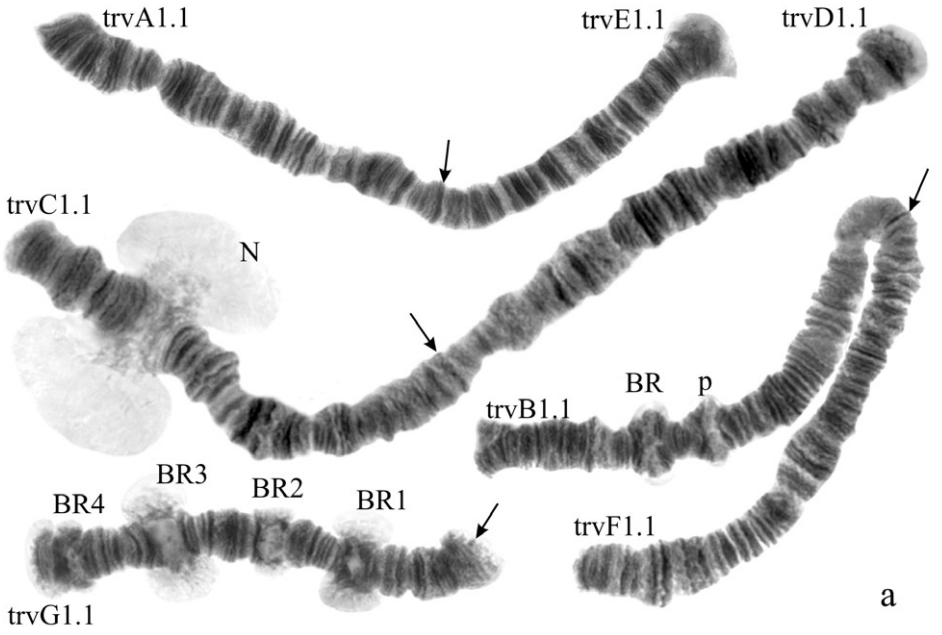

Figure 2a. Karyotype of *Chironomus transvaalensis*. **p** – puff and the designations are the same as in Fig. 1

trvC1 1a-2e 11d-12d 2f-6b 13a-15e 8a-11c 6gh 17a-16a 7d-a 6f-c 17b-22g
hyp 3 1a-2e 11d-12d 6b-2f 13a-15e 8a-11c 6gh 17a-16a 7d-a 6f-c 17b-22g
hyp 2 1a-2e 2f-6b 12d-11d 13a-15e 8a-11c 6gh 17a-16a 7d-a 6f-c 17b-22g
hyp 1 1a-2e 2f-6b 11d-12d 13a-15e 8a-11c 6gh 17a-16a 7d-a 6f-c 17b-22g
lonC1 1a-2e 2f-6b 11c-8a 15e-13a 12d-11d 6gh 17a-16a 7d-a 6f-c 17b-22g

Arm D (Fig. 2, e) has the sequence trvD1 differing from pigST by four inversion steps.
trvD1 1a-2h 4b-7e 12d-7f 19h-13a 4a-3a 20a-24g
hyp 2 1a-2h 4b-7e 7f-12d 13a-19h 4a-3a 20a-24g
hyp 1 1a-2h 19h-13a 12d-7f 7e-4b 4a-3a 20a-24g
pigST 1a-2h 3a-4a 4b-7e 7f-12d 13a-19h 20a-24g

Arm B (Fig. 2, a) not mapped, monomorphic. BR is well developed.

Arm F (Fig. 2, f) has the banding sequence trvF1 differing from cosmopolitan basic pigST by three inversion steps.
trvF1 1a-2a 10d-3f 14f-16g 14e-11a 2b-3e 17a-23f
hyp 2 1a-2a 10d-3f 16g-14f 14e-11a 2b-3e 17a-23f
hyp 1 1a-2a 10d-3f 3e-2b 11a-14e 14f-16g 17a-23f
pigST 1a-2a 2b-3e 3f-10d 11a-14e 14f-16g 17a-23f

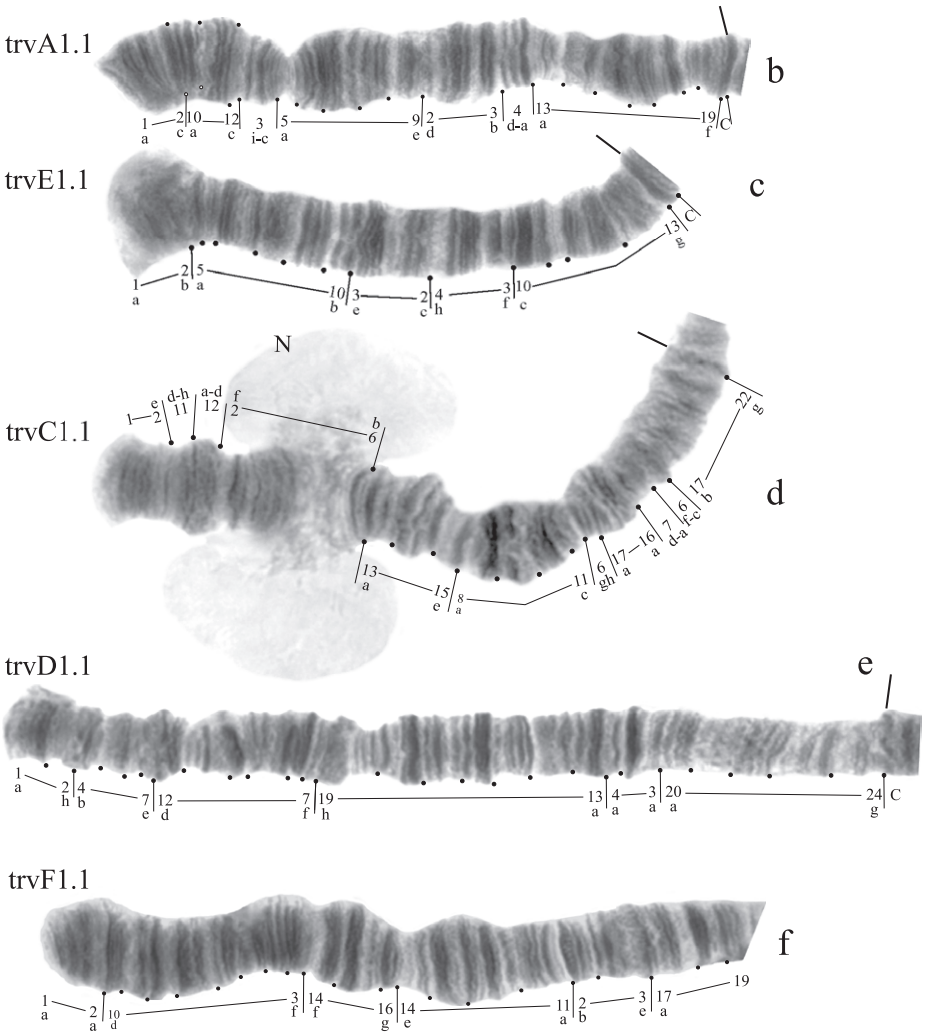

Figure 2b-f. Homozygous banding sequences of *Chironomus transvaalensis* in arms A, E, C, D and F.

Arm G (Fig. 2, g-i) has three banding sequences, trvG1, trvG2, and trvG3. The sequence trvG2 differs from trvG1 by a short inversion in the region BR1 (Fig. 2, h); the sequence trvG3 – by long inversion of central part of arm G (Fig. 2, i). Both last sequences were found as heterozygotes. There are four Balbiani rings.

In total, the banding sequence pool of *Ch. transvaalensis* contains 10 sequences, all of them are Ethiopian endemic sequences.

Larva: tubuli laterales at abdominal segment VII. Other characters - Mc Lachlan, 1969.

Distribution: various places in Africa, Freeman (1957); Blantyre Malawi (Mc Lachlan), Wülker, 1957: pool east Lake Victoria, Kikuwi-river, Tanzania (J. Grunewald), Pretoria South Africa, Israel (Martin, personal communication).

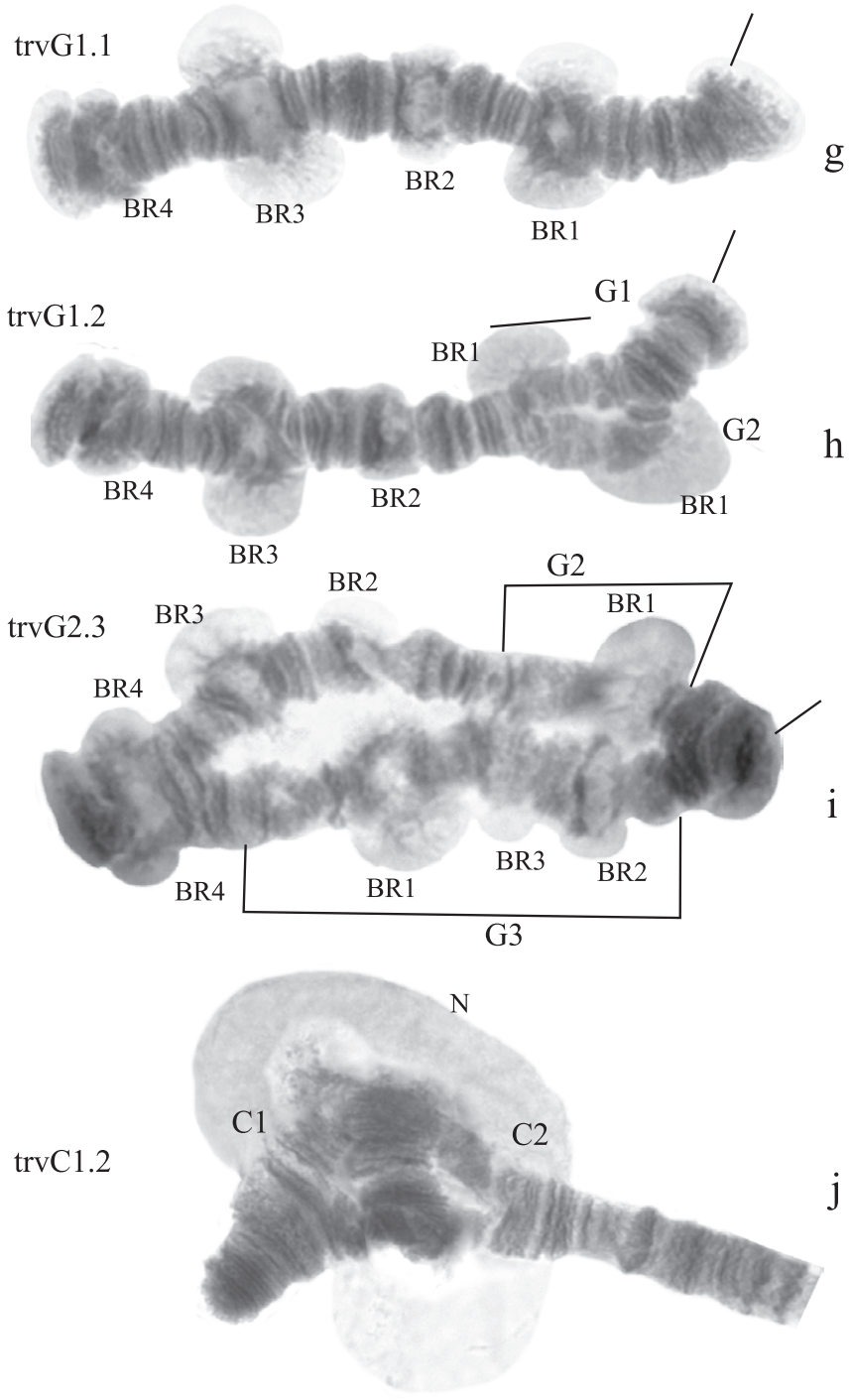

Figure 2g-j. Homozygous and heterozygous banding sequences of *Chironomus transvaalensis* in arm G (**g–i**) and heterozygous inversion in arm C (**j**). Brackets above arms indicate the localization of inversions. The designations are the same as in Fig. 1.

Chironomus sp. Nakuru

Previous report: Wülker, 1980, banding pattern of arms A, E, and F. This species was not identified as well as *Ch*. sp. Kisumu because there was no additional possibility to collect larvae for rearing. However, the study of *Ch*. sp. Nakuru karyotype was very important for comparative analysis of Ethiopian *Chironomus* banding sequences with *Chironomus* sequences of the other continents.

Karyotype (Fig. 3, a). Haploid number n=4, arm combination AE CD BF G ("pseudothummi" cytocomplex), centromeric bands not heterochromatinized, nucleoli on arms F and G, Balbiani rings on arms G, B, and A. Chromosomal polymorphism was not recorded.

Banding sequences (Fig 3, b-f).

Arm A (Fig. 3, b) has the banding sequence nakA1 identical with cosmopolitan basic sequence found in many species (*Ch. holomelas, Ch. melanescens*, etc.)

nakA1 1a-2c 10a-12c 3i-2d 9e-4a 13a-19f

Arm E (Fig. 3, c) has banding sequence nakE1 differing by two inversion steps from the cosmopolitan basic sequence lonE1 (*Ch. longistylus, Ch. anthracinus* etc.).

nakE1 1a-3e 12g-a 5a-10b 4h-3f 10c-11d 13a-g
hyp1 1a-3e 12g-a 11d-10c 3f-4h 10b-5a 13a-g
lonE1 1a-3e 5a-10b 4h-3f 10c-11d 12a-g 13a-g

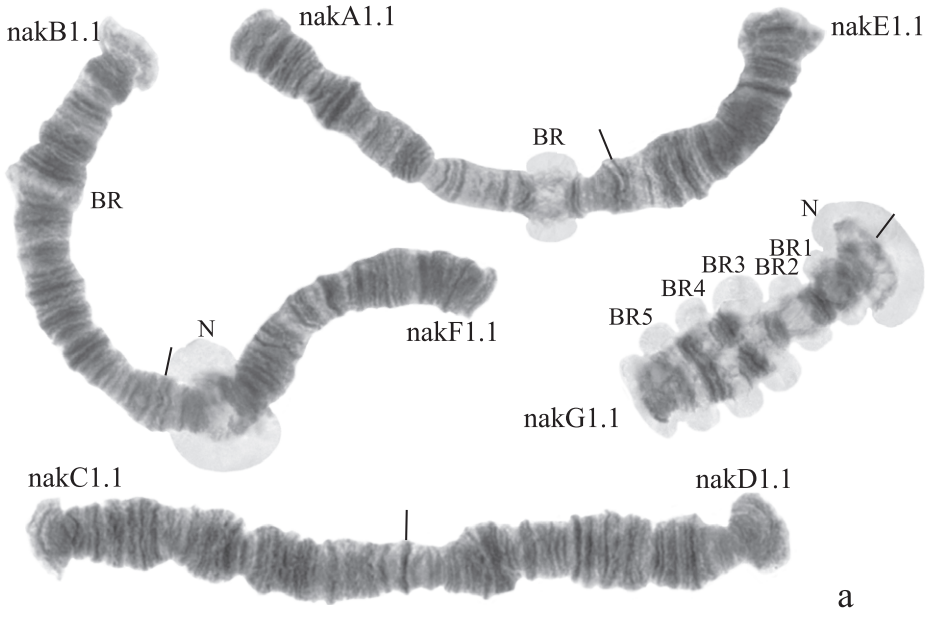

Figure 3a. Karyotype of *Chironomus* sp. Nakuru. The designations are the same as in Fig. 2a.

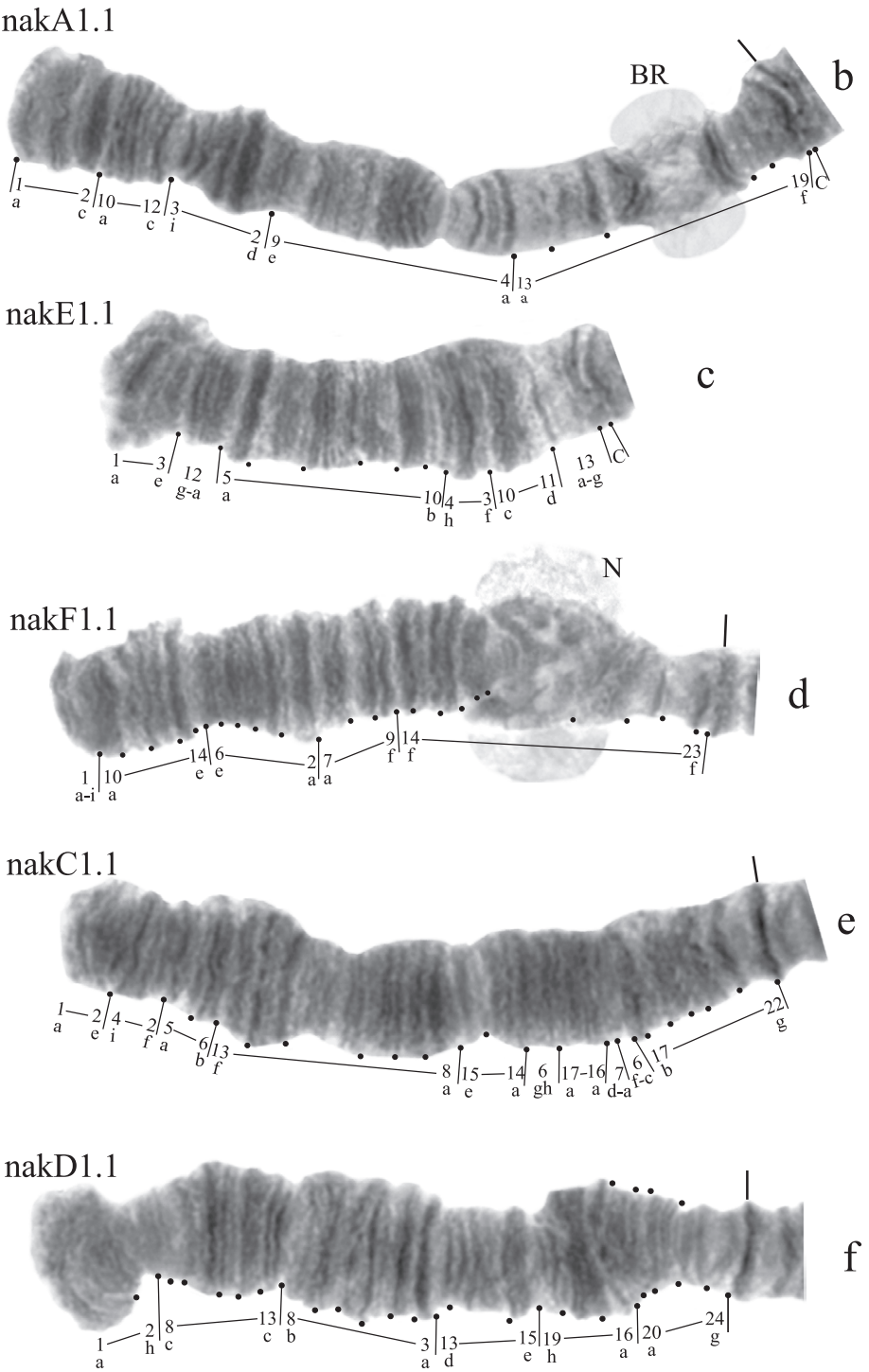

Figure 3b-f. Homozygous banding sequences of *Chironomus* sp. Nakuru in arms A, E, F, C and D. The designations are the same as in Fig. 1.

Arm C (Fig. 3, e) has the sequence nakC1 differing by four inversion steps from basic pattern lonC1 (*Ch. longistylus, Ch. anthracinus*, etc.) and by seven inversion steps from *Chironomus piger* ST (Fig. 7, b).

nakC1 1a-2e 4i-2f 5a-6b 13f-11d 11c-8a 15e-14a 6gh 17a-16a 7d-a 6f-c 17b-22g
hyp1+2 1a-2e 4i-2f 5a-6b 11d-13f 14a-15e 8a-11c 6gh 17a-16a 7d-a 6f-c 17b-22g
lonC1 1a-2e 2f-4i 5a-6b 11c-8a 15e-14a 13f-11d 6gh 17a-16a 7d-a 6f-c 17b-22g

Arm D (Fig. 3, f) has the banding sequence nakD1 differing from pigST by three inversion steps.

nakD1 1a-2h 8c-13c 8b-3a 13d-15e 19h-16a 20a-24g
hyp 1 1a-2h 13c-8c 8b-3a 13d-15c 16a-19h 20a-24g
pigST 1a-2h 3a-8b 8c-13c 13d-15c 16a-19h 20a-24g

Arm B (Fig. 3, a) not mapped, monomorphic. The common BR is not developed.

Arm F (Fig. 3, d) has the banding sequence nakF1 formed by four inversion steps from pigST.

nakF1 1a-i 10a-14e 6e-2a 7a-9f 14f-23f
hyp 3 1a-i 10a-14e 2a-6e 7a-9f 14f-23f
hyp 2 1a-i 10a-14e 9f-2a 14f-23f
hyp 1 1a-i 14e-10a 9f-2a 14f-23f
pigST 1a-i 2a-9f 10a-14e 14f-23f

The arm F of *Chironomus* sp. Nakuru has a nucleolus in region 17–19.

Arm G (Fig. 3, a) has the banding sequence nakG1. It differs from the most of *Chironomus* species arm G by numerous Balbiani rings. It is possible to suggest that some of them can be nucleoli. But it is often impossible to differentiate nucleoli and Balbiani rings without electron microscopy or in situ hybridization.

In total, seven banding sequences are found in sequence pool of *Ch*. sp. Nakuru, six chromosomal arms have Ethiopian endemic sequences, and one arm (A) a cosmopolitan basic sequence.

Larva: long tubuli laterales at abdominal segment VII, extremely long antenna, gula light, no dark stripe on clypeus.

Distribution: brook to SE of Lake Nakuru, Kenya

***Chironomus formosipennis* Kieffer, 1908**

http://species-id.net/wiki/Chironomus_formosipennis

Previous reports: Kieffer 1908, imago.
Freeman 1957, imago.
Dejoux 1970, imago.

Dejoux 1970, pupa.
Dejoux 1970, larva.

Karyotype (Fig. 4). Haploid number n=4, arm combinations AB CD EF G ("thummi" cytocomplex), centromeric bands not heterochromatinized, nucleoli in arms A and G, Balbiani ring in arm G. Chromosomal polymorphism was not recorded.

Banding sequence was determined only in arm E. The sequence frmE1 was identical with the cosmopolitan basic pattern, aprE1 (as in *C. aprilinus* Meigen, 1818)

frmE1 1a-3e 10b-3f 10c-13g

Larva: long tubuli laterales at abdominal segment VII. Other characters - Dejoux, 1970.

Distribution: Lake Naivasha, Kenya, Zigi-river, Tanzania, running waters.

Chironomus prope *pulcher* Wiedemann, 1830

Previous reports: Wiedemann 1830, imago.
Freeman 1957, imago.
Dejoux 1968, imago, pupa, larva.

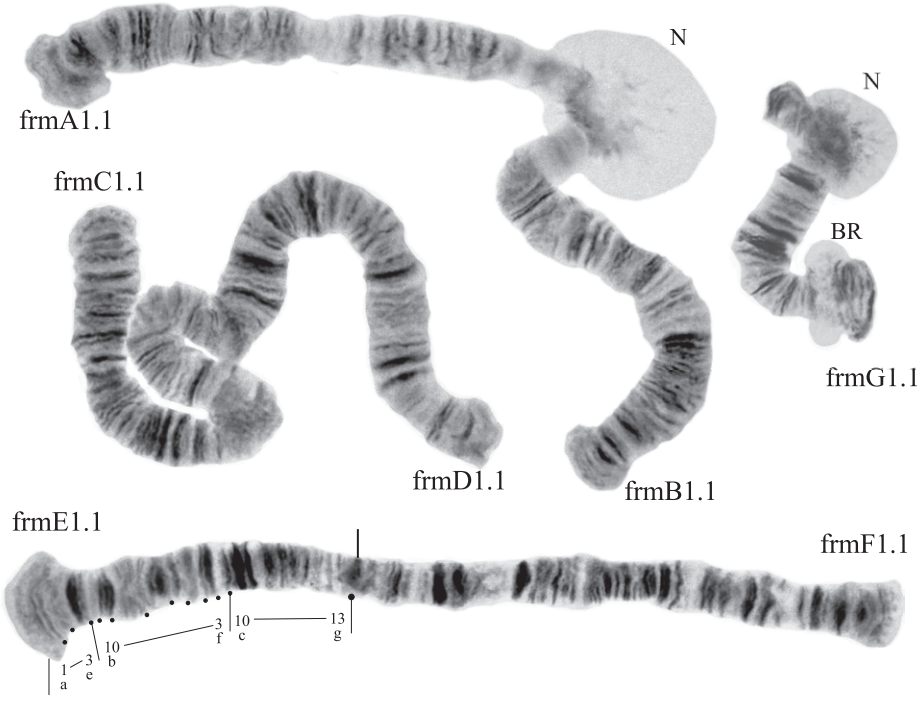

Figure 4. Karyotype of *Chironomus formosipennis*. The designations are the same as in Fig. 1.

The association to this species is based on one male adult from the collecting sites of the larvae.

Karyotype (Fig. 5, a). Haploid number n=3, arm combination AB CD FEG (modified "thummi" cytocomplex), centromeric bands not heterochromatinized, nucleolus in arm F (at the very telomeric end) and nucleolus-like bodies at the ends of arms A, B, E; Balbiani rings are in arms G and B. Chromosomal polymorphism in arm C (Fig. 5, a).

Banding sequences (Fig. 5, a, b-e).

Arm A (Fig. 5, b) has the banding sequence pulA1, formed by four inversions from pigST

pulA1 1a-3i 8g-6a 16d-17h 11e-9a 4ab 5e-4c 16c-12a 18a-19f
hyp 3+4 1a-3i 8g-6a 16d-17h 11e-9a 4abcd-5e 16c-12a 18a-19f
hyp 1+2 1a-3i 8g-6a 5e-4a 9a-11e 17h-16d 16c-12a 18a-19f
pigST 1a-3i 4a-5e 6a-8g 9a-11e 12a-17h 18a-19f

Arm B (Fig. 5, a) not mapped, monomorphic. It has a sequence pulB1. The common BR is well developed.

Arm C (Fig. 5, a) not mapped. It has two banding sequences pulC1 and pulC2 differing by a simple inversion, which involved practically the whole central part of arm C.

Arm D (Fig. 5, c) has the sequence pulD1, formed by five inversion steps from pigST

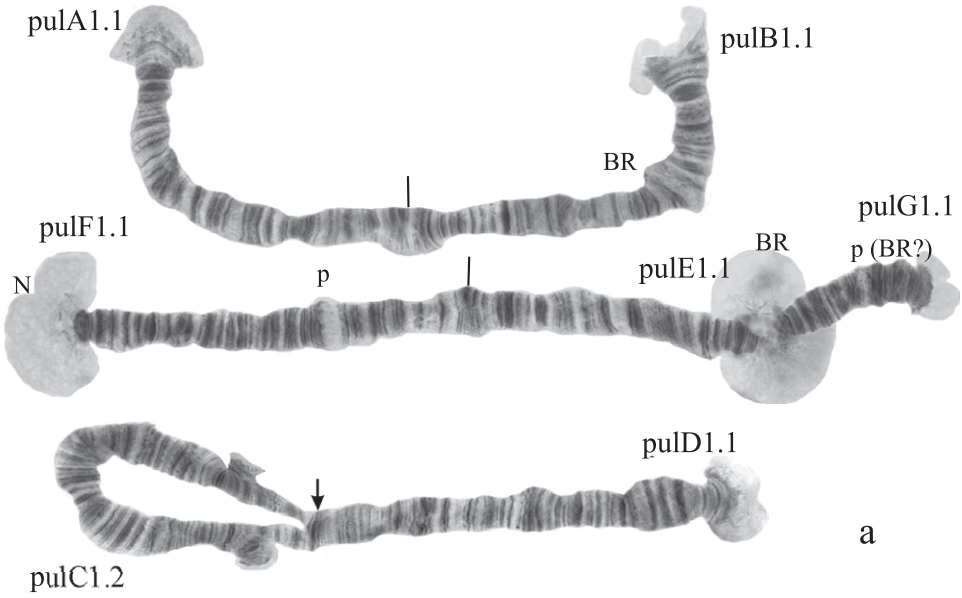

Figure 5a. Karyotype of *Chironomus* prope *pulcher.* The designations are the same as in Fig. 1.

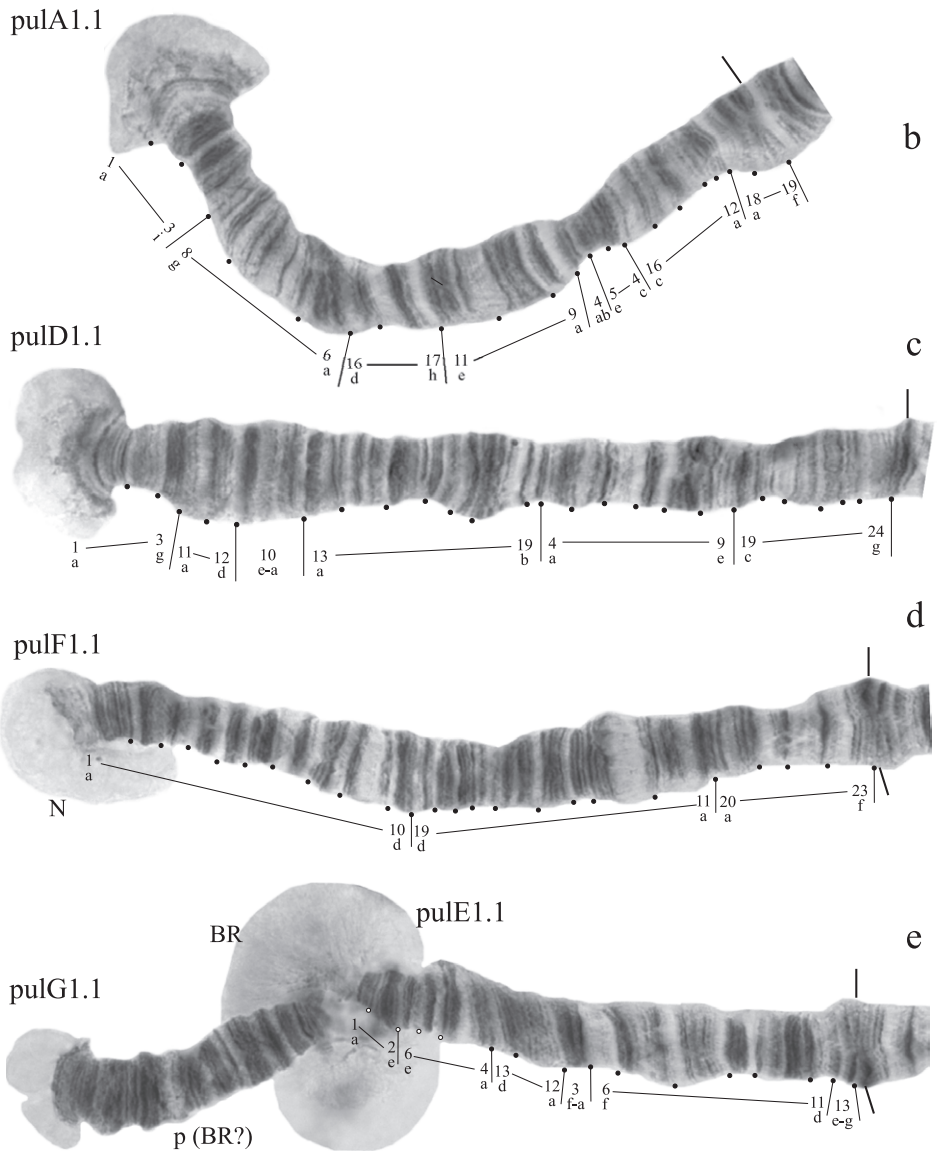

Figure 5b-e. Homozygous banding sequences of *Chironomus* prope *pulcher* in arms A, D, E, F, and G.

pulD1	1a-3g 11a-12d 10e-a 13a-19b 4a-9e 19c-24g
hyp 4	1a-3g 10a-e 12d-11a 13a-19b 4a-9e 19c-24g
hyp 2+3	1a-3g 10a-e 11a-12d 13a-19b 4a-9e 19c-24g
hyp 1	1a-3g 19b-13a 12d-11a 10e-a 9e-4a 19c-24g
pigST	1a-3g 4a-9e 10a-e 11a-12d 13a-19b 19c-24g

Arm E (Fig. 5, e) has the banding sequence pulE1, formed by three inversion steps from pigST.

pulE1 1a-2e 6e-4a 13d-12a 3f-a 6f-11d 13e-g
hyp 2 1a-2e 6e-4a 13d-12a 11d-6f 3a-f 13e-g
hyp 1 1a-2e 6e-4a 3f-a 6f-11d 12a-13d 13e-g
pigST 1a-2e 3a-f 4a-6e 6f-11d 12a13d 13e-g

Arm F (Fig. 5, d) has the sequence pulF1, formed by one simple inversion from pigST.

pulF1 1a-10d 19d-11a 20a-23f
pigST 1a-10d 11a-19d 20a-23f

The characteristic of arm F in *Ch.* prope *pulcher* is the presence of the nucleolus at the telomeric end, which is a rare event among *Chironomus* species.

Arm G (Fig. 5, e) is joined with arm E. There is large Balbiani ring near the site of fusion, and a small Balbiani ring or puff in the center of arm G. A small nucleolus is possibly developed at the telomeric end of arm G.

In total, eight banding sequences were recorded in the *Ch.* prope *pulcher* banding sequence pool. All of them are endemic for Ethiopia. There are no basic sequences.

Larva: long tubuli laterales on abdominal segment VII. Other characters - Dejoux, 1968.

Distribution: two pools within a short distance, River Athi south of Nairobi, Kenya.

Chironomus sp. Kisumu

Karyotype (Fig. 6, a). Haploid number n=4, arm combination AC BF DE G ("parathummi" cytocomplex), centromeric bands not heterochromatinized, nucleoli in arms E and G, Balbiani rings in arms B and G. Chromosomal polymorphism was not recorded.

Banding sequences (Fig. 6, b-f)

Arm A (Fig. 6, b) has the sequence kisA1, formed by 3 inversion steps from pigST.

kisA1 1a-k 19d-16b 10a-16a 2a-9e 19ef
hyp 1 1a-k 19d-16b 16a-10a 9e-2a 19ef
pigST 1a-k 2a-9e 10a-16a 16b-19d 19ef

Arm C (Fig. 6, c) has the sequence kisC1, formed by 8 inversion steps from pigST.

kisC1 1a-2e 5d-6f 22e-17a 2f-5c 16h-14a 11f-13f 6g-11e 22fg
hyp 4+7 1a-2e 5d-6f 22e-17a 2f-5c 6g-11e 11f-13f 14a-16h 22fg
hyp 3 1a-2e 5d-6f 22e-17a 16h-14a 13f-11f 11e-6g 5e-2f 22fg
hyp 2 1a-2e 5d-6f 6g-11e 11f-13f 14a-16h 17a-22e 5e-2f 22fg

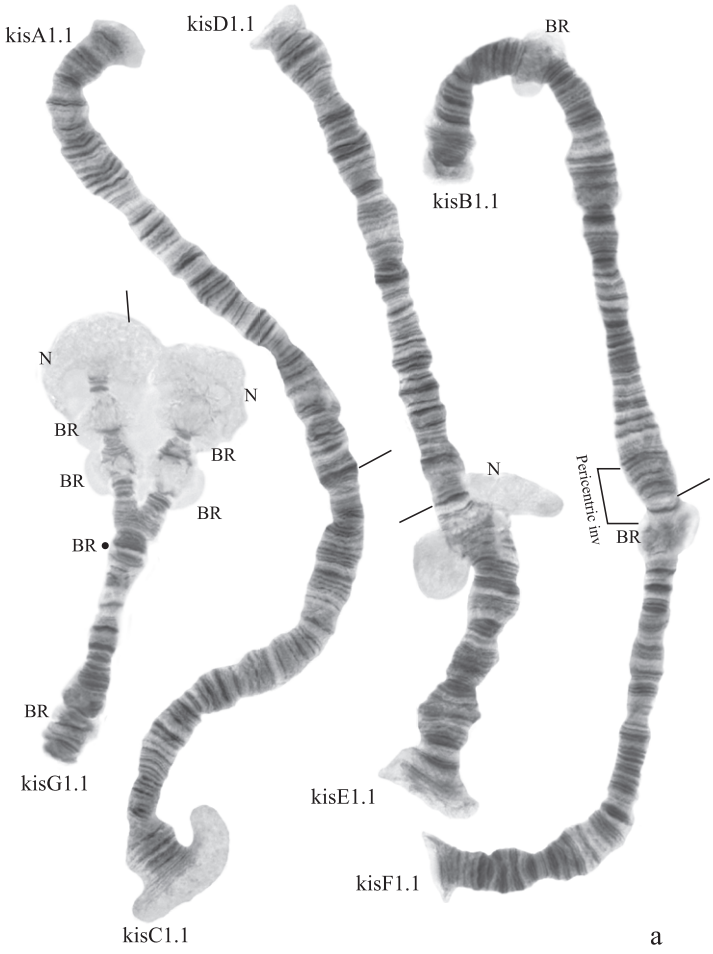

Figure 6a. Karyotype of *Chironomus* sp. Kisumu.

hyp 1 1a-2e 22e-17a 16h-14a 13f-11f 11e-6g 6f-5d 5e-2f 22fg
pigST 1a-2e 2f-5c 5d-6f 6g-11e 11f-13f 14a-16h 17a-22e 22fg

Arm D (Fig.6, d) has the sequence kisD1, formed by 6 inversion steps from *Chironomus piger* ST:

kisD1 1a-e 3g-1f 18c-16a 14c-15e 23d-18d 8d-4a 14b-9a 23e-24g
hyp 4+5 1a-e 3g-1f 18c-16a 15e-14c 23d-18d 8d-4a 14b-9a 23e-24g
hyp 3 1a-e 3g-1f 14c-15e 16a-18c 18d-23d 8d-4a 14b-9a 23e-24g
hyp 1+2 1a-e 3g-1f 4a-8d 23d-18d 18c-16a 15e-14c 14b-9a 23e-24g
pigST 1a-e 1f-3g 4a-8d 9a-14b 14c-15e 16a-18c 18d-23d 23-24g

Arm E (Fig. 6, e) has the sequence kisE1, formed by two inversion steps from *Chironomus piger* ST

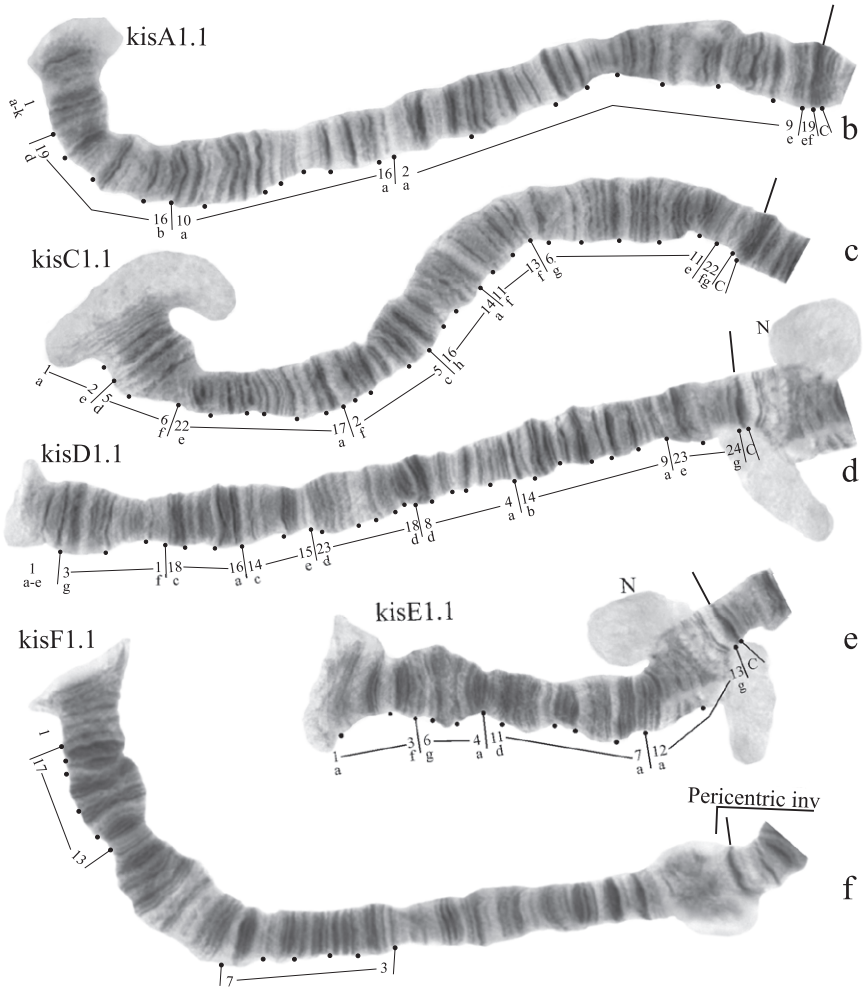

Figure 6b-f. Homozygous banding sequences of *Chironomus* sp. Kisumu in arms A, C. D, E and F.

kisE1 1a-3f 6g-4a 11d-7a 12a-13g
pigST 1a-3f 4a-6g 7a-11d 12a-13g

Presence of a nucleolus in region 13 in arm E is a great characteristic of the *Ch*. sp. Kisumu karyotype.

Arm B (Fig. 6, a) not mapped. It has one sequence – kisB1. The common BR is well developed.

Arm F (Fig. 6, f) has the sequence kisF1. It was mapped only fragmentarily because of complex inversions in comparison with *Chironomus piger* ST. The presence of a large Balbiani ring situated just near the centromeric band is a characteristic of arm F in the *Ch*. sp. Kisumu karyotype. There is pericentric inversion in the chromosome BF (Fig. 6, a, f).

Arm G (Fig. 6, a) is longer than usual in *Chironomus* species. There is a nucleolus and four Balbiani Rings on arm G. One of Balbiani Rings, noted by the black dot in Fig. 6, a, was developed only in some cells of the salivary gland cells.

In total, seven Ethiopian endemic banding sequences are found in the sequence pool of *Ch*. sp. Kisumu. All these sequences differ from *Ch. parathummi* Keyl, 1961 sequences.

Larva: long tubuli laterales on abdominal segment VII.

Distribution: near Victoria lake, Kenya.

Discussion

Karyotypes of six African *Chironomus* species were studied. Four of these karyotypes were described for the first time (*Ch.* sp. Nakuru*, Ch. formosipennis, Ch.* prope *pulcher, Ch.* sp. Kisumu). Detailed photomaps of arms A, C, D, E, and F were presented, also for the first time, for *Ch*. *alluaudi*, *Ch. transvaalensis*, and *Ch.* sp. Nakuru.

Among the species studied, three species (*Ch. transvaalensis*, *Ch*. prope *pulcher*, *Ch*. sp. Kisumu) have only endemic Ethiopian banding sequences in their karyotypes, while cosmopolitan basic banding sequences were discovered in the karyotypes of the other species, along with endemic sequences (*Ch. alluaudi, Ch.* sp. Nakuru*, Ch. formosipennis*). The presence of these basic sequences indicates a relationship of African *Chironomus* species to *Chironomus* species from other continents before their separation (Kiknadze et al. 2008).

The results on African species are relevant the problem whether or not the chromosome arm combination of the "thummi" cytocomplex is rare in Southern continents. At the moment, one species in South America (*Chironomus* sp. Las Brisas, Wülker, Morath, 1989), one species in India (*Ch. javanus* Kieffer, 1924), and two species in Australia (*Ch. javanus, Ch. queenslandicus* Martin, 2005) are known to have this "thummi" cytocomplex chromosome arm combination (Martin 2010, Martin, pers. comm. and this paper).

Earlier it was demonstrated (Wülker 1980), that the presence of basic sequences in arms A, E, F of some *Chironomus* species of the "thummi" and "pseudothummi" cytocomplexes supports an idea that the basic sequences existed in hypothetical stem species before the separation of the complexes. The results of this paper contribute to the understanding of chromosome arms C and D in phylogeny in both cytocomplexes, in addition to data on arms A, E and F published earlier (Wülker 1980, 2010, Kiknadze et al. 2008).

Keyl (1962) established the hypothesis, that "the hypothetical species, which crossed the border between "thummi" and "pseudothummi" cytocomplexes" had most probably three banding patterns in arm E (in Keyl's terms): standard as *Ch. piger* Strenzke, 1959, pattern as *Ch. aprilinus* Meigen, 1838 and others, pattern as *Ch. aberratus* Keyl,1961 and others. We can ask, whether these three patterns are known today in both cytocomplexes. This is indeed so (Fig. 7, a) with the exception of the fact that

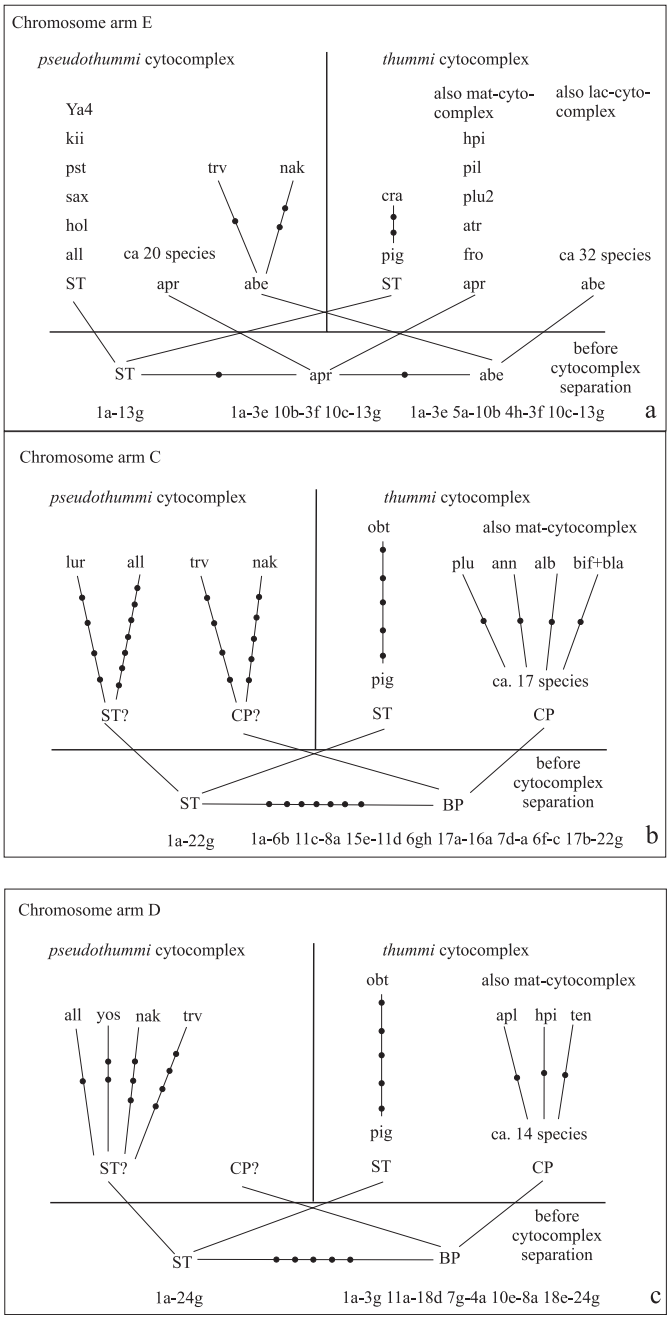

Figure 7a-c. Relations of recent species and hypothetical "basic" species before separation of the cytocomplexes in arm E (**a**), arm C (**b**), and arm D (**c**). The data of Keyl (1962) and Kiknadze (unpublished) were also used. **Dots** – inversion steps between banding sequences; **ST** – piger standard after Keyl (1962) and Dévai et al. (1989). **all** – *alluaudi*, **atr** – *atrella*, **apr** – *aprilinus*, **abe** – *aberratus*, **cra** – *crassicaudatus*, **fro** – *frommeri*, **hol** – *holomelas*, **hpi** – *heteropilicornis*, **kii** – *kiiensis*, **pil** – *pilicornis*, **plu** – *plumosus*, **pst** – *pseudothummi*, **sax** – saxatilis, **trv** – *transvaalensis*.

the pattern of *Ch. aberratus* itself is not known in the "pseudothummi" cytocomplex, but there are the sequences trvE1 and nakE1 which differ only by 1–2 inversions from abeE (Fig. 7, a).

In arms C and D, an accumulation of species with the identical sequences was previously observed only in the "thummi" cytocomplex (Wülker, 2010). With the data of this paper we can propose that chromosome arms C and D had also two patterns before separation of the cytocomplexes (*Chironomus piger* ST sequence sensu Keyl, 1962 and basic pattern sensu Wülker, 1980, 2010). Fig. 7, b shows that pattern ST and basic themselves are not found in "pseudothummi" cytocomplex (question marks in Fig. 7, b), but there are several species, which have banding patterns differing only by a few inversions from ST and basic. The African species (*Ch. alluaudi, Ch. transvaalensis, Ch*. sp. Nakuru, *Ch. formosipennis*) play an important role in the development of the arm C and D phylogeny. Fig. 7, c demonstrates that there are ST and basic patterns in the 'thummi' cytocomplex, but only patterns close to ST were found in the "pseudothummi" cytocomplex: allD1 only by one, yosD1 by two, nakD1 by three, and trvD1 by four inversions from ST.

A great peculiarity of some African *Chironomus* karyotypes is the presence of large numbers of functionally active chromosome sites, especially Balbiani rings. For example 5 BRs were found in *Ch. transvaalensis* (Figs 2, a, 2, g-i), 6 BRs in *Ch.* sp. Nakuru (Fig. 3, a). Most *Chironomus* species have two or three visible BRs since e.g. many species have the gene for BR4 but do not express it, and the number seen may also vary with developmental stage.

Acknowledgements

Travel of W.W. was sponsored by Deutsche Forschungsgemeinschaft. In Nairobi, family B. and R. Seelmann-Eggebert offered great hospitality and cooperation, Prof. Schneider and Prof. Kaib gave important informations. Prof. Dr. Vareschi, Drs. Reyer, Pater N. Cox, Oltmanns, d'Oliveira and J. Grunewald helped in collection of the larvae. Wülker's family contributed encouragement, car driving and food. Many thanks to all of them.

The work was supported by the Presidium of the Russian Academy of Sciences under the program "The Origin and Evolution of Biosphere", subprogram 2 project "The Role of Chromosome Rearrangements in Speciation and Evolution"; the program "Biodiversity and Gene Pool Dynamics", subprogram "The Dynamics of Plant, Animal, and Human Gene Pools", project "The Mechanisms of Genetic Variation of Diptera Populations and Species" and by the grant of RFBR No 09-04-01440.

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