© Comparative Cytogenetics, 2008 . Vol. 2, No. 1, P. 37-55. ISSN 1993-0771 (Print), ISSN 1993-078X (Online)

The revision of chromosome I (AB) mapping in *Chironomus plumosus* group (Diptera: Chironomidae)

V.V. Golygina[1], I.I. Kiknadze[2]

Institute of Cytology and Genetics SB RAS, Prosp. akademika Lavrentieva 10, Novosibirsk 630090, Russia.
E-mails: [1]nika@bionet.nsc.ru, [2]kiknadze@bionet.nsc.ru

Abstract. The revision of main and alternative banding sequences in chromosome I (AB) have been made for all 14 species of the *Chironomus plumosus* (Linnaeus, 1758) group. New version of mapping had been suggested for 14 out of 22 banding sequences of arm A. Mapping of 18 banding sequences in arm B have been made for the first time according to Maximova-Shobanov system. Phylogenetic relationships of the banding sequences of chromosome I are discussed.

Key words: *Chironomus plumosus*, karyotype, banding sequence, chromosome I, mapping.

Introduction

Chironomus plumosus (Linnaeus, 1758) group of sibling species is a unique object for the study of patterns in linear reorganization of the genome during speciation. Among 14 presently known species of the group there are species with wide geographic ranges that cover almost all the Holarctic with all its difference in natural conditions (Butler et al., 1999, Kiknadze et al., 2000) and species with small ranges, such as endemics of Japanese islands (Golygina et al., 2003; Golygina, Ueno, 2005). Species of *Ch. plumosus* group also differ greatly in the level of chromosomal polymorphism in natural populations: some of them are almost completely monomorphic whereas the others are highly polymorphic. These facts together with the presence of polytene chromosomes with clear banding sequences and highly conservative banding structure, which is characteristic for the genus *Chironomus* Meigen, 1803 in general, allow us to use *Ch. plumosus* group for the study of contribution of chromosomal rearrangements to a divergence of species in all stages of speciation.

For the cytogenetic analysis of chromosomal rearrangements, specifically for the exact localization of inversion breakpoints, it is very important to have high-quality photographic maps of karyotypes and unified mapping system of polytene chromosomes. Earlier the maps were created for all species of *Ch. plumosus* group (Maximova, 1976; Dévai et al., 1983; Ryser et al., 1983; Kiknadze et al., 1986a, b, 1987, 1991a, b, d, 1996a, 2000, 2005; Kerkis at al., 1988, 1989; Shobanov, Djomin, 1988; Shobanov, 1994; Djomin, Shobanov, 1990; Loginova, Beljanina, 1994; Golygina, 1999; Butler et al., 1999; Golygina, Kiknadze, 2001; Golygina et al., 2003), however the res-

olution of some of them was not to the present time standard and there were contradictions between mappings of identical banding sequences in different papers. Our long-term study had shown that for the reliable mapping it is necessary to produce maps with higher resolution. Another problem for any researcher, working with *Ch. plumosus* group, is the existence of several mapping systems: some authors work with Keyl-Dévai system (Keyl, 1962; Dévai et al., 1989) whereas others prefer Maximova (1976) or Maximova-Shobanov system (Shobanov, 1994a).

H.-G. Keyl was the first who created standard maps for arms A, E, and F of polytene chromosomes of *Ch. piger* Strenzke, 1959 and mapped banding sequences of some other species of the genus *Chironomus* (Keyl, 1962). Later Dévai with coauthors (Dévai et al., 1989) developed standard maps for arms B, C, and D; so, at present it is possible to work with 6 chromosomal arms. Keyl-Dévai mapping system is used for the mapping of all species from the genus *Chironomus* with banding sequences of *Ch. piger* as a standard. In this mapping system chromosomal arm is divided into regions, which are designated by numbers, and letters designate bands inside every region. Thus, every band of the chromosomal arm has the exact designation. However, due to significant interspecific divergence of banding sequences in arms B and G it was impossible to make complete maps of these arms for all *Chironomus* species up to now and thus an effective mapping in Keyl-Dévai system could be made for 5 chromosomal arms – A, C, D, E, and F. This mapping system was used for mapping of most of *Chironomus* species.

In Maximova system (Maximova, 1976) banding pattern of *Ch. plumosus* is standard for mapping of other species from *Ch. plumosus* group. Although standard maps were created for all 7 chromosomal arms the Maximova system has grave disadvantage, as there is no exact designation for single bands as it is in Keyl-Dévai mapping system. As a result, in all cases when inversion breakpoints fall inside a region instead of its border, the resulted parts of this regions were designated arbitrary, for example 15a and 15b or 15a and 15bc, depending on the length of the parts and the will of an author. Thus the exact mapping of inversions existed only if inversion breakpoints fall on the regions borders. In 1994 N.A. Shobanov made the revision of Maximova mapping system and gave exact designation to all the bands. Nevertheless, converting of banding sequences from one mapping system into the other is difficult because of a difference between the number of designated bands in Maximova-Shobanov and Keyl-Dévai mapping systems. The difference in the number of bands detailed in two mapping system is possible do to the fact that chromosomes could have different level of compactization that result in a slight difference in the number of visible bands. As a result, it is impossible to compare banding sequences of *Ch. plumosus* sibling species that were published in different mapping systems without comparison of chromosome photos. Besides, to include banding sequences mapped in Maximova-Shobanov mapping system into phylogenetic analysis of banding sequences of *Chironomus* genus it is also necessary to convert them to Keyl-Dévai system. All this significantly complicates the work with this group of siblings. Thus it is necessary to conduct a revision of banding sequence mapping of species of *Ch. plumosus* group both in way of improving of mapping accuracy and bringing all available data to agreement. As was mentioned above, the complete mapping of arms B and G in Keyl-Dévai mapping system was impossible, nevertheless it is preferable to use this system whenever possible as it is allows direct comparison of

banding sequences with all other mapped species of the genus *Chironomus*. We believe that at present it is optimal to map arms A, C, D, E, and F of *Ch. plumosus* siblings in Keyl-Dévai system and arms B and G – in Maximova-Shobanov system.

As main and alternative banding sequences, the most frequent in species populations (for definition of banding sequence types see Golygina et al., 2007), are ancestral for all other banding sequences (rare and unique) that could be found in species pools of banding sequences, the first part of our study was to revise their mapping in all species of *Ch. plumosus* group and to analyse their phylogenetic relationships. In this paper we present the result of the revision of banding sequences in chromosome I (AB).

Material and methods

Revision of chromosome I mapping was conducted for *Ch. plumosus* sibling species: *Chironomus agilis* Shobanov et Djomin, 1988, *Chironomus* sp. prope *agilis* (working name "*Ch. agilis* 2") (Kiknadze et al., 1991a), *Ch. balatonicus* Dévai, Wülker et Scholl, 1983, *Ch. bonus* Shilova et Dzhvarsheishvili, 1974, *Ch. borokensis* Kerkis, Filippova, Shobanov, Gunderina et Kiknadze, 1988, *Ch. entis* Shobanov, 1989, *Ch. muratensis* Ryser, Scholl et Wülker, 1983, *Ch. nudiventris* Ryser, Scholl et Wülker, 1983, *Ch. plumosus* (Linnaeus, 1758), *Ch. sinicus* Kiknadze, Wang, Istomina et Gunderina, 2005, *Chironomus* sp. J (Kiknadze et al, 1991d), *Chironomus* sp. K (Golygina, Ueno, 2005), *Ch. suwai* Golygina et Martin, 2003, *Ch. usenicus* Loginova et Belyanina, 1994. For mapping of all examined banding sequences high-resolution photomaps were created.

Mapping of arm A was done according to Keyl-Dévai mapping system (Keyl, 1962), arm B was mapped according to Maximova-Shobanov mapping system (Maximova, 1976; Shobanov, 1994a).

Each banding sequence in each chromosomal arm is numbered according to the order of its discovery; these numbers prefixed by an abbreviation of the species name (for example, agi – for *Ch. agilis*, bal – for *Ch. balatonicus*, etc.), with a further prefix p' for Palearctic sequences, n' for Nearctic sequences, or h' for Holarctic sequences (e.g. p'agiA1, n'pluA9, h'pluB1, etc.).

Numbering of banding sequences of arm A in *Ch. plumosus* is done according to Butler et al. (1999).

Equipment of the Centre of Microscopy analysis of biological objects SB RAS in the Institute of Cytology and Genetics (Novosibirsk) was used in accomplishment of this work: microscope "Axioskop" 2 Plus, CCD-camera AxioCam HRc, software package AxioVision 4 (Zeiss, Germany).

Results

Arm A

Mapping of banding sequences of *Ch. plumosus* sibling species according to Keyl-Dévai system that was published till now is shown in the Table 1. In total 22 main and alternative banding sequences are considered in this study. Phylogram of banding sequences constructed on the basis of this mapping is shown in Fig. 1, a, where main banding sequences are written in bold and alternative – in italic. As could be seen, most of banding sequences of different species were considered identical to either p'pluA1 (6 main and 1 alternative banding sequences) or h'pluA2 (3 main and 3 alternative banding sequences). Seven banding sequences were considered species specific.

According to our analysis, 14 banding sequences of 10 species require a revision of mapping. The most important changes should

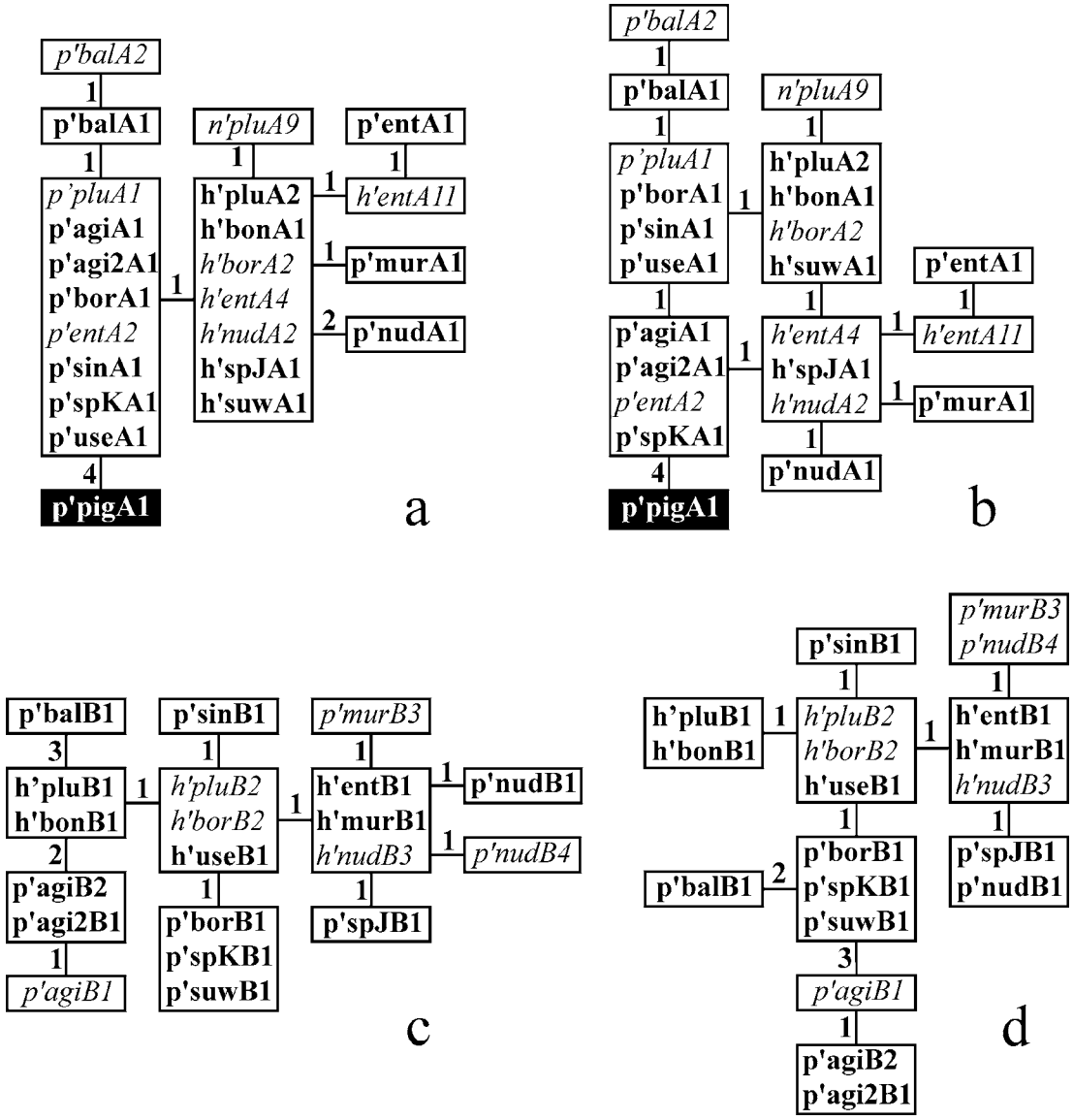


Fig. 1, a-d. Phylogenetic relationship of main and alternative banding sequences in arms A and B before (**a, c**) and after (**b, d**) the revision. Main banding patterns are written in bold, alternative – in italic. Identical banding sequences enclosed in boxes, figures near the lines that connect banding sequences indicate numbers of inversion steps between them.

be made in mapping of banding sequences of species *Ch. balatonicus*, *Ch. bonus*, *Ch. borokensis*, *Ch. plumosus*, *Ch. sinicus*, *Ch. suwai* and *Ch. usenicus* where we have found a microinversion, characteristic of all banding sequences of these species. In case of *Ch. entis*, *Ch. muratensis* and *Ch. nudiventris* it was necessary to define more precisely the breakpoints of inversions, which differ p'entA1, p'murA1 and p'nudA1 from the ancestral banding sequences. Our version of mapping of banding sequences in arm A is shown in Fig. 2.

Table 1. Mapping of arm A main and alternative banding sequences (BS) in *Ch. plumosus* group before the revision. [1] papers with given version of the mapping are shown in parenthesis.

Designation of BS	Mapping of banding sequence
p'agiA1	1a-2c 10a-12c 3i-2h 4d-9e 2d-g 4c-a 13a-19f C (Shobanov, Djomin, 1988; Kerkis et al., 1989; Kiknadze et al., 1991d, 1996b, 2004)[1]
p'agi2A1	1a-2c 10a-12c 3i-2h 4d-9e 2d-g 4c-a 13a-19f C (Kiknadze et al., 1991a, 2004)
p'balA1	1a-2c 9e-4d 2h-3i 12c-10a 2d-g 4c-a 13a-19f C (Devai et al., 1983, Kiknadze, Kerkis, 1986; Golygina et al., 1996)
p'balA2	1a-2c 9e-7c 15e-13a 4a-c 2g-d 10a-12c 3i-2h 4d-7b 16a-19f C (Devai et al., 1983; Golygina et al., 1996)
h'bonA1	1a-2c 10a-12a 13b-13a 4a-c 2g-d 9e-4d 2h-3i 12cb 13c-19f C (Kerkis et al., 1989; Kiknadze et al., 1991d)
p'borA1	1a-2c 10a-12c 3i-2h 4d-9e 2d-g 4c-a 13a-19f C (Kerkis et al., 1988; Kiknadze et al., 1991d, 1996b)
h'borA2	1a-2c 10a-12a 13b-13a 4a-c 2g-d 9e-4d 2h-3i 12cb 13c-19f C (Kerkis et al., 1988; Kiknadze et al., 1991d, 1996b)
p'entA1	1a-2c 10a-12a 13ba 14f-13c 12bc 3i-2h 4a-c 2g-d 9e-4d 14g-19f C (Golygina, 1999; Kiknadze et al., 2000)
p'entA2	1a-2c 10a-12c 3i-2h 4d-9e 2d-g 4c-a 13a-19f C (Shobanov, Djomin, 1990)
h'entA4	1a-2c 10a-12a 13b-13a 4a-c 2g-d 9e-4d 2h-3i 12cb 13c-19f C (Kiknadze et al., 1991c, 2000, 2004)
h'entA11	1a-2c 10a-12a 13ba 14f-13c 12bc 3i-2h 4d-9e 2d-g 4c-a 14g-19f C (Golygina, 1999; Kiknadze et al., 2000)
p'murA1	1a-2c 13f-c 12bc 3i-2h 4d-9e 2d-g 4c-a 13ab 12a-10a 14a-19f C (Ryser et al., 1983; Kiknadze, Kerkis, 1986; Kiknadze et al., 1991b)
p'nudA1	1a-2c 10a-12a 13ba 13dc 12bc 3i-2h 4d-6e 8f-7a 8g-9e 2d-g 4c-a 13ef 14a-19f C (Ryser et al., 1983)
p'nudA2	1a-2c 10a-12a 13b-13a 4a-c 2g-d 9e-4d 2h-3i 12cb 13c-19f C (Kiknadze et al., 1987)
p'pluA1	1a-2c 10a-12c 3i-2h 4d-9e 2d-g 4c-a 13a-19f C (Keyl, 1962, Kiknadze et al., 1991d, 1996b, 2004; Shobanov, 1994)
h'pluA2	1a-2c 10a-12a 13b-13a 4a-c 2g-d 9e-4d 2h-3i 12cb 13c-19f C (Keyl, 1962, Kiknadze et al., 1991d, 1996b, 2004; Shobanov, 1994)
n'pluA9	1a-2a 17h-13c 12b-12c 3i-2h 4d-9e 2d-g 4c-a 13a-13b 12a-10a 2cb 18a-19f C (Butler et al., 1999; Golygina, 1999; Golygina, Kiknadze, 2001)
p'sinA1	1a-2c 10a-12c 3i-2h 4d-9e 2d-g 4c-a 13a-19f C (Kiknadze et al., 2005)
p'spJA1	1a-2c 10a-12a 13b-13a 4a-c 2g-d 9e-4d 2h-3i 12cb 13c-19f C (Kiknadze et al., 1991d)
p'spKA1	1a-2c 10a-12c 3i-2h 4d-9e 2d-g 4c-a 13a-19f C (not published)
h'suwA1	1a-2c 10a-12a 13b-13a 4a-c 2g-d 9e-4d 2h-3i 12cb 13c-19f C (Golygina et al., 2003; Kiknadze et al., 2004)
p'useA1	1a-2c 10a-12c 3i-2h 4d-9e 2d-g 4c-a 13a-19f C (Loginova, Beljanina, 1994)

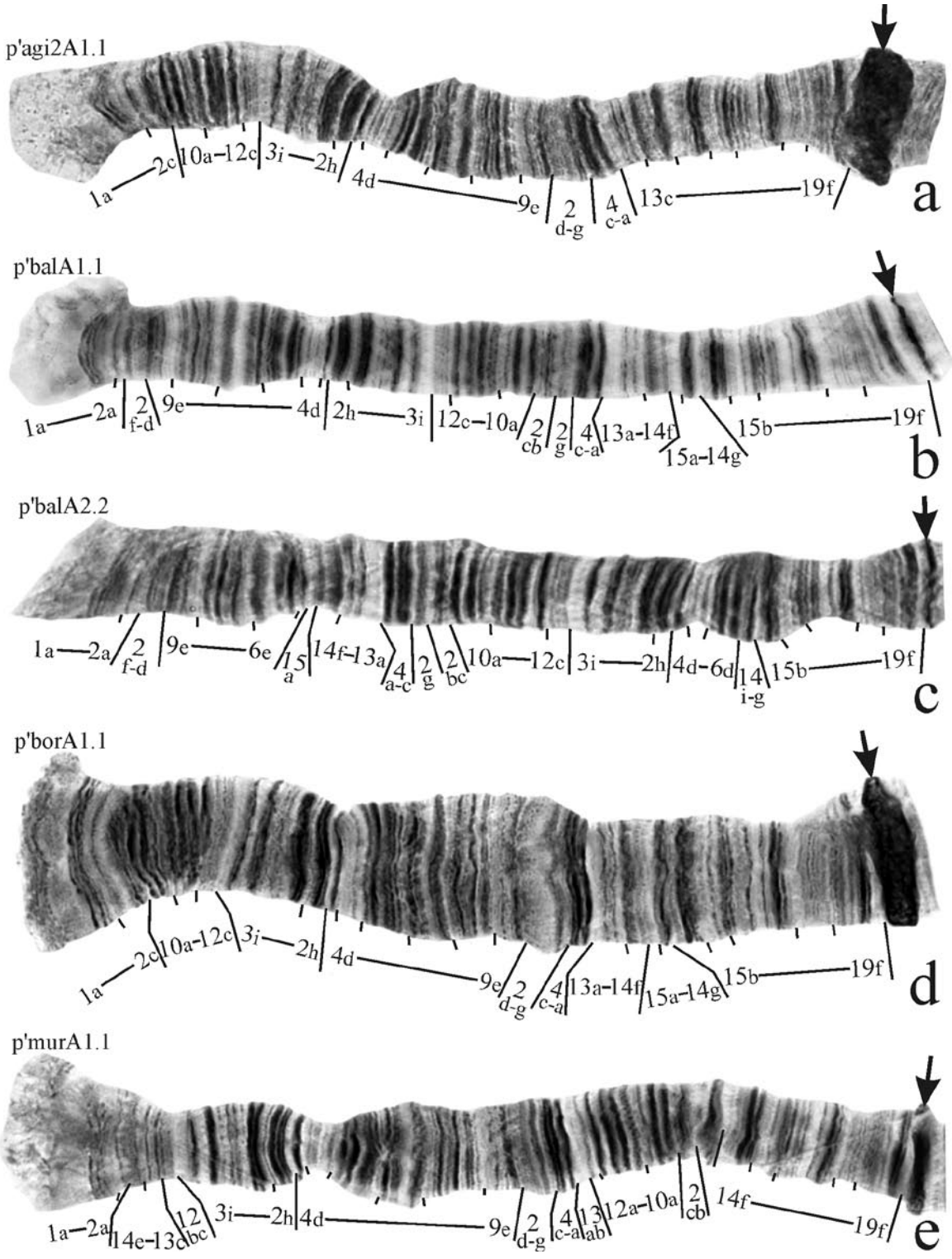


Fig. 2, a-e. Mapping of banding sequences of *Ch. plumosus* sibling species in arm A. **a -** p'agi2A1.1. **b -** p'balA1.1. **c -** p'balA2.2. **d -** p'borA1.1. **e -** p'murA1.1.

1. The revision of arm A mapping of *Ch. balatonicus*, *Ch. bonus*, *Ch. borokensis*, *Ch. plumosus*, *Ch. sinicus*, *Ch. suwai*, and *Ch. usenicus*.

It was considered previously that banding sequences in regions 14-15 of all *Ch. plumosus* sibling species are identical to banding sequence of *Ch. piger* (the only exception was *Ch. entis* as this region was divided by inversions in banding sequences p'entA1 and h'entA11). However, our study had shown that half of the species from *Ch. plumosus* group – *Ch. balatonicus*, *Ch. bonus*, *Ch. borokensis*, *Ch. plumosus*, *Ch. sinicus*, *Ch. suwai* and *Ch. usenicus* – have a microinversion that include several bands from regions 14 and 15 (Fig. 3, a). Comparison of banding sequences p'agiA1 (without microinversion) and p'balA1 (with microinversion) with each other and with banding sequence of p'pigA1 is shown in Fig. 3, b. It is clear that banding sequence in regions 14 and 15 of p'agiA1 completely identical to such of p'pigA1 whereas in p'balA1 bands 14g-15a arranged in reverse order. The same situation can be observed in banding sequences of *Ch. bonus*, *Ch. borokensis*, *Ch. plumosus*, *Ch. sinicus*, *Ch. suwai*, and *Ch. usenicus*. New mapping of the banding sequences of these species is shown in Table 2 and in Fig. 2, b-d, j, k.

In the case of *Ch. balatonicus* in addition to the change in mapping of the region 14-15 it is necessary to revise the mapping of inversions, which form banding sequences p'balA1 and p'balA2. Banding sequence p'balA1 differs from p'pluA1 in the simple inversion. It was considered that inversion breakpoints are placed between bands 2c and 10a on the left, and bands 9e and 2d on the right (Table 1) that is the regions 2a-c and 2d-g aren't split up by the inversion. However, on the both ends of the inversion banding sequences do not look like they should have if the previous mapping is correct. Comparison of p'balA1 with banding sequences of other species of *Ch. plumosus* group had shown that in p'balA1 regions 2a-c and 2d-g were split up by the inversion so that bands 2bc and 2d-f can be found on the right and left borders of inversion, respectively (Fig. 4, a). Mapping of p'balA1 after the revision is shown in Table 2 and in Fig. 2, b.

Banding sequence p'balA2 originated from p'balA1 by simple inversion and also require a revision. We suggest that p'balA2 has the banding sequence that is shown in Table 2 and in Fig. 2, c.

2. The revision of arm A mapping of *Ch. entis*, *Ch. muratensis*, and *Ch. nudiventris*.

Among the four banding sequences of *Ch. entis* considered in this study only p'entA1 required a revision. Until now it was considered that the region 2k-h remains undivided in p'entA1 (Table 1). However, the concerned region of p'etnA1 has atypical structure: whereas in other banding sequences the region 2k-h looks like compact group of dark dense bands, in p'entA1 this region consists of 2 bands divided by the wide light interband (Fig. 4, b). Comparison of p'entA1 with other banding sequences had shown that the real inversion breakpoints fall inside the region 2h-k on the one side and between bands 14g and 15b on the other (Table 2, Fig. 2, f).

The banding sequence p'murA1 originated from h'entA4 (which was formally considered identical to h'pluA2) and differs from it in simple inversion. According to previous mapping (Ryser et al., 1983) regions 2a-c and 14a-f was intact (Table 1), but parts of the chromosome that should correspond to these regions if previously published mapping was correct are atypical. Comparison of p'murA1 with p'murA1 and p'agi2A1, which have most clear banding structure in the regions concerned (Fig. 4, c), had shown that inversion borders fall inside regions 2a-c and 14a-f:

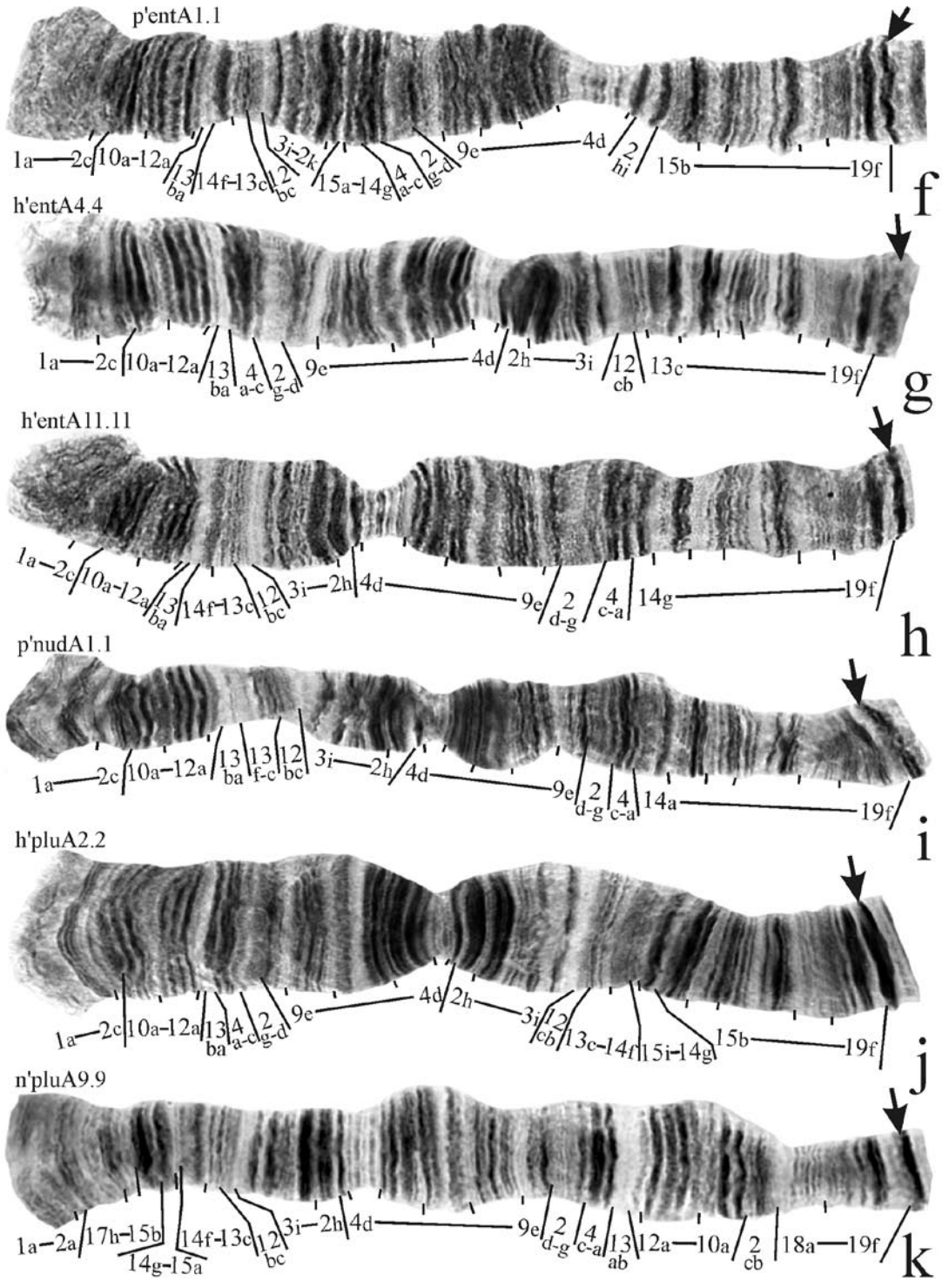


Fig. 2, f-k. Mapping of banding sequences of *Ch. plumosus* sibling species in arm A. **f -** p'entA1.1. **g -** h'entA4.4. **h -** h'entA11.11. **i -** p'nudA1.1. **j -** h'pluA2.2. **k -** n'pluA9.9. Centromeric bands designated by arrows.

Table 2. Mapping of arm A main and alternative banding sequences (BS) in *Ch. plumosus* group after the revision. [1]parts of the sequences highlighted in bold indicate regions which mapping had been changed as a result of the revision.

Designation of BS	Mapping of banding sequence
p'agiA1	**=p'entA2**
p'agi2A1	**=p'entA2**
p'balA1	1a-2a **2f-d** 9e-4d 2h-3i 12c-10a 2cb **2g** 4c-a 13a-14f **15a-14g** 15b-19f C[1]
p'balA2	1a-2a **2f-d** 9e-**6e 15a** 14f-13a 4a-c **2g** 2bc 10a-12c 3i-2h 4d-6d **14i-g** 15b-19f C
h'bonA1	**=h'pluA2**
p'borA1	**=p'pluA1**
h'borA2	**=h'pluA2**
p'entA1	1a-2c 10a-12a 13ba 14f-13c 12bc 3i-2k **15a-14g** 4a-c 2g-d 9e-4d **2hi** 15b-19f C
p'entA2	1a-2c 10a-12c 3i-2h 4d-9e 2d-g 4c-a 13a-19f C
h'entA4	1a-2c 10a-12a 13ba 4a-c 2g-d 9e-4d 2h-3i 12cb 13c-19f C
h'entA11	1a-2c 10a-12a 13ba 14f-13c 12bc 3i-2h 4d-9e 2d-g 4c-a 14g-19f C
p'murA1	1a-2a **14e**-13c 12bc 3i-2h 4d-9e 2d-g 4c-a 13ab 12a-10a 2cb 14f-19f C
p'nudA1	1a-2c 10a-12a 13ba **13f-c** 12bc 3i-2h **4d-9e** 2d-g 4c-a 14a-19f C
p'nudA2	**=h'entA4**
p'pluA1	1a-2c 10a-12c 3i-2h 4d-9e 2d-g 4c-a 13a-14f **15a-14g** 15b-19f C
h'pluA2	1a-2c 10a-12a 13ba 4a-c 2g-d 9e-4d 2h-3i 12cb 13c-14f **15a-14g** 15b-19f C
n'pluA9	1a-2a 17h-15b **14g-15a** 14f-13c 12bc 3i-2h 4d-9e 2d-g 4c-a 13ab 12a-10a 2cb 18a-19f C
p'sinA1	**=p'pluA1**
p'spJA1	**=h'entA4**
p'spKA1	**=p'entA2**
h'suwA1	**=h'pluA2**
p'useA1	**=p'pluA1**

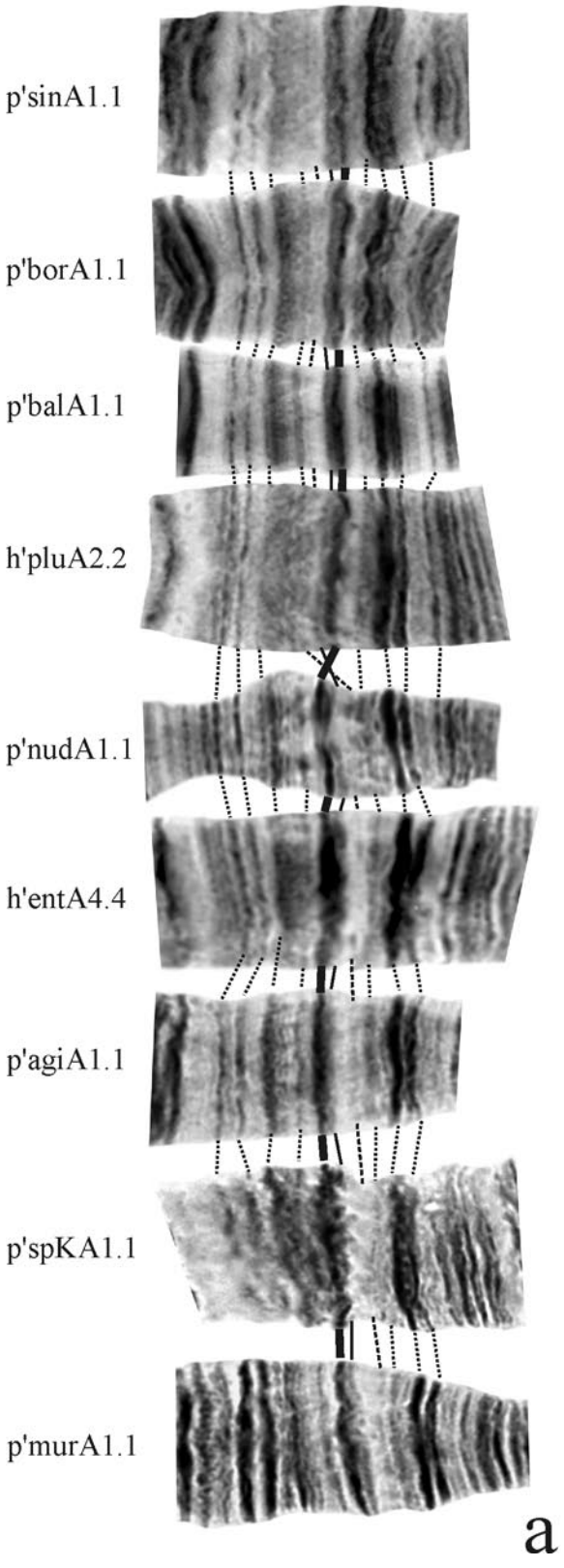
bands 2bc go with the region 10 to the proximal part of the arm, and bands 14a-e move to the distal part with the region 13c-f (Table 2, Figs 2, e; 4, c).

From the two banding sequences of *Ch. nudiventris* that are examined in this paper only p'nudA1 needed a revision. Ryser et al. (1983) described this sequence as differed from the p'pluA1 by included inversion (Table 1). However, comparison of the p'nudA1 and p'pluA1 had shown that they have identical banding sequence in the region 4d-9e, which mean that p'nudA1 differs from p'pluA1 by one simple inversion. Besides, our mapping of inversion breakpoints is different (Table 2, Fig. 2, i).

Phylogenetic relationships of revised banding sequences in arm A of *Ch. plumosus* group's species are shown in Fig. 1, b.

Arm B

Mapping of banding sequences in arm B was done according to Maximova system in all papers published before 1994. As a result, the exact mapping of inversion breakpoints was impossible in most cases. The mapping according to Maximova-Shobanov system was performed for *Ch. plumosus* and *Ch. en-*



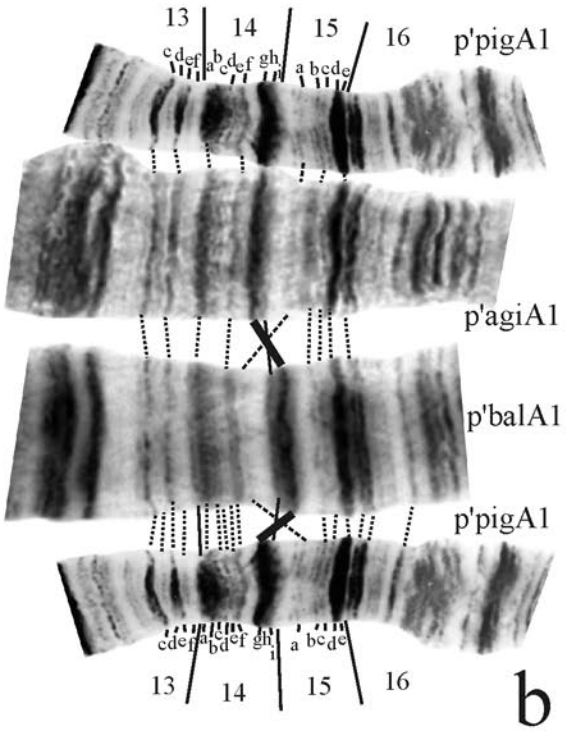


Fig. 3, a-b. Comparison of banding sequences in regions 14 and 15 of arm A in species of *Ch. plumosus* group. **a -** comparison of banding sequences in 9 species of Ch. plumosus group. **b -** comparison of banding sequences in regions 14-15 in *Ch. agilis* and *Ch. balatonicus* from *Ch. plumosus* group with standard *Ch. piger*.

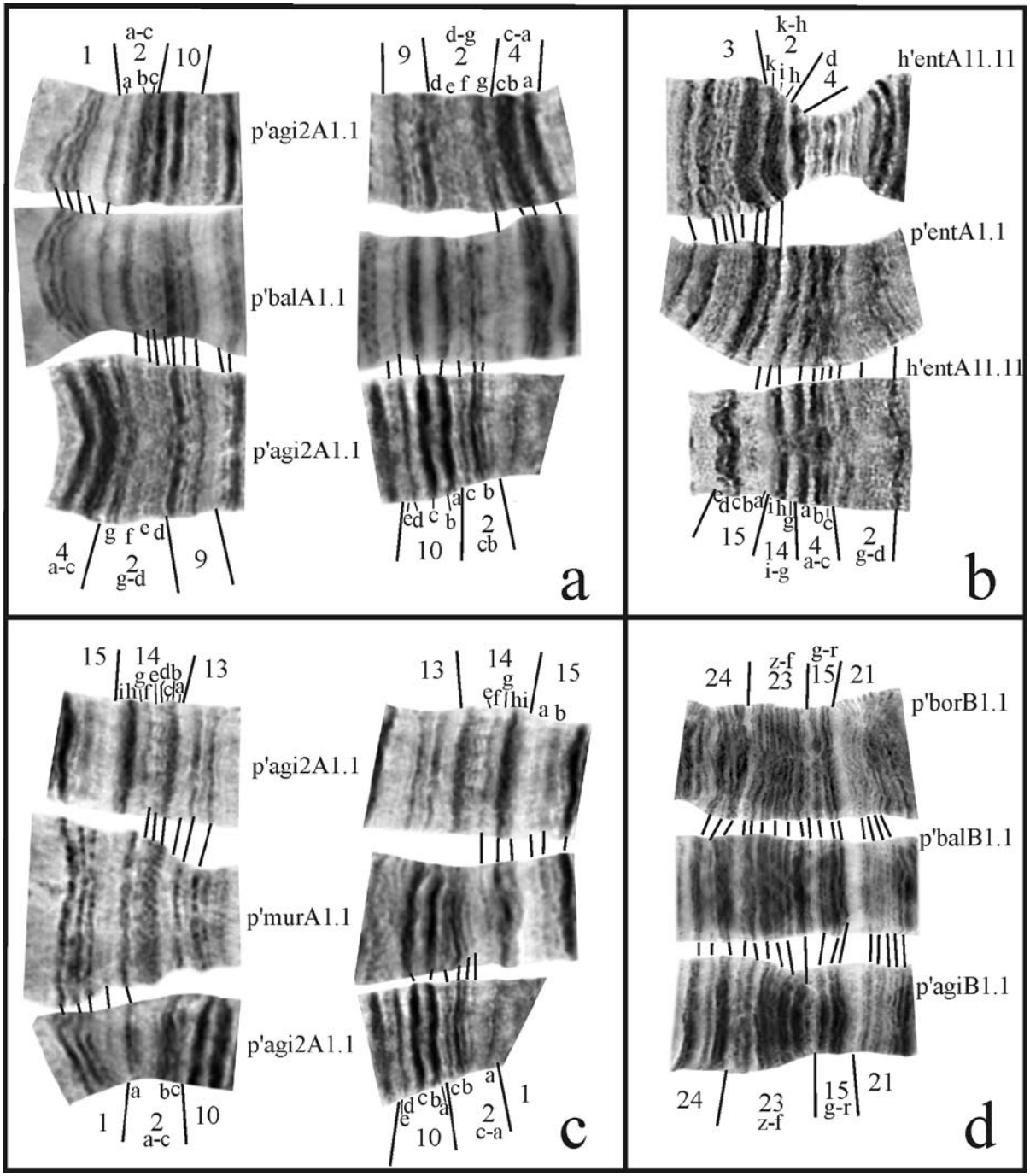


Fig. 4, a-d. Mapping of some inversion breakpoints in species of *Ch. plumosus* group. **a -** comparison of proximal (on the right) and distal (on the left) parts of p'balA1.1 with parts of p'agi2A1.1 that contain bands, homologous to p'balA1. **b -** comparison of part of p'entA1.1 with parts of h'entA11.11. **c -** comparison of proximal (on the right) and distal (on the left) parts of p'murA1.1 with parts of p'agi2A1.1 that contain bands, homologous to p'murA1. **d -** comparison of parts of banding sequences of p'balB1.1 and p'agiB1.1 with p'borB1.1.

Table 3. Mapping of arm B main and alternative banding sequences (BS) in *Ch. plumosus* group before the revision. [1]papers with given version of the mapping are shown in parenthesis; [2]it was said in the paper that banding sequence agi2B1 and agiB2 are identical but the mapping of agi2B1 given in the paper differs from that of agiB2, published previously; [3]it was said in the paper that banding sequence useB1 is identical to pluB2 but the mapping of useB1 given in the paper differs from that of pluB2, published by other authors.

Designation of BS	Mapping of banding sequence
p'agiB1	25 18-15 25-22 18-22 14-12 (Shobanov, Djomin, 1988)[1] 25 17-15c 22 24 23 18-21 15b-12 (Kerkis et al., 1989)
p'agiB2	25 17-16 21-18 23-24 22 15-12 (Kerkis et al., 1989)
p'agi2B1	25 18-16 21-18 22-25 15-12[2] (Kiknadze et al., 1991a)
p'balB1	25c 21b-15c 22c-21c 23-25b 15b-12 (Kiknadze et al., 1991d)
h'bonB1	25s-12v (Kerkis et al., 1989)
p'borB1	25-23 15c 21-16 22 15b-12 (Kerkis et al., 1988; Kiknadze et al., 1991d)
h'borB2	25-23 15c-22 15b-12 (Kerkis et al., 1988; Kiknadze et al., 1991d)
h'entB1	25s-e 15r-g 23f-25d 16a-23e 15f-12v C (Golygina, 1999)
h'murB1	25 23-24 15c-22 15b-12 (Kiknadze, Kerkis, 1986)
p'murB3	25 23a 15ab 22-15c 24-23b 14-12 (Kiknadze et al., 1991b)
p'nudB1	25d-b 21-15c 24-23 25 22 15b-12 (Kiknadze et al., 1991d)
h'nudB3	25 23-24 15c-22 15b-12 (Kiknadze et al., 1987)
p'nudB4	25 15ab 22-15c 24-23 14-12 (Kiknadze et al., 1987)
h'pluB1	25s-a 24s-a 23z-a 22u-a 21t-a 20l-a 19p-a 18o-a 17m-a 16m-a 15r-a 14r-a 13z-a 12y-v C (Shobanov, 1994)
h'pluB2	25s-23f 15g-23e 15f-12v C (Shobanov, 1994; Golygina, 1999; Butler et al., 1999; Golygina, Kiknadze, 2001)
p'sinB1	not mapped, differs from **h'pluB2** by simple inversion (Kiknadze et al., 2005)
p'spJB1	25 21-15c 24-22 15b-12 (Кикнадзе и др., 1991d)
p'spKB1	25-23 15c 21-16 22 15b-12 (mapping not published)
p'suwB1	25-23 15c 21-16 22 15b-12 (Golygina et al., 2003; Kiknadze et al., 2004)
p'useB1	25-23 15c-22 15b-12[3] (Loginova, Beljanina, 1994)

tis only (Shobanov, 1994a; Golygina, 1999; Golygina, Kiknadze, 2001). Mapping that was published so far is shown in Table 3. Phylogenetic relationship of banding sequences based on this mapping is shown in Fig. 1, c.

The comparison of main and alternative banding sequences in arm B of species of *Ch. plumosus* group resulted in phylogram presented in Fig. 1, d. As could be seen, the central part of the phylogram occupied by h'pluB2 and identical to it h'borB2 and h'useB1. It should be especially noted that when N.A. Shobanov made the revision of *Ch. plumosus* banding sequences he suggested different mapping of h'pluB2 then it was accepted earlier and we agree with his version. As h'pluB2 is in fact fundamental for the mapping of other banding sequences in these group, it means that all the banding sequences of arm B (except h'pluB1 and identical h'bonB1) require a revision.

As could be seen in Fig. 1, d, four groups of banding sequences originated from h'pluB2 by simple inversions. Two of these groups are the source of all other banding sequences of

Ch. plumosus siblings.

1. The revision of arm B mapping of *Ch. borokensis*, *Ch. suwai* and *Chironomus* sp. K.

Between two *Ch. borokensis* banding sequences considered in this paper only p'borB1 require a revision. It originated from h'borB2=h'pluB2, which mapping was changed during the revision of *Ch. plumosus* banding sequences (Shobanov, 1994a), therefore it was necessary to correct the mapping of p'borB1 and identical banding sequences p'suwB1 and p'spKB1 (Fig. 1, d). New mapping is shown in Table 4 and on Figure 5, d.

2. The revision of arm B mapping of *Ch. agilis*, *Chironomus* sp. prope *agilis* and *Ch. balatonicus*.

Earlier all banding sequences of these species were derived from the p'pluB1 directly as it is the standard banding sequence in *Ch. plumosus* group, however, comparative analysis of these sequences had shown that they derived from the p'borB1. The fact is that h'pluB1 and p'borB1 are very similar in banding orders but differs by included inversion. As a result, in p'borB1 bands 15g-r are places in distal part of the arm between regions 23z-f and 21, and bands 22a-23e are situated between regions 16 and 15f-a in the proximal part of the arm (Fig. 5, d). We performed the analysis of banding sequences of *Ch. balatonicus*, *Ch. agilis*, and *Chironomus* sp. prope agilis and found out that above-mentioned regions could be found in these species in the same neighbourhood as in p'borB1 (Fig. 4, d).

Banding sequence p'balB1 (Fig. 5, c) differs from p'borB1 by two inversion steps.

Banding sequences in arm B of *Ch. agilis* and *Chironomus* sp. prope agilis are most difficult for mapping as they differ from the closest p'borB1 by several inversion steps and some breakpoints of these inversions are located near the telomere or Balbiany Ring where the banding structure is usually very unclear. The closest to p'borB1 is p'agiB1, which differs from it by 3 inversion steps (Table 4, Fig. 5, a).

Banding sequences p'agiB2 and p'agi2B1 are identical and originate from the p'agiB1 by simple inversion (Table 4, Fig. 5, b).

3. The revision of arm B mapping of *Ch. entis*, *Ch. muratensis*, *Ch. nudiventris*, and *Chironomus* sp. J.

Comparison of main and alternative banding sequences of these species had shown that they could be grouped into 3 blocks of identical banding sequences (Fig. 1, d). As was mentioned in earlier publications, h'entB1, h'murB1 and h'nudB1 are identical (Fig. 1, c). Mapping of h'entB1 according to Maximova-Shobanov system was suggested by Golygina (1999) and does not need further revision. This last version differs from mapping published previously by the mapping of inversion which differ h'entB1 from h'pluB2: it was considered previously that h'entB1 differs from h'pluB2 by the inversion of regions 23-24 but the analysis had shown that the real inversion is larger and include part of the region 25 and the region 15g-r. This version of mapping is shown in Fig. 5, f by the example of h'murB1.

According to the data published previously, breakpoints of simple inversions that differ banding sequences p'murB3 and p'nudB4 from h'entB1=murB1=nudB3 are slightly different, however, comparison of these banding sequences to each other had shown that they are identical. Thus p'murB3 and p'nudB4 have banding sequences that are shown in Table 4 and in Fig. 5, h.

Identical banding sequences p'nudB1 and p'spJB1 also can be derived from h'entB1=h'nudB3 by one simple inversion (Table 4, Fig. 5, g).

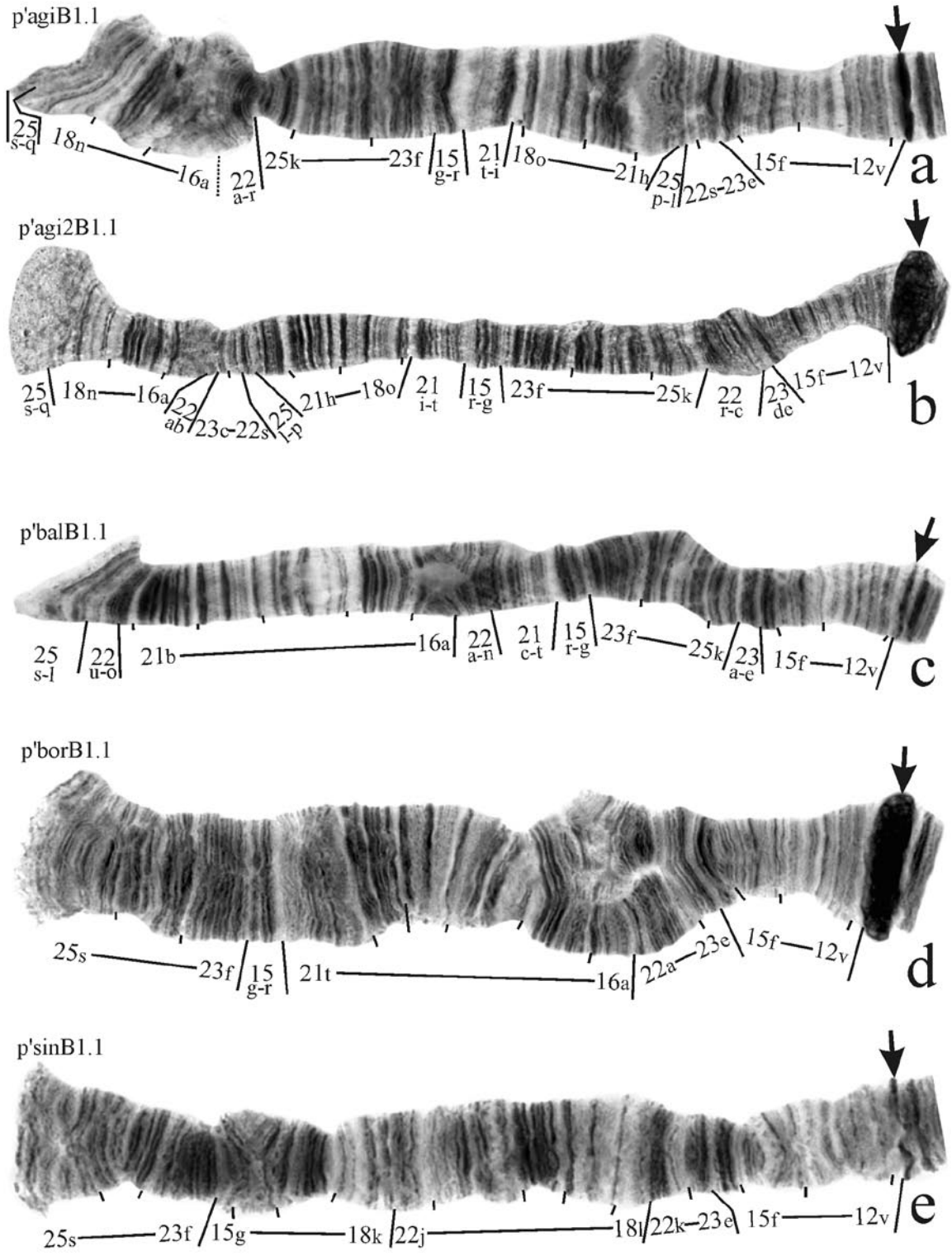


Fig. 5, a-e. Mapping of banding sequences of *Ch. plumosus* sibling species in arm B. **a -** p'agiB2.2. **b -** p'agi2B1.1. **c -** p'balB1.1. **d -** p'borB1.1. **e -** p'sinB1.1.

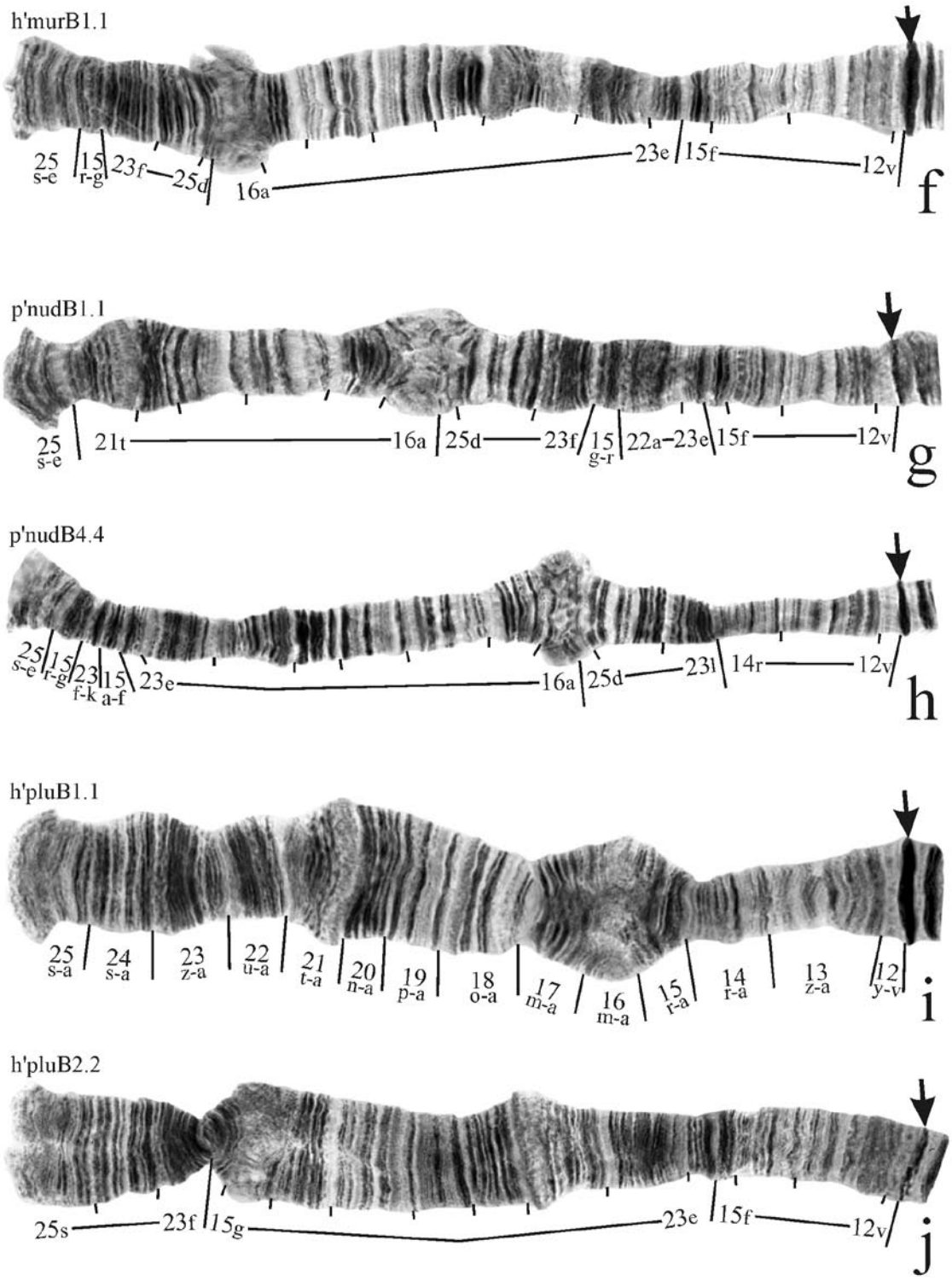


Fig. 5, f-j. Mapping of banding sequences of *Ch. plumosus* sibling species in arm B. **f -** h'murB1.1. **g -** p'nudB1.1. **h -** p'nudB4.4. **i -** h'pluB1.1. **j -** h'pluB2.2. Centromeric bands designated by arrows.

Table 4. Mapping of arm B main and alternative banding sequences (BS) in *Ch. plumosus* group after the revision.

Designation of BS	Mapping of banding sequence
p'agiB1	25s-q 18n-16a 22a-r 25k-23f 15g-r 21t-i 18o-21h 25p-l 22s-23e 15f-12v C
p'agiB2	25s-q 18n-16a 22ab 23c-22s 25l-p 21h-18o 21i-t 15r-g 23f-25k 22r-c 23de 15f-12v C
p'agi2B1	**=p'agiB2**
p'balB1	25s-l 22u-o 21b-16a 22a-n 21c-t 15r-g 23f-25k 23a-e 15f-12v C
h'bonB1	**=h'pluB1**
p'borB1	25s-23f 15g-r 21t-16a 22a-23e 15f-12v C
h'borB2	**=h'pluB2**
h'entB1	25s-e 15r-g 23f-25d 16a-23e 15f-12v C
h'murB1	**=h'entB1**
p'murB3	25s-e 15r-g 23f-k 15a-f 23e-16a 25d-23l 14r-12v C
p'nudB1	25s-e 21t-16a 25d-23f 15g-r 22a-23e 15f-12v C
h'nudB3	**=h'entB1**
p'nudB4	**=p'murB3**
h'pluB1	25s-a 24s-a 23z-a 22u-a 21t-a 20n-a 19p-a 18o-a 17m-a 16m-a 15r-a 14r-a 13z-a 12y-v C
h'pluB2	25s-23f 15g-23e 15f-12v C
p'sinB1	25s-23f 15g-18k 22j-18l 22k-23e 15f-12v C
p'spKB1	**=p'borB1**
p'spJB1	**=p'nudB1**
p'suwB1	**=p'borB1**
h'useB1	**=h'pluB2**

4. Mapping of p'sinB1.

Banding sequence p'sinB1 originated from h'pluB2 and differs from it in simple paracentric inversion (Table 4, Fig. 5, e).

Discussion

Our revision of banding sequences in chromosome AB of *Ch. plumosus* sibling species made considerable change to the conception of banding sequence evolution in the group. Comparative analysis of banding sequences in arm A had shown that their phylogenetic relationships are more complex than it was presumed previously. It was considered before that the most ancient banding sequence in the *Ch. plumosus* group is p'pluA1 (and other identical to it banding sequences) as it was the closest to the pigA1 (Fig. 1, a). Another 6 banding sequences were considered identical to h'pluA2. However, our data indicate that there is a greater degree of divergence between banding sequences in arm A. Thus, half of the species have microinversion in regions 14-15 and because of this banding sequences which were grouped into 2 large clusters now divided into 4 clusters with 3-4 banding sequences in each (Fig. 1, b). The most ancient banding sequence should be considered p'agiA1 and identical to its banding sequences of *Chironomus* sp. prope agilis, *Ch. entis*, and *Chi-*

ronomus sp. K. Banging sequences identical to p'pluA1 or h'entA4 could be derived from p'agiA1 by simple inversions (Fig. 1, b), and combination of these two inversions in one sequence lead to origination of h'pluA2 that, supposedly, happened due to crossing-over. It can be noted that most fruitful was banding sequences of h'entA4 cluster, which gave existence to another 4 banding sequences.

In general analysis of phylogeny of banding sequences in arm A had shown that the degree of divergence in this arm is rather low as most of the main and alternative banding sequences are not species specific (Fig. 1, b). Actually only arm A of *Ch. balatonicus* and *Ch. muratensis* diverged completely since these species have no banding sequences that are identical to banding sequences of other species.

Until now the phylogenetic relationship between banding sequences in arm B could not be freely analysed, as it was impossible to map banding sequences precisely. Moreover, as in most cases comparison of banding sequences of newly described species was done only with h'pluB1 or h'pluB2 of standard in Maximova-Shobanov species *Ch. plumosus*, it made an impression that they are ancestral to other sequences in the group (Fig. 1, c).

Our analysis of arm B banding sequences had shown that h'pluB1 in fact is a lateral branch whereas the central and, apparently, most ancient are h'pluB2 and p'borB1, each of them originate banding sequences of several species of the group (Fig. 1, d). Thus, p'borB1 and not h'pluB1 originates banding sequences of *Ch. balatonicus*, *Ch. agilis* and *Chironomus* sp. prope agilis. It could be also noted that the degree of divergence of banding sequences in the arm B of *Ch. entis*, *Ch. muratensis*, *Ch. nudiventris* and *Chironomus* sp. J appeared to be a little bit lower than was assumed previously, as p'murB3 and p'nudB4 that were considered species specific are, in fact, identical.

In general, the level of divergence in both arms is rather similar and only slightly higher in arm B. Thus, the number of species with identical banding sequence decreased in arm B in comparison to arm A (from 4 in arm A to 3 in arm B), also there are sequences in arm B that originated by complex inversions whereas all sequences in arm A differs from their ancestors by simple inversion. Yet, like in arm A, only 2 species have completely divergent arm B: banding patterns of *Ch. balatonicus* and *Ch. sinicus* are species-specific (Fig. 1, d). In case of all other species either main or alternative banding sequences could be found in banding sequence pools of one or several other species of the group.

In conclusion we would like to note that the revision of mapping of banding sequences in chromosome AB allowed us to significantly improve the accuracy of banding sequences and lead to qualitative modification of their phylogeny. We analysed banding sequences in other chromosomal arms and came to a conclusion that it is necessary to revise mapping of banding sequences in all arms, especially in arm F. These data will be published in forthcoming papers whereupon it will be possible to make thorough analysis of chromosomal evolution in *Ch. plumosus* group.

Acknowledgements

Financial support was provided by the grants from the Presidium of Russian Academy of Sciences: "Biodiversity and dynamics of gene pools" and "Origin and evolution of biosphere".

References

Butler M.G., Kiknadze I.I., Golygina V.V., Martin J., Istomina A.G., Wülker W.F., Sublette J.E., Sublette M.F. 1999. Cytogenetic differentiation between Palearctic and Nearctic populations of *Chironomus plumosus* L. (Diptera, Chironomidae) // *Genome*. 42(5): 797-815.

Dévai Gy., Wülker W., Scholl A. 1983. Revision der Gattung *Chironomus* Meigen (Diptera). IX. *Ch. balatonicus* sp. n. aus dem Flachsee Balaton (Ungarn) // *Acta Zool. Acad. Sci. Hungarica.* 29(4): 357-374.

Dévai Gy., Miskolczi M., Wülker W. 1989. Standartization of chromosome arms B, C and D in *Chironomus* (Diptera: Chironomidae) // *Acta Biol. Debr. Oecol. Hungarica*. 2(1): 79-92.

Dyomin S.Yu., Schobanov N.A. 1990. Karyotype of *Chironomus entis* Shobanov from the *plumosus* group (Diptera, Chironomidae) living in the European part of the Soviet Union // *Tsitologiya*. 32(10): 1046-1054. (In Russian).

Golygina V.V. 1999. Divergence of karyotypes of Holarctic *Chironomus* species from *Ch. plumosus* group in Paleartic and Nearctic (Diptera, Chironomidae). Ph.D. Dissertation, Institute of Cytology and Genetics, Russian Academy of Sciences. Novosibirsk. 132 p. (In Russian).

Golygina V.V., Istomina A.G., Rakisheva A.Zh., Kiknadze I.I. 1996. New banding sequences in the *Chironomus balatonicus* karyofund (banding sequence pool) // *Tsitologiya*. 38(8): 869-883.

Golygina, V.V., Kiknadze I.I. 2001. Karyofund (banding species pool) of *Chironomus plumosus* (Diptera, Chironomidae) in Palearctic // *Tsitologiya*. 43(5): 507-519. (In Russian).

Golygina V.V., Martin J., Kiknadze I.I., Siirin M., Ivanchenko O.V., Makarchenko E.A. 2003. *Chironomus suwai*, a new species of the *plumosus* group (Diptera, Chironomidae) from Japan // *Aquatic Insects*. 25(3): 177-189.

Golygina V.V., Ueno R. 2005. *Chironomus* sp. n. K – a new member of *plumosus*-group from Japan (p. 39) // *Abstr. the 3rd Intern. Symp. Aquatic Entomol. East Asia (AESEA). June 17-20, 2005, Nankai University, China.* Tianjin. 72 p.

Golygina V.V., Kiknadze I.I., Istomina A.G., Gunderina L.I., Miroshnichenko L.A., Gusev V.D. 2007. Cytogenetic divergence of genomes in *Chironomus plumosus* group (Diptera: Chironomidae) // *Comp. Cytogenet*. 1(1): 17-32.

Kerkis I.E., Filippova M.A., Shobanov N.A., Gunderina L.I., Kiknadze I.I. 1988. Karyological and genetico-biochemical characteristics of *Chironomus borokensis* sp. n. from the *plumosus* group (Diptera, Chironomidae) // *Tsitologiya*. 30(11): 1364-1372. (In Russian).

Kerkis I.E., Kiknadze I.I., Istomina A.G. 1989. A comparative karyotype analysis of three *Chironomus* sibling species of the *plumosus* group (Diptera, Chironomidae) // *Tsitologiya*. 31(6): 713-720. (In Russian).

Keyl H.-G. 1962. Chromosomenevolution bei *Chironomus*. II. Chromosomenumbauten und phylogenetische Beziehungen der Arten // *Chromosoma*. 13(4): 464-514.

Kiknadze I.I., Kerkis I.E. 1986. Comparative analysis of the polytene chromosome banding patterns in sibling-species *Chironomus balatonicus* and *Ch. muratensis* // *Tsitologiya*. 28(4): 430-436. (In Russian).

Kiknadze I.I., Kerkis I.E., Ruzanova A.I. 1987. Chromosomal polymorphism of *Chironomus nudiventris* Ryser, Scholl et Wülker (Diptera, Chironomidae) // *Tsitologiya*. 28(10): 1161-1168. (In Russian).

Kiknadze I.I., Siirin M.T., Filippova M.A., Gunderina L.I., Kalachikov S.M. 1991a. The change of the pericentromeric heterochromatin mass is one of important ways of the chironomid evolution // *Tsitologiya*. 33(12): 90-98. (In Russian).

Kiknadze I.I., Filippova M.A., Izvekova E.I. 1991b. Karyological characteristics of the *plumosus* sibling species-group in Mozhaiskoe reservoir // *Sibirskiy Biol. Zh.* 6: 9-18. (In Russian).

Kiknadze I.I., Filippova M.A., Siirin M.T., Kerkis I.E. 1991c. Chromosomal polymorphism in *Chironomus entis* (Diptera, Chironomidae) // *Zool. Zh.* 70(8): 106-120. (In Russian).

Kiknadze I.I., Shilova A.I., Kerkis I.E., Shobanov N.A., Zelentzov N.I., Grebenjuk L.P., Istomina A.G., Prasolov V.A. 1991d. Karyotypes and morphology of larvae in the tribe Chironomini. Novosibirsk. 113 p. (In Russian).

Kiknadze I.I., Golygina V.V., Istomina A.G. 1996a. The mapping of chromosomal arms C and D in the midge *Chironomus balatonicus* // *Tsitologiya*. 38(7): 674-680. (In Russian).

Kiknadze I.I., Istomina A.G., Gunderina L.I., Aimanova K.G., Salova T.A., Savvinov D.D. 1996b. Chironomid karyofunds (banding species pools) of Yakutian cryolitozone (permafrost). Novosibirsk. 166 p. (In Russian).

Kiknadze I.I., Butler M.G. Golygina V.V., Martin J., Wülker W.F., Sublette J.E., Sublette M.F. 2000. Intercontinental karyotypic differentiation of *Chironomus entis* Shobanov, a Holarctic member of the *C. plumosus* group (Diptera, Chironomidae) // *Genome.* 43(5): 857-873.

Kiknadze I.I., Golygina V.V., Istomina A.G., Gunderina L.I. 2004. Pattern of chromosomal polymor-

phism during population and species divergence in *Chironomus* (Diptera, Chironomidae) // *Sibirskiy Ecol. Zh.* 11(5): 635-652. (In Russian).

Kiknadze I.I., Wang X., Istomina A.G., Gunderina L.I. 2005. A new *Chironomus* species of the *plumosus*-group (Diptera, Chironomidae) from China // *Aquatic Insects*. 27(3): 199-211.

Loginova N.V., Belyanina S.I. 1994. A new species of the genus *Chironomus* from the group *plumosus* - *Chironomus usenicus* sp. n. (Diptera, Chironomidae) // *Zool. Zh.* 73(11): 93-105. (In Russian).

Maximova F.L. 1976. The karyotype of *Chironomus plumosus* from the Ust'-Izhora wild population of Leningrad region // *Tsitologiya*. 18: 1264-1269. (In Russian).

Ryser H.M., Scholl A., Wülker W. 1983. Revision der Gattung *Chironomus* Meigen (Diptera). VII. *Ch. muratensis* n. sp. und *Ch. nudiventris* n. sp., Geschvisterarten aus der *plumosus*-Gruppe // *Rev. Suisse Zool.* 90(2): 299-316.

Shobanov N.A. 1994a. Karyofund of *Chironomus plumosus* (L.) (Diptera, Chironomidae). I. Standartization of bands according to the Maximova system // *Tsitologiya*. 36(1): 117-122. (In Russian).

Shobanov N.A. 1994b. Karyofund of *Chironomus plumosus* (L.) (Diptera, Chironomidae). II. Band patterns of chromosomal arms // *Tsitologiya.* 36(1): 123-128. (In Russian).

Shobanov N.A., Djomin S.Yu. 1988. *Chironomus agilis* – new species of group *plumosus* (Diptera, Chironomidae) // *Zool. Zh.* 67(10): 1489-1497. (In Russian).

Received March 10, 2008.
Accepted by I.A. Gavrilov, May 25, 2008.
Published June 30, 2008.