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Populational and Karyological Analysis of the Phytophilous Chironomid *Endochironomus tendens* F. (Diptera, Chironomidae). 1. New Chromosomal Sequences in the Species' Karyotype Pool

A. A. Ogledneva[a],* and N. A. Durnova[a]

[a] *V.I. Razumovsky Saratov State Medical University, Saratov, 410012 Russia*

**e-mail: cucurbita25@gmail.com*

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Abstract—A karyological analysis of the phytophilous chironomids *Endochironomus tendens* F. (Diptera, Chironomidae) from six water bodies of Saratov and Volgograd provinces has been carried out. The karyotype of *E. tendens* has 2*n* = 6, with morphologically well-defined centromeric zones represented by large heterochromatin blocks. In chromosome I (EF), arm E is characterized by five banding sequences: *tend*E1, *tend*E2, *tend*E3, *tend*E4, and *tend*E5; the band order for sequence *tend*E5 is described for the first time. Arm F comprises three previously described sequences: *tend*F1, *tend*F3, and *tend*F4. In chromosome II (CDG), sequence *tend*C1 occurs in most of the studied larvae, while *tend*C2 and *tend*C3 are rare sequences. Three previously described sequences, *tend*(DG)1, *tend*(DG)2, and *tend*(DG)3, occur in arm DG; the band order of the previously described sequence *tend*(DG)4 is clarified. Two new sequences, *tend*(DG)5 and *tend*(DG)6, are described for the first time. In chromosome III (AB), arm A comprises four known sequences: *tend*A1, *tend*A2, *tend*A3, and *tend*A4. Arm B comprises only two sequences: *tend*B1 and *tend*B2. In total, 24 chromosome banding sequences have been discovered in the studied *E. tendens* populations.

Keywords: polytene chromosome, chromosomal sequence, *Endochironomus tendens*

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The role of chromosomal polymorphism in microevolution of chironomid midges (Diptera, Chironomidae) has been repeatedly considered in members of the genera *Chironomus* and *Camptochironomus* with benthic larvae. In particular, the effect of intercontinental isolation on cytogenetic divergence was demonstrated for the natural populations of these species (Gunderina et al., 1996; Kiknadze et al., 1996, 2006), and a correlation was revealed between chromosomal variability and certain living conditions of their larvae (Shobanov, 1994a, 1994b; Petrova et al., 2000). Studying chromosomal polymorphism in phytophilous chironomids, in particular the members of *Endochironomus*, *Glyptotendipes*, *Phaenopsectra*, and some other genera is important since the zone of periaquatic vegetation provides a unique diversity of ecological niches; therefore, colonization of different macrophytes by chironomid larvae even within the same water body may be related to changes in the spectrum and incidence of inversion sequences.

Endochironomus tendens (Fabricius, 1794) is a phytophilous chironomid species which may serve as a good model for studying interpopulation chromosomal polymorphism. First, as shown earlier, in Saratov Province the larvae of this species are the most common miners of macrophytes, well adapted to development in both living and decomposing parts of various plants (Durnova et al., 2011, 2012). Second, the reference photomap of its polytene chromosomes has been previously created, and data on the inversion polymorphism in its populations have been obtained. The karyotype of *E. tendens* was originally described from Bulgaria and Hungary (Michailova and Gercheva, 1982); two cytotypes (I, II) were distinguished in its populations, and a high level of chromosomal polymorphism due to homo- and heterozygous inversions was observed (Michailova and Gercheva, 1982; Michailova, 1989). Later, the available data on the karyotype variation of *E. tendens* in different geographic zones were summarized (Belyanina, 1978, 1983; Michailova and Gercheva, 1982; Michailova, 1987, 1989, 1992), a more detailed photomap of its polytene chromosomes was proposed, and 22 banding sequences were described in the larval karyotypes of the species from the territory of Russia (Durnova, 2009a).

The populations of *E. tendens* from Bulgaria and Hungary (Michailova, 1992) revealed a certain correlation between some homozygous inversions and the occurrence of larvae in different plant species: the larvae of cytotype I preferred mining the leaves and stems of *Typha latifolia* L., while those of cytotype II more often mined the tissues of *Trapa natans* L. and very rarely developed in *T. latifolia*. A detailed description of the banding sequences in the karyotype pool of *E. tendens*, corresponding to the reference photomap, is needed for studying the composition of inversions in different populations and for analyzing the chromosomal polymorphism of the species in relation to the occurrence of its larvae in different macrophytes.

The objective of this work was to study the karyotype and to describe the chromosome banding sequences and their combinations in *E. tendens* larvae collected in different water bodies of Saratov and Volgograd provinces.

MATERIALS AND METHODS

Our material comprised larvae from 22 samples collected in the summer seasons of 2012–2015 in several water bodies of Saratov and Volgograd provinces of Russia (Table 1). Karyotypes were studied in the IV instar larvae collected from partly decomposed leaves of *Butomus umbellatus* L., *Sparganum erectum* L., *Typha angustifolia* L., *Sagittaria sagittifolia* L., and *Stratiotes aloides* L. Altogether, 673 individuals were collected and studied.

The larvae were identified by morphological characters (Makarchenko and Makarchenko, 2006). Preparations of polytene chromosomes from salivary gland cells were made using the ethyl-orcein method (Demin and Shobanov, 1990) and examined using an Axioskop2 Plus microscope, an AxioCamHRc CCD camera, and the AxioVision 4 software (Zeiss, Germany), at 10 × 20; 10 × 40, and 10 × 100 magnifications. The images were processed in Adobe Photoshop CS6 and Corel Draw 13.

The banding sequences were described and named according to the reference photomap for this species (Durnova, 2009a).

RESULTS

The karyotype of *E. tendens* ($2n = 6$) is characterized by the following chromosome arm combination: I (EF), II (CDG), III (AB) (Michailova and Gercheva, 1982; Michailova, 1987, 1989). The centromeric zones are morphologically well defined and represented by large heterochromatin blocks.

Chromosome I (EF). Five banding sequences were previously described in arm E: *tend*E1, *tend*E2, *tend*E3, *tend*E4, and *tend*E5. Sequence *tend*E5 was earlier found only in the heterozygous state *tend*E1.5 and therefore remained unmapped (Durnova, 2009a). We found this inversion in the larvae mining *B. umbellatus* in the Tereshka River (Table 1), both in the homogygous state *tend*E5.5 (Fig. 1, *a*) and in the heterozygous one *tend*E4.5 (Fig. 1, *b*). Sequence *tend*E5 probably originated from *tend*E4 and differs from the latter in a simple inversion in the segment comprising regions 14–17a–d (since the break occurred within region 17, this region was additionally divided into subregions a–e):

*tend*E4: 1 2 3 4 5 6 7 8 9 10 11 12 13 $\underline{\text{14 15 16 17abcd}}$ e

*tend*E5: 1 2 3 4 5 6 7 8 9 10 11 12 13 $\underline{\underline{\text{17dcba 16 15 14}}}$ 17e

The combination *tend*E1.5 of two sequences, E1 and E5, was described for the first time in the studied populations. The conjugated homologs formed a complex double loop (Fig. 1, *c*).

Of the four known banding sequences of arm F (Durnova, 2009a), we found three previously described sequences *tend*F1, *tend*F3, and *tend*F4 and their combinations: *tend*F1.3, *tend*F1.4, *tend*F3.3, and *tend*F4.4.

Chromosome II (CDG). Arm C in most of the studied larvae contained the sequence combination *tend*C1.1, while *tend*C1.2 and *tend*C3.3 were rare combinations; they were described by us for the first time. The nucleolar organizer region was located in region 10 of arm C (Fig. 2; Fig. 3). A characteristic feature of arm DG was the varying level of activity of BR_1, BR_2, and p regions, depending on the inversions affecting different parts of this arm. All the four sequences that have been described previously for arm DG, namely *tend*(DG)1, *tend*(DG)2,

Table 1. Localities and dates of collection of *Endochironomus tendens* F. larvae

No.	Substrate	Collection locality	Number of ind. collected	Collection date
1	*Sparganum erectum* L.	Volgograd Province, Danilovskii District, Danilovka, Medveditsa River	30	21.VII.13
2	*Sparganum erectum* L.	Volgograd Province, Frolovskii District, Frolovo, Medveditsa River	11	21.VII.13
3	*Sparganum erectum* L.	Saratov Province, Rtishchevskii District, Potma, Khoper River	64	22.VII.13
4	*Sparganum erectum* L.	Saratov Province, Balashovskii District, Repnoe, Khoper River	27	17.VIII.12
5	*Sparganum erectum* L.	Saratov Province, Lysogorskii District, Lysye Gory, Medveditsa River	13	20.VII.13
6	*Butomus umbellatus* L.	Saratov Province, Khvalynskii District, Kulatka, Tereshka River	27	18.VII.13
7	*Butomus umbellatus* L.	Saratov Province, Lysogorskii District, Lysye Gory, Medveditsa River	53	29.VII.12
8	*Sparganum erectum* L.	Saratov Province, Arkadakskii District, env. of Arkadak, Khoper River	61	22.VII.13
9	*Typha angustifolia* L.	Saratov Province, Lysogorskii District, Lysye Gory, Medveditsa River	50	29.VII.12
10	*Sparganum erectum* L.	Saratov Province, env. of Chardym, Volga River	28	29.VII.13
11	*Sagittaria sagittifolia* L.	Saratov Province, Atkarskii District, Prirechnoe, Medveditsa River	37	15.VIII.12
12	*Butomus umbellatus* L.	Saratov Province, Atkarskii District, Prirechnoe, Medveditsa River	15	15.VIII.12
13	*Sparganum erectum* L.	Rostov Province, Khmelnitskoe, Don River	72	14.IX.12
14	*Butomus umbellatus* L.	Volgograd Province, env. of Khmelnitskoe, Don River	8	15.IX.12
15	*Butomus umbellatus* L.	Saratov Province, Balashovskii District, env. of Almazovo, Khoper River	9	22.VII.13
16	*Butomus umbellatus* L.	Saratov Province, Voskresenskii District, Chardym, branch of Volga River	6	1.VIII.13
17	*Sparganum erectum* L.	Saratov Province, Engelsskii District, Shumeika, Saratovka River	35	31.VIII.14
18	*Sparganum erectum* L.	Saratov Province, Engelsskii District, Shumeika, Saratovka River	59	3.VIII.15
19	*Typha angustifolia* L.	Saratov Province, Engelsskii District, Shumeika, Volga River	27	29.VIII.14
20	*Sagittaria sagittifolia* L.	Saratov Province, Engelsskii District, Shumeika, Saratovka River	23	3.VIII.15
21	*Sagittaria sagittifolia* L.	Saratov Province, Lysogorskii District, Lysye Gory, Medveditsa River	12	27.VII.15
22	*Stratiotes aloides* L.	Saratov Province, Lysogorskii District, Nevezhkino, Medveditsa River	6	12.VIII.13

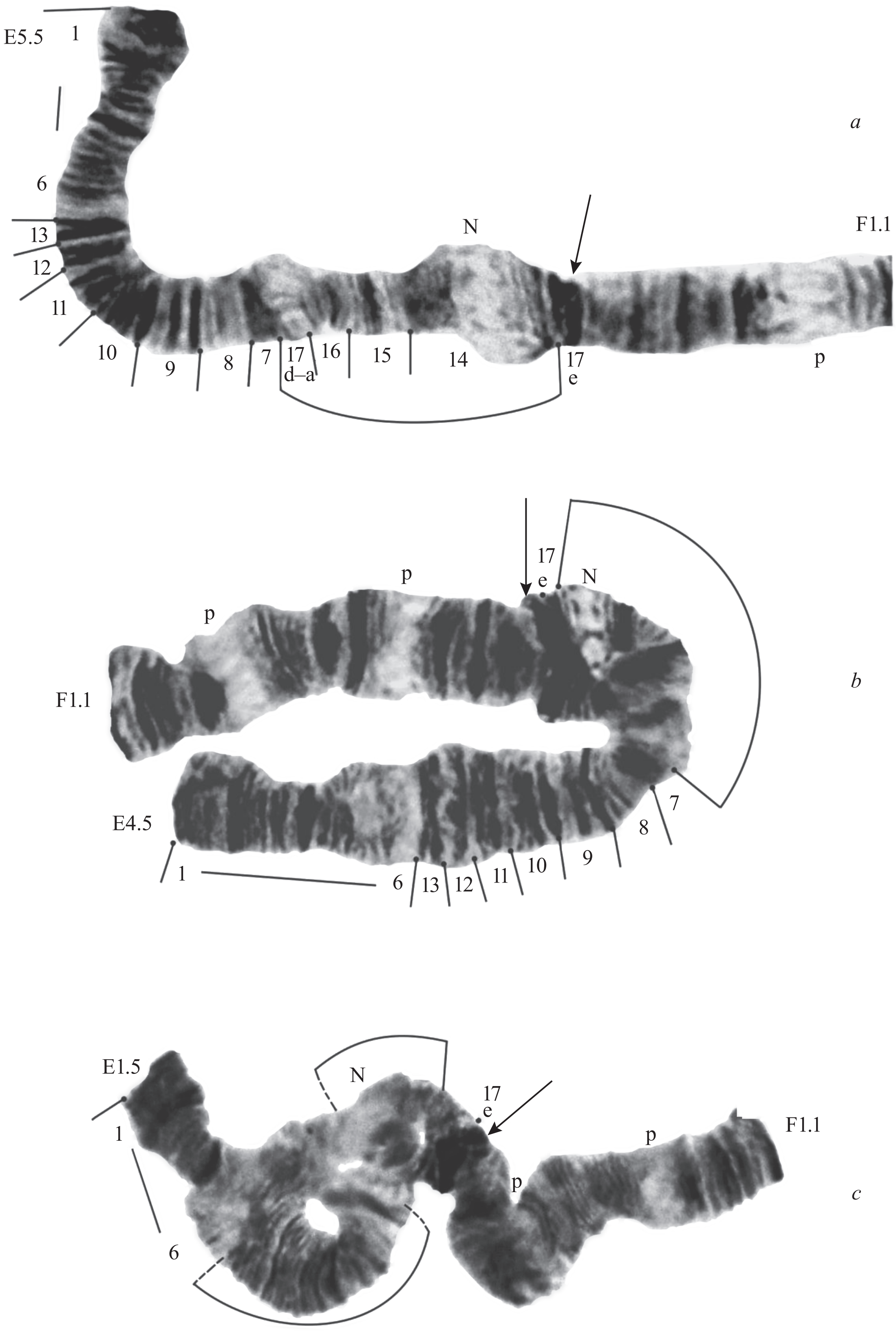

Fig. 1. Homo- and heterozygous inversions in chromosome I (EF) of *E. tendens*: (*a*) sequence combination E5.5F1.1; (*b*) E4.5F1.11; (*c*) E1.5F1.1; N, nucleolus; BR, Balbiani ring; p, puff; arrows mark the centromeres.

tend(DG)3, and *tend*(DG)4 (Durnova, 2009a), were recorded in our material in different combinations. Banding sequence *tend*(DG)4 was recorded for the first time in the homozygous state in the population developing in *B. umbellatus* (Fig. 2, *b*); the previous mapping of this sequence should be regarded as preliminary, because this sequence was earlier found only in the heterozygous states *tend*(DG)1.4 and *tend*(DG)2.4 (Durnova, 2009a). Sequence *tend*(DG)4 probably originated from *tend*(DG)2 as the result of a simple inversion of region 16–20 (since the break occurred within region 16, this region was additionally divided into subregions a–e):

tend(DG)2: 11 12 13 14 15 16abced 17 18 26 25 24 23 22 21 20 19 27

tend(DG)4: 11 12 13 14 15 16abc 20 21 22 23 24 25 26 18 17 16de 19 27

This sequence was found by us not only in the homozygous state *tend*(DG)4.4 (Fig. 2, *b*), but also in various heterozygous combinations: *tend*(DG)1.4 (Fig. 3, *a*), *tend*(DG)2.4 (Fig. 2, *c*), *tend*(DG)4.5 (Fig. 3, *b*), and *tend*(DG)4.6 (Fig. 3, *c*).

Sequence *tend*(DG)5 was found by us for the first time. It probably originated from *tend*(DG)4 as the result of a simple inversion of region 23-16ed:

tend(DG)4: 11 12 13 14 15 16abc 20 21 22 23 24 25 26 18 17 16ed 19 27

tend(DG)5: 11 12 13 14 15 16abc 20 21 22 16de 17 18 26 25 24 23 19 27

Sequence *tend*(DG)6 was found for the first time and only in the heterozygous state *tend*(DG)4.6 (Fig. 3, *c*). It probably originated from *tend*(DG)4 as the result of an inversion of region 12b-25a:

tend(DG)4: 11 12ab 13 14 15 16abc 20 21 22 23 24 25abc 26 18 17 16ed 19 27

tend(BG)6: 11 12a 25a 24 23 22 21 20 16cba 15 14 13 12b 25bc 26 18 17 16ed 19 27

Combination *tend*DG3.4 of two inversion sequences was described for the first time from the studied populations (Fig. 4, *a*–*c*). These sequences originated by inversions in different parts of sequence *tend*(DG)2. When the two inversions co-occurred in the chromosome, the homologs formed a complex heterozygous inversion; the conjugated segments are marked with brackets in Fig. 4, *a*, *b*.

Chromosome III (AB). Four sequences were previously described in arm A: *tend*A1, *tend*A2, *tend*A3, and *tend*A4 (Durnova, 2009a). The populations studied by us did not reveal any new sequences; *tend*A2 and *tend*A3 were found both in homogygous states and in heterozygous ones: *tend*A2.2, *tend*A2.3, and *tend*A3.3. A new combination *tend*A1.3 was recorded in the population inhabiting *B. umbellatus* in the Tereshka River (Fig. 5).

Arm B in the studied populations included only two previously described sequences *tend*B1 and *tend*B2 (Durnova, 2009a) and the following combinations: *tend*B1.1, *tend*B1.2, and *tend*B2.2.

DISCUSSION

The level of chromosomal polymorphism varies greatly between chironomid species and depends on different factors. For instance, chromosomal transformations are almost completely absent in some narrowly specialized species, such as *Xenochironomus xenolabis* (Kieffer, 1916) and *Demeijerea rufipes* (Linnaeus, 1761) developing as miners of sponges, and also *Stenochironomus gibbus* (Fabricius, 1794) inhabiting the bast layer of submerged tree trunks and branches; this fact may be related to the relatively stable living conditions of these species (Durnova, 2009b, 2010a, 2010b).

Comparative analysis of karyotype pools in the species with a high level of chromosomal polymorphism (members of the genera *Chironomus*, *Camptochironomus*, and *Glyptotendipes*) is difficult due to the non-uniform level of knowledge of these species, in particular, the number of the populations examined. To date, the highest level of chromosomal polymorphism (estimated only by the number of chromosome banding sequences) has been observed in the benthic species *Ch. balatonicus* (Devai et al., 1983) and *Ch. plumosus* (Linnaeus, 1758), with 60 and 54 sequences recorded in the Palaearctic, respectively (Kiknadze, 2008). The larvae of *Ch. plumosus* from the Rybinsk Reservoir revealed 15 sequences forming 64 genomic combinations, with 1.3–2.2 heterozygous inversions per individual on average. The appearance of various genomic combinations was probably related to the microenvironmental conditions (Bolshakov and Shobanov, 2017).

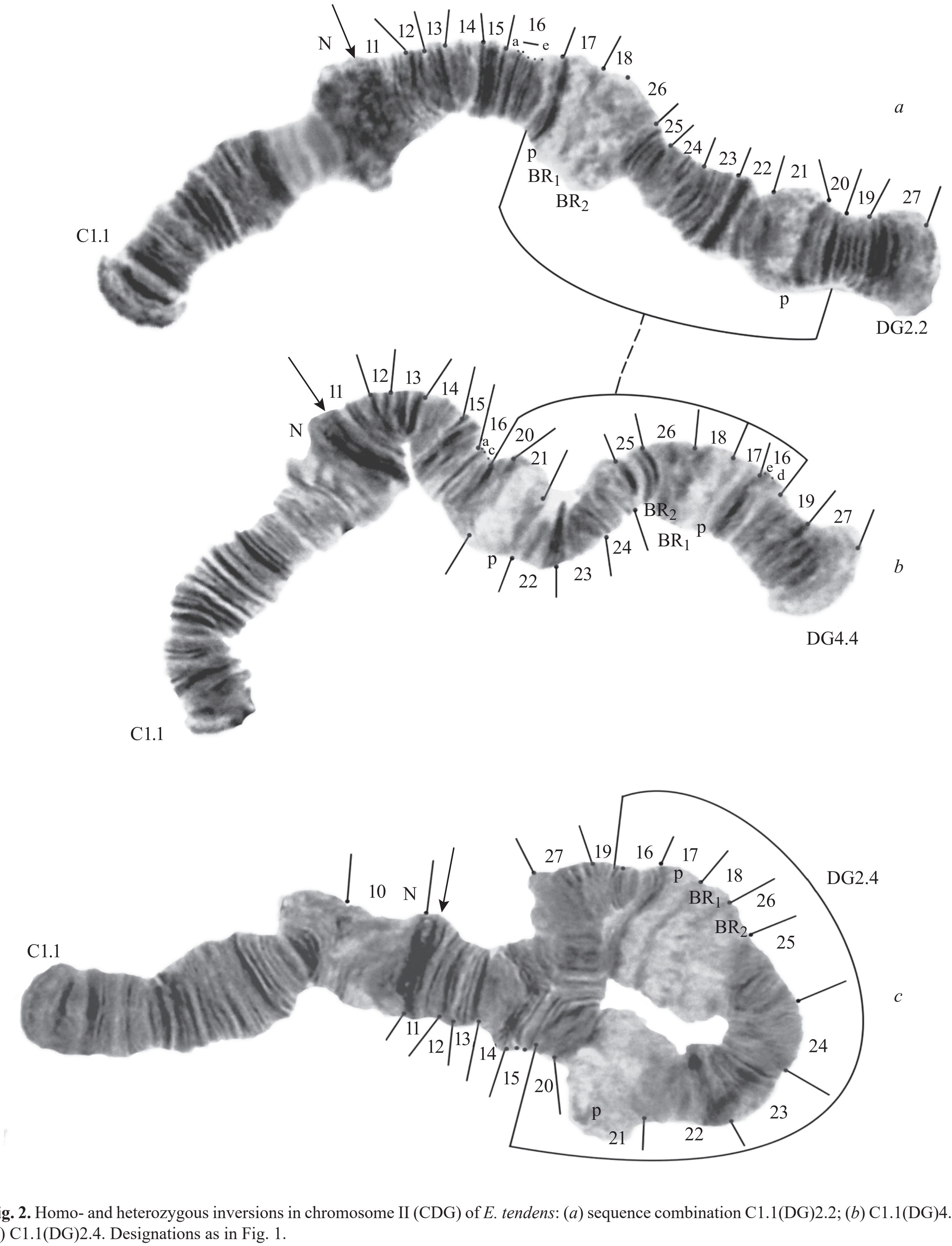

Fig. 2. Homo- and heterozygous inversions in chromosome II (CDG) of *E. tendens*: (*a*) sequence combination C1.1(DG)2.2; (*b*) C1.1(DG)4.4; (*c*) C1.1(DG)2.4. Designations as in Fig. 1.

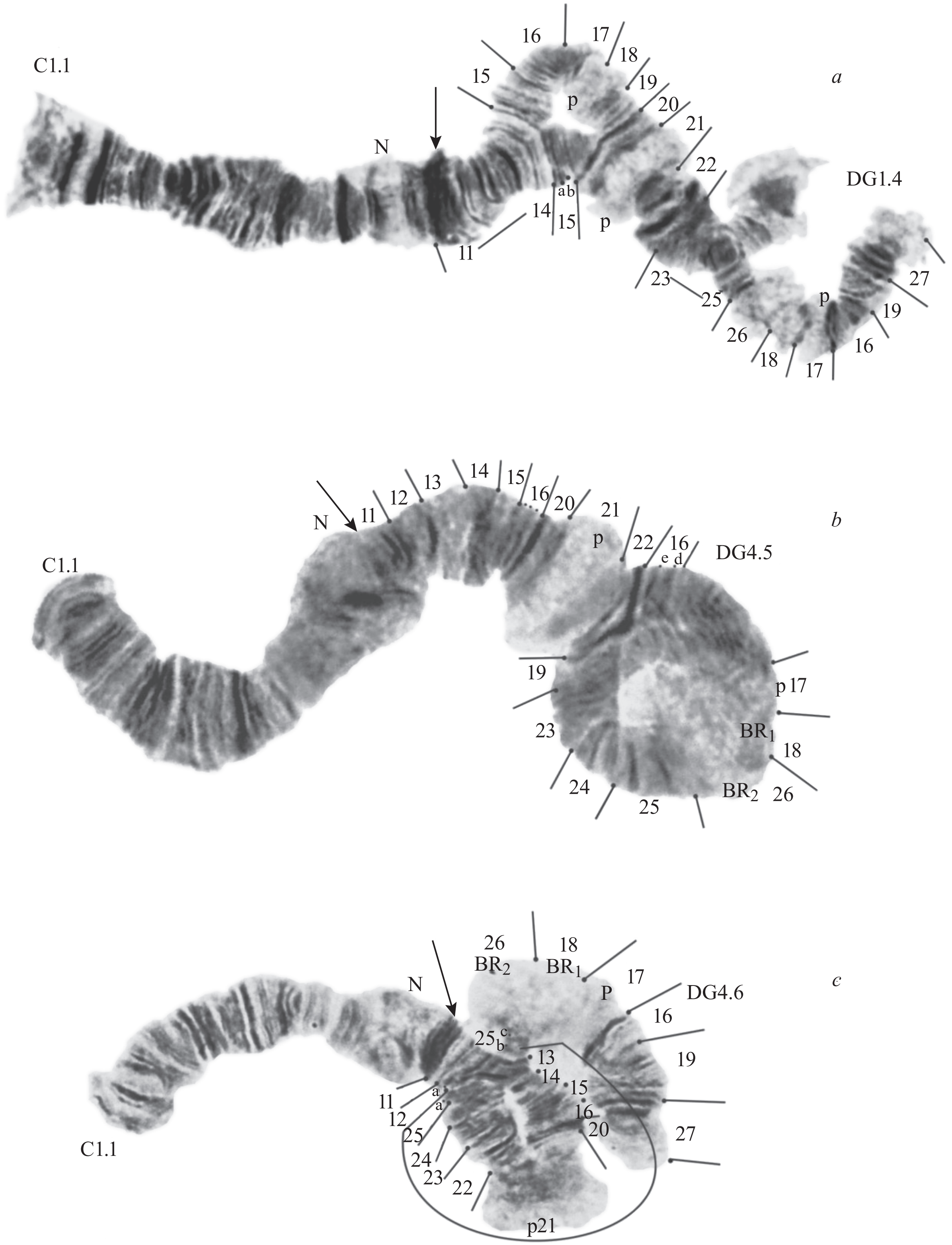

Fig. 3. Homo- and heterozygous inversions in chromosome II (CDG) of *E. tendens*: (*a*) sequence combination C1.1(DG)1.4; (*b*) C1.1(DG)4.5; (*c*) C1.1(DG)4.6. Designations as in Fig. 1.

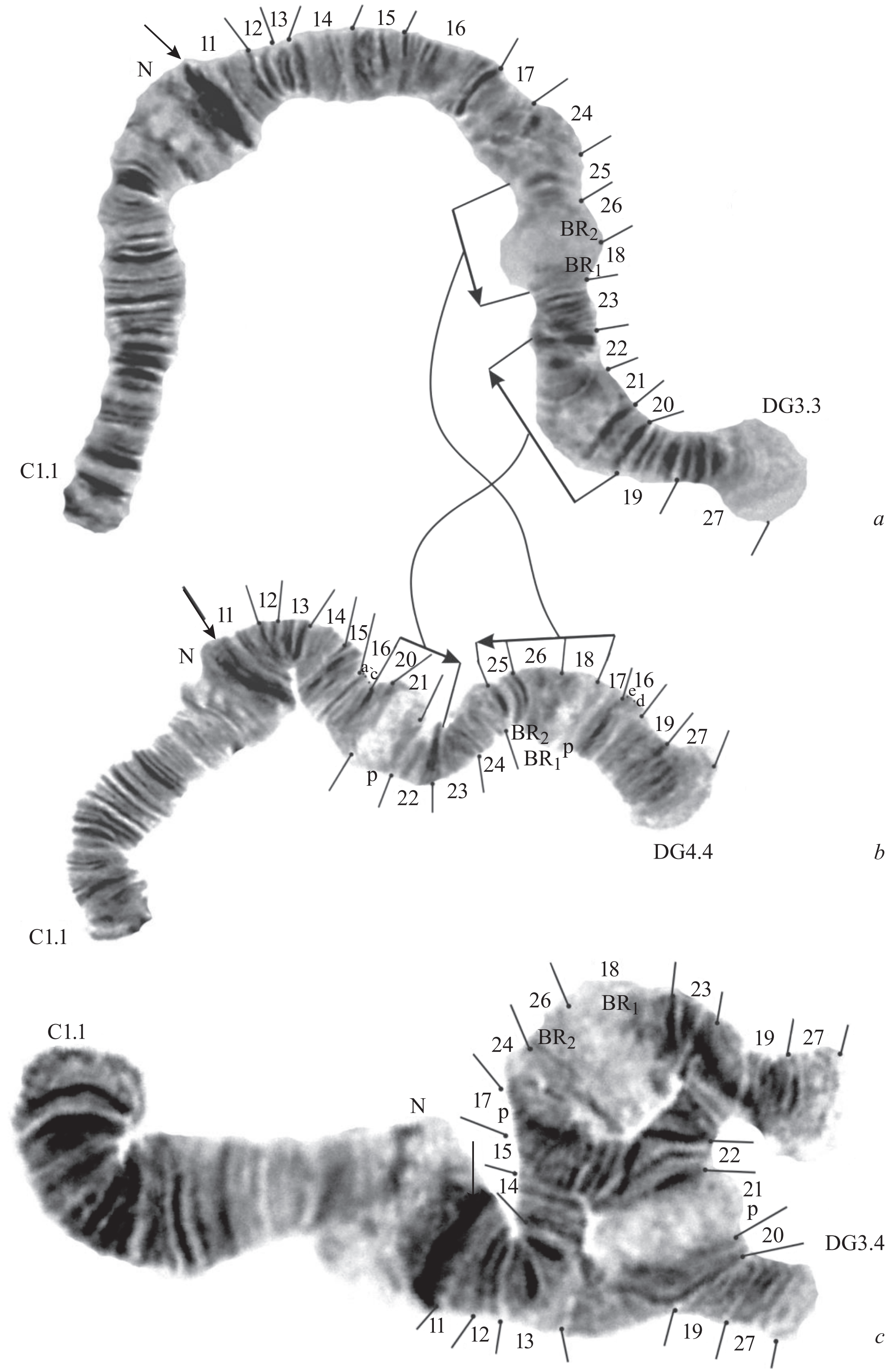

Fig. 4. Homo- and heterozygous inversions in chromosome II (CDG) of *E. tendens*: (*a*) sequence combination C1.1(DG)3.3; (*b*) C1.1(DG)4.4; (*c*) C1.1(DG)3.4. Designations as in Fig. 1.

Table 2. Frequency of occurrence of chromosome banding sequences in *E. tendens* F. from water bodies of Saratov and Volgograd provinces

Sequence	Samples																					
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
E1	0.52	0.77	0.56	0.54	0.63	0.63	0.53	0.53	0.52	0.85	0.47	0.13	0.72	0.75	0.39	0.67	0.29	0.20	0.91	0.94	0.83	0.83
E2	0	0	0.01	0	0.08	0.18	0.27	0.03	0.30	0	0.04	0.63	0.04	0	0	0.33	0.11	0.34	0.09	0.09	0.13	0
E3	0	0.14	0.07	0	0	0.10	0.09	0	0.11	0	0	0.02	0.01	0	0	0	0.03	0.02	0	0	0.21	0
E4	0.45	0.09	0.25	0.32	0.12	0	0.12	0.30	0.07	0.11	0.47	0.17	0.19	0.13	0.55	0	0.03	0.05	0	0.02	0	0.27
E5	0.03	0	0.17	0.15	0.08	0.04	0.05	0.14	0	0.02	0	0	0.04	0.12	0.05	0	0.86	0.64	0	0	0	0
F1	0.48	0.56	0.66	0.74	0.69	0.82	0.8	0.75	0.80	0.65	0.81	0.73	0.79	0.57	0.11	0.67	1	0.98	0.59	0.50	0.33	0.91
F2	0	0	0	0	0	0	0	0	0	0	0	0	0.01	0	0	0	0	0.02	0	0	0	0
F3	0.28	0.46	0.34	0.26	0.31	0.17	0.2	0.26	0.20	0.33	0.19	0.20	0.21	0.25	0.89	0.33	0	0	0.41	0.46	0.67	0.19
F4	0.03	0	0	0	0	0.04	0	0	0	0.02	0	0	0	0	0	0	0	0	0	0.04	0	0
C1	0.93	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	0	1	1	1	1
C2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
C3	0.07	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
(DG)1	0.07	0.09	0.01	0	0.04	0.44	0.18	0	0.04	0	0.14	0	0.01	0	0	0.17	0	0	0	0.09	0.13	0.30
(DG)2	0.55	0.32	0.28	0.20	0.27	0.35	0.68	0.31	0.52	0.63	0.83	0.83	0.56	0.38	0.39	0.83	0	0.02	0.74	0.67	0.67	0.56
(DG)3	0.03	0.18	0.23	0.17	0.15	0	0.39	0.29	0	0	0.01	0	0	0	0	0	0	0.01	0	0.07	0	0
(DG)4	0.32	0.23	0.45	0.56	0.46	0.20	0.09	0.36	0.44	0.19	0.01	0.04	0.29	0.49	0.44	0	0	0	0.26	0.15	0.17	0.24
(DG)5	0.02	0.18	0	0	0.08	0	0	0	0	0.17	0	0	0.15	0.13	0	0	0.17	0.27	0	0.02	0	0
(DG)6	0	0	0.02	0.06	0	0	0	0.04	0	0.02	0	0	0	0	0.17	0	0.51	0.44	0	0	0	0
A1	0	0.05	0	0	0	0.11	0.3	0	0.01	0.24	0	0	0	0	0	0	0	0	0.02	0	0.08	0.22
A2	0.52	0.46	0.53	0.52	0.58	0.57	0.76	0.36	0.65	0.28	0.89	0.97	0.43	0.25	0.22	0.67	0	0	0.50	0.65	0.63	0.11
A3	0.46	0.50	0.44	0.46	0.58	0.32	0.2	0.53	0.33	0.46	0.11	0.03	0.50	0.61	0.64	0.33	0	0	0.48	0.33	0.25	0.67
A4	0.07	0	0.03	0.02	0	0	0.04	0.11	0.01	0.02	0	0	0.07	0.13	0.11	0	0.29	0.80	0	0.02	0.17	0
B1	0.48	0.64	0.59	0.50	0.65	0.70	0.47	0.49	0.62	0.93	0.26	0.33	0.41	0.33	0.25	1	0.66	0.20	0.86	0.61	0.75	0.67
B2	0.52	0.36	0.41	0.50	0.35	0.30	0.53	0.51	0.38	0.07	0.74	0.67	0.59	0.67	0.75	0	0.09	0	0.18	0.39	0.25	0.33

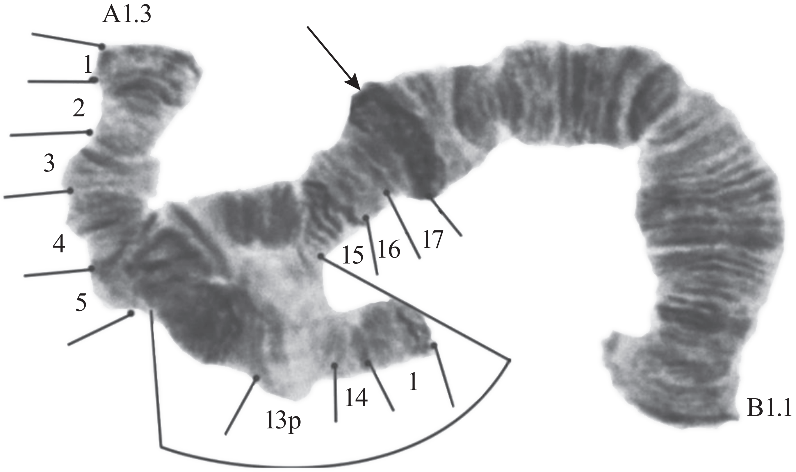

Fig. 5. A heterozygous inversion in arm A of chromosome III (AB) of *E. tendens*: A1.3.

The phytophilous chironomids developing in various plant substrates were also shown to have a varying level of chromosomal polymorphism. For example, the eurybiotic species *Glyptotendipes glaucus* (Meigen, 1818), inhabiting various submerged substrates, revealed 20 chromosome banding sequences forming 30 genomic combinations in Saratov and Kaliningrad provinces; the mean number of heterozygous inversions per individual was 0.55–0.93 (Belyanina and Durnova, 1998; Durnova et al., 2012, 2014; Vinokurova et al., 2016). In the highly specialized *G. mancunianus* (Edwards, 1929), inhabiting only living plant tissues, 17 sequences were described, the mean number of heterozygous inversions per individual being 0.11–0.53 (Durnova and Oglezneva, 2015). In the genus *Endochironomus*, the karyotype pool of *E. albipennis* (Meigen, 1830) was found to comprise 17 chromosome banding sequences and 20 zygotic combinations; that of *Endochironomus* sp. comprised 6 sequences (Durnova et al., 2015).

Altogether, 24 chromosome banding sequences were described for *E. tendens* larvae collected from the water bodies of Saratov and Volgograd provinces (Table 2); the results of analysis of their chromosomal polymorphism will be reported in the next communication. Two sequences, *tend*(DG)5 and *tend*(DG)6, were found and described for the first time; they occurred in both homo- and heterozygous states. The new data have allowed us to clarify the banding pattern of sequence *tend*(DG)4 and to map sequence *tend*E5; both sequences were previously found only in the heterozygous state.

Our karyological analysis of *E. tendens* has shown that this species is the most polymorphic of all the presently studied phytophilous chironomids, in terms of the number of banding sequences and their combinations.

COMPLIANCE WITH ETHICAL STANDARDS

The authors declare that they have no conflict of interest. All the applicable international, national, and/or institutional guidelines for the care and use of animals were followed. All the procedures performed in studies involving animals were in accordance with the ethical standards of the institution or practice at which the studies were conducted.

REFERENCES

Belyanina, S.I., Larval karyotypes of *Endochironomus tendens* F. from different regions of the Volga River, *Tsitologiya*, 1978, vol. 20, no. 5, p. 596.

Belyanina, S.I., Karyotypic analysis of chironomids (Diptera, Chironomidae) in the Fauna of the USSR, *Doctoral Dissertation in Biology*, Moscow, 1983.

Belyanina, S.I., The karyotype of the Volga chironomid *Demeijerea rufipes* L., an endoparasite of sponges, in *Kariosistematika bespozvonochnykh zhivotnykh II. Sbornik nauchnykh trudov* (Karyosystematics of Invertebrates II: Collected Papers), St. Petersburg, 1993, p. 55.

Belyanina, S.I. and Durnova, N.A., Morphology and chromosomes of phytophilous *Glyptotendipes glaucus* (Diptera, Chironomidae) from reservoirs of Saratov Region: 2. Karyotypic analysis, *Zool. Zh.*, 1998, vol. 78, no. 12, p. 243.

Bolshakov, V.V. and Shobanov, N.A., The effects of microenvironmental conditions on the cytogenetic population structure of *Chironomus plumosus* in the Rybinsk Reservoir, *Tr. Inst. Biol. Vnutr. Vod*, 2017, vol. 80, p. 20.

Demin, S.Yu. and Shobanov, N.A., Karyotype of *Chironomus entis* of the *plumosus* group in the European part of the USSR, *Tsitologiya*, 1990, vol. 32, no. 10, p. 1046.

Durnova, N.A., Chromosome polymorphism and cytotypes of *Endochironomus tendens* F. (Diptera, Chironomidae) from Saratov and Samara regions, *Tsitologiya*, 2009a, vol. 51, no. 7, p. 592.

Durnova, N.A., Chromosome analysis of chironomids (Diptera: Chironomidae) – obligatory miners of freshwater sponges (Porifera), *Comp. Cytogenet.*, 2009b, vol. 3, no. 1, p. 25.

Durnova, N.A., Cytogenetic characteristics of *Stenochironomus gibbus* (Fabricius, 1794) (Diptera, Chironomidae), an obligate miner of submerged wood, *Tsitologiya*, 2010a, vol. 52, no. 7, p. 883.

Durnova, N.A., Chironomids in the periphyton of Saratov Province: ecological features, morphology, and cytogenetics (Diptera, Chironomidae, Chironomini), *Extended Abstract of Doctoral Dissertation in Biology*, St. Petersburg: Zool. Inst., 2010b.

Durnova, N.A. and Oglezneva, A.A., Recent data on chromosome polymorphism of phytophilous *Glyptotendipes mancunianus* Mg. (Diptera, Chironomidae) from different reservoirs of the Saratov Province, in *Entomologicheskie i parazitologicheskie issledovaniya v Povolzh'e. Sbornik nauchnykh trudov* (Entomological and Parasitological Research in the Volga Basin: Collected Papers), *Issue 12*, Saratov: Saratov Univ., 2015, p. 27.

Durnova, N.A., Voronin, M.Yu., and Sukhova, E.I., Biotopic distribution of periphytic Chironomidae in water reservoirs of Saratov region, *Povolzh. Ekol. Zh.*, 2011, vol. 3, p. 304.

Durnova, N.A., Voronin, M.Yu., and Oglezneva, A.A., Selectivity of settling certain types of macrophytes by chironomid miners (Diptera, Chironomidae), in *Entomologicheskie i parazitologicheskie issledovaniya v Povolzh'e. Sbornik nauchnykh trudov* (Entomological and Parasitological Research in the Volga Basin: Collected Papers), *Issue 10*, Saratov: Saratov Univ., 2012, p. 55.

Durnova, N.A., Voronin, M.Yu., and Oglezneva, A.A., Chromosomal polymorphism and cytogenetic divergence o *Glyptotendipes glaucus* (Meigen) (Diptera: Chironomidae) in the reservoirs of Saratov Province, *Tr. Russ. Entomol. Ob-va*, 2014, vol. 85, no. 2, p. 19.

Durnova, N.A., Sigareva, L., and Sinichkina, O., Chromosome analysis of *Endochironomus albipennis* Meigen, 1830 and morphologically similar *Endochironomus* sp. (Diptera, Chironomidae) from water bodies of the Volga region, Russia, *Comp. Cytogenet.*, 2015, vol. 9, no. 4, p. 579.

Gunderina, L.I., Aimanova, K.G., Proviz, V.I., Rakisheva, A.Zh., Kiknadze, I.I., Istomina, A.G., Salova, T.A., and Butler, M.G., Cytogenetic differentiation of natural and laboratory populations of *Camptochironomus tentans* (Fabricius) (Chironomidae: Diptera), *Genetika*, 1996, vol. 32, p. 53.

Kiknadze, I.I., The role of chromosomal polymorphism in divergence of populations and species of the genus *Chironomus* (Diptera), *Zool. Zh.*, 2008, vol. 87, no. 6, p. 686.

Kiknadze, I.I., Butler, M.G., Aimanova, K.G., Gunderina, L.I., and Cooper, J.K., Geographic variation in the polytene chromosome banding pattern of the Holarctic midge *Chironomus* (*Camptochironomus*) *tentans* (Fabricius), *Can. J. Zool.*, 1996, vol. 74, no. 1, p. 171.

Kiknadze, I.I., Michailova, P., Istomina, A.G., Golygina, V.V., Int Panis, L., and Krastanov, B., The chromosomal polymorphism and divergence of populations in *Chironomus nuditarsis* Str. (Diptera, Chironomidae), *Tsitologiya*, 2006, vol. 48, no. 7, p. 595.

Makarchenko, E.A. and Makarchenko, M.A., Family Chironomidae: non-biting midges, in *Opredelitel' nasekomykh Dal'nego Vostoka Rossii* (Key to Insects of the Russian Far East), Vol. 6, Part 4: *Dvukrylye i blokhi* (Diptera and Siphonaptera), Vladivostok: Dalnauka, 2006, p. 204.

Michailova, P.V., Comparative karyological analysis of species of genus *Endochironomus* Kieff., *Entomology*, 1987, vol. 29, p. 105.

Michailova, P.V., The polytene chromosomes and their significance to the systematic and phylogeny of the family Chironomidae, Diptera, *Acta Zool. Fenn.*, 1989, vol. 186, p. 1.

Michailova, P.V., *Endochironomus tendens* (F.) (Diptera, Chironomidae) an example of stasipatric speciation, *Neth. J. Aquat. Ecol.*, 1992, vol. 26, p. 173.

Michailova, P.V. and Gercheva, P., Cytotaxonomical characteristics of the genus *Endochironomus* Kieff. (Diptera, Chironomidae), *Caryologia*, 1982, vol. 35, p. 33.

Petrova, N., Michailova, P., and Bovero, S., Cytogenetic characteristics of *Chironomus nuditarsis* Str. (Chironomidae, Diptera) and its relationship with species from the *plumosus* group, in *Late 20th Century Research on Chironomidae: an Anthology from the 13th Int. Symp. on Chironomidae, Freiburg, 5–9 September 1997*, Aachen: Shaker, 2000, p. 201.

Shobanov, N.A., The karyotype pool of *Chironomus plumosus* L. (Diptera, Chironomidae). Intra- and interpopulation polymorphism, *Tsitologiya*, 1994a, vol. 36, no. 11, p. 1129.

Shobanov, N.A., The karyotype pool of *Chironomus plumosus* L. (Diptera, Chironomidae). III. The dependence of the karyotype pool parameters on the sample size and the population structure, *Tsitologiya*, 1994b, vol. 36, no. 3, p. 289.

Vinokurova, N.V., Kalinina, E.A., and Stol, E.E., Karyotype and inversion polymorphism of natural populations *Glyptotendipes glaucus* Meigen, 1818 (Diptera, Chironomidae) from the small reservoirs of Kaliningrad, *Ecol. Genet.*, 2016, vol. 14, no. 4, p. 41.